

Theoretical and Computational Studies of Hydrodynamics-based Separation of Particles and Polymers in Microfluidic Channels

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Summary

The advent of microfluidic technology presents many difficulties but also many opportunities for separation science. Leveraging the potential of micro- and nanofluidic geometries is not only a matter of shrinking systems. Miniaturization can shift the relative importance of physical phenomena leading to separation. Theoretical and computational studies into the consequences of miniaturization are vital. Mesoscopic, multi-particle collision dynamics simulations are performed on polyelectrolytes and hard, colloidal solutes. Multiple variations of this simulation algorithm are implemented to achieve versatility for simulating non-equilibrium flows and dispersed solutes. The algorithm is extended to simulate the effects of finite Debye layers on the electro-hydrodynamics of electrophoresing macromolecules and used to study the electrophoresis of charged oligomers, polyelectrolytes and polyampholytes in both free-solution and confined geometries. Multi-particle collision dynamics simulations of hydrodynamic chromatography and field-flow fractionation are also performed to test the predictions of the derived unified, ideal retention theory. This unified, ideal retention predicts the transitions between multiple operational modes, including Faxén-mode FFF. Simulations and the theory show that increases in drag due to hydrodynamic interactions with microfluidic channel walls perturb the retention curves from the ideal predictions at large particle sizes. Further complications to field-flow fractionation including undesirable forces perpendicular to the flow direction, slip at channel walls and rectangular channel geometries are investigated. These theoretical studies lead to the proposal of several novel fractionation techniques, namely adverse-mode FFF, slip-mode FFF and polymer/depletant HC.

Sommaire

Le développement des technologies microfluidiques présente de nombreuses difficultés, mais présente également plusieurs opportunités pour le design de nouveaux procédés de séparation. L'utilisation du potentiel des géométries micro et nanofluidiques n'a pas seulement comme but de réduire la taille des systèmes. En effet, la miniaturisation peut même changer l'importance relative des phénomènes physiques qui entrent en jeu la séparation. Des études théoriques et informatiques visant à comprendre les conséquences de la miniaturisation sont essentielles. Des simulations mésoscopiques de dynamiques de particules à collision multiples ont été utilisées pour étudier des polyélectrolytes et des solutés colloïdaux durs dans divers cas hydrodynamiques. De plus, l'algorithme a été modifié pour simuler l'effet de couches de Debye finies sur l'électrodynamique de macromolécules durant l'électrophorèse, en particulier l'électrophorèse d'oligomères, de polyelectrolytes et de polyampholytes chargés, tant dans des solutions libres que dans des géométries confinées. Des simulations de chromatographie hydrodynamique et de fractionnement flux-force ont aussi été exécutées pour tester les prévisions de la théorie de rétention idéale unifiée que nous proposons. Cette nouvelle théorie prédit des transitions entre de nombreux modes opérationnels, dont le mode Faxén. Les simulations et la théorie montrent que les augmentations de freinage causées par les interactions hydrodynamiques avec les parois du canal microfluidiques perturbent les courbes de rétention prévues pour des particules de grandes tailles. D'autres complications du fractionnement flux-force, y compris des forces indésirables perpendiculaires à la direction du débit, des glissements sur les parois du canal et des géométries de canal rectangulaires ont aussi été examinées. Ces études théoriques mènent à la proposition de plusieurs techniques de fractionnement novatrices.

Dedicated to Amanda,
to whom every thesis ever written should be dedicated to.

Acknowledgments

This thesis would never have existed if it were not for the support of countless people in my life.

Principally, I would like to thank Gary Slater but I do not have elegant enough words to express how much of an impact you have had on me. You are a good scientist, a good supervisor and a good person. I have learned so much from you.

I would never have had the opportunity to do this research if it was not for the generous support of NSERC. This support included the chance to visit Pai-Yi Hsiao's research group in Taiwan for which I am very grateful. I have had the good fortune of having many such senior collaborators who have guided me along the way, including Mark Whitmore and especially Michel Godin, whom I respect greatly. James Harden has in many ways provided my graduate physics education and I could not have asked for a better teacher.

The Slater Research Group has been filled with so many inspiring and creative members. In particular, working with Owen Hickey shaped the way that I approach research. Likewise, Hendrick de Haan has taught me what it means to be a real scientist and David Sean has shown me the power of always asking "why?" The group has been a remarkable place to explore polymer physics and that is because of all the excellent scientists that have been members over the years. This thesis required a lot of editing and Martin Bertrand, David Sean and Amanda Shendruk greatly improved it by their critical readings. Amanda also offered endless help creating the schematics.

Finally, I'd like to thank my family. You've always supported me in everything that I have done. Thank you for always being there for me.

Thank you, everyone.

Publication List

1. *Hydrodynamic Chromatography and Field Flow Fractionation in Finite Aspect Ratio Channels*, **T.N. Shendruk**, G.W. Slater, *Journal of Chromatography A*, Submitted October 3, 2013.
2. *Field-flow fractionation and hydrodynamic chromatography on a microfluidic chip*, **T.N. Shendruk**, R. Tahvildari, N. Catafard, L. Andrzejewski, C. Gigault, A. Todd, L. Gagne-Dumais, G.W. Slater, M. Godin, *Analytical Chemistry*, **85**, 12, 5981-5988 (2013).
3. *Structure of polyelectrolyte brushes subject to normal electric fields*, Y.-F. Ho, **T.N. Shendruk**, G.W. Slater and P.-Y. Hsiao, *Langmuir*, **29**, 7, 2359-2370 (2013).
4. *Controlling grafted polymers inside cylindrical tubes*, T. Suo, **T.N. Shendruk**, O.A. Hickey, G.W. Slater and M. Whitmore, *Macromolecules*, **46**, 3, 1221-1230 (2013).
Citations: 1¹
5. *Simulations of the free-solution electrophoresis of polyelectrolytes with a finite Debye length using the Debye-Hückel approximation*, O.A. Hickey, **T.N. Shendruk**, J.L. Harden and G.W. Slater, *Physical Review Letters* **109**, 9, 098302 (2012).
Citations: 3¹
6. *Can slip walls improve field-flow fractionation or hydrodynamic chromatography?*, G.W. Slater and **T.N. Shendruk**, *Journal of Chromatography A* **1256**, 206-212 (2012).
Citations: 2¹
7. *Electrophoresis: When hydrodynamics matter*, **T.N. Shendruk**, O.A. Hickey, G.W. Slater and J.L. Harden, *Current Opinion in Colloid & Interface Science* **17**, 2, 74-82 (2012).
Citations: 12¹

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8. *Operational-modes of field-flow fractionation in microfluidic channels*, **T.N. Shendruk** and G.W. Slater, *Journal of Chromatography A* 1233, 100-108 (2012).

Citations: 3¹

9. *Modeling the separation of macromolecules: A review of current computer simulation methods*, G.W. Slater, C. Holm, M.V. Chubynsky, H.W. de Haan, A. Dubé, K. Grass, O.A. Hickey, C. Kingsburry, D. Sean, **T.N. Shendruk** and L. Zhan, *Electrophoresis* 30, 792-818 (2009).

Citations: 49¹

¹Number of citations as of December 14, 2013.

Statement of Originality

I hereby declare that this thesis results from my own investigations and that, to the best of my knowledge, they constitute original research performed under the supervision of Professor Gary Slater.

In producing this work, I have collaborated with many scientists, both internal to the Slater Research Group and external. While I wrote the multi-particle collision dynamics (MPCD) code (§ 3) from square one, Dr. Owen Hickey and I coupled the algorithm to a molecular dynamics program (§ 3.5.2) that was originally written by Dr. Frédéric Tessier. Dr. Hickey proposed the idea of the mean-field MPCD-MD Debye-Hückel algorithm and together we implemented it (§ 4.2) and published a peer-reviewed letter [1]. In this letter, our mesoscopic simulations are compared to the results of explicit ion simulations and experimental data that were provided to us by Professor Christian Holm and Dr. Kai Grass. In collaboration with Dr. Martin Bertrand, the mesoscopic algorithm was applied to the electrophoresis of confined polyelectrolytes (§ 4.4). My knowledge of electrophoresis in microfluidic devices and the literature of the field greatly benefited from the opportunity to pen a review article with Dr. Hickey and Professor James Harden [2]. The Slater Research Group reviewed computational methods for simulating the separation of macromolecules in 2009 and I contributed section 2.3 on mesoscopic models of fluids and section 3.2 on free-solution electrophoresis (with visiting researcher Dr. Kai Grass) to this review [3].

To verify my theoretical work on unifying field flow fractionation [4], Professor Michel Godin and his research group designed a 18 μm tall microfluidic channel, from which they obtained video microscopy data. These experiments were compared to my multi-particle collision dynamics simulations and to a modified retention theory that I worked out. This theory relies on the results from studies using multipole expansion method by Pasol *et. al.* [5]. The combined experimental, theoretical and computational results are reported in a manuscript co-authored by Radin Tahvildari [6]. Also included in the manuscript (and this

thesis) are various experimental results from the literature of the retention ratio about the steric-inversion point (Figure 7.1.1). These were reproduced in Reference [6] with permission from Dr. Thadeus Schauer [7], Dr. Myeong Hee Moon [8, 9], Dr. Luo Jian [10]), and Dr. Josef Janča [11], and have been reproduced here with the permission of the journal.

Further complications to field-flow fractionation were considered by Professor Slater and I. The slip-FFF work is published [2] and the investigation into the effects of rectangular channels is currently under review. The article proposing device selectivity is also submitted. This article resulted out of comments from referee # 3 of Reference [2] and discussions with David Sean and Dr. Hendrick de Haan. Discussions with Charlie Ponyik were quite useful as well.

In the course of my studies, I have been given the opportunity to collaborate on many projects that are not directly a part of the work presented here. With Dr. Hickey, we collaborated with Professor Mark Whitmore and Dr. Tongchuan Suo to investigate polymer brushes in capillaries [12]. We provided molecular dynamics simulations for comparison to their self-consistent mean-field theory. I also participated in research on polyelectrolyte brushes led by Professor Pai-Yi Hsiao and his student Yu-Fan Ho [13]. I aided in theoretical interpretation of Ho's simulation results and the production of the manuscript. These papers have been appended here with the permission of Professor Hsiao and Professor Whitmore.

The text and many of the figures are based heavily on the published articles and permission to reproduce them was obtained from the various journals. Figures 2.4.1 and 2.4.3 were made in part with David Sean and I am indebted to Amanda Shendruk for endless help producing many of the schematics included in this thesis.

Statement of Contribution

The following researchers contributed to sections 4.2-4.3: Owen Hickey proposed the mean-field MPCD-MD Debye-Hückel algorithm. Tyler Shendruk provided the MPCD code and worked with Owen Hickey to couple it to a pre-existing MD program. They co-coded the mean-field MPCD-MD Debye-Hückel algorithm. James Harden co-supervised this work.

The following researchers contributed to section 4.4: Martin Bertrand began studying confined polyelectrolytes in his Masters project under the supervision of Gary Slater. Tyler Shendruk applied the mean-field MPCD-MD Debye-Hückel algorithm to this system and analyzed the results.

The following researchers contributed to chapter 6: David Sean and Hendrick de Haan collaborated on the development of these thoughts with the author.

The following researchers contributed to section 7: Radin Tahvildari led the experimental work; Nicolas M.-Catafard measured density; Lukasz Andrzejewski developed tracking software; Andrew Todd helped to design the microfluidic devices; Laurent Gagné-Dumais and Christian Gigault aided with the data analysis and experimental work; Michel Godin supervised the experimental aspects. Tyler Shendruk was responsible for the simulations and for determining the form of the wall-induced lag.

The following researchers contributed to chapter 8: Martin Bertrand (under the supervision of James Harden) and Hendrick de Haan ran simulations. Tyler Shendruk performed the theoretical work and corresponding analysis of simulation data.

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It would be dramatic to say that saloon noises and shootings kept me awake that night, but in fact it was a banging radiator that disturbed my night's rest . . . I spent most of the remainder of the night thinking about ways to improve and implement [field-flow fractionation].

J. Calvin Giddings [14]

1

Introduction

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Each morning while writing this thesis, I poured myself a cup of coffee before beginning my work. Each morning I would add a drop of cream and watch the eddies for less than a moment before a flick of my spoon mixed them out of existence. Each morning the coffee and the cream mingled together into a single smooth, homogeneous mixture. Each morning this was done with less effort than is worth mentioning.

Yet, the constituent lipids and protein complexes of the cream remained intact. They had only been dispersed throughout the hot water, just as the coffee grinds before them. Dispersion was easy, but consider attempting to extract the cream after mixing. What a monumental task that would be. How would you even do such a thing? To what lengths would you have to go just to undo a lazy swish of my spoon?

1.1 Foreword

In our everyday world, mixing and chaos can feel natural and, at times even inevitable. On the other hand, separation and sorting those mixed systems can require a concerted effort. This thesis is about separation science. It reports on the projects that I have contributed to throughout my doctoral studies that have attempted to advance separation science — particularly fractionation within microfluidic devices.

Separation scientists have developed a legion of techniques to purify, fractionate and separate mixed ensembles of dispersed entities. My work has focused on the separation of solutes (dispersed constituent particles) in a solvent (fluid medium). In particular, this thesis concerns itself with separation processes that occur on a micro- or nanofluidic device and rely in some way on hydrodynamic interactions. The field focuses on three primary goals:

1. To identify if a specific constituent species is present in a solution.
2. To separate a solution into its constituent parts.
3. To measure and/or compare the properties of one or more constituent populations.

Since systems are inclined to exist in a mixed state, physical phenomena must be intelligently applied to engineer separation.

The need for separation techniques is ageless. Indeed, separation systems have been used by humanity for thousands of years. In order to process ochre (iron-rich earth) into pigments,

prehistoric South Africans added marrow fat, which was extracted from bones by heating [15]. Neolithic Europeans separated cheese curds from whey through pottery sieves [16]. From these humble beginnings evolved separation science, which was using filter paper to separate amino acids by the middle of the last century [17].

Much of separation science remains focused on large, industrial scale applications such as separation in petroleum processing, in food processing, in metallurgy, *etc.* But much of it also occurs in laboratories and is done with extreme finesse. Micro- and nanofluidics are one of many very promising avenues for advancing our ability to perform such highly precise fractionation procedures. Such “lab-on-a-chip” devices hold the potential to deal with small samples in an automated and integrated manner, reducing time and costs. It has been suggested that the impact of mature microfluidic techniques could be as substantial as the advent of integrated circuits once was for electronics [18].

Miniaturizing separation techniques is not as simple as just making smaller plumbing. Physical phenomena can be drastically different and, at times, non-intuitive when occurring at microscopic length scales. Miniaturization requires theoretical and computational thought.

1.2 Summary

This thesis reports the theoretical and computational investigations into miniaturizing separation techniques that my supervisor Professor Gary Slater and I have undertaken in recent years with our collaborators. In particular, we studied in which situations and in what manner long-range hydrodynamic interactions play an important role in microfluidic separation methods. The work performed is theoretical and computational. It often seeks issues that arise within microfluidic systems, issues that would not have been present or have been of negligible importance in the equivalent traditional or macroscopic separation system. However, it is not pessimistic. The results of these studies have led us to propose ideas for novel separation techniques.

The majority of this work has been published in the peer-reviewed scientific literature and we have every expectation that the remainder will be published shortly. These papers are appended to the end of this dissertation. Therefore, why write a thesis? Why read a thesis? It was my intent to tailor this manuscript to graduate students who are just launching their own studies into the separation systems discussed here, and to researchers who may wish to

extend this work. These readers can find the individual published articles in Appendix E but the main body of this thesis offers the thread of thought that bridges them. With this audience in mind, this thesis has been written with somewhat of a storyarc.

It begins with a background chapter (§ 2). This chapter presents separation science from the perspective of transport phenomena, giving a unified framework for each technique. The thesis next presents the multi-particle collision dynamics algorithm. With the hope that future students will take advantage of this versatile code, this chapter describes the implementation in some detail. It is not focused on the computer science side of producing the code, but rather on giving the reader an understanding of the models used. After introducing the tools that we used in these first two chapters, the thesis reports our investigations of specific systems.

The first phenomenon considered is electrophoresis. Applying an electric field to move charged macromolecules (such as DNA) through a solvent is a very natural idea. However, the results of doing this simple act are often surprising. In order to study the non-intuitive behaviour of these chains, a novel mesoscopic simulation technique is developed. This technique captures the electro-hydrodynamic interactions within the chain in a coarse-grained manner. This is work that I did with Owen Hickey and Professor James Harden.

This algorithm is used to simulate the electrophoretic mobility of charged macromolecules in a variety of situations, including chains with different Debye layers and chains with different charge distributions. Martin Bertrand and I have applied it to study the effect of confinement chains' mobility.

The second set of investigations revolve around hydrodynamic chromatography. In my opinion, this is a wonderful topic. The system is amazingly simple and yet Professor Slater and I were able to see such rich behaviour. When I write “hydrodynamic chromatography” in this thesis I specifically mean the separation technique in which solutes are carried through an open channel by a flowing solvent. As solutes are carried along by the flow, their centres are excluded from the near-wall regions by steric repulsion. Larger solutes have a larger exclusion zone and are subject to the faster flowing velocity in the the centre region of the channel than the smaller solutes. Field-flow fractionation is hydrodynamic chromatography but with an external field applied perpendicular to the flow that establishes a non-homogeneous concentration distribution.

There are multiple regimes of field-flow fractionation, which were previously treated by distinct theories. In macroscopic systems this is acceptable since scientists stick to a single

regime for a set of experiments. In microfluidic systems, however, it is far more likely that a sample can span multiple regimes. In response to this motivation, Professor Slater unified the ideal theory describing separation. We derived a single theory that encompasses all of the previous theories. In fact, in doing so we even predicted a previously unsuspected regime, which we named Faxén-mode field-flow fractionation.

Since the unified theory is quite idealized, we considered many complications. Simulations were performed and theory was extended to account for increased drag due to the ubiquitous presence of microfluidic walls. This work was done in collaboration with Professor Michel Godin and his student Radin Tahvildari, who performed meticulous experiments to confirm our unified theory. Radin and I co-authored a paper presenting the culmination of theoretical, computational and experimental work.

One of the issues that the experimental work revealed was that the channels have a finite width that the ideal theory does not account for. Therefore, we considered field-flow fractionation in rectangular channels. We found that a finite aspect ratio does not affect the transport of solutes as much as one might guess. This null result is interesting because it suggests that microfluidic channels can be designed with little concern for channel width.

We also considered walls with a finite slip. This is a complication that may result from the porous channel walls used in some macroscopic field-flow fractionation apparatus. Surprisingly, our theoretical investigations discovered an unpredicted separation method. We named this method slip-mode field-flow fractionation.

We attempted an extremely simplified model to predict the transport of solutes when lift plays a non-negligible role. Although this model is likely of little use because it is so over-simplified, it led to yet another proposal, which we named adverse-mode field-flow fractionation.

With so many new techniques being proposed, we had to ask ourselves what the best way to compare between them might be. Many conversations with David Sean and Hendrick de Haan led to a manuscript in which we questioned the traditional metrics used by the field-flow fractionation community to judge separation power and proposed modified versions. These metrics are device oriented (rather than solute oriented). They preserve many of the advantages of the traditional definitions but avoid much of the difficulties.

To end this thesis, I present our work on coil/globule transitions induced in simple bacterial chromosomes by small depletant particles. Much simulation work was done by Hendrick de Haan and Martin Bertrand in order to produce the data that led us to develop the simple

but robust framework for discussing the transition from a swollen coil to a collapsed globule. Whether the action of these inert depletant particles is sufficient to cause collapse and what the nature of any such collapse would be was not certain at the onset of this work and so this chapter has much necessary overhead. However, it is proposed that the transition can be used in conjunction with hydrodynamic chromatography in order to glean information about the internal structure of chromosome chains.

*Fundamentals means old stuff because that's now
fundamental.*

Dave Weitz [19]

2

Background: Separation Science

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Separation science is a wide-ranging topic, which requires broad foundations. Before discussing its novel contributions to the field of separation science, this thesis will establish a general perspective and vocabulary. Although the fundamentals are disparate, this introduction seeks to cast the topic as a uniform, theoretical framework, which will prove valuable when considering the specific examples of microfluidic separation devices discussed within this work (electrophoresis, hydrodynamic chromatography and field-flow fractionation).

Theoretical separation science in microfluidic devices requires key concepts from a variety of fields (Figure 2.0.1):

- Primarily, separation science is **statistical** in nature since it most often involves large ensembles of solute particles (or analytes) that thermally sample many states.
- However, these particles are being acted on specifically to drive their distributions out of equilibrium, which makes separation science a branch of **non-equilibrium thermodynamics**.
- Many fractionation techniques separate particles based on some transport property that differs between species of analytes. Such techniques are called **chromatographic** and are examples of **transport phenomena**, specifically **mass transport**.
- In all cases, the ensembles of solutes are dispersed in a liquid medium (called the **solvent** or carrier fluid), the velocity fields of which are described by **fluid dynamics**.
- Finally, ions abound within solvents and they interact with the surfaces of microfluidic walls and/or the particles, which themselves are quite often charged. When electric fields are applied, these free ions **electrophorese**, dragging the fluid medium along with them and generating fields that obey **electro-hydrodynamics**.

Although this is a large amount of academic material, reviewing it contributes a theoretical framework that naturally incorporates each of these seemingly distinct topics into a body of fundamental principles.

This introduction stresses that although thermodynamic systems can show spontaneous phase segregation, very fundamental reasons underlie systems' preference for mixing (§ 2.1). Work must be performed to combat mixing and these concepts lead directly to a general description of transport phenomena within a dispersive medium (§ 2.2). The fluid medium itself plays an essential role in each of the systems that will be discussed and its very nature affects the transport of dispersed particulates in microfluidic devices (§ 2.3). Accounting for

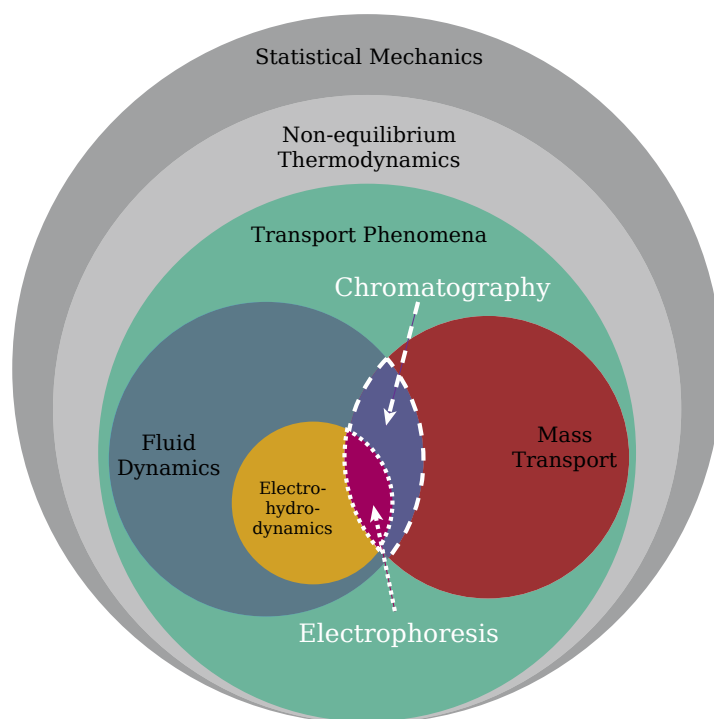


Figure 2.0.1: Chromatographic separation science is a subset of mass transport (§ 2.2.2) and fluid dynamics (§ 2.3). In the case of electrophoresis, electro-hydrodynamics are necessary to describe electrophoresis of polyelectrolytes (§ 2.4). Both mass transport and fluid dynamics can be described as a field of transport phenomena (§ 2.2), which is a branch of non-equilibrium thermodynamics (§ 2.1.2).

hydrodynamic interactions propagated through the fluid medium can be a computationally expensive endeavor because they are long-range. If the solvent is an electrolyte, then the freely diffusing ions within the fluid medium can rearrange their distribution around charged analytes or solutes, which not only screens Coulombic interactions but also, to a certain extent, hydrodynamic interactions (§ 2.4).

Topics will be approached from a continuum perspective, although microscopic processes lie at the heart of each and many of the subjects considered here can be conveniently viewed from a microscopic perspective. Since this chapter provides background information, its content can, of course, be found elsewhere, though perhaps not with such a strong focus on separation processes involving hydrodynamic and electro-hydrodynamic interactions. Readers familiar with the relevant background information are encouraged to skip directly to their sections of interest and utilize the list of variables and the glossary provided (§ D).

2.1 Beating Entropy

Why is mixing generally easier than separation? It is a child-like question that has a simple and fundamental answer:

Entropy is greater when species are mixed than when they are ordered into well-resolved, separate volumes (Figure 2.1.1).

This is because there are more states available to the system. Of course, mixing does not always occur. In some mixtures, interactions between the particles of different species can lead to phase segregation. An everyday example is the segregation of oil and water that arises from enthalpic interactions specific to pairs of species but a more interesting example is the entropically mediated depletion attractions that are discussed in § 8. Although a subpopulation of constituent particles within such systems may be inclined to be more ordered, the system as a whole has more states available to it. The entropically motivated propensity toward increased disorder and mixing is universal.

2.1.1 Equilibrium Thermodynamics and Entropy of Mixing

Though it is the flux of particles from one subsystem to another that is the primary interest of this thesis, any macroscopic, extensive variable X_i may be tied to the entropy of the system S . The entire set of all relevant generalized coordinates will be represented as $\mathbf{X} = \{X\} = \{\text{particle number } (N), \text{ internal energy } (U), \text{ volume } (V), \text{ length, area, magnetization, polarization, etc.}\}$. A generalized coordinate $X = \vec{X}$ may itself be a vector, but for clarity's sake this will not be explicitly written, unless discussing a specific example.

Entropy, Generalized Coordinates and Thermodynamic Forces

The entropy of the system depends on each of the generalized thermodynamic coordinates and the relationship

$$S = S(\mathbf{X}) \tag{2.1}$$

is called the *fundamental equation of thermodynamics* (in the entropy representation, as one can always do a Lagrange transformation to a different thermodynamic potential such as

energy, enthalpy, free energy [Gibbs or Helmholtz] or grand potential). The total entropy of a system is the sum of the entropy of each subsystem, which is to say that entropy is *extensive*. Although individual particles may possess some momentum at any given time, in the situations considered here their exact positions in momentum phase-space rarely contribute to the entropic propensity to mix and so in these cases only the *conformational entropy* is considered.

Conjugated to each generalized coordinate X_i is an intensive *generalized thermodynamic force* F_i . For instance, chemical potential $F_i = \mu$ is the thermodynamic force conjugated with particle number $X_i = N$ but generally speaking F_i is defined by considering the differential form of the fundamental equation of thermodynamics

$$\begin{aligned} dS &= \sum_i \left. \frac{\partial S}{\partial X_i} \right|_{\{X\}_{j \neq i}} dX_i \\ &= \sum_i F_i dX_i \end{aligned}$$

such that the generalized forces are defined as

$$F_i \equiv \left. \frac{\partial S}{\partial X_i} \right|_{\{X\}_{j \neq i}}. \quad (2.2)$$

As a short hand, it is elegant to replace summations by vector operations on the sets of thermodynamic variables. For instance, the differential entropy can be written concisely as

$$dS = \mathbf{F} \cdot d\mathbf{X}. \quad (2.3)$$

Writing the generalized forces as a function of the generalized coordinates $F_i = F_i(\mathbf{X})$ defines the system's *equation of state*. For the example list of generalized coordinates given previously, the conjugate set of generalized forces is $\mathbf{F} = \{\text{chemical potential } (\mu), \text{ pressure } (P), \text{ tension, surface tension, magnetic field, electric field, etc.}\}$. For instance, if one considers a multi-component gas of various species of molecules denoted by subscript A the fundamental equation would be

$$TdS = dU + PdV - \sum_A \mu_A dN_A. \quad (2.4)$$

Notice that:

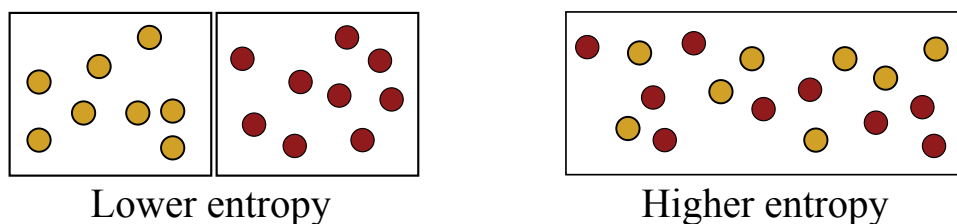


Figure 2.1.1: Mixed states possess higher entropy than when different species are ordered into well-resolved separated volumes.

1. Generalized thermodynamic forces do not need units of force
2. It is the differences in thermodynamic forces or *affinities* $\mathcal{F} = \mathbf{F}_{\text{final}} - \mathbf{F}_{\text{initial}}$ that drive a system toward equilibrium, not $\mathbf{F}$. A relevant example is a container of non-interacting solute particles that has been partitioned into two subsystems (Figure 2.1.1). The total conformational entropy of the system in this unmixed state is less than if the partition were removed (Figure 2.1.1). If the partition were to be removed then the system would be momentarily out of equilibrium; however, after the particles diffuse for a sufficient period of time, equilibrium would once again be established and the particles are equally likely to be found anywhere in the space available. The conformational entropy is then greater than in the original state. The difference is called the *entropy of mixing*.

Equilibrium is Maximized Entropy

The entropy of mixing example demonstrates that if the particles had remained segregated in their subsystems after the partition was removed then the entropy would have been lower than the equilibrium value of the mixed state. This illustrates the *principle of maximum entropy*. The differential entropy (Equation (2.3)) suggests that equilibrium occurs when entropy is at an extrema point. When combined with the second law of thermodynamics (that entropy cannot decrease) this means entropy is maximized in equilibrium (Figure 2.1.2). When a system is out of equilibrium, the affinities will produce entropy, driving the system towards a new equilibrium. In terms of mixing, this means that whenever there are ensembles of particles, the difference between the current and equilibrium chemical potential will drive the system toward a homogeneously mixed state, in which each of the particle species is equally likely to be found at any point in the system.

Entropy is maximized in equilibrium, but how are the dynamics of entropy production and the transition toward equilibrium described?

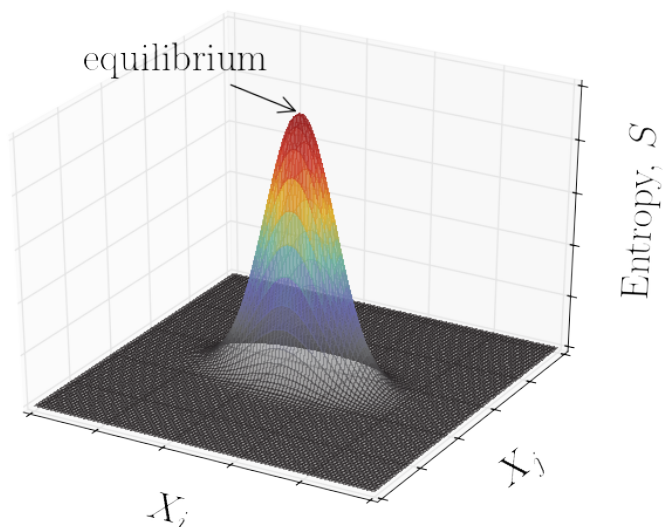


Figure 2.1.2: Equilibrium occurs at the point of maximum entropy. An example system that depends on only two generalized coordinates is shown.

2.1.2 Non-equilibrium Thermodynamics

Sorting a mixed state into segregated volumes requires external work to counteract the effects of entropy maximization. Any external work causes the extensive properties to evolve in time t and be transported in space $\vec{x}$. Therefore, *non-equilibrium thermodynamics* (i.e. the thermodynamics of irreversible processes) is required in order to consider the transport phenomena that are implicitly involved in separation techniques. The theory of non-equilibrium thermodynamics rests on four simplifying postulates:

1. near equilibrium
2. linear transport
3. Curie's postulate
4. Onsager's reciprocal relations

Two of these are vitally important to this work and will be discussed in detail (postulates 1 and 2). The others will be stated but not thoroughly discussed, as they are not essential to this thesis.

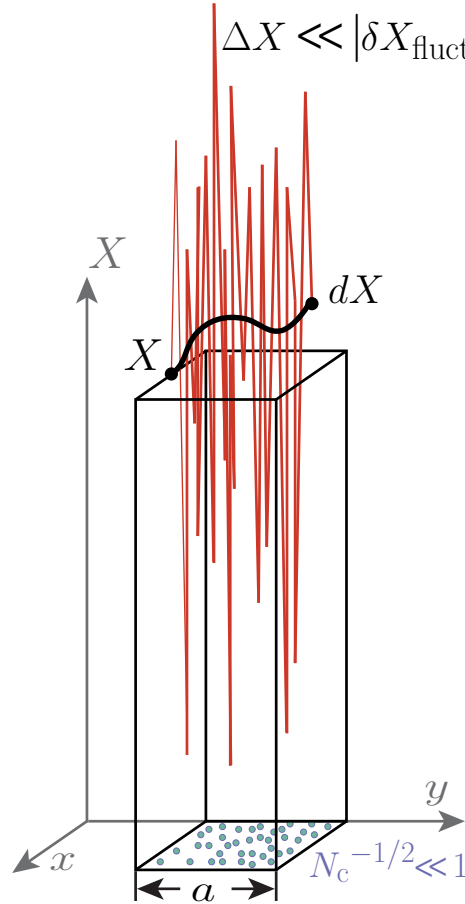


Figure 2.1.3: Postulate I of non-equilibrium thermodynamics is the quasi-equilibrium assumption that changes in generalized coordinates are small relative to fluctuations (Equation (2.6)), while remaining in the thermodynamic limit (Equation (2.7)).

Postulate I: Quasi-equilibrium

The primary postulate of non-equilibrium thermodynamics limits the theory to systems that are not too far out of equilibrium.

Quasi-equilibrium: Non-equilibrium systems can be subdivided into cells between which parameters $\mathbf{X}$ and $\mathbf{F}$ vary slowly but within which equilibrium thermodynamics apply. This requires that affinities are not too large. In other words, the system is *near equilibrium*.

The quasi-equilibrium postulate formulates itself in terms of time and length scales. A system can only be divided into subsystems which are in open contact with their neighbours

but are locally in equilibrium (referred to as *local equilibrium cells*) if there exists some inherent division of time and length scales. The quasi-equilibrium postulate itself consists of three requirements:

- *Fluctuations* decay with some *relaxation time scale* $[\tau]_{\text{fluct}}$ that is rapid compared to the time characterizing the evolution of the whole system $[\tau]_{\text{ev}}$. Quasi-equilibrium time scales Δt are those in the range

$$[\tau]_{\text{fluct}} \ll \Delta t \ll [\tau]_{\text{ev}}. \quad (2.5)$$

- Each local equilibrium cell is open: Entropy, energy, particles or any relevant extensive variables can be transported in or out, which means there can exist gradients ∇X_i . If the cell has a length scale a then the change in X_i is

$$\Delta X_i \approx a |\nabla X_i|.$$

Only when any ΔX_i is small compared to the fluctuations of X_i within the cell ($|\delta X_{i,\text{fluct}}|$)

$$\Delta X_i \ll |\delta X_{i,\text{fluct}}| \quad (2.6)$$

can one say the system is near equilibrium (Figure 2.1.3). Then small changes occur against a backdrop of relatively much larger fluctuations. Since thermodynamic fluctuations are time-reversal invariant and a system's response to such a fluctuation is to reversibly return to equilibrium, perturbations ΔX_i smaller than the natural fluctuations within a cell appear *locally reversible*. Therefore, when Equation (2.6) is met, the equilibrium point can evolve reversibly since it moves through a sequence of equilibrium states. These reasonable statements have only been formally proven for very simple systems [20–22].

- For each cell to have a thermodynamically meaningful local equilibrium, it must be in the *thermodynamic limit* (Figure 2.1.3). One way of defining the thermodynamic limit is to say that fluctuations must be small compared to the average value. Therefore, it is required that

$$|\delta X_{i,\text{fluct}}| \ll X_i. \quad (2.7)$$

Fluctuations are generally small (an exception being near a phase transition where fluctuations and associated correlations can span the entire system). This is seen by considering a small subsystem represented by $\mathbf{X}$ that is in contact with a reservoir (with macroscopic extensive parameters $\mathbf{X}_{\text{res}}$). The correlation of pairs of fluctuations of variables X_i from the reservoir values is [23]

$$\langle \delta X_{i,\text{fluct}} \delta X_{j,\text{fluct}} \rangle = -k_B \frac{\partial X_i}{\partial F_j}. \quad (2.8)$$

Since X_i is extensive, its value within a cell increases as $X_i \sim N_c^1$, while F_j is intensive and so does not change with the size of the local ensemble ($F_j \sim N_c^0$). For this simple reason, it is generally true that the fluctuations are small and go to zero with ensemble size as

$$\frac{|\delta X_{i,\text{fluct}}|}{X_i} = \frac{1}{X_i} \sqrt{k_B \frac{\partial X_i}{\partial F_i}} \sim N_c^{-1/2}. \quad (2.9)$$

Combining local reversibility (Equation (2.6)), the thermodynamic limit (Equation (2.7)) and the size of fluctuations (Equation (2.9)) causes the quasi-equilibrium postulate to become

$$\boxed{\frac{a |\nabla X_i|}{X_i} \ll N_c^{-1/2} \ll 1}. \quad (2.10)$$

In summary, if the system is large enough and the variation in general coordinates is small enough then the quasi-equilibrium postulate is met and the system can be divided into local equilibrium cells.

Entropy Production In the entropy of mixing example (from § 2.1.1) the system moved from one equilibrium state to one of higher entropy once the separating partition was removed. The analysis only treated these equilibrium states and not the manner in which entropy increased. Since the separation systems considered by this thesis are driven further and further from equilibrium, it is essential to understand the rate of *entropy production*.

Since the concept of local equilibrium cells has already been introduced, entropy in a system of discrete subsystems is treated first. A continuous treatment follows.

Discrete Cells Imagine that the system has been subdivided into two local equilibrium cells (say A-subsystem and B-subsystem as in Figure 2.1.1). The set of extensive variables

for the whole system ($\mathbf{X}$) do not change in time (are conserved) but the local equilibrium cells are open and the parameters can certainly evolve in each subsystem

$$\mathbf{X} = \mathbf{X}_A(t) + \mathbf{X}_B(t) = \text{constant in time}$$

and the instantaneous entropy of the entire system (which is not conserved) is

$$S(t) = S_A(\mathbf{X}_A(t)) + S_B(\mathbf{X}_B(t)).$$

Since $\mathbf{X}$ is fixed, it is possible to say that $\mathbf{X}_B$ is completely determined by $\mathbf{X}_A$ (or vice versa) and therefore, so too is the entropy. Thus, the change of entropy can be stated as the rate of change with respect to $\mathbf{X}_A$. This rate of change is called the *instantaneous affinity* and is given by

$$\left. \frac{\partial S(t)}{\partial X_{A,i}} \right|_{X_i} = F_{A,i}(t) - F_{B,i}(t) = \mathcal{F}_i(t). \quad (2.11)$$

This definition will be altered when continuous systems are considered.

In the case of entropy of mixing, the affinity is only given as the difference in thermodynamic generalized forces (in that case chemical potential) but in non-equilibrium thermodynamics it can be defined instantaneously as in Equation (2.11). Once equilibrium is established, the set of affinities reaches zero, $\mathcal{F} = 0$.

Since the entropy is a function of the instantaneous extensive parameters, the evolution toward entropy maximization (entropy production) can be given by

$$\begin{aligned} \dot{S} &= \frac{d}{dt} S(\mathbf{X}) = \sum_i \left(\frac{\partial S}{\partial X_i} \right) \left(\frac{d}{dt} X_i(t) \right) \\ &= \mathcal{F} \cdot \dot{\mathbf{X}} \end{aligned} \quad (2.12)$$

where $\dot{X}_i \equiv dX_i/dt$ is the rate of change of a generalized coordinate or a *generalized displacement*. This term is sometimes called the flux but is more accurately described as current. Equation (2.12) demonstrates how entropy production toward equilibrium is driven by non-zero affinities and requires generalized displacements within the discretized system.

Continuous Systems If an inhomogeneous system meets the requirements of quasi-equilibrium, the parameters can be given as a function of the position of each cell $\vec{x}$ at any time t

$$\mathbf{X} = \mathbf{X}(\vec{x}, t).$$

Notice the two types of vector notation: $\mathbf{X} = \{X_i\}$ is the set of extensive parameters, while $\vec{x} = (x, y, z)$ is a spatial vector.

If each cell is exceedingly small compared to the size of the system ($a \ll [L]$) then $\vec{x}$ is safely approximated as a continuous position, leading the generalized coordinates to become fields. Since the fields depend on the arbitrary volume of each cell (arbitrary so far as they must meet the requirements of the quasi-equilibrium postulate), it is prudent to consider their densities

$$\mathbf{x}(\vec{x}, t) = \frac{\mathbf{X}(\vec{x}, t)}{a^{\mathbf{d}}}, \quad (2.13)$$

where $\mathbf{d}$ is the dimensionality of the system. Throughout this chapter, a lower case letter corresponds to the density of the uppercase extensive quantity. In this notation the differential local entropy density is

$$ds(\vec{x}, t) = \sum_i \frac{\partial s(\vec{x}, t)}{\partial \mathbf{x}_i(\vec{x}, t)} d\mathbf{x}_i(\vec{x}, t) = \mathbf{F}(\vec{x}, t) \cdot d\mathbf{x}(\vec{x}, t). \quad (2.14)$$

Since each infinitesimal cell is open, the total entropy production in a local equilibrium cell is

$$\psi_S = \frac{d}{dt} s_{\text{INT}}(\vec{x}, t) + \frac{d}{dt} s_{\text{EX}}(\vec{x}, t), \quad (2.15)$$

where s_{INT} is the internal creation of entropy, while the second term refers to the exchange of entropy between neighbours. The entropy density production is sometimes called the *entropy production rate* or the dissipation term. The balance equation for entropy is qualitatively intuitive (transport theory will be discussed in detail in § 2.2):

- The *local internal entropy density production rate* is simply found in terms of the generalized forces and fluxes through Equation (2.14)

$$\frac{d}{dt} s_{\text{INT}}(\mathbf{x}(\vec{x}, t)) = \mathbf{F}(\vec{x}, t) \cdot \dot{\mathbf{x}}(\vec{x}, t). \quad (2.16)$$

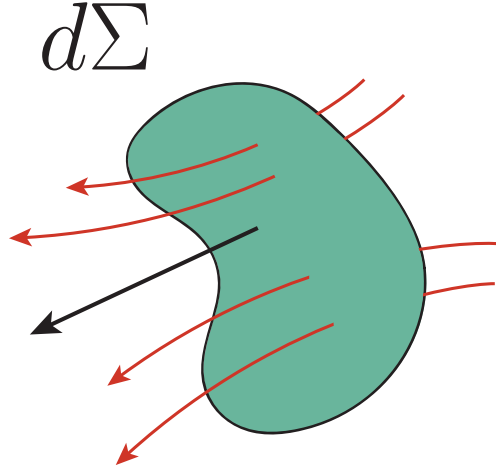


Figure 2.1.4: Flux of X is the flow per unit time through a unit area $d\Sigma$ (Equation (2.18)) or equivalently the amount of X transported across the surface is the flux normal to that surface (Equation (2.19)).

- The *exchange entropy density rate* is no less simple. The exchange entropy density must flow in from neighbouring cells and intuitively is controlled by the flux of entropy, $\vec{j}_S$. The divergence of entropy flux is the entropy current per unit volume, which is precisely equivalent to the exchange entropy density rate into the infinitesimal local equilibrium cell

$$\frac{d}{dt} s_{\text{EX}}(\vec{x}, t) = \vec{\nabla} \cdot \vec{j}_S. \quad (2.17)$$

Flux is fundamental transport theory and so a brief description is warranted:

- Generally speaking, the vector flux of any extensive parameter X_i is the flow of X_i per unit time through some area Σ (in d -dimensions with normal vector $\hat{n}$) as shown in Figure 2.1.4 or mathematically

$$\vec{j}_{X_i} \equiv \frac{d\dot{X}_i}{d\Sigma} = \frac{d}{d\Sigma} \left(\frac{dX_i}{dt} \right), \quad (2.18)$$

which means that the amount of X_i transferred through a bounding surface in a given time $t_2 - t_1$ is

$$X_i = \int_{t_1}^{t_2} \int_{\Sigma} \vec{j}_{X_i}(\vec{x}, t) \cdot \hat{n}(\vec{r}) d\Sigma dt. \quad (2.19)$$

- Sometimes the flux is referred to as the flux density; however, $\vec{j}_{X_i}$ is the flux of the quantity itself (X_i) and certainly not the flux of the density of the given quantity ($\mathbf{x}_i$). In this context, flux density is an ambiguous term and so is avoided here.
- Since the flux at each point is associated with a surface that has an orientation defined by its normal vector, the flux intrinsically has direction.

The entropy flux results from the flux of the extensive generalized coordinates (as can be seen by inserting Equation (2.12) into Equation (2.18) while remembering that the forces are intensive)

$$\vec{j}_S(\vec{x}, t)c = \frac{d\dot{S}}{d\Sigma} = \mathbf{F}(\vec{x}, t) \cdot \mathbf{j}_X(\vec{x}, t). \quad (2.20)$$

Therefore, the exchange in entropy density is

$$\frac{d}{dt}s_{\text{EX}}(\vec{x}, t) = \vec{\nabla} \cdot \vec{j}_S = \sum_i \left[\left(\vec{\nabla} F_i \right) \cdot \vec{j}_{X_i} + F_i \left(\vec{\nabla} \cdot \vec{j}_{X_i} \right) \right], \quad (2.21)$$

where the summation notation is momentarily preferred over the vector notation.

Now the local entropy density production rate is obtained by substituting the internal (Equation (2.16)) and the exchange (Equation (2.21)) into (Equation (2.15)). This gives the entropy production in terms of the system properties to be

$$\psi_S = \sum_i \left(\vec{\nabla} F_i \right) \cdot \vec{j}_{X_i} + F_i \dot{\mathbf{x}}_i + F_i \left(\vec{\nabla} \cdot \vec{j}_{X_i} \right). \quad (2.22)$$

The second and third terms cancel because the rate of change ($\dot{\mathbf{x}}_i$) is equal and opposite to the incoming/outgoing amount ($\vec{\nabla} \cdot \vec{j}_{X_i}$). Therefore, the entropy density production is simply

$$\psi_S = \sum_i \left(\vec{\nabla} F_i \right) \cdot \vec{j}_{X_i}. \quad (2.23)$$

The equation for the entropy production in the discrete system has the strikingly similar form of $\dot{S} = \mathcal{F} \cdot \dot{\mathbf{X}}$ (Equation (2.12)). By comparison to the discrete form, the **affinities for a continuous system** are **defined** to be the gradient of the thermodynamic generalized forces

$$\boxed{\mathcal{F}_i \equiv \vec{\nabla} F_i}. \quad (2.24)$$

Thus, entropy production is once again the sum of all affinities times each conjugate flux

$$\boxed{\psi_S = \mathcal{F} \cdot \mathbf{j}_X}. \quad (2.25)$$

By utilizing only the quasi-equilibrium postulate of non-equilibrium thermodynamics, the dynamics of how a system moves from an unmixed state to a mixed state can be understood; whereas, mixing was only understood in terms of the final mixed state when only equilibrium thermodynamics were employed. In a continuous system, entropy production is driven by the gradient of the thermodynamic forces and necessarily leads to a flux.

Postulate II: Linearity

Although the quasi-equilibrium postulate is the primary foundation of non-equilibrium thermodynamics, flux is the base quantity in transport phenomena. Mixing and transport are tied to the flux. What determines the flux of a generalized density field? This section states the second postulate of non-equilibrium thermodynamics, which relates thermodynamic fluxes to thermodynamic affinities.

Linear transport: All thermodynamic fluxes in a system are linearly related to each of the affinities.

This is the second postulate of non-equilibrium thermodynamics and is itself closely tied to the fact that the system is near equilibrium (Postulate I). Being near equilibrium allows the flux to be expressed as a Taylor expansion of all the affinities about equilibrium

$$\begin{aligned} \vec{j}_{X_i}(\vec{x}, t) &= \vec{j}_{X_i}(\mathcal{F}(\vec{x}, t), \mathbf{F}(\vec{x}, t)) \\ &\approx \vec{j}_{\text{eq}, X_i} + \left(\sum_j \gamma_{ij} \mathcal{F}_j \right) + \frac{1}{2!} \left(\sum_{j,k} \gamma_{ijk} \mathcal{F}_j \mathcal{F}_k \right) + \dots \end{aligned} \quad (2.26)$$

Here $\gamma_{ij} = \gamma_{ij}(\mathbf{F}_{\text{eq}})$ and $\gamma_{ijk} = \gamma_{ijk}(\mathbf{F}_{\text{eq}})$ are called the *kinetic coefficients* and in this formalism they are functions of the equilibrium values of the thermodynamic forces. As a reminder, the indices i and j correspond to the **set** of thermodynamic variables, not the spatial dimensionality. Therefore, the kinetic coefficients form a tensorial set $\underline{\gamma}$, the elements of which can themselves be tensors $\hat{\gamma}_{ij}$. For instance, if the fluxes and affinities are vectors

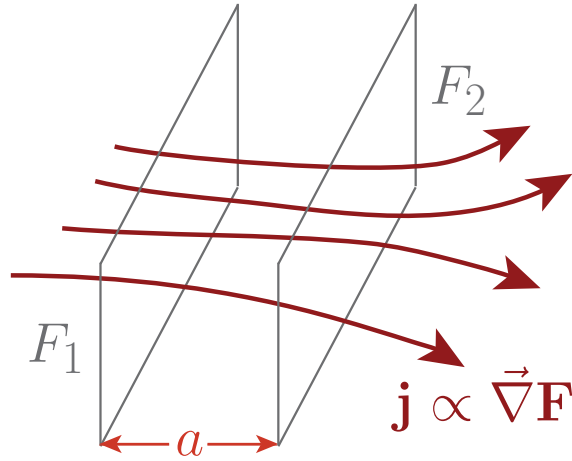


Figure 2.1.5: Postulate II of non-equilibrium thermodynamics is the linear transport assumption that fluxes are linearly proportional to affinities (Equation (2.27)).

then the kinetic coefficients form rank-two tensors, while if the fluxes and affinities are rank-two tensors then the kinetic coefficients are rank-four tensors.

In equilibrium, the net fluxes are zero (unless the entire system translates as a whole, which shall be dealt with in § 2.2) so $\mathbf{j}_{\text{eq}} \equiv 0$. Near equilibrium, the affinities are small (the gradients of the thermodynamic generalized forces are small from Equation (2.24)) and near enough to equilibrium the first term suffices. Thus, the linearity postulate is

$$\mathbf{j}(\vec{x}, t) = \underline{\gamma} \cdot \mathcal{F}(\vec{x}, t) \quad (2.27)$$

where the set of fluxes and affinities are written in vector notation, which causes the kinetic coefficients to be a tensorial set. Notice that generally Equation (2.27) relates the flux of a certain field $\vec{j}_{X_i}$ not only to its own conjugate affinity but to **all** of the relevant affinities $\mathcal{F}$. In the simplest cases (such as mass transport), the flux depends only on its own affinity, but this need not be true.

Examples of Linear Transport

Before linearity between flux and affinity was framed as a postulate of non-equilibrium thermodynamics it had a long history as phenomenological laws for many diverse systems. A sample of these phenomenological laws is presented below with respect to Equation (2.27). In each example the flux of a quantity depends on its own affinity and no other.



Figure 2.1.6: Fourier's law of heat flux. Thermal energy flows from a hot surface (red) through a medium defined by a thermal conductivity κ . Thermal conductivity is an example of a linear kinetic coefficient.

Fourier's Law of Heat Conduction (1822) The phenomenological law for heat flux (Figure 2.1.6) reads

$$\vec{j}_U = \kappa \vec{\nabla} T, \quad (2.28a)$$

where $\vec{j}_U$ is the energy flux density. This form appears consistent with Equation (2.27); however, the generalized force conjugate to energy is not temperature T but rather inverse temperature. Therefore, the affinity and kinetic coefficients for Fourier's law are recognized to be

$$\vec{\mathcal{F}}_U = \vec{\nabla} \left(\frac{1}{T} \right) \quad ; \quad \hat{\gamma}_{UU} = \hat{\kappa} T^2,$$

where the thermal conductivity can be a rank-two tensor since the flux and affinity are vectors. If the medium is isotropic then a single kinetic coefficient suffices, $\hat{\gamma}_{UU} = \kappa T^2 \hat{1}$.

Newton's Law of Viscous Dissipation (1687) For the simplest, noncompressible fluids, the stress Π that results from some strain rate in a single direction $\dot{\epsilon}_x = \partial u / \partial x$ is

$$\hat{\Pi} = \eta \frac{\partial u}{\partial x}. \quad (2.29a)$$

This is the phenomenological law for an isotropic incompressible fluid (Figure 2.1.7). The stress is the flux of momentum and since momentum is a vector, the stress is a rank-two tensor that is linearly connected to the strain tensor by the rank-four viscosity tensor $\hat{\eta}$. Newton's law will be discussed in greater detail in § 2.3.1.

Ohm's Law of Electrical Conduction (1826) The phenomenological law for electrical current reads

$$\vec{j}_Q = \sigma_{\text{cond}} \vec{E}, \quad (2.30a)$$

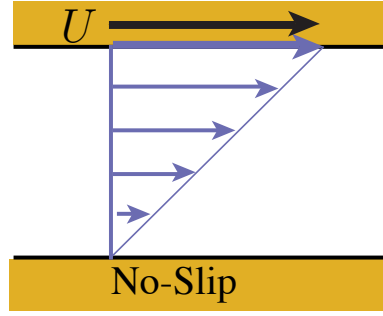


Figure 2.1.7: Shear flow between two plates. No-slip boundary conditions at each plate result in a velocity gradient within the fluid. Newtonian fluid. The velocity variation results in internal, viscous stress. Newton's law of viscous dissipation describes the case when viscous stress is linearly related to the velocity gradient.

where $\vec{j}_Q$ is the flux of unit charges Q , σ_{cond} is the electrical conductivity and $E = -\vec{\nabla}\Psi$ is the electric field, which is itself the gradient of the electrostatic potential Ψ . In a sense, Ohm's law already has the form of Equation (2.27) but generalized thermodynamic forces in the entropy representation are per temperature so in non-equilibrium thermodynamics the generalized force in Ohm's law is the electrochemical potential

$$\vec{\mathcal{F}}_Q = \vec{\nabla} \left(\frac{Q^2 \Psi}{T} \right)$$

and the kinetic coefficients can be a rank-two tensor since the flux and affinity are vectors. If the medium is isotropic then a single kinetic coefficient once again suffices

$$\hat{\gamma}_{QQ} = \frac{T}{Q^2} \hat{\sigma}_{\text{cond}} = \frac{T \sigma_{\text{cond}}}{Q^2} \hat{1}.$$

Darcy's Law of Porous Flow (1856) The phenomenological law for the discharge rate Q of fluid through a porous medium is linearly related to the pressure gradient (Figure 2.1.8)

$$\vec{j}_Q = -\frac{k}{\eta} \vec{\nabla} P. \quad (2.31a)$$

The coefficients are the permeability of the porous medium k and viscosity η of the fluid.

From Equation (2.27), the form of Darcy's law could have been predicted: The discharge rate is the volume of fluid per unit time. The conjugate thermodynamic force to volume is

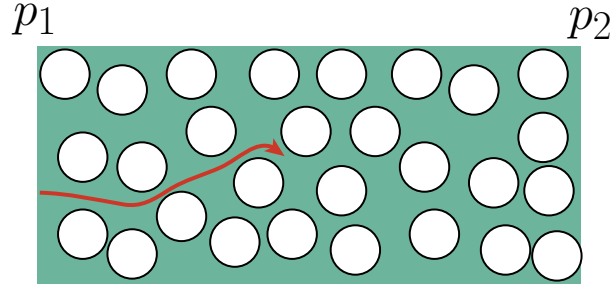


Figure 2.1.8: An illustration of a system for which the volumetric flow rate would be described by Darcy's law. A system of packed beads creates a series of effective channels, through which fluid flows. Darcy's law states that the volumetric flow rate of the fluid is linearly proportional to the pressure drop per unit length.

pressure per temperature (Equation (2.4)) so the affinity is

$$\vec{\mathcal{F}}_{\mathcal{Q}} = \vec{\nabla} \left(\frac{P}{T} \right).$$

This suggests that the kinetic coefficients are

$$\hat{\gamma}_{\mathcal{Q}\mathcal{Q}} = -\frac{T}{\eta} \hat{k} = -\frac{Tk}{\eta} \hat{\mathbb{1}},$$

where a single kinetic coefficient suffices if the medium is once again assumed to be isotropic.

Fick's First Law of Particle Diffusion (1855) The phenomenological law for diffusion is

$$\boxed{\vec{j}_N = -\mathcal{D} \vec{\nabla} n}, \quad (2.32a)$$

where n is the number density (or concentration) of particles and $\mathcal{D}$ is the diffusion coefficient. Fick's first law appears to be in the form of Equation (2.27) but $\vec{\nabla} n$ is not an affinity — n is an generalized density. Chemical potential is the generalized force conjugated with changes in particle number. However, if the other intensive parameters do not change, the gradient of chemical potential can be related to concentration gradients:

$$\begin{aligned} \vec{j}_N &= \hat{\gamma}_{NN} \cdot \vec{\nabla} \left(\frac{\mu}{T} \right) \\ &= \hat{\gamma}_{NN} \cdot \left(\frac{1}{T} \frac{\partial \mu}{\partial n} \Big|_{\mathbf{F} \neq \mu} \vec{\nabla} n \right). \end{aligned} \quad (2.32b)$$

This is Fick's phenomenological law if the rank-two diffusion tensor is identified to be

$$\widehat{\mathcal{D}} = \frac{1}{T} \frac{\partial \mu}{\partial n} \Big|_{\mathbf{F} \neq \mu} \widehat{\gamma}_{NN}.$$

As always, if the medium is isotropic a single diffusion coefficient suffices. Fick's first law can be conveniently derived by considering the random walks of non-interacting Brownian particles.

Nernst-Einstein Relation for Electrical Mobility (1905 & 1906) Both Ohm's law for electrical flux and Fick's law for particle flux have been considered above. However, in solutions, the charges in Ohm's law are carried by solutes (such as polyelectrolyte macromolecules or colloids of total charge Q) moving with some velocity $\mathcal{V}$. The kinetic coefficients relating velocity to affinity field are generally referred to as mobilities (many mobilities will be considered in this thesis):

$$\vec{\mathcal{V}} = \widehat{\mu}_{\text{el}} \cdot \vec{E}. \quad (2.33a)$$

In the absence of any electrical screening at all (see § 2.4.1), the electric flux in terms of the electrophoretic mobility of the charged molecules is then

$$\vec{j}_Q = Q\vec{j}_n = Qn \left(\widehat{\mu}_{\text{el}} \cdot \vec{E} \right).$$

Therefore, the unscreened electrical conductivity of Ohm's law is identified as $\widehat{\sigma}_{\text{cond}} = nQ\widehat{\mu}_{\text{el}}$. Since the charge is carried by the particles, the kinetic coefficients from Ohm's law and those from Fick's first law must be equivalent. This means that the free diffusion of particles is related to their mobility when subject to a force:

$$\begin{aligned} \widehat{\gamma}_{NN} &= \widehat{\gamma}_{QQ} \\ \widehat{\mathcal{D}} &= \widehat{\mu}_{\text{el}} \frac{n}{Q} \frac{\partial \mu}{\partial n} \Big|_{\mathbf{F} \neq \mu}. \end{aligned}$$

If the charged molecules are dilute enough (this thesis limits itself to the infinitely dilute case) then they can be treated as an ideal gas for which $\partial \mu / \partial n|_{\mathbf{F} \neq \mu} = k_B T / n$. A relation between diffusion and unscreened electrophoretic mobility coefficients can then be written

$$\widehat{\mathcal{D}} = \widehat{\mu}_{\text{el}} \frac{k_B T}{Q}. \quad (2.33b)$$

This relationship may appear rather pedestrian as it simply relates two kinetic coefficients. However, the diffusion coefficient characterizes random fluctuations **about equilibrium**, while the electrophoretic mobility describes the response to an external force that drives the system **out of equilibrium**. This important conclusion extends beyond electrophoretic mobility and can be derived generally for any mobility by the fluctuation-dissipation theorem as will be done in § 2.3.5.

Postulates III & IV: Curie's Principle and Onsager's Reciprocal Relations

The first two postulates of non-equilibrium thermodynamics (quasi-equilibrium [§ 2.1.2] and linearity [§ 2.1.2]) were discussed in some detail in the previous section. That is because this work rests primarily on these first two but non-equilibrium thermodynamics rests on four postulates. The final two relate to the nature of the kinetic coefficients.

Curie's symmetry principle: Any effect should have the same symmetry as the causes.

In terms of transport phenomena, Curie's principle means that in an isotropic system fluxes and forces of different tensorial nature cannot couple. So while heat transport and electrical conduction can couple (resulting in cross-terms $\hat{\gamma}_{UQ}$ and $\hat{\gamma}_{QU}$), heat transport and stress within a fluid cannot ($\hat{\gamma}_{U\epsilon} = 0$). They can both occur but there can be no cross terms in the kinetic coefficient tensor set.

Onsager's reciprocal relations: $\hat{\gamma}_{ij} = \hat{\gamma}_{ji}$

Onsager's reciprocal relations make a statement about the symmetry of the set tensor $\underline{\gamma}$, which says that if there are cross-terms they are symmetric. For example, if energy is transported because of both a temperature gradient ($\vec{\mathcal{F}}_U = \vec{\nabla}T^{-1}$) and a chemical potential gradient ($\vec{\mathcal{F}}_N = \vec{\nabla}(\mu/T)$) the flux would be

$$\vec{j}_U = \hat{\gamma}_{UU} \cdot \vec{\mathcal{F}}_U + \hat{\gamma}_{UN} \cdot \vec{\mathcal{F}}_N.$$

This necessarily means that particle transport exists in the system

$$\vec{j}_N = \hat{\gamma}_{NN} \cdot \vec{\mathcal{F}}_N + \hat{\gamma}_{NU} \cdot \vec{\mathcal{F}}_U$$

with the same cross-term $\hat{\gamma}_{NU} = \hat{\gamma}_{UN}$. Such indirect transport phenomena involving cross terms do not play a role in the subjects treated in this thesis and so Curie's principle and Onsager's relations do not contribute.

Summary of Thermodynamics

Separation science has its formal roots in non-equilibrium thermodynamics. Fractionation techniques must compete against entropy production (Equation (2.25)) that moves quasi-equilibrium systems (Equation (2.5) and Equation (2.10)) toward equilibrium via all relevant affinities (Equation (2.24)). In the case of solute mixing, the entropy difference between unmixed and mixed states is called the entropy of mixing and the affinity is the difference in chemical potentials. The production of entropy drives thermodynamic fluxes (Equation (2.18)), including particles fluxes. Near equilibrium, the fluxes are linearly related to the driving affinities (Equation (2.27)) through kinetic coefficients. The linear kinetic coefficient relating to particle fluxes is Fick's first law of diffusivity (Equation (2.32a)). Separation science must design systems that produce controllable fluxes, which are more significant than the randomizing diffusivity.

2.2 Transport Theory

The previous section (§ 2.1) demonstrated that the flux of intensive quantities (such as mass, charge, momentum, energy or even entropy) is a natural consequence of being out of equilibrium. Separation science must compete against these fluxes that drive the system toward maximum entropy and equilibrium, mixing species of dispersed solutes in the process. However, separation science can also harness fluxes. The fractionation techniques considered in this thesis are chromatographic, meaning that they rely on the ability to differentiate particles and macromolecules based on transport properties.

A theoretical framework for transport-based separation science is necessarily a transport phenomena theory. In particular, it is a mass transport theory. In this section, the mass transport theory will be derived and written in a form that stresses the different limits.

Mass transport is described by the convection-diffusion mass transport equation, which reduces to the continuity equation when diffusion is negligible and reduces to Fick's second law when convection is absent. A dimensionless number (called the Péclet number) describes the competition between the two transport limits.

2.2.1 Transport Equations of Irreversible Thermodynamics

To account for the evolution of continuous quantities in a system, a framework that allows for dynamic inhomogeneity is needed. If a quantity is not conserved (like entropy) then the framework must account for sources and/or sinks. If the density can vary then compressibility must also be included. Because these ideas are universal concepts and not tied to specific materials, the *transport equation* is sometimes described as the **balance equation**.

The transport equation is extremely simple to say in words:

What enters a space (*INPUT*) plus what is created there (*SOURCE*) must exit (*OUTPUT*), be destroyed (*SINK*) or remain (*ACCUMULATION*).

That is to say

$$\text{ACCUMULATION} = (\text{SOURCE} - \text{SINK}) + (\text{INPUT} - \text{OUTPUT}), \quad (2.34)$$

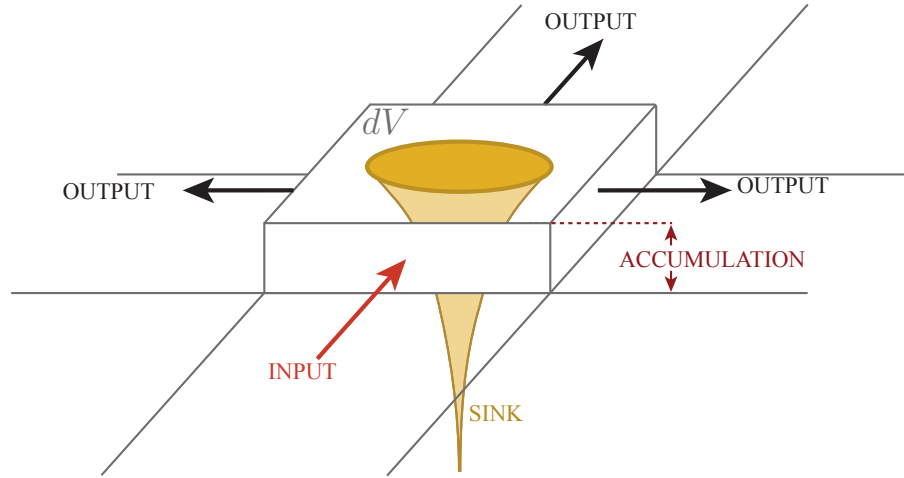


Figure 2.2.1: Schematic representation of the balance equation (Equation (2.34) and Equation (2.43)). Whatever enters (less what exits) plus any sources (less sinks) accumulates within the local volume dV .

which is simply the *conservation concept* (Figure 2.2.1). The transport equation shall now be stated mathematically in terms of field density and flux.

In § 2.1, extensive fields were denoted by X_i (with corresponding densities $\mathbf{x}_i$), with entropy taking a special place. Now let $X(\vec{x}, t)$ be **any** extensive quantity that can vary from place to place within the system and is not necessarily conserved. Let $\vec{j} = \vec{j}_X$ be the flux of X *i.e.* the subscript is dropped for clarity. Consider some arbitrary (though bounded) space containing the field $X(\vec{x}, t)$ within it. Without loss of generality, the arbitrary volume can be chosen to be an infinitesimal cube of volume dV . In Cartesian coordinates the volume is $dV = dx dy dz$ and the area of each face is $d\Sigma_{xy} = dx dy$, *etc.* The transport equation then results from writing each term in Equation (2.34) for the volume.

ACCUMULATION The accumulation is simply the total rate of change of X :

$$\text{ACCUMULATION} = \frac{\partial X}{\partial t} = \frac{\partial \mathbf{x}}{\partial t} dV. \quad (2.35)$$

It can be either positive or negative.

SOURCE-SINK Generation and destruction may occur, therefore let the difference between any sources and sinks simply be denoted

$$\text{SOURCE} - \text{SINK} = \psi dV \quad (2.36)$$

and physical arguments foreign to transport science must inform the value of the net *production rate* ψ .

INPUT-OUTPUT Let the net flux through three perpendicular surfaces of the infinitesimal cube be called the “INPUT”:

$$\text{INPUT} = j_x dydz + j_y dxdz + j_z dxdy. \quad (2.37)$$

Notice that this so-called “INPUT” flux can be either positive or negative. The input into a cubic volume through the $dxdy$ face is shown in Figure 2.2.2 along with the corresponding output through the opposite face. The flux through these three surfaces can be changed by accumulation or sources and sinks within the volume, such that by the time each component reaches the opposite surface it has become

$$\begin{aligned} \text{OUTPUT} = & \left(j_x + \frac{\partial j_x}{\partial x} dx \right) dzdy + \left(j_y + \frac{\partial j_y}{\partial y} dy \right) dzdx \\ & + \left(j_z + \frac{\partial j_z}{\partial z} dz \right) dydx \end{aligned} \quad (2.38)$$

simply by the definition of the derivative. The flux through these three surfaces is labeled “OUTPUT” and can also be either positive or negative. The reason why this derivation is so nonchalant about which is incoming flux and which is outgoing, is because only the difference matters. The difference is

$$\begin{aligned} \text{INPUT} - \text{OUTPUT} &= - \left[\frac{\partial j_x}{\partial x} + \frac{\partial j_y}{\partial y} + \frac{\partial j_z}{\partial z} \right] dx dy dz \\ &= - \left(\vec{\nabla} \cdot \vec{j} \right) dV. \end{aligned} \quad (2.39)$$

This shown schematically in Figure 2.2.2.

Each of these three terms can be inserted into Equation (2.34) to find that the general transport equation for any extensive field is

$$\boxed{\frac{\partial \mathbf{x}}{\partial t} = \psi_X - \vec{\nabla} \cdot \vec{j}_X}. \quad (2.40)$$

This general transport equation can be applied to balance any extensive field. A good example is entropy production. When the entropy production was determined in § 2.1.2, the derivation was convoluted because a general form of the transport equation had not yet been

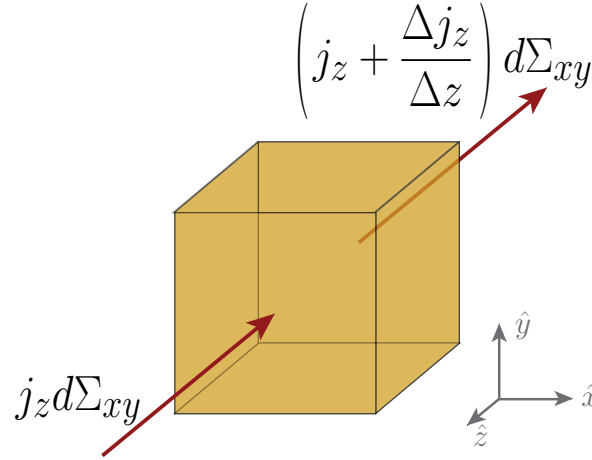


Figure 2.2.2: Input and output through the xy -planes of a cubic control volume.

introduced. Comparing Equation (2.40) for general transport phenomena to Equation (2.15) with Equation (2.16) and Equation (2.17) shows their equivalence.

While discussing the general transport equation (Equation (2.40)), scientists often divide the flux into *molecular transport* and *convective transport* [24, 25]. By molecular transport they mean entropy producing fluxes which are driven by thermodynamic affinities of the form discussed in § 2.1:

$$\vec{j}_{\text{ent}} = \hat{\gamma} \cdot \vec{\mathcal{F}} = \hat{\gamma} \cdot \vec{\nabla} F, \quad (2.41)$$

where more precisely speaking $\hat{\gamma} = \hat{\gamma}_{XX}$. By convective transport they mean a net motion of the whole local cell (which was the $j_{\text{eq},X}$ term in Equation (2.26)). Assuming that the entire local equilibrium cell at $(\vec{x}, t)$ translates with the velocity $\vec{v}(\vec{x}, t)$ this flux is simply

$$\vec{j}_{\text{conv}} = \mathbf{x} \vec{v}. \quad (2.42)$$

The total flux is decomposed in this manner because these two types of flux are quite different in nature, but both are universally present in transport phenomena. For this reason, the total flux is rather generally written

$$\vec{j} = \vec{j}_{\text{conv}} + \vec{j}_{\text{ent}} = \mathbf{x} \vec{v} + \hat{\gamma} \cdot \vec{\nabla} F$$

and the transport equation becomes

$$\boxed{\frac{\partial \mathbf{x}}{\partial t} = \psi - \vec{\nabla} \cdot [\mathbf{x}\vec{v} + \hat{\gamma} \cdot \vec{\nabla} F]} \quad (2.43)$$

The transport equation can be written in many different ways (including using the material derivative) but this form is sufficient for the needs of this thesis. It is the mathematical version of Equation (2.34) that can be read as, “Per unit volume, the change in a field is given by the net sum of all interior sources and sinks plus the flux from the exterior, which can include both convective and entropic components.”

2.2.2 Mass Transport

The primary utility of the transport equation (Equation (2.43)) for separation science, is its application to **mass**. In this section, two simple limits are considered first and then the general mass transport equation is presented.

Continuity Equation

Consider a fluid of particles in which the particle concentration n is in equilibrium ($\vec{\nabla} F = \vec{\nabla} \mu = 0$), such that the only flux is due to the macroscopic fluid flow velocity $\vec{u}(\vec{x}, t)$. If no chemical reactions occur to increase or decrease n then $\psi_n = 0$. If the particles each have a mass m then the density of the fluid is $\varrho(\vec{x}, t) = mn(\vec{x}, t)$. Applying the transport equation (Equation (2.43)) to mass in this limit produces the *continuity equation*

$$\boxed{\frac{\partial \varrho}{\partial t} + \vec{\nabla} \cdot (\varrho \vec{u}) = 0}, \quad (2.44)$$

which is often encountered in fluid dynamics. The continuity equation is the continuum expression for the conservation of mass.

An important class of fluids are *incompressible* liquids in which the density is fixed in both time and space, $\varrho(\vec{x}, t) = \varrho$. For such fluids the continuity equation becomes simply

$$\vec{\nabla} \cdot \vec{u} = 0. \quad (2.45)$$

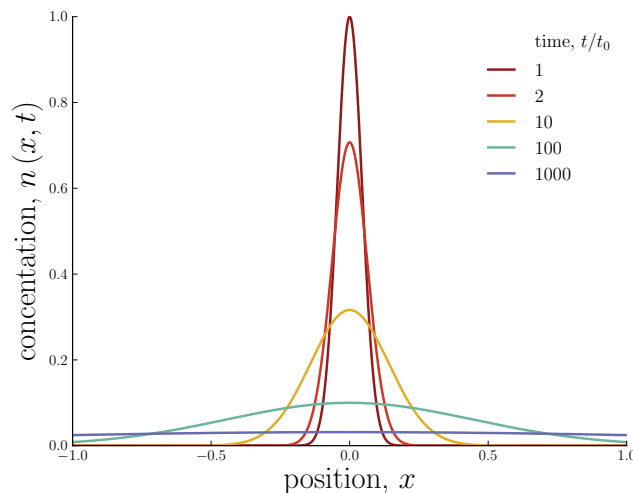


Figure 2.2.3: The 1D solution of Fick's second law (Equation (2.47)), for an initial distribution of $n(x, t) = \exp[-x^2/4\mathcal{D}t_o]$ in a closed system of size $2x_o$.

Fick's Second Law

Next, consider the opposite limit. If a distribution of particles $n(\vec{x}, t)$ undergoes no net convective motion but is not in equilibrium then diffusive flux obeying Fick's first law (Equation (2.32a)) results. The transport equation (Equation (2.43)) becomes

$$\frac{\partial n}{\partial t} = \vec{\nabla} \cdot (\hat{\mathcal{D}} \cdot \vec{\nabla} n) \quad (2.46)$$

This is *Fick's second law*, which (if $\hat{\mathcal{D}}(\vec{x}, t)$ is a constant scalar) becomes

$$\boxed{\frac{\partial n}{\partial t} = \mathcal{D} \nabla^2 n}. \quad (2.47)$$

Figure 2.2.3 shows the evolution of $n(\vec{x}, t)$ in one dimension having begun as $n = n_o \delta(x)$ where $\delta(\vec{x})$ is the Dirac-delta function. The solution to Equation (2.47) is then a Gaussian distribution (Figure 2.2.3). As was true for Fick's first law (§ 2.1.2), these conclusions can be derived from a microscopic, random walk perspective as well.

Convection-Diffusion Equation

Generally speaking, chromatographic methods use convection to compete against entropic diffusion and so both fluxes must be included. In this work, the total number of analytes is always conserved, although this is certainly not the case for all fractionation techniques.

Therefore, the source term ψ_n is kept for the time being. The full *convection-diffusion transport equation* for a concentration distribution of analytes convecting with a velocity $\vec{\mathcal{V}}$ is

$$\frac{\partial n}{\partial t} + \vec{\nabla} \cdot (n \vec{\mathcal{V}}) = \vec{\nabla} \cdot (\hat{\mathcal{D}} \cdot \vec{\nabla} n) + \psi_n. \quad (2.48)$$

The convection-diffusion equation completes the basis of separation science from a theoretical standpoint. In a sense, further work (including this thesis) seeks to determine the proper flows and kinetic coefficients for a given separation system and to understand what Equation (2.48) reveals about the ability to fractionate previously mixed distributions of analytes in different situations. The convection-diffusion equation hides many complications: Flow fields can be complicated, external fields can act on the solutes, diffusivity can vary for many reasons. Furthermore, there are complications to the analysis of Equation (2.48): It is not always possible to analytically find the concentration at all points in space and time. Sometimes only certain moments can be found as a function of time. However, these can often be useful since these can reveal the average position of the solutes or how coherent an eluting distribution is (eluting distributions are at times called *zones*).

2.2.3 Péclet Number

Not all the terms in the convection-diffusion equation (Equation (2.48)) are equally important in all situations. Dropping insignificant terms can make the convection-diffusion take much simpler forms, such as the continuity equation or Fick's second law. To understand the significance of each term, Equation (2.48) will be non-dimensionalized.

If the system has some characteristic size $[L]$ and the flow has some characteristic speed $[U]$ then the terms in Equation (2.48) can be given in terms of their non-dimensional values:

$$\begin{aligned} \tilde{\nabla} &\equiv [L] \nabla & \tilde{\mathcal{V}} &\equiv \frac{\mathcal{V}}{[U]} \\ \tilde{n} &\equiv [L]^3 n & \tilde{t} &\equiv \left(\frac{[U]}{[L]} \right) t. \end{aligned}$$

Throughout this thesis, $\tilde{\cdot}$ denotes the dimensionless values. Inserting these into Equation (2.48) gives the non-dimensionalized convection-diffusion equation

$$\frac{\partial \tilde{n}}{\partial \tilde{t}} + \tilde{\nabla} \cdot (\tilde{n} \tilde{\mathcal{V}}) = \frac{1}{\text{Pé}} \tilde{\nabla}^2 \tilde{n} + \tilde{\psi}. \quad (2.49)$$

On the right-hand side two dimensionless numbers were introduced. The first is the *Péclet number*

$$\text{Pé} \equiv \frac{[U][L]}{\mathcal{D}}, \quad (2.50)$$

which represents the competition between convective transfer and diffusive processes. The second number $\tilde{\psi} \equiv [L]^4 \psi_n / [U]$ is the ratio of the species generation rate to the convection. When there is no generation (as will always be the case in this work), the Péclet number controls the nature of the transport.

In the limit of $\text{Pé} \ll 1$ diffusion dominates over convection and the convection-diffusion equation reduces to Fick's second law (Equation (2.48) $\rightarrow$ Equation (2.47)). When $\text{Pé} \gg 1$ the flow is dominant and the diffusion is negligible so that the convection-diffusion equation becomes the continuity equation (Equation (2.48) $\rightarrow$ Equation (2.44)).

The Péclet number can be interpreted in a number of ways:

1. The Péclet number is a ratio of time scales. A particle of size $[L]$ subject to a flow $[U]$ will travel its body length in roughly $[\tau]_{\text{conv}} \sim [L] / [U]$ but it diffusively relaxes from such perturbations in a time $[\tau]_{\text{diff}} \sim [L]^2 / \mathcal{D}$ (Figure 2.2.4a). The Péclet number is the ratio

$$\frac{[\tau]_{\text{diff}}}{[\tau]_{\text{conv}}} = \frac{[U][L]}{\mathcal{D}} = \text{Pé}. \quad (2.51a)$$

2. The Péclet number is a dimensionless distance that a solution must elute before it is fully mixed. Solutes within a channel of width $[L]$ require $[\tau]_{\text{diff}} \sim [L]^2 / \mathcal{D}$ to diffuse across (Figure 2.2.4b). In that time, a flow $[U]$ could carry the sample a distance $[L]_{\text{conv}} \sim [U][\tau]_{\text{diff}}$. The Péclet number is

$$\frac{[L]_{\text{conv}}}{[L]} = \frac{[U][\tau]_{\text{diff}}}{[L]} = \frac{[U][L]^2 / \mathcal{D}}{[L]} = \frac{[U][L]}{\mathcal{D}} = \text{Pé}. \quad (2.51b)$$

3. The Péclet number is the ratio of the potential energy drop to the thermal energy for a distribution of sedimented particles (Figure 2.2.4c). The thermal energy is $k_B T$. As will be seen in § 2.3.5, the diffusion coefficient and the friction coefficient ζ are related as $\mathcal{D} = k_B T / \zeta$ through the Stokes-Einstein equation (Equation (2.91)). Although strange, the thermal energy can thus be written $k_B T = \mathcal{D}\zeta$. The drop in potential energy is

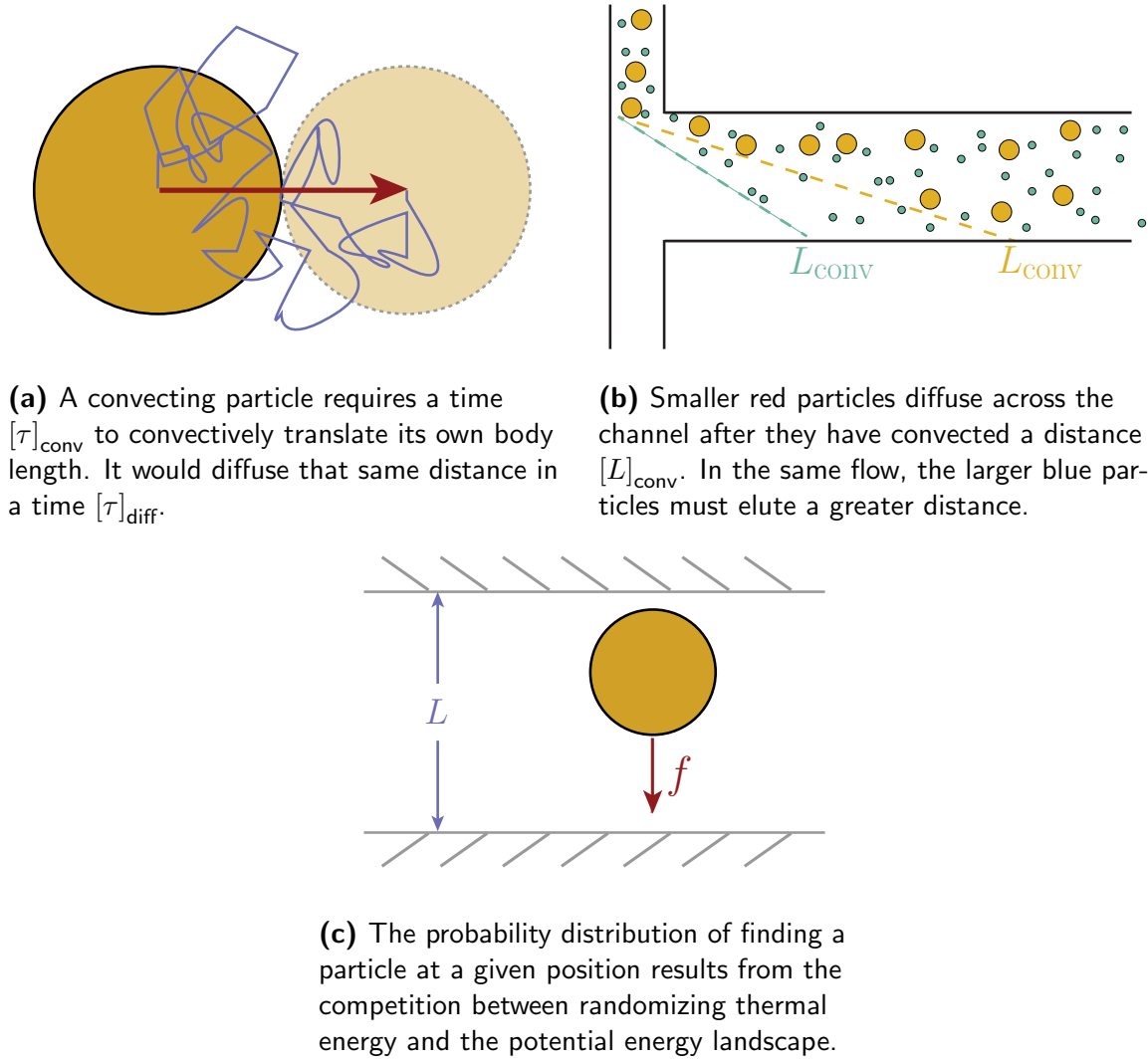


Figure 2.2.4: Schematic representations of the different, though equivalent, Péclet number interpretations.

$f[L]$. Since viscosity causes the motion to be over-damped, the force f acting on the particles and the velocity $\mathcal{V}$ of the particles are related through the Stokes drag $f = \zeta \mathcal{V}$ (Equation (2.80) in § 2.3.5). Therefore, the ratio of the potential energy drop and the thermal energy is

$$\frac{f[L]}{k_{\text{B}}T} = \frac{\zeta \mathcal{V}[L]}{\mathcal{D}\zeta} = \frac{\mathcal{V}[L]}{\mathcal{D}} = \text{Pé}. \quad (2.51\text{c})$$

This form will be particularly meaningful when discussing the separation technique field-flow fractionation (§ 5). As one would expect, the situation dictates the appropriate interpretation.

Summary of Transport Theory

The transport equation (Equation (2.43)) follows from the universal concept of conservation, “Whatever enters plus whatever is created must either exit, be destroyed or remain.” It can be applied to energy, entropy or any extensive quantity. The convection-diffusion equation of mass transport (Equation (2.48)) is the primary transport equation of separation science. The behaviour of the convection-diffusion equation is controlled by the dimensionless Péclet number (Equation (2.50)), which describes the competition between diffusion and convection. The limits of $Pé \ll 1$ and $Pé \gg 1$ correspond to the Fick’s second law (Equation (2.47)) and the continuity equation (Equation (2.44)), respectively. The continuity equation plays a significant role in the description of fluid dynamics and the transport of dispersed analytes occurring within a carrier fluid medium. The motion of the analytes can perturb the carrier fluid and cause long-range hydrodynamic interactions. The next section applies the transport equation to fluid momentum and arrives at the Navier-Stokes equation of fluid dynamics.

2.3 Hydrodynamic Interactions

In this thesis a *solution* is composed of particles (the *solutes*) that are dispersed within a carrier fluid (the *solvent*). Flows within the solvent apply forces to the solutes and inversely the motion of solutes perturbs the solvent. These perturbations can propagate long distances at speeds much greater than any relevant velocity scale and so propagation is assumed to be instantaneous. Thus the motion of one solute particle generates long-range, nearly instantaneous *hydrodynamic interactions* that enact forces on neighbouring solutes. This effectively leads to instantaneous communication between all solutes. Hydrodynamic interactions are ubiquitous and often significant in microfluidic separation methods, though at other times they are effectively screened as in free-solution electrophoresis (§ 2.4.4 and § 4).

In this section, the equations governing fluid dynamics are derived using the transport equation from § 2.2. Since this work focuses on microfluidic systems, the equations take a simplified form, in which hydrodynamic interactions have the possibility of being analytically determined. The conclusion is that the nature of microfluidic systems can be very concisely and yet precisely defined by a pair of non-dimensional numbers.

Likewise, the drag relationship between the force applied to an arbitrarily shaped solute particle (or even an ensemble of solutes) and the resulting velocity can be understood. This section presents an argument for the form of this relationship and the specific equations for many specific particle shapes and conformations. The drag of microscopic solutes subject to external forces through a viscous medium is intimately tied to the random diffusive motion of the solute. This conclusion has important ramifications for determining the mobility of solutes through microfluidic devices.

2.3.1 Navier-Stokes Equation

Applying the transport equation (Equation (2.43)) to the flux of momentum (here the generalized thermodynamic density is the mass density times the fluid velocity field: $\mathbf{x} \rightarrow \varrho \vec{u}$) results in

$$\frac{\partial \varrho \vec{u}}{\partial t} = \psi_{\varrho u} - \vec{\nabla} \cdot \left[(\varrho \vec{u}) \vec{u} + \hat{\gamma}_{\varrho u \varrho u} \cdot \vec{\nabla} F \right].$$

Unfortunately, each of the terms on the right-hand side are rather uninformative in this form. Foremost amongst these is the molecular transport component of the flux of momentum

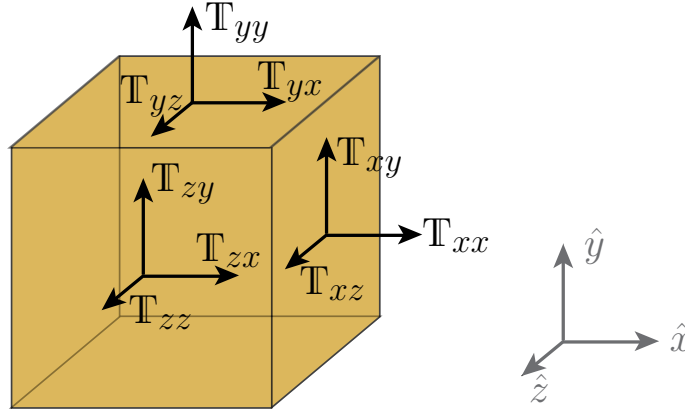


Figure 2.3.1: Stress elements (momentum flux) on each surface of a Cartesian volume.

$\hat{\gamma}_{\varrho u \varrho u} \cdot \vec{\nabla} F$ (see Equation (2.41)). For a moment, let the flux of momentum be written as the *stress tensor*, $\hat{\mathbb{T}} \equiv -\hat{\gamma}_{\varrho u \varrho u} \cdot \vec{\nabla} F = -\hat{j}_{\varrho u, \text{ent}}$. The source term $\psi_{\varrho u}$ is also rather obtuse. The rate of production of momentum is an acceleration and so the source can be more clearly written as $\psi_{\varrho u} \equiv \varrho \vec{g}$, where $\vec{g}$ is the acceleration of the fluid due to an external field such as (but not limited to) gravity. Let it be assumed that the fluid density ϱ is constant. Finally, the convective flux term, $\vec{\nabla} \cdot (\varrho \vec{u} \vec{u})$, can be simplified if the derivation is limited to incompressible fluids. For such fluids, the continuity equation has the simple form $\vec{\nabla} \cdot \vec{u} = 0$ (Equation (2.44) → Equation (2.45)). Therefore, the convective flux term becomes

$$\vec{\nabla} \cdot (\varrho \vec{u} \vec{u}) = \varrho \vec{u} \cdot (\vec{\nabla} \vec{u}).$$

Inserting these more informative forms into the momentum transport equation results in *Cauchy's equation* of motion

$$\varrho \left[\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot (\vec{\nabla} \vec{u}) \right] = \varrho \vec{g} + \vec{\nabla} \cdot \hat{\mathbb{T}}. \quad (2.52)$$

Cauchy's equation applies to all incompressible, continuous materials. Fluid media are characterized by the form of the stress tensor $\hat{\mathbb{T}}$.

Stress Tensor: Isotropic Incompressible Fluid

An element of stress $\mathbb{T}_{ij}$ may be interpreted as the flux of the j -component of the momentum density in the $\hat{i}$ -direction or equivalently as the force in the $\hat{j}$ -direction on a unit area

perpendicular to the $\hat{i}$ -direction (Figure 2.3.1). For this reason, it is natural to break the stress into two parts:

- *normal stresses* for which $i = j$
- *shear stresses* for which $i \neq j$.

The total stress within an infinitesimal element of fluid results from normal pressures but also entropy-producing, dissipative stresses. Therefore, the total stress is commonly written

$$\hat{\mathbb{T}} = \hat{P} + \hat{\Pi}, \quad (2.53)$$

where $\hat{P} = P\hat{\mathbb{I}}$ is the *hydrostatic pressure* (which in a sense belongs with $\varrho\vec{g}$ since it is non-dissipative or elastic) and $\hat{\Pi}$ is the deviatoric stress tensor or, commonly, the *viscous stress* tensor.

The viscous stress arises because of internal friction within the fluid that is generated as the fluid particles are displaced at some rate. The displacement in continuous media is best defined by the *Cauchy strain tensor*

$$\hat{\epsilon} = \frac{1}{2} \left[\vec{\nabla} \delta\vec{x} + \left(\vec{\nabla} \delta\vec{x} \right)^{\top} \right], \quad (2.54)$$

where $\delta\vec{x}$ is a small displacement. Defined in this way, the strain is symmetric. The viscous stress is directly related to the rate of deformation (the strain rate $\hat{\epsilon}$). This is the generalized form of *Newton's law*, which was introduced in § 2.1.2:

$$\hat{\Pi} = \hat{\eta} : \hat{\epsilon}. \quad (2.55)$$

For clarity, a given element of stress is specifically

$$\Pi_{ij} = \sum_k \sum_{\ell} \eta_{ijk\ell} \frac{\partial u_k}{\partial x_{\ell}}, \quad (2.56)$$

which relates the viscous stress to the fluid velocity field $\vec{u}$.

Because η is a rank-four tensor, it potentially possesses **81** distinct kinetic coefficients. Amazingly, symmetry arguments reduce this number from 81 to only two for isotropic fluids. The two remaining coefficients are the *shear viscosity* η (sometimes called the dynamic

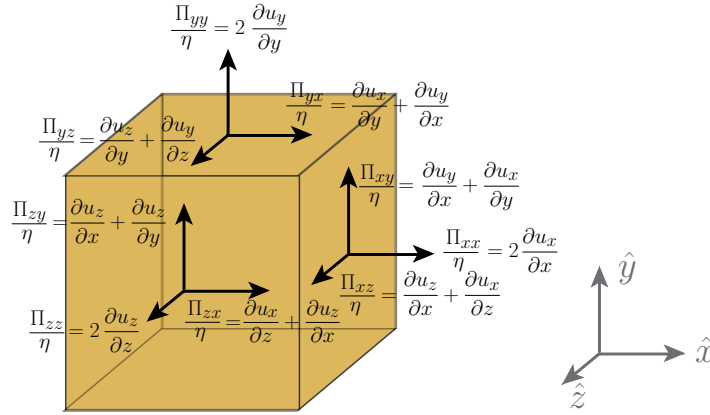


Figure 2.3.2: Viscous stress elements on each surface of a Cartesian volume for an incompressible, Newtonian fluid (after Kirby [26]).

viscosity) and the *dilation viscosity* η' (sometimes called the bulk viscosity) [27]. The remaining stress is

$$\hat{\Pi} = \eta \left[\vec{\nabla} u + (\vec{\nabla} u)^T \right] + \left(\frac{2}{3} \eta - \eta' \right) (\vec{\nabla} \cdot u) \hat{\mathbf{1}}$$

but the second term (involving the dilation viscosity) is proportional to the divergence of the fluid velocity, which by the continuity equation for an incompressible fluid (Equation (2.45)) must be zero. Therefore, although the kinetic coefficient η' exists, it is strictly non-consequential to an incompressible fluid. The generalized Newton's law (Equation (2.55)) is thus reduced to

$$\hat{\Pi} = \eta \left[\vec{\nabla} u + (\vec{\nabla} u)^T \right] \quad (2.57)$$

$$\Pi_{ij} = \eta \left[\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right], \quad (2.58)$$

for an isotropic, incompressible fluid (Figure 2.3.2). Inserting this simplification into Cauchy's equation (Equation (2.52)) produces the equation of motion for an isotropic, incompressible Newtonian fluid

$$\boxed{\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot (\vec{\nabla} \vec{u}) = \vec{g} - \frac{\vec{\nabla} p}{\varrho} + \frac{\eta}{\varrho} \nabla^2 \vec{u}.} \quad (2.59)$$

This is the *Navier-Stokes equation* for fluid dynamics.

Since the pressure is non-dissipative, the pressure gradient and the external acceleration are equivalent and one often speaks of the total effective force density

$$\vec{f}^* \equiv -\vec{\nabla} P \equiv \varrho \vec{g} - \vec{\nabla} P. \quad (2.60)$$

Reynolds Number

As was done to the convection-diffusion equation (Equation (2.48)) in § 2.2.2, the Navier-Stokes equation can be non-dimensionalized into

$$\text{Re} \left[\frac{\partial \vec{u}}{\partial \tilde{t}} + \vec{u} \cdot \left(\tilde{\nabla} \vec{u} \right) \right] = \tilde{\nabla}^2 \tilde{u} - \tilde{\nabla} \tilde{P}. \quad (2.61)$$

There are two dimensionless numbers in Equation (2.61). The first is the *Reynolds number*

$$\text{Re} \equiv \frac{\varrho [L] [U]}{\eta}, \quad (2.62)$$

which controls the nature of the flow. The second dimensionless number defined is a dimensionless pressure $\tilde{P} \equiv P [L] / \eta [U]$. This is the appropriate non-dimensionalization for pressures at low velocities.

Just as with the Péclet number, the Reynolds number can be interpreted in many equivalent ways [28].

1. The Reynolds number is the ratio of typical force densities within a fluid flowing at a characteristic speed $[U]$. The inertial force density is $f_{\text{inert}} = \varrho \vec{u} \cdot \left(\vec{\nabla} \vec{u} \right) \sim \varrho [U]^2 / [L]$, while the viscous force density is $f_{\text{visc}} = \eta \nabla^2 \vec{u} \sim \eta [U] / [L]^2$. The ratio is

$$\frac{f_{\text{inert}}}{f_{\text{visc}}} \sim \frac{\varrho [U]^2 / [L]}{\eta [U] / [L]^2} = \frac{\varrho [L] [U]}{\eta} = \text{Re}. \quad (2.63a)$$

2. It is also the ratio of the characteristic time required for viscosity to dissipate momentum, $[\tau]_{\text{visc}} = \mathcal{D}_{\text{visc}} [L]^2 = \varrho [L]^2 / \eta$, and the time it would take the fluid to flow the

same distance $[\tau]_{\text{conv}} = [L] / [U]$

$$\frac{[\tau]_{\text{visc}}}{[\tau]_{\text{conv}}} \sim \frac{\varrho [L]^2 / \eta}{[L] / [U]} = \frac{\varrho [L] [U]}{\eta} = \text{Re}. \quad (2.63b)$$

3. If an external force $f_{mg} = mg \sim \varrho [U]^2 [L]^2$ acts on a body of size $[L]$ then the resistive, viscous drag force is $f_{\text{drag}} \sim \eta [L] [U]$ (this is Stokes' law [Equation (2.80)], which will be discussed in § 2.3.5). The ratio is

$$\frac{f_{mg}}{f_{\text{drag}}} \sim \frac{\varrho [U]^2 [L]^2}{\eta [L] [U]} = \frac{\varrho [U] [L]}{\eta} = \text{Re}. \quad (2.63c)$$

4. A particle of size $[L]$ and mass $m_p \sim \varrho_p [L]^3$ that is initially traveling with a velocity $[U]$ will decelerate to a stop over some distance $d \sim [U]^2 / g_p$ due to Stokes drag $\sim \eta [L] [U]$ (Equation (2.80)). The deceleration is $g_p = f_{\text{drag}} / m_p \sim [\eta [L] [U] / (\varrho_p [L]^3)]$. The ratio of the stopping distance to the size of the particle is

$$\frac{d}{[L]} \sim \frac{[U]^2 / g_p}{[L]} = \frac{[U]^2}{[L]} \frac{\varrho_p [L]^3}{\eta [L] [U]} = \varrho_p \left(\frac{[U] [L]}{\eta} \right) = \frac{\varrho_p}{\varrho} \text{Re}. \quad (2.63d)$$

5. The fluid medium itself has a characteristic force

$$f_{\text{fluid}} = \frac{\eta^2}{\varrho}.$$

According *Purcell's interpretation*, any body acted on by a force $f = f_{\text{fluid}}$ will have a Reynolds number of unity, **by definition**. This can occur in two ways:

- (a) If the body is acted on by a viscous force:

$$\frac{f_{\text{drag}}}{f_{\text{fluid}}} = \frac{\eta [U] [L]}{\eta^2 / \varrho} = \frac{\varrho [U] [L]}{\eta} = \text{Re} \quad (2.63e)$$

- (b) or if the body is acted on by an inertial force

$$\sqrt{\frac{f_{\text{inert}}}{f_{\text{fluid}}}} = \left(\frac{\varrho [U]^2 [L]^2}{\eta^2 / \varrho} \right)^{1/2} = \frac{\varrho [U] [L]}{\eta} = \text{Re}. \quad (2.63f)$$

As with the Péclet number, the situation once again dictates what interpretation is valid. No matter what interpretation is employed, the consequence to the Navier-Stokes equation

is the same. In high Re flows the inertial terms (left-hand side of Equation (2.59) and Equation (2.61)) dominate over insignificant viscous effects (right-hand side). In low Re flows the inertial forces are negligible and the viscous terms control the velocity field. In the limits of high or low Re, the complexity of the full Navier-Stokes equation (Equation (2.59)) is reduced.

2.3.2 High and Low Reynolds Number Flows

The velocity field as described by the Navier-Stokes equation is highly non-linear, which causes immense mathematical difficulties. Modern fluid dynamics makes heavy use of computational techniques to solve Equation (2.59) but traditionally, the simplifications to Navier-Stokes equation were considered. Flows for which inertial terms dominate the Navier-Stokes equation ($\text{Re} \gg 1$) can be described by Euler's equation, while viscous flows in which inertia is insignificant ($\text{Re} \ll 1$) can be analyzed using the Stokes equation. The Reynolds number (Equation (2.62)) signals whether an analysis can be simplified by considering one limit or the other. Microfluidic flows are safely described by the Stokes equation.

Stokes Equation of Creeping Flow: $\text{Re} \ll 1$

If $\text{Re} \ll 1$, then viscous forces dominate over inertial forces and the Navier-Stokes equations reduces to the very relevant *Stokes equation*

$$\boxed{\eta \nabla^2 \vec{u} = \vec{\nabla} P}. \quad (2.64)$$

This much simpler, linear equation plays a prominent role in separation processes on microfluidic chips. All flows that obey Stokes' equation (Equation (2.64)) share qualities that are vital for understanding low Re in microfluidic situations.

2.3.3 Microfluidics

The class of separation technology that this thesis is interested in is fractionation occurring within microfluidic chips. Microelectromechanical systems (MEMS) have been important due to their ability to work with small sample volumes and their potential for as portable devices. However, building systems with sizes on the scale of microns has general consequences for the physical behaviour of the solution.

Microfluidics are both diffusive (near-unity Péc) and laminar (low Re). Both convective flows and thermal noise play non-negligible roles.

Inertial Forces are Negligible The Reynolds number scales linearly with system size and flow speed (Equation (2.62)). Microfluidic devices have a primary characteristic size between $\sim 1 \mu\text{m}$ and $\sim 1000 \mu\text{m}$ (say $\sim 100 \mu\text{m}$). For room temperature water with density $\rho \approx 1000 \text{ kg m}^{-3}$ and viscosity $\eta \approx 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$, the Reynolds number is $\text{Re} \approx [U] \times \mathcal{O}(10 \text{ s m}^{-1})$. This suggests that a flow speed of about $[U] \sim 0.1 \text{ m s}^{-1}$ in order to have $\text{Re} = \mathcal{O}(1)$. Although this may not appear difficult at first glance, a meter is $\times 10^5$ larger than the characteristic system size of $10 \mu\text{m}$. That is to say, a flow would be required to traverse the characteristic length of the device 10,000 **times per second** just to achieve a Reynolds number of only order unity. Typical velocities are actually in the range $1 \mu\text{m s}^{-1}$ - $10^4 \mu\text{m s}^{-1}$ [18] (say $10^2 \mu\text{m s}^{-1}$) yielding an unsurprising Reynolds number $\text{Re} = \mathcal{O}(10^{-3})$. The conclusion is that the microfluidic world is a

$$\text{Re} \ll 1 \tag{2.65}$$

world and this will assume that $\text{Re} \ll 1$ is valid throughout. As a consequence, in microfluidic devices hydrodynamics are well described by the Stokes equation (Equation (2.64)) rather than requiring the full, non-linear Navier-Stokes equation (Equation (2.59)).

Thermal Noise Is Significant Through the fluctuation-dissipation theorem (§ 2.3.5), the diffusion coefficient is known to be $\mathcal{D} = k_B T / \zeta$ and through the discussion about Stokes drag for a sphere (§ 2.3.5), the friction coefficient is expected to be $\zeta = 6\pi\eta r \approx 20\eta r$. Therefore, the Péclet number for a solute is $\text{Pé} = \frac{[U][L]}{\mathcal{D}} \approx [U] \frac{20\eta r^2}{k_B T}$. In a microfluidic system the solute size is smaller than the characteristic size of the device, so it plays the role of characteristic size. Defining a “typical solute” is difficult since dispersed particle sizes can span many orders of magnitude. Ions are near-nanometer in size (10^{-9} m), while pollen and living cells can be larger than the size of the microfluidic device ($\sim 10^{-6} \text{ m}$). Since the solutes can range so widely in size, the Péclet number can too. Rather than defining a “typical” size, consider the extreme situations:

- In room temperature water flowing at a typical speed of $10^2 \mu\text{m s}^{-1}$, the Péclet number falls in the range $0.01 \lesssim \text{Pé} \lesssim 10^6$.

While it is certainly not the middle of the range,

$$Pé \sim 1 \tag{2.66}$$

describes the fact that the systems considered here require that both convection and diffusion must be accounted for. Specific systems will have greater or lesser Péclet numbers, but they are qualitatively described as “moderate” or near-unity.

Miniaturization

Thus, microfluidic separation devices are summarized as *low Re* and *near-unity Pé* systems. Is this good for fractionation techniques? The absence of turbulent mixing is certainly appealing, as is having systems in which convective motion is more significant than diffusive mixing, but this is not where the main benefit lies. The value of microfluidic systems is in the fact that analysis by separation or purification can exist inline with other processing and that sample volumes are small. The benefit of small sample volumes is twofold:

- Material costs are reduced since samples can be quite expensive or even irreplaceable.
- Portability is possible. Microfluidic components can be incorporated together on a microfluidic chip and integrated into hand-held devices.

Miniaturization does indeed present many challenges; however, this thesis highlights that it also offers opportunities for novel separation techniques.

2.3.4 Consequences of the Stokes Equation

What are the consequences of low Re on the hydrodynamic flows that occur within microfluidic devices? A series of very general but practical conclusions can immediately be drawn.

Linearity: Laminar Flows Flows at large Reynolds numbers are not well-behaved. The non-linearity of the Navier-Stokes equation causes unpredictable turbulent flow fields to exist as solutions of Equation (2.59). Irregularities occur in the velocity fields and flows are unstable and characterized by rapid, intense fluctuations. This churning and turning of fluid

elements greatly enhances mixing, which (if purely diffusive) is a strikingly slow process. Even flows that everyday experience would define as “smooth” possess irregular currents that have a significant impact on mixing.

On the other hand, the Stokes equation is linear and flows are well-behaved. Such flows are described as *laminar*. In laminar flows, motion is orderly and without eddies, such that streamlines do not cross. There can be no lateral mixing of fluid.

Linearity: Superimposing Solutions Because the Stokes equation is linear, solutions for complex situations can be obtained by **superimposing** solutions to simpler situations. The ability to superimpose solutions is quite important. Linearity ensures that all interactions occur in a pair-wise fashion.

Long-Range: Hydrodynamic Interactions Solute particles moving through a carrier fluid perturb the velocity field. These perturbations persist for quite long distances $|\vec{x}| = \rho$. In fact, perturbations decay only as $\sim \rho^{-1}$. As an immediate consequence of this, solutes have long-range interactions. For instance, the extent of coupling between distinct segments of a charged polymer chain is examined in § 4 and increased drag on solutes due to hydrodynamic interactions with channel walls are considered in § 7.1. The long-range nature of hydrodynamic interactions plays a paramount role in this thesis and so the far-field behaviour is explicitly demonstrated below.

Consider a single point force $\vec{f}(\vec{x}, t) = \vec{f}_o \delta(\vec{x})$. Stokes’ equation for such a force is

$$\eta \nabla^2 \vec{u} = \vec{\nabla} P - \vec{f}_o \delta(\vec{x}). \quad (2.67)$$

The solution flow field that results from Equation (2.67) is called a *stokeslet*. To solve for the stokeslet, the Stokes equation is cast in Fourier space. Each term in Equation (2.67) can be Fourier transformed such that the point force Stokes equation and the continuity equation in Fourier space are

$$-\eta q^2 \text{FT}[\vec{u}] = i\vec{q} \text{FT}[P] - \text{FT}[\vec{f}_o] \quad (2.68)$$

$$\vec{q} \cdot \text{FT}[\vec{u}] = 0. \quad (2.69)$$

By solving the Stokes equation for velocity in terms of pressure and substituting into the continuity equation, the pressure in Fourier space can be found, which can then be substituted back into the Fourier space velocity. Performing the inverse Fourier transform on these

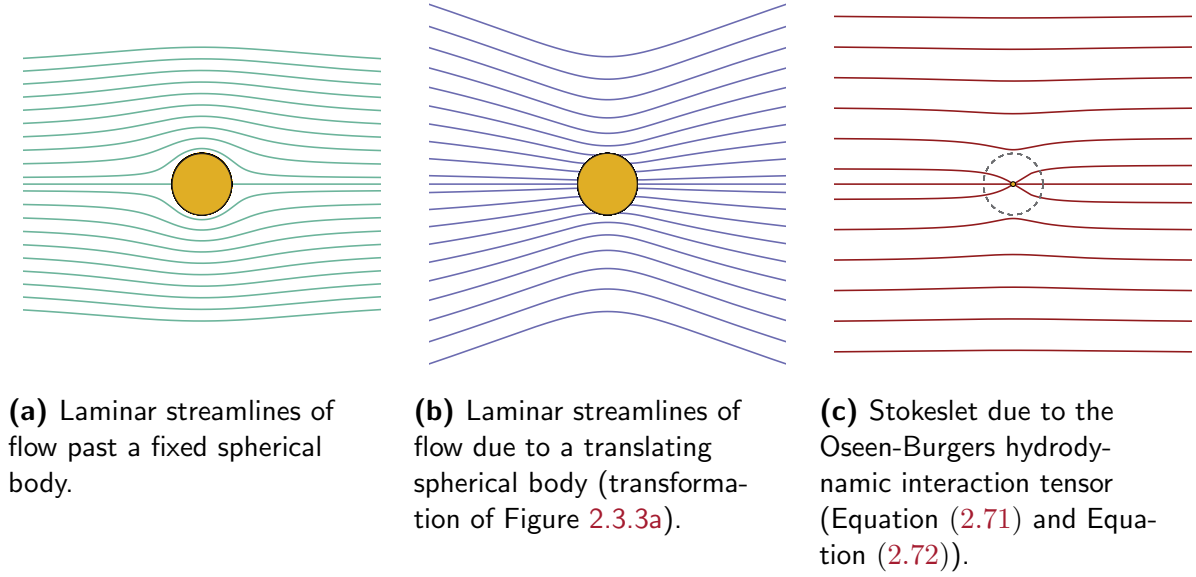


Figure 2.3.3: Laminar flow fields associated with spherical and point-like solutes.

produces the pressure field

$$P(\vec{x}) = \frac{\vec{x} \cdot \vec{f}_o}{4\pi\rho^3} \quad (2.70)$$

and stokeslet velocity field

$$\vec{u}(\vec{x}) = \hat{\mathcal{H}}_{\text{OB}}(\vec{x}) \cdot \vec{f}_o, \quad (2.71)$$

which has been written in terms of a Green's function for the *hydrodynamic interaction tensor* for a point force (which is specifically called the *Oseen-Burgers tensor*)

$$\hat{\mathcal{H}}_{\text{OB}}(\vec{x}) = \frac{1}{\rho} \left(\hat{\mathbf{1}} + \frac{\vec{x} \vec{x}}{\rho^2} \right) \left(\frac{1}{8\pi\eta} \right). \quad (2.72)$$

A series of important conclusions can be drawn about and from the stokeslet:

- The stokeslet (Equation (2.71)) has the form of a linear transport phenomenon like all the examples discussed in § 2.1.2. That is to say that the Oseen-Burgers tensor is the rank-two linear kinetic coefficient tensor relating the generalized flux field (fluid velocity) to the affinity (an actual force, in this case).
- The Oseen-Burgers hydrodynamic interaction tensor scales as ρ^{-1} . Thus, the stokeslet

decays languidly with distance from the point of perturbation and hydrodynamic perturbations are long-range (Figure 2.3.3c).

- Just as any arbitrary distribution of charges can be effectively represented as a single point charge when viewed from sufficiently large distances in electrostatics, so too the velocity field far enough from any force distribution is adequately described by a stokeslet. The precise hydrodynamic interaction tensor $\hat{\mathcal{H}}$ approaches $\hat{\mathcal{H}}_{\text{OB}}$ when viewed in the far-field limit. Figure 2.3.3a shows the flow field around a solid sphere of radius r . The corresponding flow field of a sphere translating through stagnant fluid is most easily found by a change of reference frames (Figure 2.3.3b). The stokeslet (Figure 2.3.3c) is dissimilar to the flow due to a moving sphere in the vicinity of the sphere but they become comparable for radial distances $\rho \gg r$.
- Because solutions can be superimposed, complicated force distributions can be constructed as multipole expansions of stokeslets. Consider a rigid solute particle moving through stagnant fluid. Since the surface of the particle enacts a continuous stress force of $\hat{\Pi}(\vec{x}') \cdot \hat{n} \, d\Sigma_{\vec{x}'}$ on the fluid at each point $\vec{x}'$ of the surface, superimposing solutions gives the resulting velocity at some distant point $\vec{x}$ far from the surface to be

$$\vec{u}(\vec{x}) = - \oint_{\Sigma} \left[\hat{\Pi}(\vec{x}') \cdot \hat{n} \right] \cdot \hat{\mathcal{H}}_{\text{OB}}(\vec{x} - \vec{x}') \, d\Sigma_{\vec{x}'} . \quad (2.73)$$

Identifying the total force on the particle within Equation (2.73) suggests that the form of Equation (2.71) is universal to any force distribution or an arbitrary particle shape at low Re:

$$\vec{u}(\vec{x}) = \hat{\mathcal{H}}(\vec{x}) \cdot \vec{f} . \quad (2.74)$$

Except for the simplest shapes, Equation (2.73) is too complex to be useful. But, again as in electrostatics, the hydrodynamic interaction tensor can be approximated (to arbitrary precision) by a *multipole expansion*

$$\hat{\mathcal{H}}(\vec{x} - \vec{x}') = \sum_{n=0}^{\infty} \frac{(-1)^n}{n!} \left(\vec{x}' \cdot \vec{\nabla} \right)^n \hat{\mathcal{H}}_{\text{OB}}(\vec{x}) . \quad (2.75)$$

A Stokes dipole is produced by superimposing two stokeslets of equal and opposite force separated by an infinitesimal distance. Summing two such Stokes dipoles oriented perpendicular to each other creates a so-called stresslet interaction tensor, while subtracting two Stokes dipoles oriented perpendicular to each other creates a rotlet.

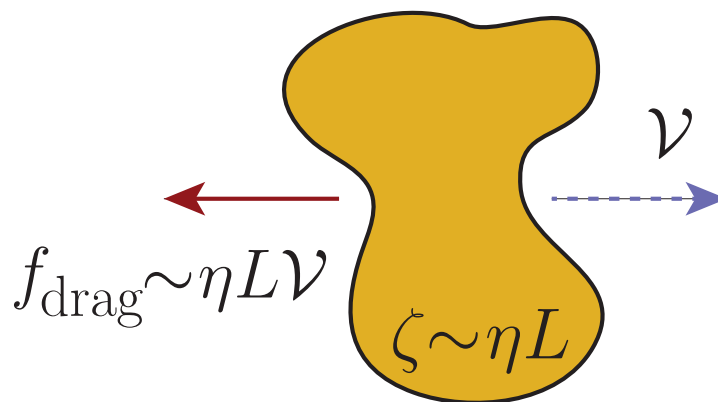


Figure 2.3.4: The drag on any body scales as the velocity $\mathcal{V}$ times the friction coefficient ζ at low Reynolds number. For an arbitrarily shaped body the friction coefficient must scale as the viscosity of the fluid medium η times the characteristic size of the body $[L]$.

While the specific forms of these Stokes multipoles are unimportant, the results of a multipole expansion method (Equation (2.75)) will be utilized to accurately describe the motion of a large colloid eluting through a microfluidic channel in § 5.

2.3.5 Stokes Drag

The previous section was concerned with the velocity fields that result due to the perturbative motion of an analyte through the fluid medium but, of course, the fluid applies an equal and opposite force on the solute. The total resistive force enacted by the fluid on any solute particle moving at velocity $\mathcal{V}$ (the *drag force*) is

$$f_{\text{drag}} = - \oint_{\Sigma} \left[\hat{\Pi}(\vec{x}') \cdot \hat{n} \right] d\Sigma. \quad (2.76)$$

Rather than attempting to re-derive analytical solutions for the drag on various shapes of solute particles, this section presents simple arguments for the form of the drag force and lists (without derivation) several specific cases.

The stokeslet (Equation (2.71)) suggests that an arbitrarily shaped particle moving with velocity $\mathcal{V}$ produces a force on the solvent that scales as $f_{\text{drag}} \sim \mathcal{H}^{-1} \mathcal{V}$ (as shown schematically in Figure 2.3.4). For points near the surface of a solute particle of size $[L]$, the form of the Oseen tensor (Equation (2.72)) suggests that the actual hydrodynamic interaction tensor

scales as $\mathcal{H} \sim 1/\eta [L]$. Together, these facts immediately suggest that the drag force on a particle scales as

$$f_{\text{drag}} \sim \eta [L] \mathcal{V}. \quad (2.77)$$

This scaling result could have just as easily been predicted from dimensional analysis. Since the drag force is due to viscous dissipation, one expects $f_{\text{drag}} \sim \eta$. Simple dimensional analysis then requires $f_{\text{drag}} \sim \eta [L]^2 / [\tau]$. However, there is no intrinsic time-scale except that defined by the particle velocity $[\tau] = [L] / \mathcal{V}$ such that the drag force scales as

$$f_{\text{drag}} \sim \frac{\eta [L]^2}{[\tau]} = \frac{\eta [L]^2}{[L] / \mathcal{V}} = \eta [L] \mathcal{V}.$$

Both arguments lead to the same form.

Indeed, this scaling is strictly true at low Re and in analogy to the linear transport theory of § 2.1.2, the drag force created due to a particle moving at a velocity $\vec{\mathcal{V}}$ is written as

$$f_{\text{drag}}^{\vec{}} = \hat{\zeta} \cdot \vec{\mathcal{V}}, \quad (2.78a)$$

where $\hat{\zeta}$ is a rank-two kinetic coefficient tensor called the *friction coefficient tensor*. It is pedantic, but rather than considering the force on the surrounding fluid due to the motion of the particle, one can consider the force $\vec{f}$ that must be applied to move the particle with a velocity $\vec{\mathcal{V}}$. Then the equivalent linear relationship would be written

$$\vec{\mathcal{V}} = \hat{\mu} \cdot \vec{f}, \quad (2.78b)$$

where the kinetic coefficient tensor $\hat{\mu}$ in this form is the *hydrodynamic mobility*. Obviously, the two relationships are inverses and so the friction and the hydrodynamic mobility are as well:

$$\hat{\mu} = \hat{\zeta}^{-1}. \quad (2.78c)$$

Hydrodynamic mobility and friction coefficients are used interchangeably in both the literature and this thesis.

Since the drag scales with viscosity and particle size (Equation (2.77)) as well as velocity



Figure 2.3.5: The drag on a spherical body of radius r moving through a fluid of viscosity η at low Reynolds numbers (small speed v) has a friction coefficient that obeys Stokes' law $\zeta = 6\pi\eta r$ (Equation (2.80)).

(Equation (2.78a)), the friction coefficient is expected to scale as

$$\zeta \sim \eta [L].$$

For this reason, friction coefficients are often written as

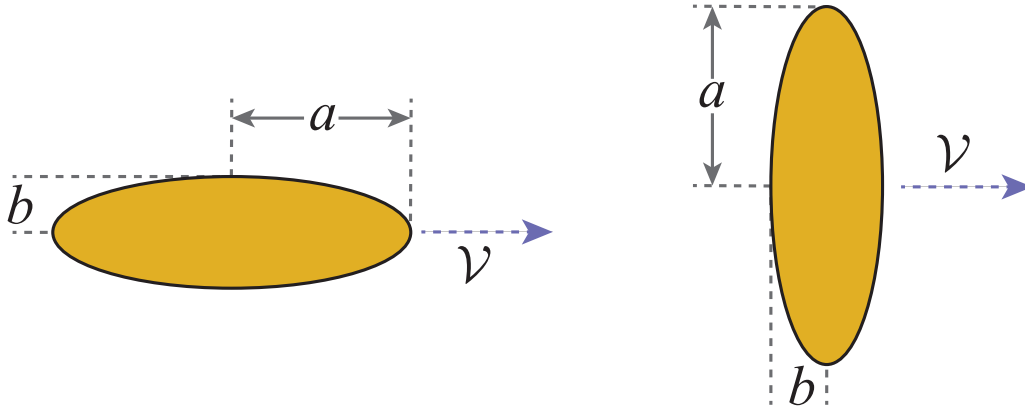
$$\hat{\zeta} \equiv 6\pi\eta [L] \hat{K}. \quad (2.79)$$

This is an appealing form because *Stokes' law* for the drag on a spherical obstacle of radius r with no-slip boundary conditions (Figure 2.3.5) is

$$\boxed{\zeta = 6\pi\eta r}. \quad (2.80)$$

Thus, the dimensionless *resistance tensor* $\hat{K}$ (sometimes called the translation tensor) gives the correction to Stokes' law. The functions for the inverse of the resistance tensor K^{-1} are called *slip functions* [29].

Since the focus of this thesis is the controlled motion of solutes through microfluidic devices for separation purposes, hydrodynamic mobility plays a paramount role. Understanding the true hydrodynamic mobility in terms of the mobility of simplified bodies proves valuable at times.



(a) Ellipsoid oriented with the major axis (of length a) oriented parallel to the direction of motion.

(b) Ellipsoid oriented with the major axis oriented perpendicular to the direction of motion.

Figure 2.3.6: The friction coefficient of an ellipsoid oriented parallel to its direction of motion (a) is approximately half that of an ellipsoid oriented perpendicular (b), as shown in Equation (2.82c).

Resistance Coefficient Examples

The resistance for many symmetric bodies (for which $\hat{K} = K\hat{\mathbf{1}}$) are known. For example [30]

$$K = \begin{cases} 1 & \text{Spherical with no-slip} \\ \frac{2}{3} & \text{Spherical with perfect slip} \\ \left(\frac{br+2\eta}{3\eta+br} \right) & \text{Spherical with sliding friction, } b \\ \frac{1+\frac{2}{3}\eta_i/\eta_o}{1+\eta_i/\eta_o} & \text{Spherical droplet (i=inside, o=outside of droplet)} \\ \left(1 + \frac{3}{16}\text{Re} \right) & \text{Spherical with no-slip, finite Re (Oseen's correction).} \end{cases} \quad (2.81a)$$

When the object is asymmetric, K becomes a tensor. Of particular utility are the resistance coefficients for spheroids. Most important for this work are ellipsoids since the polymers considered in § 4 can be roughly viewed as chains of rod-like segments. The components of the resistance tensor for an ellipsoid (Figure 2.3.6) are [31]

$$K = \begin{cases} \frac{8}{3a} \frac{a^2-b^2}{(2a^2-b^2)\mathcal{S}-2a} & \text{Ellipsoid oriented parallel to } a \\ \frac{16}{3a} \frac{a^2-b^2}{(2a^2-3b^2)\mathcal{S}+2a} & \text{Ellipsoid oriented perpendicular to } a \text{ (along } b), \end{cases} \quad (2.82a)$$

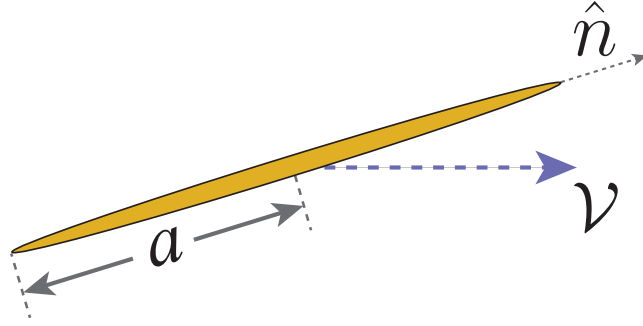


Figure 2.3.7: The friction coefficient of a rod-like body is orientation dependent. A rod oriented perpendicular to its direction of motion has a friction coefficient that is roughly double that of a rod oriented along its direction of motion.

where a is the major axis, b is the semi-axis and

$$\mathcal{S} = \begin{cases} 2(a^2 - b^2)^{-1/2} \ln \left[\frac{a + (a^2 - b^2)^{1/2}}{b} \right] & \text{if prolate } (a > b) \\ 2(b^2 - a^2)^{-1/2} \tan^{-1} \left[\frac{a + (b^2 - a^2)^{1/2}}{a} \right] & \text{if oblate } (a < b). \end{cases} \quad (2.82b)$$

By taking the limit $a \gg b$, one finds the resistance tensor for a rod of length $L = 2a$. The resistance coefficients of a rod parallel and perpendicular to the major axis are then

$$K \approx \begin{cases} K_{\parallel, \text{rod}} = \frac{4}{3} \left[2 \ln \left(\frac{2a}{b} \right) - 1 \right]^{-1} \\ K_{\perp, \text{rod}} = \frac{8}{3} \left[2 \ln \left(\frac{2a}{b} \right) + 1 \right]^{-1} \approx 2K_{\text{rod}, \parallel}, \end{cases} \quad (2.82c)$$

which means that the friction tensor in the direction of motion for a rod of length $2a$ and width b , whose major axis is aligned in a direction $\hat{\mathbf{n}}$ with respect to the flow direction, is [28]

$$\hat{\zeta} = \zeta_{\parallel} \hat{\mathbf{n}} \hat{\mathbf{n}} + \zeta_{\perp} \left(\mathbb{1} - \hat{\mathbf{n}} \hat{\mathbf{n}} \right), \quad (2.82d)$$

where

$$\zeta_{\parallel} \approx \frac{4\pi\eta a}{\ln \left(\frac{2a}{b} \right)} \quad \text{and} \quad \zeta_{\perp} \approx 2\zeta_{\parallel}. \quad (2.82e)$$

The friction coefficient of a long rod oriented perpendicular to a low Re flow is roughly twice as great as a rod aligned with the flow. It takes twice as much force to drag a rod perpendicularly as it does to drag it along its length.

One can also calculate the drag on an ensemble of spheres as a single friction coefficient

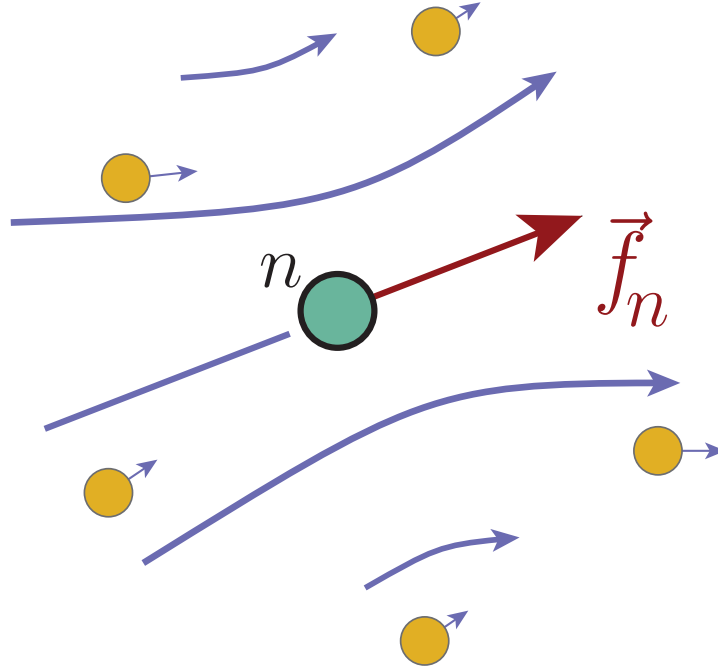


Figure 2.3.8: The motion of a solute n subject to a force perturbs the surrounding fluid. The resulting flow enacts a hydrodynamic-mediated force on neighbouring solutes. Likewise, the motion of all the neighbouring solutes enacts a net force on the n^{th} monomer. This is the idea of long-range hydrodynamic interactions (after Doi[32]).

cient/hydrodynamic mobility (Figure 2.3.8). Say that the i^{th} solute particle is acted upon by a force $\vec{f}$ such that if it were alone, moving through stagnant solvent, it would move with a velocity $\vec{\mathcal{V}}_i = \hat{\mu}_i \cdot \vec{f}$ (Equation (2.78b)). If the surrounding fluid moves with some velocity $\vec{u}(\vec{x}, t)$ then by superposition the particle's velocity becomes $\vec{\mathcal{V}}_i = \hat{\mu}_i \cdot \vec{f} + \vec{u}$. The flow field $\vec{u}$ can be due to boundary conditions or pressure gradients. This is the unperturbed velocity field. Let the unperturbed velocity field be represented by $[\vec{U}](\vec{x}, t)$. However, the flow field can also be due to the motion of the neighbouring solutes. The motion of these neighbours produces the flow field $\vec{u} = \sum_{j \neq i} \hat{\mathcal{H}}_j(\vec{x}_i - \vec{x}_j) \cdot \vec{f}_j$ where $\vec{f}_j$ is the force on the j^{th} neighbour and $\hat{\mathcal{H}}_j$ is the hydrodynamic interaction tensor appropriate to its shape.

The velocity of the i^{th} particle is then

$$\vec{\mathcal{V}}_i = [\vec{U}] + \hat{\mu}_i \cdot \vec{f}_i + \sum_{j \neq i} \hat{\mathcal{H}}_j \cdot \vec{f}_j. \quad (2.83a)$$

One can concisely write the velocity of all the solutes using the set notation introduced in

§ 2.1.1 to be

$$\mathbf{v} = [\mathbf{U}] + \underline{\boldsymbol{\mu}} \cdot \mathbf{f}, \quad (2.83b)$$

where $\mathbf{v}$ is the set of all solute velocities, $[\mathbf{U}]$ is the set of unperturbed flow velocities at each point and $\mathbf{f}$ is the set of all forces on the solutes. The hydrodynamic mobility tensorial set is

$$\underline{\boldsymbol{\mu}} = \begin{pmatrix} \hat{\mu}_1 \delta(\vec{x}_1) & \hat{\mathcal{H}}_{12}(\vec{x}_1 - \vec{x}_2) & \hat{\mathcal{H}}_{13}(\vec{x}_1 - \vec{x}_3) & \dots \\ \hat{\mathcal{H}}_{21}(\vec{x}_2 - \vec{x}_1) & \hat{\mu}_2 \delta(\vec{x}_2) & \hat{\mathcal{H}}_{23}(\vec{x}_2 - \vec{x}_3) & \dots \\ \vdots & & \ddots & \end{pmatrix}.$$

This seems complicated but if the external force on each solute particle is the same then things appear more transparent. In that case, the velocity of the i^{th} solute becomes

$$\begin{aligned} \vec{v}_i &= [\vec{U}] + \hat{\mu}_i \cdot \vec{f}_i + \sum_{j \neq i} \hat{\mathcal{H}}_j \cdot \vec{f}_j \\ &= [\vec{U}] + \hat{\mu}_{\text{eff}} \cdot \vec{f} \end{aligned} \quad (2.84)$$

where $\hat{\mu}_{\text{eff}} \equiv \hat{\mu} + \sum_{j \neq i} \hat{\mathcal{H}}_j$ is the effective hydrodynamic mobility of the i^{th} particle that accounts for hydrodynamic interactions between solutes. This reveals that there is nothing fundamentally different when hydrodynamic interactions between solutes are present but rather that one can simply discuss an effective mobility. Additional complications can arise due to the presence of walls:

1. The effective mobility of each particle will be modified by hydrodynamic interactions with the walls in much the same spirit as with interactions with other particles;
2. but walls may also screen hydrodynamic interactions between monomers.

Walls will screen interactions on length scales greater than the length scale associated with the walls. For instance, when polymers are confined to a slit, hydrodynamic interactions are screened on distances greater than the spacing between the walls [33–35].

If the distance between neighbours is assumed to be much larger than the characteristic size of the solutes then things simplify further since $\hat{\mathcal{H}} \rightarrow \hat{\mathcal{H}}_{\text{OB}}$. This corresponds to the infinitely dilute limit assumed in this thesis. Hydrodynamic interactions have been included in a pre-averaged manner in neutral polymer dynamics [32, 36, 37] and have been used to

calculate the resistance coefficient for sets of spheres of radius r separated by a distance ℓ . To first order, the slip functions are [30, 38, 39]:

$$K^{-1} = \begin{cases} \left[1 + \frac{r}{\ell}\right] / 2 & \text{Two spheres in a line} \\ \left[1 + \frac{10}{3} \frac{r}{\ell} - \frac{1}{4} \left(\frac{r}{\ell}\right)^2\right] / 3 & \text{Three spheres in a line} \\ \left[1 + \frac{13}{2} \frac{r}{\ell} - \frac{9}{8} \left(\frac{r}{\ell}\right)^2\right] / 4 & \text{Four spheres in a line} \\ \left[1 + 2.7 \frac{r}{\ell} - 0.04 \left(\frac{r}{\ell}\right)^2\right] / 4 & \text{Four spheres on the corners of a square} \\ \left[1 + 5.7 \frac{r}{\ell} - 0.34 \left(\frac{r}{\ell}\right)^2\right] / 8 & \text{Eight spheres on the corners of a cube,} \end{cases} \quad (2.85a)$$

where these have been averaged over all orientations and assume that the distance between the centres of the spheres is large ($\ell \gg r$). When particle interactions are allowed (but ℓ is still large) and orientation is held fixed then the slip functions are approximately [30, 39–41]

$$K^{-1} = \begin{cases} 1 + \frac{3}{2} \frac{r}{\ell} - \left(\frac{r}{\ell}\right)^3 & \text{Two spheres } \parallel \text{ to flow} \\ 1 + \frac{3}{4} \frac{r}{\ell} + \frac{1}{2} \left(\frac{r}{\ell}\right)^3 & \text{Two spheres } \perp \text{ to flow.} \end{cases} \quad (2.85b)$$

In particular, notice that the resistance coefficient (and so, too, the drag force) of two spheres in either conformation is less than double that of a single sphere. Furthermore, the resistance coefficient for two spheres aligned with the flow is less than for two spheres held perpendicular to the flow. This qualitatively agrees with what was seen for rod orientation.

The large number of examples for the resistance (friction) coefficients of many different objects suspended in a low Re carrier fluid has been given above in order to stress that they are all near-unity corrections to Stokes' law (Equation (2.80)). The shape and the connectivity (be it physical or through hydrodynamic interactions) affects only the form of K or equivalently ζ and not the dependence $f_{\text{drag}} \sim \eta r \mathcal{V}$ that was predicted by scaling.

Stokes-Einstein Equation

The friction coefficient describes a solute particle's resistance to motion through a low Re fluid while subject to an external force. The diffusion coefficient describes a solute's sensitivity to thermal forces. One deals with non-random external forces, while the other deals with random thermal forces. However, they both treat a solute's propensity to move subject to force and they are intimately tied together, as was already hinted at for diffusivity and electrostatic mobility (§ 2.1.2).

In order to see this in an elegant (though informal) way, consider a particle diffusing in a one dimensional harmonic well of potential $U = kx^2/2$. The particle is subject to white, thermal noise $\xi(t)$ of amplitude A and so its position $x(t)$ obeys a Langevin equation

$$\zeta \frac{\partial x}{\partial t} = -kx + \xi(t). \quad (2.86)$$

Since the noise is symmetric, its first moment must be zero ($\langle \xi(t) \rangle = 0$) and the average solution to Equation (2.86) is an exponential decay. This suggests that the general solution to Equation (2.86) is an exponential and that the appropriate Green's function in response to a perturbation is $G(t, t') = \Theta(t - t') \exp[-(t - t') / [\tau]]$ where $[\tau] = \zeta/k$ is the characteristic time scale. This is enough information to calculate the average squared displacement

$$\begin{aligned} \langle \Delta x^2 \rangle &= \left\langle \left[\frac{1}{\zeta} \int_0^t \xi(t') dt' \right] \left[\frac{1}{\zeta} \int_0^t \xi(t'') dt'' \right] \right\rangle \\ &= \frac{A}{\zeta^2} \frac{[\tau]}{2} \left[1 - \exp\left(-2\frac{t}{[\tau]}\right) \right]. \end{aligned} \quad (2.87)$$

Why is this form useful? It is useful because it can be compared to the physically known limits to the average squared displacement:

1. At short times, the particle simply diffuses, unaware that it is confined in a harmonic trap. This means that the average squared displacement obeys a *random walk*: $\langle \Delta x^2 \rangle = 2\mathbf{d}\mathcal{D}t$.
2. At long times, the particle is trapped within the well and its energy must obey the *equipartition theorem*, meaning $\langle U(\vec{x}) \rangle = \mathbf{d}k_{\text{B}}T/2$. Of course, $\langle U(\vec{x}) \rangle = k \langle \Delta x^2 \rangle / 2$ as well. Together the physical limits read

$$\langle \Delta x^2 \rangle = \begin{cases} 2\mathbf{d}\mathcal{D}t & t \rightarrow 0 \\ \frac{\mathbf{d}k_{\text{B}}T}{k} & t \rightarrow \infty. \end{cases} \quad (2.88)$$

If the long-time limit of the response (Equation (2.87)) is investigated, one finds $\lim_{t \rightarrow \infty} \langle \Delta x^2 \rangle = A[\tau] / (2\zeta^2)$, which can be compared to Equation (2.88) to see that the amplitude of the noise is

$$A = 2\zeta k_{\text{B}}T. \quad (2.89)$$

Likewise, by Taylor expanding Equation (2.87) the short-time behaviour is $\lim_{t \rightarrow 0} \langle \Delta x^2 \rangle =$

At/ζ^2 . Inserting the amplitude and comparing to Equation (2.88) reveals that

$$\mathcal{D} = \frac{k_B T}{\zeta}. \quad (2.90)$$

This is the *Einstein relation* that relates the diffusion coefficient $\mathcal{D}$ to the friction coefficient ζ . It does for the friction coefficient what the Nernst-Einstein relation did for electrophoretic mobility (Equation (2.33b)). It is a very relevant example of the *fluctuation-dissipation theorem*, which quite generally says that fluctuations in equilibrium are intimately tied to dissipation as a perturbed system returns to equilibrium [42, 43].

It is clear that through the fluctuation-dissipation theorem the friction coefficient and hydrodynamic mobility are equivalent to the diffusivity. Furthermore, through the Stokes drag (Equation (2.80)), the Einstein relation trivially becomes the *Stokes-Einstein equation* for a spherical, no-slip solute

$$\mathcal{D} = \frac{k_B T}{\zeta} = \frac{k_B T}{6\pi\eta r}, \quad (2.91)$$

which ties the hydrodynamic mobility and diffusion coefficient to the particle's hydrodynamic radius r .

Summary of Hydrodynamic Interactions

Separation science seeks to separate different species of solutes that are mixed and dispersed within a fluid medium. The dynamics of the fluid medium are controlled by the Navier-Stokes equation (Equation (2.59)), which can be non-dimensionalized (Equation (2.61)) to reveal that the behaviour is controlled by the dimensionless Reynolds number (Equation (2.62)). The Reynolds number can be interpreted in many ways that compare the relative importance of inertia and viscous effects. In low Reynolds number situations, such as is nearly always the case in microfluidic systems, the non-linear Navier-Stokes equation reduces to the linear Stokes equation (Equation (2.64)).

Particles moving through a fluid feel a drag force. The drag force is related to the velocity through a kinetic coefficient tensor called the friction coefficient, the inverse of which is called the hydrodynamic mobility. The friction coefficient tensor very generally depends on the viscosity of the medium and the size and shape of the solute. For a spherical particle the friction coefficient obeys Stokes' law (Equation (2.80)). The friction coefficients of many more

complicated bodies can be given as a correction (called the resistance tensor) to Stokes' law. Friction coefficients are tied to diffusion coefficients through the Stokes-Einstein equation (Equation (2.90)), which is a classic example of the fluctuation-dissipation theorem.

The inverse of considering the drag force on a particle is to consider the fluid velocity field due to the force exerted on it by the particle. Moving particles perturb the fluid medium and when viewed from sufficiently far away, the flow field due to any solute particle looks like the perturbation due to a point force (Equation (2.71)). A kinetic coefficient tensor called the hydrodynamic interaction tensor relates the resulting fluid velocity field to the force distribution. For a point force this is called the Oseen-Burgers hydrodynamic interaction tensor (Equation (2.72)). These perturbations (and therefore also the hydrodynamic interactions between solute particles) are long-range.

The presence of charges in the solvent can dramatically change these hydrodynamic interactions. Redistribution of counterions not only screens Coulombic interactions but also hydrodynamic interactions.

2.4 Electro-Hydrodynamics

Bodies (both surfaces and solutes) have a tendency to be charged in microfluidic systems. Having presented the theoretical framework for the flux of solute particles (§ 2.2) and the dynamics of the carrier fluid (§ 2.3), the most significant of the (many) remaining complications is the behaviour of locally non-zero charge distributions. One may be tempted to think that long-range electrostatic repulsion (or attraction) between objects is the principle effect caused by the fact that bodies (both surfaces and solutes) immersed in solvents tend to be charged; however, this is untrue. The electrostatic potential of the surfaces attracts ions of the opposite charge in the fluid medium (*counterions*). This establishes a distribution of free ions that screens Coulombic interactions between highly charged bodies. In fact, not only are Coulombic interactions screened but so too are hydrodynamic interactions due to electrophoretic motion.

2.4.1 Electric Double Layer

Surface charge densities establish electrostatic potentials that act on the free, mobile ions in solution. The potential modifies the distribution of ions until the surface potential, the entropy and the interactions between counterions are in equilibrium. The resulting charge distribution is called the *electric double layer* because it is mainly composed of two layers in the immediate surface region, followed by the homogeneous bulk value distribution of charges far from the surface.

Stern Layer The inner region is approximately a monolayer of counterions that have adsorbed to a fraction of available sites on the surface and so have changed the surface charge density from $\sigma_{\text{el},0}$ to a lower value. This creates an effective surface charge density of $\sigma_{\text{el},\delta}$. As a consequence, the potential at the surface changes from Ψ_0 to Ψ_δ . This *Stern layer* has a thickness of δ (Figure 2.4.1). The thickness is often ill-defined in that charges in the Stern layer have adsorbed to the surface.

At some point slightly beyond δ the charged fluid molecules are again mobile. This point is called the *slipping plane* (Figure 2.4.1). The electric potential at the slipping plane is called the *zeta-potential* (ζ -potential) and it is common to assume that

$$\Psi(\delta) = \Psi_\delta \approx \zeta,$$

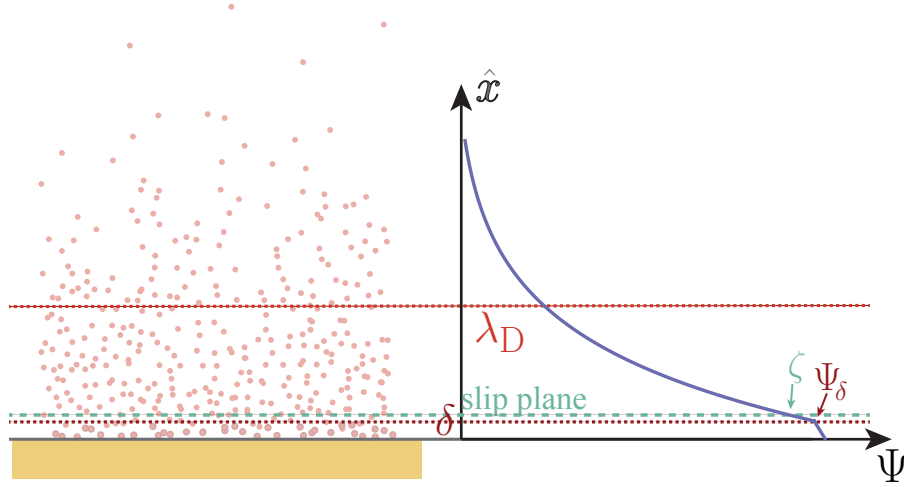


Figure 2.4.1: Schematic of electric double layer.

since experimental techniques are able to measure the ζ -potential but it is difficult to measure Ψ_δ . Equivalence $\Psi_\delta \approx \zeta$ is assumed throughout.

Diffuse Layer Outside of the compact Stern layer, electrostatics compete with diffusion until equilibrium is established. This region of the double layer is named the *diffuse layer* (or *Debye layer*) (Figure 2.4.1). The potential is found through the *Gouy-Chapman model*. This assumes that the ions are point charges of type- i each with a number density $n_{i,0}$ in bulk and each with a charge Q_i . The ion density of each type in the diffuse layer is found via the transport theory developed in § 2.2.

The convection-diffusion equation (Equation (2.48)) with no source term ($\psi_{n_i} = 0$) and a constant diffusion coefficient can be applied to each species of ion to find the equilibrium distribution ($\partial n_i / \partial t = 0$). If the curvature of the surface is supposed to be small compared to the thickness of the diffuse layer, then the concentration as a function of distance x from the surface is

$$n_i(x) = n_{i,0} \exp \left[\int_0^x \frac{v_i}{\mathcal{D}_i} dx \right].$$

The integrand can be recognized as a Péclet number ($\text{Pé}_\parallel = v_i dx / \mathcal{D}_i$ where the subscript emphasizes that this is the Péclet number relevant to the distribution in the direction of the force field). From the Einstein relation (Equation (2.90)) $\mathcal{D}_i = k_B T / \zeta_i$ but of course the net velocity of the ensemble of counterions must be zero in steady-state. So what is the velocity

v ? Concurrent to their diffusive motion, each ion is subject to an external electric force due to the surface charge, $f_{\text{el}}(x) = -\vec{\nabla}[Q_i\Psi(x)]$. Even if there is no net motion this force must be balanced by the drag and results in the microscopic velocity $v_i(\vec{x}) = f_{\text{el}}(\vec{x})/\zeta_i$. The concentration therefore obeys the Boltzmann distribution:

$$n_i(x) = n_{i,0} \exp \left[\int_0^x \frac{f}{k_{\text{B}}T} dx \right], \quad (2.92)$$

which in this case ($f = f_{\text{el}}$) is

$$n_i(x) = n_{i,0} \exp \left[-\frac{Q_i\Psi(x)}{k_{\text{B}}T} \right]. \quad (2.93)$$

This thesis will only consider *symmetrical electrolytes* i.e. $Q_- = -Q_+ = -Q$ and $n_{-,0} = n_{+,0} = n_0$. For a symmetrical electrolyte the charge density within the solvent takes the particularly elegant solution of

$$\varrho_{\text{el}}(x) = \sum_i Q_i n_i(x) = -2Qn_0 \sinh \left(\frac{Q\Psi(x)}{k_{\text{B}}T} \right) \quad (2.94)$$

which allows the potential $\Psi(x)$ to be found through the Poisson equation

$$\nabla^2\Psi = -\frac{\varrho_{\text{el}}}{\varepsilon_0\varepsilon_r}. \quad (2.95)$$

Substituting ϱ_{el} from Equation (2.94) into the Poisson equation gives the *Poisson-Boltzmann* equation:

$$\nabla^2\Psi = \frac{2Qn_0}{\varepsilon_0\varepsilon_r} \sinh \left(\frac{Q\Psi(x)}{k_{\text{B}}T} \right). \quad (2.96)$$

Debye-Hückel Approximation

The solution of the Gouy-Chapman model (Equation (2.96)) is a complete description of the diffuse layer. However, it is not convenient. For small potentials ($\Psi_\delta \ll k_{\text{B}}T/Q$), the hyperbolic sine function can be expanded as $\sinh(x) \approx x$ to give the linear, second order

differential equation

$$\nabla^2 \Psi \approx \lambda_D^{-2} \Psi(x). \quad (2.97)$$

This is the *Debye-Hückel approximation* in which the *Debye length* of the diffuse layer is defined as

$$\lambda_D \equiv \left(\sum_i \frac{n_0 (Q_i)^2}{\varepsilon_0 \varepsilon_r k_B T} \right)^{-1/2}. \quad (2.98)$$

It will become clear momentarily that the Debye length is the length scale beyond which Coulombic interactions are screened by the distribution of counterions (Figure 2.4.1). The boundary conditions of Equation (2.97) are that the potential must be the zeta-potential at the the Stern layer and that far from the surface the potential and its derivative must go to zero. It is valuable to consider planar and spherical geometries for the Debye-Hückel equation.

Planar Debye-Hückel Equation For planar geometry, there is technically no need for the Debye-Hückel approximation. A solution exists for the full Poisson-Boltzmann equation (Equation (2.96)) as a function of the distance from a lone charged plane x . The solution is

$$\Psi(x) = \frac{2k_B T}{Q} \ln \left[\frac{1 + \exp\left(-\frac{x}{\lambda_D}\right) \tanh\left(\frac{Q\zeta}{4k_B T}\right)}{1 - \exp\left(-\frac{x}{\lambda_D}\right) \tanh\left(\frac{Q\zeta}{4k_B T}\right)} \right], \quad (2.99a)$$

where $\Psi_\delta \approx \zeta$ has been assumed. By considering the limits of this solution, one can learn that the potential always decays exponentially with distance from the surface.

Strong potentials For strong potentials ($\zeta \gg k_B T/Q$), the hyperbolic tangent approaches unity. So in the strong potential limit, the potential approaches

$$\Psi(x) \approx \frac{2k_B T}{Q} \ln \left[\frac{1 + \exp\left(-\frac{x}{\lambda_D}\right)}{1 - \exp\left(-\frac{x}{\lambda_D}\right)} \right]. \quad (2.99b)$$

This strong potential in turn has two limits:

- Near the plane ($x \ll \lambda_D$), the argument of the logarithm reduces and the potential

is approximately

$$\lim_{x/\lambda_D \rightarrow 0} \Psi(x) \approx \frac{4k_B T}{Q} \ln \left(\frac{2\lambda_D}{x} \right). \quad (2.99c)$$

- Far from the planar surface ($x \gg \lambda_D$) the exponentials are small so the logarithm in Equation (2.99a) can be expanded to find

$$\lim_{x/\lambda_D \rightarrow \infty} \Psi(x) \approx \frac{4k_B T}{Q} \exp \left(-\frac{x}{\lambda_D} \right). \quad (2.99d)$$

Far from the surface, the potential appears to decay exponentially with the distance from the charged wall (technically speaking this is from the slip-plane but δ is small).

Weak potentials When the potentials are weak, the full solution (Equation (2.99a)) can be expanded or, equivalently, the Debye-Hückel equation (Equation (2.97)) can be utilized to find that

$$\Psi(x) \approx \zeta \exp \left(-\frac{x - \delta}{\lambda_D} \right) \approx \zeta \exp \left(-\frac{x}{\lambda_D} \right) \quad (2.99e)$$

since the Stern layer is thin. This solution also decays exponentially with a characteristic decay length of λ_D . The difference between the Debye-Hückel limit and the far-field limit for a strong potential is only the constant coefficient in front of the exponential decay. One can say that the far-field limit for a strong potential has an effective surface potential of $\zeta_{\text{eff}} = 4k_B T/Q$.

Spherical Debye-Hückel Equation Using the Debye-Hückel approximation (Equation (2.97)), the potential as a function of radial distance from the particle ρ can be calculated for a spherical colloid of radius r if the surface charge is small enough. If a thin Stern layer is assumed ($\delta \ll r$), the Debye-Hückel approximation (Equation (2.97)) to the Poisson equation in spherical coordinates has the solution

$$\Psi(\rho) = \zeta \frac{r}{\rho} \exp \left(-\frac{\rho - r}{\lambda_D} \right). \quad (2.100)$$

The Debye-Hückel approximation for the electrostatic potential around a sphere decays exponentially and is screened over distances greater than λ_D . By the Poisson equation (Equation (2.97))

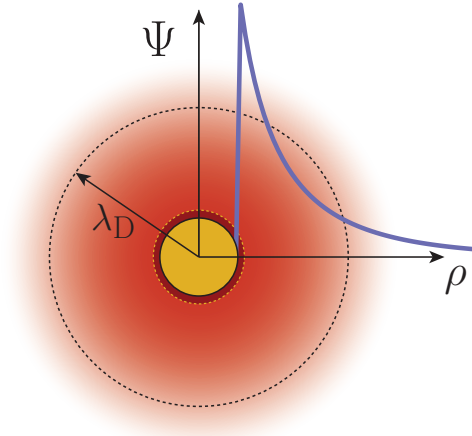


Figure 2.4.2: The Debye-Hückel approximation around a spherical, charged particle.

tion (2.95)) the charge density surrounding the charged solute particle is then

$$\varrho_{\text{el}} = -\varepsilon_0 \varepsilon_r \nabla^2 \Psi = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\lambda_D^2} \left(\frac{r}{\rho} \right) \exp \left(-\frac{\rho - r}{\lambda_D} \right). \quad (2.101)$$

As expected, far from the surface the net charge density goes to zero since the number of negative and positive charges are roughly equal. The total number of counterions in the diffuse layer must balance the surface charge. Since it is often the surface charge density σ_{el} that is unknown, one can assume net neutrality and use the Poisson equation (Equation (2.95)) to find the effective surface charge of the solute particle with a thin Stern layer to be [44]

$$\begin{aligned} \sigma_{\text{el},\delta} &= -\int_{\delta}^{\infty} \varrho_{\text{el}} d\rho = \int_{\delta}^{\infty} \varepsilon_0 \varepsilon_r \nabla^2 \Psi d\rho \\ &\approx \varepsilon_0 \varepsilon_r \zeta \left(\frac{1}{r} + \frac{1}{\lambda_D} \right). \end{aligned} \quad (2.102)$$

2.4.2 Electro-Osmotic Flow

Imagine applying an electric field $\vec{E}$ parallel to a single charged wall (Figure 2.4.3). The wall is fixed in place but the counterions can electrophorese. As they do, they drag the surrounding fluid with them. This creates a nearly continuous force field acting on the fluid. The fluid motion that results is the *electro-osmotic flow* or EOF. The force on the fluid is

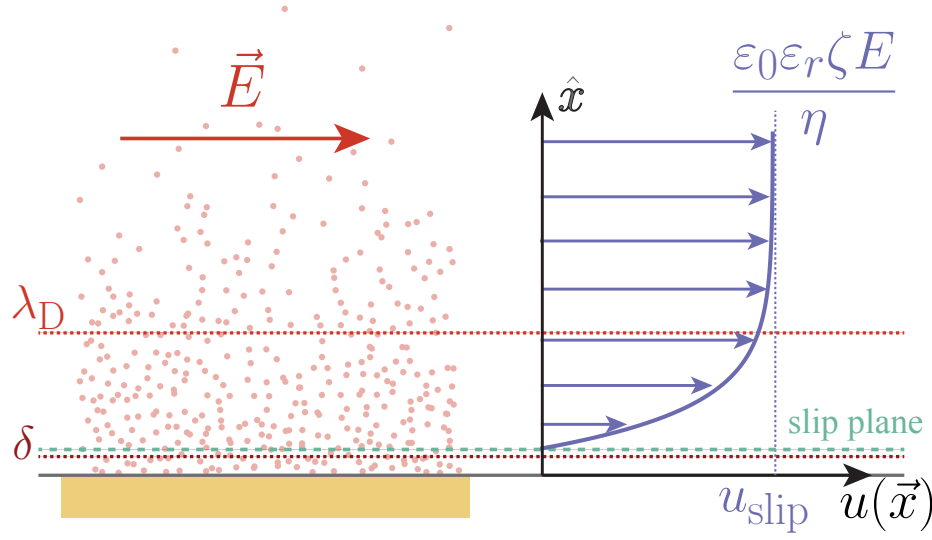


Figure 2.4.3: Schematic electro-osmotic flow parallel to a planar wall.

the force on the charge distribution

$$\vec{f}_{\text{el}}(\vec{x}, t) = \varrho_{\text{el}} \vec{E}(\vec{x}, t).$$

The Debye-Hückel approximation for the charge density $\varrho_{\text{el}}(\vec{x})$ as a function of the distance from a planar surface (Equation (2.99e)) can be substituted into the external force. The resulting force field on the fluid can be inserted into the steady Stokes equation (Equation (2.64)) to arrive at

$$-\eta \nabla^2 \vec{u} = \varrho_{\text{el}} \vec{E} = -\frac{\vec{E} \varepsilon_0 \varepsilon_r}{\lambda_D^2} \left[\zeta \exp\left(-\frac{x}{\lambda_D}\right) \right]. \quad (2.103)$$

Integrating twice (with no-slip boundary conditions) produces the velocity field parallel to the wall as a function of distance from the surface

$$u(\vec{x}) = \frac{\varepsilon_0 \varepsilon_r \zeta E}{\eta} \left[1 - \exp\left(-\frac{x}{\lambda_D}\right) \right]. \quad (2.104)$$

The electro-osmotic flow velocity above a single planar wall rises to a constant value over a distance of approximately λ_D (Figure 2.4.3). If λ_D is small then there is effectively a non-zero slip velocity at the wall. The slip velocity

$$u_{\text{slip}} = \frac{\varepsilon_0 \varepsilon_r \zeta E}{\eta} \quad (2.105)$$

is called the *Smoluchowski slip velocity*.

2.4.3 Electrophoretic Mobility

An external electric field can generate electro-osmotic motion of the solvent but it can also explicitly drive solute particles, giving them a electrophoretic velocity. Electrophoretic mobility was phenomenologically introduced in § 2.1.2 as an example of linear transport phenomena. The limiting values of the electrophoretic mobility can now be found.

The length scales r and λ_D determine the electrophoretic behaviour of a solute particle. There are two revealing limits: The Hückel ($r/\lambda_D \ll 1$) and Smoluchowski ($r/\lambda_D \gg 1$) limits.

1. The *Hückel limit* is the small particle radius/large Debye length limit of $r/\lambda_D \ll 1$. In the Hückel limit a charged solute particle has an effective charge $Q = 4\pi r^2 \sigma_{\text{el},\delta} \approx 4\pi \varepsilon_0 \varepsilon_r \zeta r$ (Equation (2.102)) and may be treated as a point charge. Balancing the driving force QE and drag force $\zeta \mathcal{V}$ leads to a velocity

$$\vec{\mathcal{V}} = \frac{Q\vec{E}}{\zeta} = \frac{4\pi \varepsilon_0 \varepsilon_r \zeta r^2}{6\pi \eta r} \left(\frac{1}{r} + \frac{1}{\lambda_D} \right) \vec{E} \approx \frac{2}{3} \frac{\zeta \varepsilon_0 \varepsilon_r}{\eta} \vec{E}. \quad (2.106)$$

The velocity per electric field defines the electrophoretic mobility $\hat{\mu}_{\text{el}} \equiv \vec{\mathcal{V}} \vec{E}^{-1}$. Equivalently, the electrophoretic mobility is the effective charge over the friction coefficient $\hat{\mu}_{\text{el}} \equiv Q^{\text{eff}} \zeta^{-1}$ and so Equation (2.106) gives the electrophoretic mobility for solute particles with a large Debye layer as

$$\mu_{\text{el}} = \frac{2}{3} \frac{\varepsilon_0 \varepsilon_r \zeta}{\eta}, \quad (2.107)$$

which is consistent with the Nernst-Einstein relation for the mobility of an unscreened ion (Equation (2.33b)) when the diffusivity is replaced by the Stokes-Einstein equation (Equation (2.91)).

2. The *Smoluchowski limit* is the opposite case of a thin Debye layer and/or large particle size such that $r/\lambda_D \gg 1$. In this case, the interface appears relatively planar to the counterions since the curvature is small compared to the length scale associated with their distribution λ_D . If the particle were fixed in space then the fact that the Debye length is small and that the solute's surface appears locally planar suggests that an

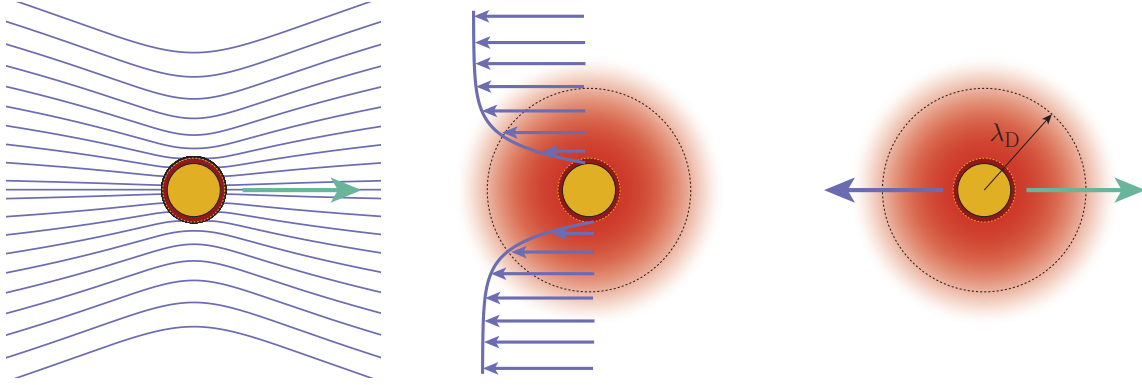


Figure 2.4.4: Schematic representation of hydrodynamic interaction screening due to the diffuse layer. The electric field pulls on the monomer and its ion sheath with equal and opposite force. To first order, the perturbation of the fluid due to the shear from the monomer is cancelled by the shear due to the counter-ions. Hydrodynamic interactions can only exist on length scales shorter than λ_D .

electro-osmotic flow would be generated that would approach the Smoluchowski slip velocity. Therefore, in the rest frame of the surface the fluid velocity must be given by Equation (2.105) but the carrier fluid is not in fact moving. The charged solute is. Therefore, it is the particle that is driven through the stagnant fluid with a mobility given by the Smoluchowski slip velocity to be

$$\mu_{\text{el}} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\eta}. \quad (2.108)$$

Since λ_D is the only relevant length scale in the Smoluchowski limit, the particle's size and shape do not enter the problem explicitly.

Notably, the electrophoretic mobility in these two limits differ only by a numerical constant. The mobility of spheres with finite Debye layers naturally fall somewhere between the Hückel and Smoluchowski limits, which suggests

$$\mu_{\text{el}} = \frac{\zeta \varepsilon_0 \varepsilon_r}{\eta} c_H, \quad (2.109)$$

where Henry's function $2/3 < c_H(r/\lambda_D) < 1$ is a function of r/λ_D that is of order 1 and satisfies both limits.

2.4.4 Electro-Hydrodynamic Screening

Imagine that an electric field is applied to a charged solute particle in solution. The particle will electrophorese through the solvent and drag the surrounding fluid with it. As discussed

in § 2.3, the perturbations to the fluid velocity field are typically long-range with $u \sim \rho^{-1}$ (Equation (2.72)), where ρ is the radial coordinate of $\vec{x}$. However, the counterions form a neutralizing sheath of thickness λ_D around the colloid (§ 2.4.1). The electric field indeed causes the particle to electrophorese, but the counterions also translate in the opposite direction. If the particle were fixed in space this would clearly generate electro-osmotic flow. As it stands, the charge of the counterion sheath is the same as the charge of the solute. This means that the forces on the diffuse layer and on the solute are equal in opposite directions, which suggests that the force shears the fluid only over the length scale $\sim \lambda_D$. Qualitatively speaking, the shearing due to the particle's motion and the counterions' motion cancel (Figure 2.4.4).

More precisely, this section will show that the $u \sim \rho^{-1}$ portion of the Oseen-Burgers tensor is cancelled, while only much shorter-ranged $\sim \rho^{-3}$ and exponentially decaying terms remain. Thus, not only electrostatic interactions but also hydrodynamic interactions are screened by the counterion sheath. However, it is important to stress that $\sim \rho^{-\alpha}$ with $\alpha = 3$ *is not* short-ranged. Interactions are technically short-ranged only when $\alpha \geq d$, where d is the dimensionality of the system [35]. Since $d = \alpha$ in this case, screened electro-hydrodynamic interactions in 3D are still long-range and can have global effects.

It is the far-field behaviour ($\rho \gg r$) that is of interest for hydrodynamic interactions. Consider a charged spherical solute for which the Debye-Hückel approximation (Equation (2.100)) applies with a thin Debye length (Smoluchowski limit). In this case of $\rho \gg r \gg \lambda_D$ the effective charge of the particle is $Q \approx 4\pi\epsilon_0\epsilon_r\zeta r$ and the charge density counterion distribution (from Equation (2.101)) is

$$\varrho_{\text{el}}(\rho) \approx -\frac{Q}{4\pi\lambda_D^2} \frac{1}{\rho} \exp\left(-\frac{\rho}{\lambda_D}\right). \quad (2.110)$$

Just as was done in developing the far-field behaviour through the Oseen-Burgers tensor (§ 2.3.4), the solute is once again modelled as a point force and the Stokes equation (Equation (2.64)) is treated in Fourier space. The Stokes equation for this situation is exactly the same as it was for the stokeslet (Equation (2.67)) but with

$$\vec{f}(\vec{x}, t) = [Q\delta(\vec{x}) + \varrho_{\text{el}}(\vec{x})] \vec{E}(\vec{x}, t) \quad (2.111)$$

rather than only $f_0 \delta(\vec{x})$. The counterion density term can be Fourier transformed into $\text{FT}[\varrho_{\text{el}}](\vec{q}) = -E\lambda_D^{-2}Q/(q^2 + \lambda_D^{-2})$, which makes the Stokes and continuity equations in

Fourier space

$$\text{FT} [\vec{u}] = \frac{1}{\eta \vec{q}^2} \left(Q \vec{E} - \vec{E} \frac{\lambda_D^{-2} Q}{q^2 + \lambda_D^{-2}} - i \vec{q} \text{FT} [p] \right) \quad (2.112)$$

$$\vec{q} \cdot \text{FT} [\vec{u}] = 0. \quad (2.113)$$

These are very similar to what they were for the stokeslet (Equation (2.68) and Equation (2.69), respectively). Solving for the velocity field in Fourier space gives $\text{FT} [\vec{u}] = \hat{\mathcal{H}}_{\text{LA}} \cdot (Q \vec{E})$. Long and Ajdari found the inverse Fourier transform that produces the hydrodynamic interaction tensor in real space [45]. The hydrodynamic interaction tensor for a charged point force with associated screening double layer is

$$\hat{\mathcal{H}}_{\text{LA}} (\vec{x}) = \frac{\exp(-\rho/\lambda_D)}{4\pi\eta\rho} \left\{ \frac{2}{3} \hat{\mathbb{1}} + \left(\frac{\lambda_D}{\rho} \right)^2 \left[1 + \frac{\rho}{\lambda_D} + \frac{\rho^2}{3\lambda_D^2} - \exp\left(\frac{\rho}{\lambda_D}\right) \right] \left(\hat{\mathbb{1}} - \frac{3\vec{x} \vec{x}}{\rho^2} \right) \right\}. \quad (2.114)$$

Indeed, it is clear that hydrodynamic interactions are screened due to the presence of the diffuse layer. The conclusion is that the diffuse layer not only screens Coulombic interactions but hydrodynamic interactions as well.

However, although the *Long-Ajdari screened hydrodynamic interaction tensor* $\hat{\mathcal{H}}_{\text{LA}}$ appears at first glance as though it decays exponentially, there is a term that scales geometrically in Equation (2.114). Dropping each exponential term reveals that in the far-field limit of $\rho \gg \lambda_D$ screened hydrodynamic interactions go as

$$\hat{\mathcal{H}}_{\text{LA}} (\vec{x}) \sim \frac{\lambda_D^2}{4\pi\eta\rho^3} \left(\frac{3\vec{x} \vec{x}}{\rho^2} - \hat{\mathbb{1}} \right). \quad (2.115)$$

This explicitly shows that the hydrodynamic interactions between distant particles are screened by their diffuse layers but not exponentially. The remaining geometric term in the screened hydrodynamic interaction goes as $\sim \rho^{-3}$.

Summary of Electro-Hydrodynamics

Surfaces of solute particles and microfluidic walls commonly possess a net charge when submersed in a solution. Although some of this charge is negated by the re-adsorption of counterions into the Stern layer, a non-zero zeta-potential remains at the surface. This electrostatic potential attracts counterions towards the surface (Equation (2.92)) establishing a

diffuse layer of characteristic thickness λ_D (Equation (2.98)). The Debye-Hückel approximation for weak surface charge (Equation (2.97)) allows the electrostatic potential to be solved in spherical coordinates (Equation (2.100)).

The electrophoretic mobility of such a charged spherical solute particle can be found in the limit of large Debye length to particle radius and the small Debye length to particle radius. The mobility is proportional to the zeta-potential divided by the viscosity and the limits only differ by a near unity coefficient.

While charged solutes electrophorese, their far-field perturbations to the fluid velocity field are screened because roughly equal and opposite forces are applied to the solute and to the surrounding fluid within its diffuse layer. The electro-osmotic flow cancels the flow due to Stokes drag. While the Oseen-Burgers hydrodynamic interaction tensor for uncharged solutes decays as $\sim \rho^{-1}$, the Long-Ajdari screened hydrodynamic interaction tensor (Equation (2.114)) approaches zero much more rapidly as $\sim \rho^{-3}$. However, it will be important to keep in mind that the residual hydrodynamic interactions are still long-range and can at times play an important, global role in electrophoretic situations.

Summary of Background

Separation science is a broad field, but this section has attempted to present a comprehensive theoretical framework that describes the foundations of the field in a unified formalism. Because separation science deals with ensembles of microscopic, dispersed solutes being actively transported, it has its formal roots in non-equilibrium thermodynamics. The thermodynamic foundations demonstrate why separation must compete against mixing: Entropy production occurs as a system attempts to return to equilibrium. The affinities that drive entropy production are necessarily accompanied by fluxes and, in the case of dispersed solute particles, a gradient in chemical potential drives the flux of particles.

Near equilibrium, kinetic coefficients relate affinities and fluxes. Examples include Fick's first law of diffusivity and also Newton's law of viscosity, Stokes' drag law and even stokeslet flows. The resulting fluxes can be balanced in a universal manner, resulting in a transport equation that can be applied to any extensive quantity whether conserved or not.

The transport equation is the basis of the convection-diffusion equation of mass transport, which is the fundamental equation of separation science. Mass transport behaviour is controlled by the Péclet number, which communicates whether convective or diffusive processes dominate. In microfluidic transport, the Péclet number has a wide range but can loosely be described as near-unity, reflecting the fact that both convection and diffusion play significant roles.

The transport equation also acts as the basis of the Navier-Stokes equation for fluid dynamics. Since mass transport in microfluidic devices occurs in a carrier fluid, it is often necessary to account for hydrodynamic interactions. Microfluidic flows can be described as low Reynolds number flows, for which hydrodynamic interactions are laminar and are described by the Stokes equation. Hydrodynamic interactions are also long range, though complex force distributions can be described accurately by multipole expansion methods.

When solute particles of any shape are forced to move through a fluid, they feel drag forces that depend on the relative velocity of the solutes, their size and the viscosity of the carrier fluid. For spheres, the friction coefficient relating drag force and velocity is given by Stokes' law. The friction coefficients of other shapes can be given as corrections to Stokes' law through a slip function. The friction and the diffusion coefficients are fundamentally connected through the fluctuation-dissipation theorem because both describe solutes' tendency to move when subjected to force.

Particle surfaces and walls have a tendency to be charged when submersed in solution. Charged surfaces pull counterions away from their bulk distributions, which has the effect of screening both the Coulombic repulsion and the hydrodynamic interactions.

This concludes the theoretical basis of the work presented here. All the results presented in this thesis fall within the purview of this content. However, to be able to investigate many of the more complicated microfluidic separation systems, virtual experiments are required. The next chapter presents the computational methods utilized in this work to study specific separation systems in the near-unity Péclet and low Reynolds numbers regime.

Navier-Stokes is a fancy way to write $F = ma$.

Paul Chaikin [46]

3

Multi-Particle Collision Dynamics

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Most separation processes on microfluidic devices involve the full gauntlet of effects described in § 2. Random, diffusive motion (including conformational effects for macromolecules), entropic forces, electrostatic, hydrodynamic and electro-hydrodynamic interactions must all be accounted for and often in complex geometries. Not all are significant in their full form in every situation, but enough of them are to warrant utilizing computational tools to explore separation phenomena.

Computer simulations allow physicists to explore numerous systems and geometries at relatively low costs and at various levels of detail. Thus, computational physics is widely seen as a third approach to scientific discovery, complementing traditional experimental and theoretical techniques. Computational physics can be roughly split into two genres:

1. Computational physicists create models that can be solved numerically. When performing such calculations, physicists can be referred to as *numerical theoreticians*.
2. Computer simulations based on simple models evolve the states of many constituents in time (which quickly becomes analytically impossible). Relatively quickly computers can calculate countless interactions between particles and produce emergent collective behaviour that would not be visible in models that average-out many degrees of freedom. When computational physicists perform such simulations they can be referred to as *in-silico experimentalists*.

In-silico experimentalists have both full control over the simulation parameters and the ability to measure every conceivable property or correlation of a system that is incorporated into the model at any instant. Having full control over objects, interactions and forces can allow (perhaps non-physical) simulations to determine which factors play what role. This strategy can lead to better fundamental understanding of phenomena.

In order to be computationally feasible, simulations of microfluidic devices must be coarse-grained. In particular, a mesoscopic method is needed that efficiently reproduces both the Navier-Stokes equation and thermal diffusion without reproducing the properties specific to a particular fluid medium. The algorithm utilized in this work is *Multi-Particle Collision Dynamics* (MPCD), which is an algorithm that propagates fluid particles in time but replaces physical interactions with a non-physical many-particle *collision operation*. This chapter explains the **implementation** of MPCD for separation phenomena, its **variants**, different **boundary conditions** and how **solute bodies** are coupled to the model solvent.

3.1 Multi-Particle Collision Dynamics Algorithm

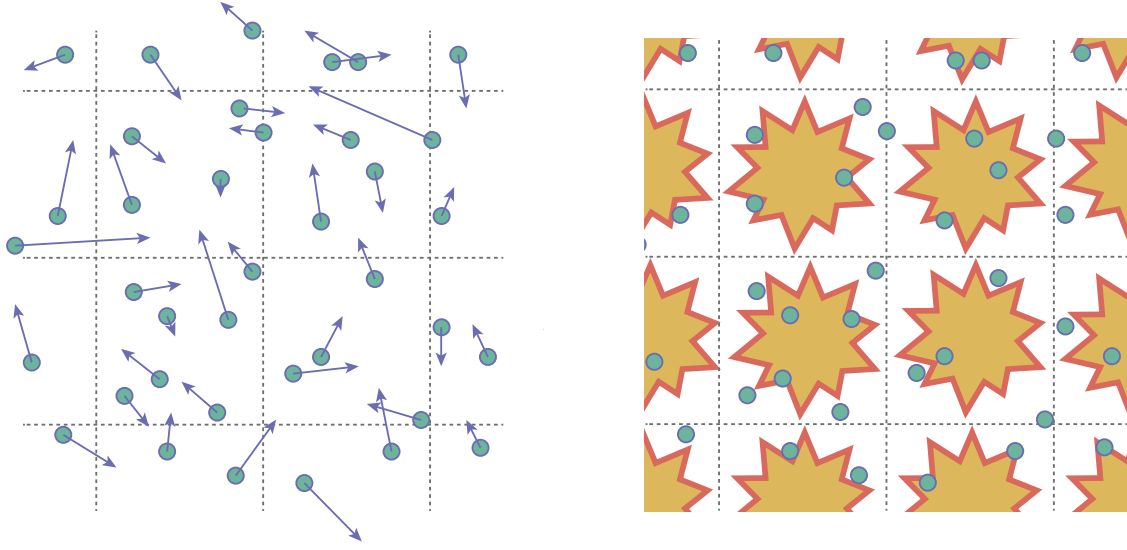
What is an appropriate simulation method for studying solutes eluting through microfluidic devices? Performing fully atomistic simulations is only appropriate to study dynamics on the nanosecond and nanometer scales. To probe larger microfluidic systems on longer time scales and include the carrier fluid medium, coarse-graining techniques are essential. Coarse-graining eschews molecular-scale details of the atoms and molecules that make up the fluid. Only a generic, coarse-grained carrier fluid that respects the Navier-Stokes equation and imparts thermal forces to solutes is required. These are generally referred to as *mesoscopic simulations* and they have dramatically reduced computational costs compared to explicitly treating the fluid as an ensemble of interacting MD particles.

Navier-Stokes solvers can certainly calculate the hydrodynamic interactions involved in elution, as can lattice-Boltzmann (LB) methods [47]. However, § 2.3.3 stressed that in the microfluidic systems of interest to this work both hydrodynamics and also thermal noise are crucial. To generate thermal motion using such techniques, an *ad hoc* random motion must be superimposed on the system. Furthermore, complicated geometries require complicated meshes, which are often required to evolve in time. Dissipative Particle Dynamics (DPD) [48, 49] is an intrinsically stochastic technique that is particle-based and so does not struggle with complicated boundaries. However, fluid properties can only be controlled indirectly [50] and the efficiency is achieved by having very large fluid particles.

This work utilizes the MPCD algorithm to coarse-grain the carrier fluid. It is especially well suited for situations involving finite Reynolds numbers and moderate Péclet numbers because both hydrodynamic interactions and thermal motion are intrinsically included in the algorithm. This makes MPCD ideal for studying separation phenomena in microfluidic devices that operate in the near-unity Péclet and low Reynolds number regime (§ 2.3.3).

In MPCD [51, 52] (which has also been called Stochastic Rotation Dynamics [53–55] and Real-Coded Lattice Gas [56, 57]) many atoms or molecules are grouped together and simulated as point-like *fluid particles*. These fluid particles are not particles of matter but rather discretized units for conserving mass, momentum and energy. Respecting conservation of these quantities is what allows the hydrodynamic equations of motion to be obeyed on sufficiently long length and time scales [58].

In essence, the MPCD algorithm consists of only two steps (Figure 3.1.1):



(a) The streaming step (Equation (3.1)).

(b) The collision step (Equation (3.2)).

Figure 3.1.1: The MPCD algorithm consists of a streaming step (Equation (3.1)) and collision (Equation (3.2)) step.

1. The first step is the *streaming step*. Each fluid particle (labelled i) of mass $m_i = m$ moves ballistically, and its position $\vec{x}_i(t)$ is updated in discrete time intervals δt . In the streaming step the particles move according to

$$\vec{x}_i(t + \delta t) = \vec{x}_i(t) + \vec{v}_i(t) \delta t. \quad (3.1)$$

If the MPCD algorithm consisted only of streaming steps then the carrier fluid would be an ideal gas.

2. The second step is the *collision step* in which momentum is transferred between fluid particles. To exchange momentum the simulation domain is partitioned into d -dimensional square cells of size a . The collision step is a simple, non-physical scheme constructed to be stochastic but also to conserve momentum within each cell. Multi-particle collisions within cells are represented by the collision operator $\mathbb{R}$ such that the velocity of each particle in a given cell evolves as

$$\vec{v}_i(t + \delta t) = \vec{v}^{\text{cm}}(t) + \mathbb{R}(\vec{v}_i(t), \vec{v}^{\text{cm}}(t)). \quad (3.2)$$

The number of fluid particles in the c^{th} cell at time t is $N_c(t)$. The number of particles in each cell may vary from one cell to another but the total number N_{tot} is conserved and the average number density is $n_{\text{MPCD}} = \langle N_c \rangle / a^d$ where d is the dimensionality of the simulation. Each cell possesses a centre of mass velocity $\vec{v}^{\text{cm}} = \left(\sum_{i=1}^{N_c} m_i \vec{v}_i \right) / \left(\sum_{i=1}^{N_c} m_i \right)$, which corresponds to the local macroscopic velocity at that point and time, $u(\vec{x}, t)$. Conservation of energy, isotropy and a Maxwell-Boltzmann velocity distribution can be achieved in the continuum limit by an appropriate choice of the collision operator $\mathbb{R}$.

The MPCD collision step replaces molecular-scale dynamics with a local, non-physical, stochastic momentum transfer operation. Thus, MPCD **does not** give correct dynamics on microscopic time or length scales. Streaming and collision events conserve the momentum and energy in each collision volume at all times and so MPCD reproduces an accurate representation of the hydrodynamics of the velocity field at large distances. The coarse-grained mesoscopic MPCD fluid provides both a thermal bath and propagates hydrodynamic interactions without having to either solve the entire Navier-Stokes equation (Equation (2.59)) for a potentially complex microfluidic system or to simulate the full molecular interactions of all fluid molecules. Therefore, it can be computationally less expensive than numerical, continuum solvers or full *Molecular Dynamics* (MD) simulations. However, this computational efficiency comes with complications:

- By discretizing the space into cells, *Galilean invariance* is broken. To understand why, consider a simulation of slow moving fluid particles (low temperatures). If the mean-free path of the molecules is much smaller than the cell size ($\lambda \ll a$) then particles will often fail to travel far enough to leave the cell and may repeatedly exchange momentum with the same set of neighbours. Thus, the implicit assumption of underlying molecular chaos fails and the fixed lattice of cells causes the behaviour in a moving reference frame to be different than a fixed reference frame, since in the moving frame the set of neighbours would be different in each (Figure 3.1.2). However, Galilean invariance can be completely reestablished by shifting the cell grid each time step by a random vector. Galilean invariance is then remedied. Practically speaking, this is implemented by shifting all the fluid particles by the same vector whose components were chosen at random from the interval $[-a/2, a/2]$.
- In this work, the MPCD fluid is used to simulate a liquid solvent; however, if the mean-free path of the fluid particles is too large ($\lambda \gg a$), the particle-based Navier-Stokes solver is more akin to a gas than a liquid. This manifests itself in two related ways:

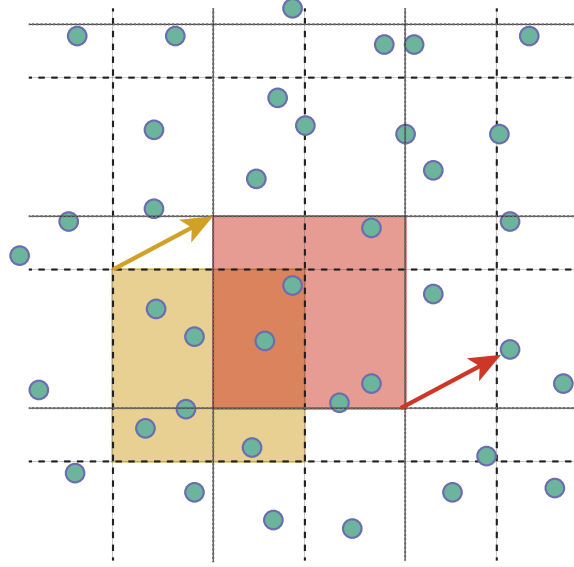


Figure 3.1.2: The structure of the MPCD cells breaks Galilean invariance. The MPCD fluid properties depend on the frame of reference because at low temperatures, a given particle can collide with a different set of neighbours depending on the frame of reference.

1. The fluid becomes quite compressible [59]. Technically, all particle-based algorithms (such as MD, DPD, LB and MPCD fluids) are more compressible than liquids. This is because a finite amount of time is required for hydrodynamic disturbances to propagate long distances. However, this is not an issue if the applied forces or pressure gradients are limited to small values.
2. The ratio of the viscosity to the diffusion coefficient (the Schmidt number, $Sc = \eta / (\rho \mathcal{D})$) is low. Typical values in a gas are $Sc \sim 1$, while actual liquids often possess Schmidt numbers in the range $Sc \sim 10^2 - 10^3$. Many particle-based simulation techniques struggle to obtain appropriately large Schmidt numbers. Traditional MD and DPD fluids have $Sc \sim 1$. While the Schmidt values for MPCD fluids are much larger (typically $Sc \sim 10^2$) and can technically be made arbitrarily large, increasing Sc comes at the cost of longer simulation times. Keeping all other simulation parameters fixed and decreasing δt increases Sc , while increasing the mean-free path causes $Sc \rightarrow 1$, as one would expect for a more gas-like fluid.

Simulations exist *in-silico* and so do not possess physical units. However, they do contain their own natural units and, when necessary, comparisons can be made to experimental results by matching dimensionless numbers such as the Schmidt number.

Basic Units			Derived Units		
Scale	Natural Unit	note	Scale	Derived Unit	note
Length	a	Cell size	Time	$a\sqrt{m/k_B T}$	Alternatively δt
Mass	m	Fluid particle mass	Diffusivity & Kinematic Viscosity	$a\sqrt{k_B T/m}$	Alternatively $a^2/\delta t$
Energy	$k_B T$	Thermal energy	Density	$\langle N_c \rangle / a^d$	$\langle N_c \rangle$ is independent

Table 3.1.1: Units associated with MPCD algorithm. After Padding [60].

The streaming portion of the MPCD algorithm constitutes an ideal, collisionless gas of point particles; therefore, there are no natural units associated with the streaming step. The natural units arise from the collision step:

- The natural unit of length for MPCD simulations is the MPCD cell size a . Throughout this thesis lengths will be given in terms of a .
- The natural unit of mass is the mass of the fluid particle, which is the discretized packet of fluid material.

One scale is left. The natural unit of time could be the discretized time step between collision operations δt and this is an acceptable choice. However, it is more convenient and more common to choose the thermal energy $k_B T$ as the fundamental unit of energy, since it plays an essential role and derives the time scale. If thermal energy is the natural unit of energy then the derived unit of time is $a\sqrt{m/k_B T}$. These natural units are summarized in Table 3.1.1.

Beyond the natural units of the simulation, independent simulation parameters include the time step δt (or thermal energy), the number of particles per cell $n_{\text{MPCD}} = \langle N_c \rangle / a^d$, the system size $[L]$ and whatever parameters are associated with the collision operation, such as rotation angle ϑ for stochastic rotation dynamics, which will be discussed next.

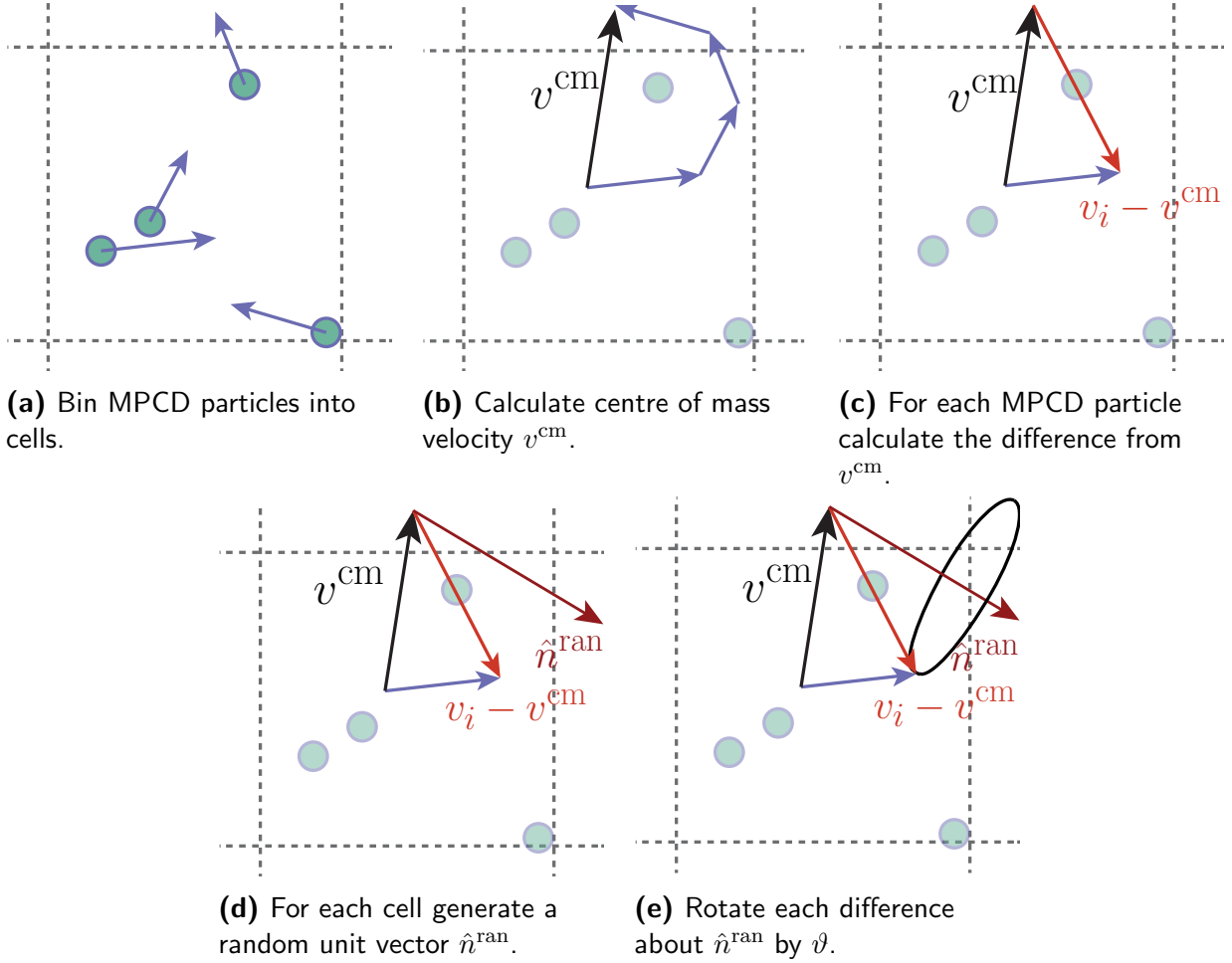


Figure 3.2.1: Schematic representing the SRD algorithm.

3.2 Multi-Particle Collision Dynamics Collision Operators

The MPCD method amounts to a judicious choice of collision operator $\mathbb{R}$ (Equation (3.2)). Anything that randomly exchanges momentum between particles, while ensuring that total momentum and energy within each cell is conserved, will do. Many options have been proposed in the literature and are implemented here.

3.2.1 Stochastic Rotation Dynamics

The original proposal was to make the collision operator $\mathbb{R}$ a rotation through an angle ϑ about a **randomly** chosen axis such that conservation of energy, isotropy and a Maxwell-

Boltzmann velocity distribution are met in the continuum limit. It is this random rotation operation that gives this MPCD variant the name *Stochastic Rotation Dynamics* (SRD). In SRD, the collision operator is

$$\mathbb{R} = \widehat{\Omega}_{\vartheta} \cdot [\vec{v}_i(t) - \vec{v}^{\text{cm}}(t)], \quad (3.3)$$

where $\widehat{\Omega}_{\vartheta}$ is the rotation tensor through an angle ϑ .

The chosen axis can either be an axis that is randomly generated for every collision operation or a random choice between a given set of predefined axes:

1. If the rotation occurs about an axis $\hat{n}^{\text{ran}} = [\hat{n}_x, \hat{n}_y, \hat{n}_z]$ that is randomly generated each time step, then the rotation tensor is

$$\widehat{\Omega}_{\vartheta}^{\text{ran}} = \begin{pmatrix} \hat{n}_x^2 + (1 - \hat{n}_x^2) \cos \vartheta & \hat{n}_x \hat{n}_y (1 - \cos \vartheta) - \hat{n}_z \sin \vartheta & \hat{n}_x \hat{n}_z (1 - \cos \vartheta) + \hat{n}_y \sin \vartheta \\ \hat{n}_x \hat{n}_y (1 - \cos \vartheta) + \hat{n}_z \sin \vartheta & \hat{n}_y^2 + (1 - \hat{n}_y^2) \cos \vartheta & \hat{n}_y \hat{n}_z (1 - \cos \vartheta) - \hat{n}_x \sin \vartheta \\ \hat{n}_x \hat{n}_z (1 - \cos \vartheta) - \hat{n}_y \sin \vartheta & \hat{n}_y \hat{n}_z (1 - \cos \vartheta) + \hat{n}_x \sin \vartheta & \hat{n}_z^2 + (1 - \hat{n}_z^2) \cos \vartheta \end{pmatrix}. \quad (3.4)$$

2. If the rotation axis is chosen from the set of orthogonal Cartesian axes $\{\hat{x}, \hat{y}, \hat{z}\}$ then the collision operator is chosen randomly from $\widehat{\Omega}_{\vartheta} \in \{\widehat{\Omega}_{\vartheta\hat{x}}, \widehat{\Omega}_{\vartheta\hat{y}}, \widehat{\Omega}_{\vartheta\hat{z}}\}$ where

$$\begin{aligned} \widehat{\Omega}_{\vartheta\hat{x}} &= \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos \vartheta & \sin \vartheta \\ 0 & -\sin \vartheta & \cos \vartheta \end{pmatrix}, \quad \widehat{\Omega}_{\vartheta\hat{y}} = \begin{pmatrix} \cos \vartheta & 0 & \sin \vartheta \\ 0 & 1 & 0 \\ -\sin \vartheta & 0 & \cos \vartheta \end{pmatrix} \\ \text{and } \widehat{\Omega}_{\vartheta\hat{z}} &= \begin{pmatrix} \cos \vartheta & \sin \vartheta & 0 \\ -\sin \vartheta & \cos \vartheta & 0 \\ 0 & 0 & 1 \end{pmatrix}. \end{aligned} \quad (3.5)$$

It is quicker and often acceptable to randomly choose one of the Cartesian unit vectors than it is to regenerate a new, random vector every time step.

By explicitly calculating the momentum and energy in a given cell before and after a collision operation, it is easy to demonstrate that SRD locally conserves momentum

$$m\vec{v}^{\text{cm}}(t + \delta t) = m\vec{v}^{\text{cm}}(t) \quad (3.6)$$

and energy

$$\frac{m}{2} [\vec{v}^{\text{cm}}(t + \delta t)]^2 = \frac{m}{2} [\vec{v}^{\text{cm}}(t)]^2 \quad (3.7)$$

in each collision cell. However, the SRD algorithm does not generally conserve angular momentum since the positions of the fluid particles within the cell during a collision operation are not taken into account [61]. While some of the other variations on the MPCD algorithm do (see § 3.2.3), in the microfluidic situations considered here (and most others [62–65]) angular momentum does not play an essential role and so this fact is inconsequential.

Transport Coefficients

The most basic estimate for the transport coefficients of an MPCD fluid should take into account a kinetic contribution and a collisional contribution [66]. For this section, the kinematic viscosity $\nu = \eta/\rho = \eta/(\langle N_c \rangle m/a^d)$ is used rather than dynamic viscosity. The kinematic viscosity of a dilute gas is dominated by the fact that as fluid particles move they carry their momentum with them to a new region of space. In a dilute gas this kinetic contribution to the transport of momentum is approximately the thermal velocity $\sqrt{k_B T/m}$ times the mean-free path $\lambda = \delta t \sqrt{k_B T/m}$ [66]

$$\nu_{\text{kin}} \sim \delta t \frac{k_B T}{m}. \quad (3.8)$$

This estimate is passable and has even been used in the literature when considering colloidal particles diffusing in an MPCD solvent [67]. However, at higher densities momentum is transported primarily through collision events and not carried by moving particles. Since collisions occur once every δt in cells of size a , simple scaling suggests that the collisions contribute to the viscosity as roughly

$$\nu_{\text{col}} \sim \frac{a^2}{\delta t}. \quad (3.9)$$

When the mean free path is small compared to the cell size, the collisional part dominates. When the mean free path is large, the kinetic term dominates. In general, the true total viscosity is the sum of both kinematic and collisional terms

$$\nu = \nu_{\text{kin}} + \nu_{\text{col}} \quad (3.10a)$$

$$\nu_{\text{kin}} = \delta t \frac{k_B T}{m} f_{\text{kin}}(\vartheta, \langle N_c \rangle, \mathbf{d}) \quad (3.10b)$$

$$\nu_{\text{col}} = \frac{a^2}{\delta t} f_{\text{col}}(\vartheta, \langle N_c \rangle, \mathbf{d}), \quad (3.10c)$$

where f_{kin} and f_{col} control the specific value of each component for various models and parameters.

Ihle, Kroll and Tüzel have calculated the total viscosity of an SRD fluid [68–71]. Through the Green-Kubo relation, Ihle and Kroll derived the generally accepted (and often verified [66, 72, 73]) expectation values for the kinetic and collisional components of the viscosity:

$$f_{\text{kin}}(\vartheta, \langle N_c \rangle, \mathbf{d}) = \begin{cases} \frac{1}{2} \left[\frac{\Xi}{\sin^2 \vartheta} - 1 \right] & \mathbf{d} = 2\text{D} \\ \frac{1}{2} \left[\frac{5\Xi}{2 - \cos \vartheta - \cos(2\vartheta)} - 1 \right] & \mathbf{d} = 3\text{D} \end{cases} \quad (3.11\text{a})$$

$$f_{\text{col}}(\vartheta, \langle N_c \rangle, \mathbf{d}) = \frac{1 - \cos \vartheta}{6\mathbf{d}\Xi} \quad (3.11\text{b})$$

where the ubiquitous term $\Xi \equiv \langle N_c \rangle / (\langle N_c \rangle - 1 + e^{-\langle N_c \rangle})$ is defined for convenience.

3.2.2 Anderson-MPCD

If there are external forces acting on the solvent particles, the SRD version of MPCD requires an external thermostat to control the system’s energy. Options for such thermostats are discussed in detail in § 3.4.2. However, mesoscale simulation techniques such as DPD have long included thermostats as an integrated part of the simulation technique. Langevin thermostats and Anderson thermostats have been elegantly incorporated directly into MPCD collision operators [62, 74]. In these algorithms $\mathbb{R}$ is both the collision operator and also the thermostat pulling the energy toward the equilibrium value, such that no velocity rescaling is required even if external fields are applied.

The Anderson thermostatted version of MPCD (*Anderson-MPCD*) uses a random collision operator

$$\mathbb{R} = \bar{v}_i^{\text{ran}} - \frac{\sum_j^{N_c} m_j \bar{v}_j^{\text{ran}}}{\sum_j^{N_c} m_j}, \quad (3.12)$$

where each component of $\bar{v}_i^{\text{ran}}$ is randomly generated from a Gaussian distribution with variance $\sqrt{k_B T / m}$. Effectively, each fluid particle in a given cell is assigned a new, randomly chosen velocity during each collision, but that particle’s velocity is the cell’s centre of mass velocity plus its new randomly assigned value minus the average of all the new random values for that cell (Figure 3.2.2). In this way, although the individual velocity of each particle has

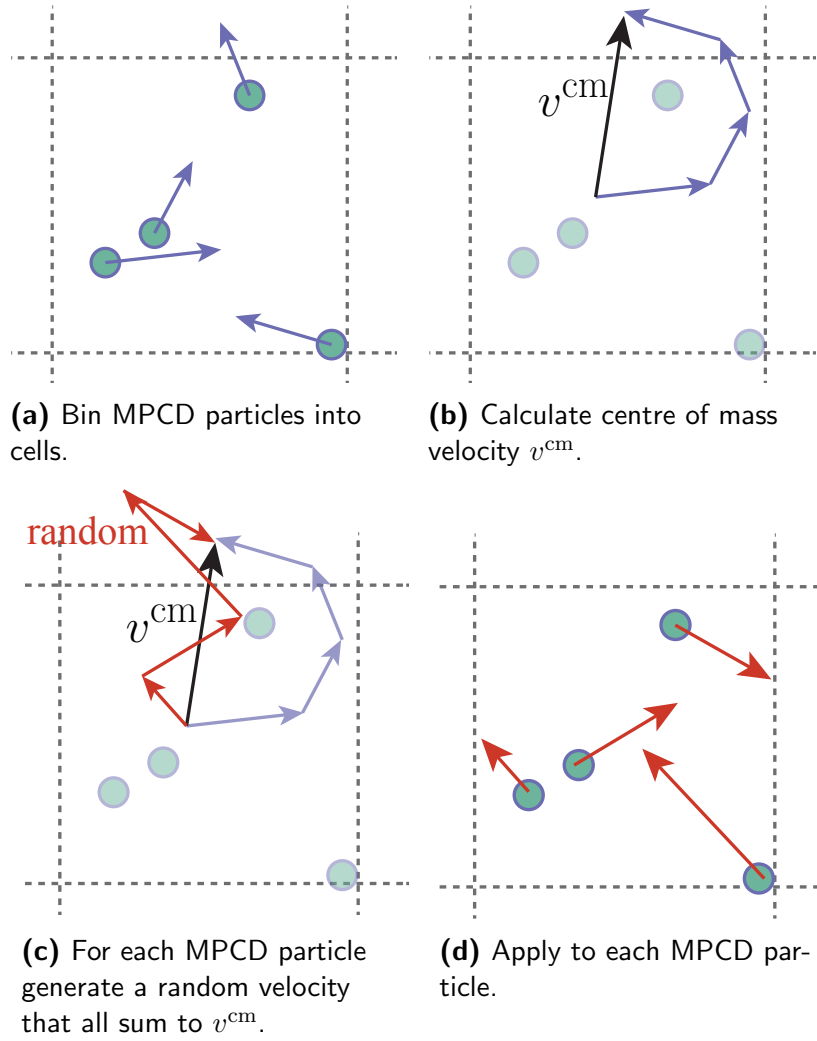


Figure 3.2.2: Schematic representing the Anderson-MPCD algorithm.

been randomly altered, the centre of mass velocity of the cell and, therefore, the momentum (if each fluid particle has the same mass) remains unchanged:

$$\vec{v}^{\text{cm}}(t + \delta t) = \vec{v}^{\text{cm}}(t). \quad (3.13)$$

Transport Coefficients

The viscosity of the thermostatted Anderson-MPCD version has been found in the same fashion for the SRD algorithm [62, 74]. The controlling functions are

$$f_{\text{kin}}(\langle N_c \rangle) = \Xi - \frac{1}{2} \quad (3.14a)$$

$$f_{\text{col}}(\langle N_c \rangle) = \frac{1}{12\Xi}. \quad (3.14b)$$

3.2.3 Angular Momentum

The Anderson-MPCD algorithm discussed so far conserves mass, energy and translational momentum but, just like the SRD collision operations, it does not conserve angular momentum. However, it can be extended to do so.

In conserving the angular momentum of the cell, the position of each fluid particle relative to the centre of mass of the cell $\vec{x}'_i = \vec{x}_i - \vec{x}^{\text{cm}}$ is important. The Anderson-MPCD algorithm presented in § 3.2.2 creates a small change in angular momentum every collision step. Let this amount be denoted $\delta\vec{L}$. This angular momentum can be exactly cancelled out by giving the entire cell an appropriate angular velocity $\vec{\omega}$. If the set of particles in the cell have a moment of inertia tensor $\hat{\mathbb{I}}$ about the centre of mass (with elements $\mathbb{I}_{ij} = \sum_k^{N_c} m_k (x'_k{}^2 \delta_{ij} - r'_{k,i} r'_{k,j})$) then the angular velocity needed to cancel the residual angular momentum is $\vec{\omega} = \hat{\mathbb{I}}^{-1} \cdot \delta\vec{L}$.

A term representing the change in linear velocity resulting from the imposed angular velocity about the centre of mass can be added to Equation (3.12):

$$\mathbb{R} = \vec{v}_i^{\text{ran}} - \frac{\sum_j^{N_c} m_j \vec{v}_j^{\text{ran}}}{\sum_j^{N_c} m_j} + \left[\hat{\mathbb{I}}^{-1} \cdot \left(\sum_j^{N_c} m_j \{ \vec{x}'_j \times (\vec{v}_j - \vec{v}_j^{\text{ran}}) \} \right) \right] \times \vec{x}'_i. \quad (3.15)$$

For situations in which angular momentum conservation within the solvent plays an important role, Equation (3.15) should be preferred over Equation (3.12).

3.2.4 Langevin-MPCD

The Langevin thermostat can also be used to create an MPCD algorithm [74]. The Langevin equation is the preeminent theoretical foothold for non-equilibrium simulations. The under-

damped Langevin equation for the i^{th} fluid particle reads

$$m_i \frac{d\vec{v}_i}{dt} = -\vec{\nabla} U_i + \vec{f}_i. \quad (3.16)$$

The final term represents the non-conservative forces. Traditionally these non-conservative forces are viewed as the only remaining effects of the background solvent on dispersed particles. Therefore, in a sense, this *Langevin-MPCD* algorithm implicitly splits the solvent into two ill-defined populations:

- Mass, momentum and energy conserving MPCD fluid particles.
- A thermodynamic reservoir of background solvent.

The reservoir enacts two main effects on the fluid particles:

1. A resistive, viscous force opposes the motion of the fluid particles. This friction is characterized by a constant γ .
2. Random kicks arising from collisions with the background solvent representing the thermal bath are characterized by white noise $\vec{\xi}$.

The Langevin force is thus $\vec{f} = -\gamma\vec{v}_i + \sqrt{\gamma}\vec{\xi}_i$. The white noise is a vector that must be different for each particle in the cell and must be regenerated for each collision operation. Integrating the traditional Langevin equation gives $\vec{v}_i(t + \delta t) = a\vec{v}_i(t) + b\vec{\xi}_i$ with the convenient notation

$$a = \frac{2m - \gamma\delta t}{2m + \gamma\delta t} \quad ; \quad b = \frac{2\sqrt{\gamma\delta t}}{2m + \gamma\delta t}. \quad (3.17)$$

When the Langevin thermostat is applied to the MPCD, the background force on the fluid particles is $\vec{f} = -\gamma(\vec{v}_i - \vec{v}^{\text{cm}}) + \sqrt{\gamma}\left(\vec{\xi}_i - \sum_j^{N_c} \vec{\xi}_j / N_c\right)$, which produces the Langevin-MPCD collision operator

$$\mathbb{R} = a(\vec{v}_i - \vec{v}_{\text{CM}}) - b\left(\vec{\xi}_i - \sum_j^{N_c} \frac{\vec{\xi}_j}{N_c}\right). \quad (3.18)$$

When the friction coefficient is chosen such that $\gamma\delta t/m = 2$, then Equation (3.18) for Langevin-MPCD is equivalent to Equation (3.12) for Anderson-MPCD simulations.

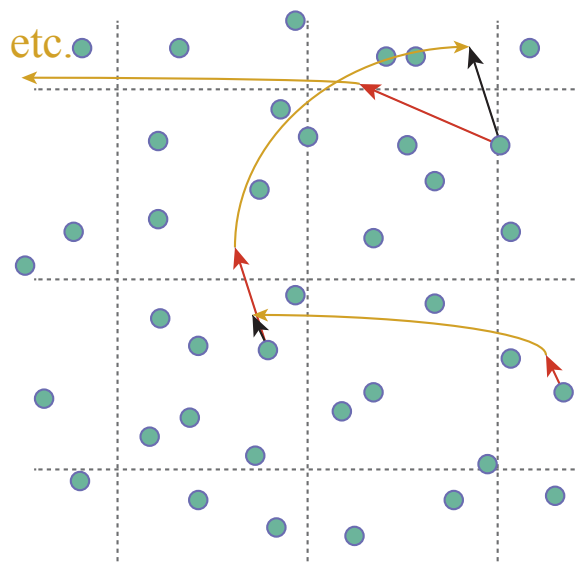


Figure 3.2.3: In order to produce a Brownian fluid with the same properties as an MPCD fluid but with no hydrodynamic interactions, the velocities of all the particles are randomly interchanged.

Transport Coefficients

The viscosity of the Langevin-MPCD versions has been determined [74] and the controlling functions are

$$f_{\text{kin}} = \Xi \frac{[1 + (\gamma \delta t / m) / 2]^2}{2 (\gamma \delta t / m)} - \frac{1}{2} \quad (3.19a)$$

$$f_{\text{col}} = \frac{1}{12\Xi} \frac{(\gamma\delta t/m)}{1 + (\gamma\delta t/m)/2}. \quad (3.19b)$$

3.2.5 Brownian-MPCD

A number of researchers have found it convenient to “turn off” hydrodynamic interactions for portions of their studies [75–81]. In situations in which many competing interactions play important roles this can be a powerful approach. However, it is difficult to compare the fluid of a *Brownian Dynamics* (BD) simulation to an MPCD fluid. An MPCD fluid can be forced to behave as a BD fluid by randomly interchanging the velocities of all the MPCD particles after each collision step (Figure 3.2.3). In this way hydrodynamic interactions are scrambled. The resulting BD simulations are highly inefficient but if it is important to keep

the fluid properties unchanged then this can be done. The specific MPCD variant algorithm used is irrelevant; this process can be done for each collision operator.

3.3 Boundary Conditions

Many variants of the MPCD algorithm for a fluid are presented in § 3.2. Separation systems are the focus of this work and so boundaries and dispersed objects are needed. Just as when one solves a differential equation, a simulation cannot be performed without proper *boundary conditions* (BCs). In computational simulations, BCs must be present to contain the *control volume*, but others can also occur within the control volume. BCs are composed of two pieces: *Boundary surfaces* and the *boundary rules* that are applied to fluid particles if they violate those surfaces.

3.3.1 Boundary Surfaces

In the program implemented here, all surfaces $\mathcal{S}(\vec{x})$ representing BCs are expressed in the form

$$\mathcal{S}(\vec{x}) = [A_x(x - Q_x)]^p + [A_y(y - Q_y)]^p + [A_z(z - Q_z)]^p - R = 0, \quad (3.20)$$

where $\vec{x}$ are points on the surface. The interpretation of $\vec{A} = [A_x, A_y, A_z]$, $\vec{Q} = [Q_x, Q_y, Q_z]$, p and R depends on the shape that the surface defines. A fluid particle at the position $\vec{x}_i(t)$ violates a boundary surface when

$$\mathcal{S}(\vec{x}_i) \leq 0. \quad (3.21)$$

As long as $\mathcal{S}(\vec{x}_i)$ is positive the fluid particle resides in the available control volume. When $\mathcal{S}(\vec{x}_i) = 0$ it is precisely at the surface. Writing BC surfaces in this way is versatile and allows many shapes to be represented:

- **Planes** are represented by Equation (3.20) when $\vec{A}$ is the plane's normal, $\vec{Q} = \vec{0}$, $p = 1$ and R is the negative of the distance from the origin. For planes, the surface equation reduces to $\mathcal{S}(\vec{x}) = \vec{A} \cdot \vec{x} - R$.
- **Ellipsoids:**
 - **Spheres** require $\vec{A} = \vec{1}$ and $p = 2$. The vector $\vec{Q}$ is the position of the sphere and R is the radius squared, $R = r^2$. The surface equation is then $\mathcal{S}(\vec{x}) = (\vec{x} - \vec{Q})^2 - r^2$.

- **Ellipsoids** are similar to spheres except $\vec{A}$ represents the inverse of the focii.
- **Cylinders** are easy to create as well. They are simply spheres but with one dimension truncated. A cylinder oriented along the $\hat{x}$ -axis simply requires $\vec{A} = [0, 1, 1]$. Thus capillaries/pipes or pillars/rods can be included in the simulations. Capillaries are created by multiplying every term on the right-hand side of Equation (3.20) by -1 .
- **Superellipsoids** have the same structure as ellipsoids except with $p \geq 4$ where p is even. *Squirrels* are useful for constructing complicated geometries because they closely approximate cubes. Squirrels can be thought of as “hyperspheres” since the surface of a squirrel is described by Equation (3.20) in the same way as a sphere except with an even power p that is greater than two. Explicitly, squirrels (of “radius” R^p and centre position $\vec{Q}$) require $\vec{A} = \vec{1}$ and $p \geq 4$. Varying $\vec{A}$ from unity creates an approximately rectangular body.
- **Hyperboloids** and **paraboloids** are also created via the appropriate choice of $\vec{A}$, though these surfaces have not been extensively tested.

Equation (3.20) allows any or all of these surfaces to be included in the simulation and many surfaces can be combined to construct complicated geometries. Alone, however, a surface is not a boundary condition.

3.3.2 Boundary Rules

The surfaces of the BCs are defined by Equation (3.20), but what is implemented if a BC-collision event occurs (Equation (3.21))? Boundary rules are needed to complete the boundary conditions. Many types of boundary rules exist. For example, periodic boundary conditions are common. Periodic boundary conditions wrap the system such that the position of a particle that violates the surface is shifted to the opposite end of the control volume (Figure 3.3.1). Other boundary rules define walls, and particles that violate these surfaces have both their positions and velocities transformed.

In order to handle a variety of boundary rules, six parameters are utilized:

- Two parameters transform the violating particle’s position:
 - A violating particle’s position is shifted normal to the boundary surface by an amount $D_{x,\hat{n}}$.

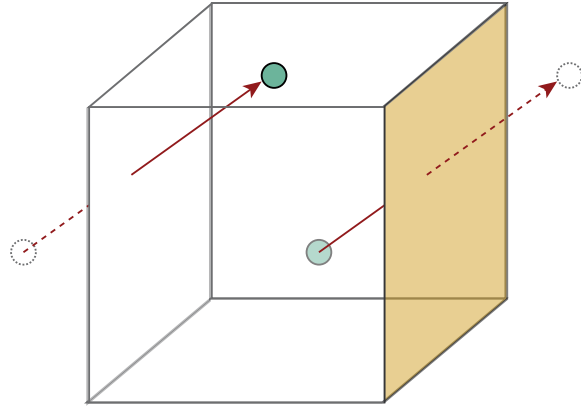


Figure 3.3.1: Periodic boundary conditions wrap the system and the positions of particles that pass the planar surface are shifted to the opposite end of the control volume.

- $D_{x,\hat{t}}$ is the tangential shift.
- Four parameters transform the violating particle's velocity. The first two are coefficients that scale the particle's velocity:
 - A violating particle's velocity normal to the boundary surface is multiplied by $M_{v,\hat{n}}$.
 - $M_{v,\hat{t}}$ is the corresponding tangential coefficient.

The remaining coefficients shift the velocity:

- $D_{v,\hat{n}}$ is the amount that a violating particle's velocity normal to the boundary surface is increased by.
- $D_{v,\hat{t}}$ is the amount added to the tangential component.

These four parameters transform a violating particle's velocity components normal and tangential to the surface at the point of the violation. At times, it may be preferable to transform the Cartesian components instead of the normal and tangential components. This can be done by replacing $D_{v,\hat{n}}$ and $D_{v,\hat{t}}$ with $D_{v,\hat{x}}$, $D_{v,\hat{y}}$ and $D_{v,\hat{z}}$.

Together, these parameters can create a wide variety of boundary rules.

Periodic Boundary Conditions

Extended geometries, such as pipe or channel flows, often use *periodic boundary conditions*. They simulate open systems. In order to achieve periodicity, a planar boundary surface

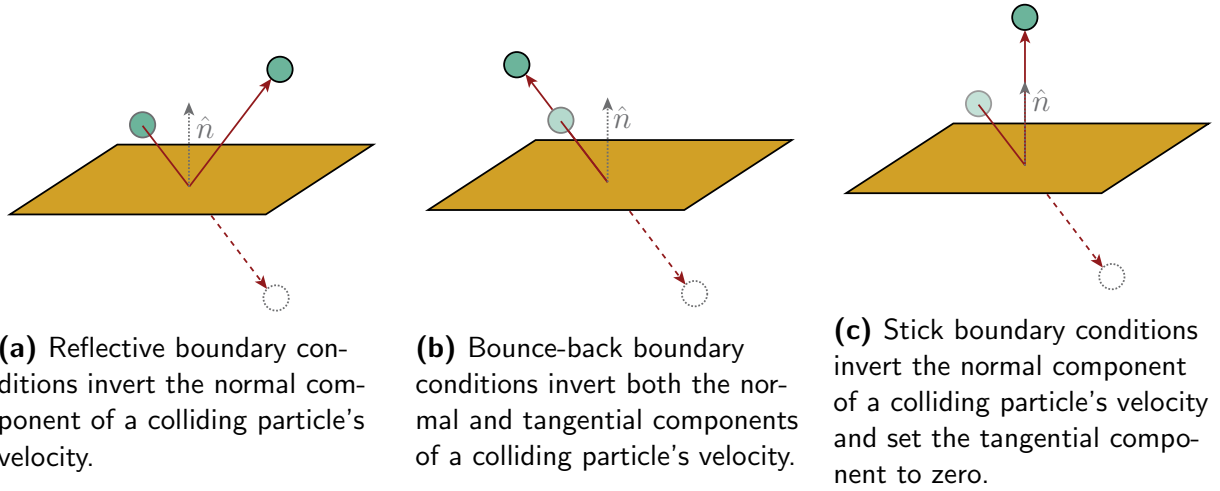


Figure 3.3.2: Schematics of boundary rules appropriate for hard wall collisions.

must be created with the transformation parameters are set to $D_{x,\hat{n}} \neq 0$, $D_{x,\hat{t}} = 0$, $M_{v,\hat{n}} = 1$, $M_{v,\hat{t}} = 1$, $D_{v,\hat{n}} = 0$ and $D_{v,\hat{t}} = 0$. The important parameter is $D_{x,\hat{n}}$, which is set to plus or minus the system size depending on the direction of the surface (Figure 3.3.1). Notice that two planar boundary conditions are needed: One at each end of the control volume.

Reflective Boundary Conditions

In order to produce a homogeneously constant velocity field profile, Allahyarov and Gompper defined an elastic collision of solvent particles with channel walls, creating *reflective boundary conditions* (Figure 3.3.2a) [82]. For an elastic BC, the component normal to the wall is inverted ($M_{v,\hat{n}} = -1$), while the tangential component remains unchanged ($M_{v,\hat{n}} = 1$). All the other/additive parameters are zero ($\{D_{x,\hat{n}}, D_{x,\hat{t}}, D_{v,\hat{n}}, D_{v,\hat{t}}\} = 0$). This results in a perfect-slip condition at the wall.

No-Slip, Bounce-Back and Stick Boundary Conditions

While simulating flow in a channel, Allahyarov and Gompper stated that they use bounce-back boundary conditions [82]. However, this is followed by a footnote emphasizing that the terms “no-slip boundary conditions”, “bounce-back boundary conditions” and “stick boundary conditions” are equivalent. This is not the terminology used in this thesis.

- In this thesis, the term *no-slip* refers to **any** impermeable boundary condition that brings the velocity of the fluid to zero at the wall. It is not a specific boundary rule.

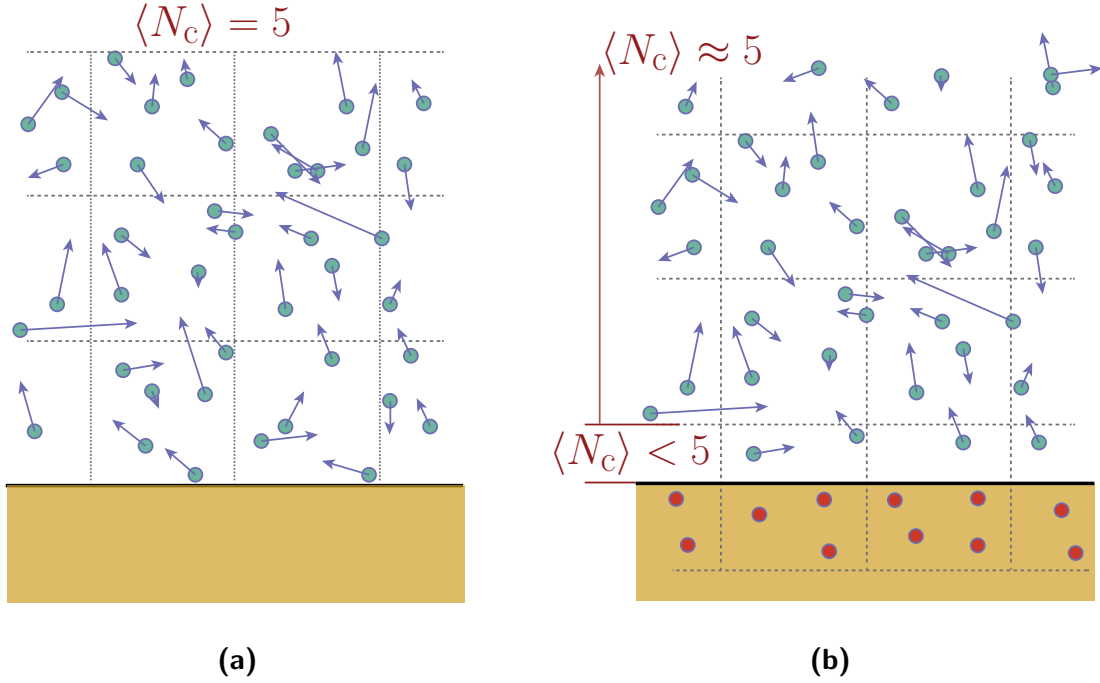


Figure 3.3.3: When a planar, bounce-back wall is flush to the neighbouring MPCD cells, no-slip boundary conditions are respected (Figure 3.3.3a). However, if the boundary surface excludes a portion of the MPCD cells, the local average number of MPCD fluid particles is less than typical in the bulk, which reduces the local viscosity (Figure 3.3.3b).

- On the other hand, *bounce-back boundary rules* cause the velocity of each violating fluid particle to be totally reversed (Figure 3.3.2b). This can be done by setting the additive parameters to zero and the multiplicative terms to $M_{v,\hat{n}} = -1$ and $M_{v,\hat{t}} = -1$.
- *Stick boundary rules* are those for which the tangential component of each violating particle's velocity is forced to zero (perhaps while the normal speed remains unaffected by the collision *i.e.* $M_{v,\hat{n}} = -1$, $M_{v,\hat{t}} = 0$ and all the rest are zero [Figure 3.3.2c]).

As is seen in the next section, neither bounce-back nor stick boundary rules are sufficient to ensure a no-slip boundary condition.

Phantom Particles

No-slip BCs require bounce-back rules and the inclusion of *phantom particles*. Bounce-back BCs are convenient and effective for ensuring no-slip at walls only when the plane coincides exactly with the boundaries of any neighbouring MPCD cells. However, in more complex geometries, walls may not coincide with or even be parallel to the neighbouring cells. When

the random, lattice-shifting procedure is implemented to produce Galilean invariance (§ 3.1), simple bounce-back BCs fail to guarantee no-slip [83].

This slip can be understood by considering the viscosity of the MPCD fluid in the MPCD cells neighbouring the walls (Equation (3.10)). For clarity, consider the viscosity of the Anderson-MPCD collision operator (Equation (3.14)). If the average number of particles is decreased then the viscosity decreases as well. For cells that overlap with a boundary surface, some portion of the cell's space is inaccessible to the fluid particles and so the cell will quite commonly be only partially filled (Figure 3.3.3). It will have an average density that is less than the bulk and, therefore, a correspondingly lower viscosity than the bulk of the fluid. This is equivalent to an inviscid film coating boundary surfaces and causing an apparent slip.

It was proposed that the section of the cell cut off by the wall be filled with $N^{\text{ghost}} = N_c - \langle N_c \rangle$ *phantom* (or virtual) particles so that the effective density is equal to the average bulk density (Figure 3.3.3). The collision step then uses the average velocity of both the real fluid and phantom particles. For stationary walls, the phantom particles are generated with a Maxwell-Boltzmann velocity distribution about zero with the same temperature as the real fluid. The centre of mass velocity of the intercepted cell is then reduced to

$$\vec{v}^{\text{cm}} = \frac{1}{N_c} \left(\sum_{i=1}^{N_c} \vec{v}_i + \sum_{j=1}^{N^{\text{ghost}}} \vec{v}_j^{\text{ghost}} \right).$$

Because they are drawn from a Maxwell-Boltzmann distribution, the properties of each phantom particle need not be determined explicitly. Let the sum of the phantom particles' velocities be $\vec{v}^{\text{ghost}} = \sum_{i=1}^{N^{\text{ghost}}} \vec{v}_i^{\text{ghost}}$. Then $\vec{v}^{\text{ghost}}$ also follows a Maxwell-Boltzmann distribution with a mean of zero but with a variance of $(\langle N_c \rangle - N_c) k_B T$. This saves computational effort. The average velocity can then be expressed as

$$\boxed{\vec{v}^{\text{cm}} = \frac{1}{N_c} \left(\sum_{i=1}^{N_c} \vec{v}_i + \vec{v}^{\text{ghost}} \right)}. \quad (3.22)$$

In essence, the phantom particles (of correct temperature but no net motion) have weighted the centre of mass velocity such that it is lowered while maintaining a stochastic temperature term. The use of Equation (3.22) eliminates slip while avoiding compressibility effects [84]. This method, called *phantom particle boundary conditions* here, is used widely [82, 85].

Thermal-Wall Boundary Conditions

Boundary conditions that can act as thermostats as well as walls also exist [66]. In these *thermal-wall boundary conditions* fluid particles that hit the boundary are assigned a random velocity drawn from a probability distribution for the normal velocity $p_{\hat{n}}(\vec{v} \cdot \hat{n})$ and for the tangential velocity $p_{\hat{t}}(\vec{v} \cdot \hat{t})$ components. The probability distributions must be constructed such that the distribution for particles near the wall remains Maxwellian. Also, $p_{\hat{t}}$ should allow both positive and negative values while $p_{\hat{n}}$ must always be positive if the particles are to scatter away from the wall and not be transmitted through it. To do this, the normal velocity is randomly drawn from a Rayleigh distribution, while the tangential velocity obeys a Gaussian distribution:

$$p_{\hat{n}}(\vec{v} \cdot \hat{n}) = \left(\frac{m |\vec{v} \cdot \hat{n}|}{k_B T} \right) \exp \left[-\frac{m (\vec{v} \cdot \hat{n})^2}{2k_B T} \right] \quad (3.23)$$

$$p_{\hat{t}}(\vec{v} \cdot \hat{t}) = \sqrt{\frac{m}{2k_B T}} \exp \left[-\frac{m (\vec{v} \cdot \hat{t})^2}{2k_B T} \right]. \quad (3.24)$$

Although this boundary rule should simultaneously produce no-slip and act as thermostat for the fluid at the boundary, it is insufficient for some flows and an additional thermostat for the bulk of the fluid might be required [66]. Setting BCs to different temperatures (perhaps with the intention of implementing a thermal gradient to study thermophoresis) should be approached with caution as the compressible nature of the MPCD fluid leads to corresponding inhomogeneous density distributions. In this sense, MPCD fluids are known to behave as ideal gases and so the density field is $\varrho(\vec{x}) = P/k_B T(\vec{x})$ [86]. Future work and algorithm development is required to utilize MPCD to study such systems without large density changes.

Boundary conditions defined by a boundary surface (§ 3.3.1) and a set of boundary rules (§ 3.3.2) are necessary components to any MPCD simulation. At the very least, 2d planes are required to set the control volume and more BCs may be included to construct various microfluidic geometries. The next section (§ 3.4) demonstrates how boundary conditions contain and drive flows within a simulation. However, boundaries need not be static. They can also be implemented as dispersed, diffusing and convecting bodies (§ 3.5).

3.4 Non-equilibrium, Flowing Systems

The MPCD algorithm from § 3.1 and § 3.2 provides the thermalized fluid, and the boundary conditions discussed in § 3.3 define the geometry of the microfluidic device. However, studying separation science requires simulations of non-equilibrium situations as discussed in § 2.1.2. In particular, macroscopic flows are often necessary and, because MPCD fluids are viscous, sustained work must be done to drive a flow. Flows must be driven and thermostats must be applied to control the system’s energy.

3.4.1 Driving Flows

Mechanically driving flows is done in two ways:

1. A local force is applied to fluid particles at a boundary.
2. An external force is applied to each MPCD fluid particle.

The first strategy uses the boundary rules from § 3.3.2 to increase the fluid velocity in a given direction. In particular, the control parameters $D_{v,\hat{x}}$, $D_{v,\hat{y}}$ and $D_{v,\hat{z}}$ are useful since they form the velocity vector that is added to a fluid particle in the event of a collision. Three cases are particularly common:

Lees-Edwards Boundary Conditions To introduce simple shear flow into their MPCD simulations, the Yeomans research group used *Lees-Edwards boundary conditions* [73, 87]. This is a variation on periodic boundary conditions that requires two parallel periodic boundary conditions. Imagine that they are in the xz -plane and one is the “top” and the other is the “bottom”. By setting $D_{v,\hat{y}}$ to some positive value for the top plane and to an equal but opposite value for the bottom plane, a shear will be generated in the $\hat{y}$ -direction (Figure 3.4.1a).

Pump Periodic Boundary Conditions Imagine a periodic boundary condition at the end of a control volume. This BC necessarily has a non-zero $D_{x,\hat{n}}$ that wraps the fluid particle position back to the other end of the control volume but also has a non-zero $D_{v,\hat{n}}$. This means that any particle that crosses the periodic BC plane will appear at the other end with an increased velocity (Figure 3.4.1b). Thus, the fluid is continuously pumped in the direction normal to the plane — a method called *pumped periodic BCs*.

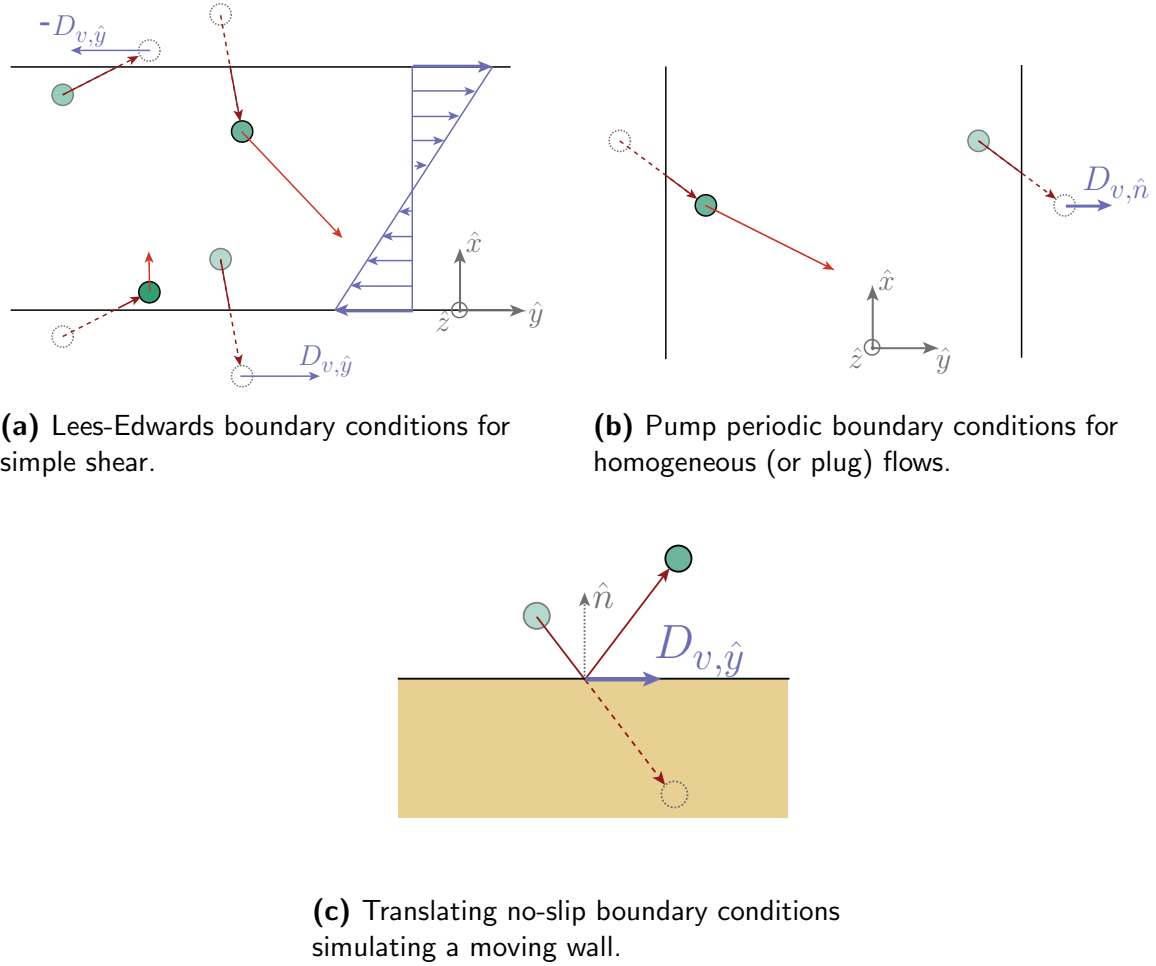


Figure 3.4.1: Schematics of flow-driving schemes.

Translating No-slip Wall Boundary Conditions A normal bounce-back BC representing a planar surface (say again in the xz -plane) can be supplemented with non-zero $D_{v,\hat{y}}$. This rule is equivalent to a hard, *translating no-slip* planar wall moving with a constant velocity $D_{v,\hat{y}}$ in the $\hat{y}$ -direction. As the wall moves it shears the fluid at its surface, creating a boundary layer (see § 2.3.4) and a flow in the $\hat{y}$ -direction (Figure 3.4.1c).

The second strategy, used to establish a pressure gradient, is to apply an external force to every fluid particle in the system. By Equation (2.60), a pressure gradient and an external acceleration are equivalent. One can always refer to an external acceleration as an effective pressure gradient: $\vec{\nabla}P \equiv -\rho\vec{g}$. In order to respect Equation (3.1), the particles' velocities are increased by $g\delta t$ immediately prior to the collision operation. The acceleration is thus essentially incorporated into the collision operator $\mathbb{R}$ (Equation (3.2)).

3.4.2 Thermostats

In driving flows, work is done. Because the MPCD fluid is viscous, the work done on the fluid is continually converted into heat by internal friction, as represented by the MPCD collision operator.

In real systems, such heating occurs but is often mediated and removed by container walls acting as reservoirs. Thermostats are algorithms that act as virtual reservoirs for the simulation. They keep the system near the desired fixed temperature even when work is being done on the fluid. Thermal-wall boundary conditions are one type of thermostat discussed in § 3.3.2 and many of the collision operators intrinsically act as thermostats (§ 3.2.2 and § 3.2.4) but others require a separate thermostat algorithm.

Velocity Rescaling

The simplest method to thermostat a system is to rescale the velocities. Since the kinetic energy of the constituents constitutes the thermal energy ($k_B T = \sum_i m_i v_i^2 / 2$), a desired temperature is achieved by scaling every velocity by $\gamma_{\text{rel}} = \sqrt{T_o / T}$. Rescaling the velocity in this way instantaneously moves the temperature to its fixed value of T_o .

Berendsen Thermostat

More realistically, the temperature should relax toward the reservoir value over a finite period of time. More aggressive scaling should be done when T/T_o is much different than 1. The Berendsen thermostat [88] defines a relaxation time, τ , that moves $T/T_o \rightarrow 1$ by rescaling the velocities by a factor

$$\gamma_{\text{rel}} = \left[1 + \left(\frac{T}{T_o} - 1 \right) \left(\frac{\delta t}{\tau} \right) \right]^{1/2}. \quad (3.25)$$

Velocity rescaling and the Berendsen thermostat share some weaknesses:

1. If the temperature increases in one region of the fluid then both of these global, velocity e-scaling thermostats will cool distant regions.

2. Even if work is being done to drive macroscopic flow in a particular direction, these thermostats will scale velocity in all directions, repressing thermal motion in the perpendicular directions in an attempt to lower the total kinetic energy. In extreme cases, all fluctuations are repressed and the fluid moves as a solid block.

The second concern has been referred to as the *flying ice cube artefact* [89]. A simple improvement is to rescale the difference between the characteristic velocity of the flow $[\vec{U}]$ and the velocity of each fluid particle $\vec{v}_i$ as

$$\vec{v}_i = [\vec{U}] + \gamma_{\text{rel}} \left(\vec{v}_i - [\vec{U}] \right).$$

Heyes Thermostat

The Heyes algorithm is a stochastic thermostat that is applied locally to each MPCD cell [66]. In a sense (like acceleration or velocity rescaling) it becomes a part of the collision operation. The Heyes thermostat enacts the following procedure:

1. A random number is homogeneously generated in the range $\xi_1 \in [1, 1 + c]$, where c is the control parameter of the thermostat and should be between 0.05 and 0.3.
2. Generate a second random number $\xi_2 \in [0, 1]$.
 - If $\xi_2 > 0.5$ then the scaling factor is $\gamma_{\text{rel}} = \xi_1$
 - Else $\gamma_{\text{rel}} = \xi_1^{-1}$

3. Generate a third (and final) random number $\xi_3 \in [0, 1]$ and let

$$A \equiv \gamma_{\text{rel}}^{d(N_c-1)} \exp \left[- \left(\frac{\gamma_{\text{rel}}^2 - 1}{2k_B T_0} \right) \sum_i^{N_c} m_i (\vec{v} - \vec{v}^{\text{cm}})^2 \right]. \quad (3.26)$$

If $\xi_3 \geq \min[1, A]$ then set $\gamma_{\text{rel}} = 1$ (*i.e.* do not rescale the velocities in the current cell).

4. Rescale the velocities in the current cell.

Variants of this procedure exist [90, 91]. Since non-equilibrium flows play such an important role in this work, the Heyes thermostat is preferred if the Anderson-MPCD or Langevin-MPCD collision operators are not utilized. The Heyes algorithm is ideal because each cell is thermostatted about its own centre of mass velocity, which allows flows with shears to be treated without risking the formation of flying ice cubes or non-local cooling.

3.4.3 Example Flows

In order to demonstrate the versatility of the current implementation of the MPCD algorithm, this section presents many simple 2D and 3D flow geometries [92]. In each simulation the Anderson-MPCD collision operator is used and phantom particles are included in each situation for which no-slip BCs are desired. The simulations presented in this section were performed on a 2.50GHz Intel Core 2 Duo processor and required several hours to obtain sufficiently smooth flow profiles.

Channel Flow

Pressure-driven flow through a 2D channel is a classical problem with a simple flow profile. The solution to the Navier-Stokes equation (Equation (2.59)) for a pressure gradient of $\partial P/\partial z$ between two parallel, no-slip plates is

$$u(\tilde{y}) = 6 \langle u \rangle (\tilde{y} - \tilde{y}^2), \quad (3.27)$$

where $\tilde{\cdot}$ denotes normalization. In this case, the channel height h normalizes the position $\tilde{y} = y/h$. The flow around various obstacles can be predicted by performing 2D simulations that include surfaces with bounce-back boundary conditions. The average velocity of the fluid in this geometry is

$$\langle u \rangle = \frac{h^2}{12\eta} \frac{\partial P}{\partial y}. \quad (3.28)$$

This flow profile is commonly referred to as *Poiseuille flow* or descriptively as parabolic flow. In the example (Figure 3.4.2), the channel height is $h = 18a$ and in 3D the channel width is $w = 100a$. In both dimensionalities, the length of the simulation control volume is $L = 100a$. In each of the following systems, a small external force drives the Anderson-MPCD fluid to flow. In terms of the natural MPCD simulation units, an external force of $10^{-3}ma/\delta t^2$ is

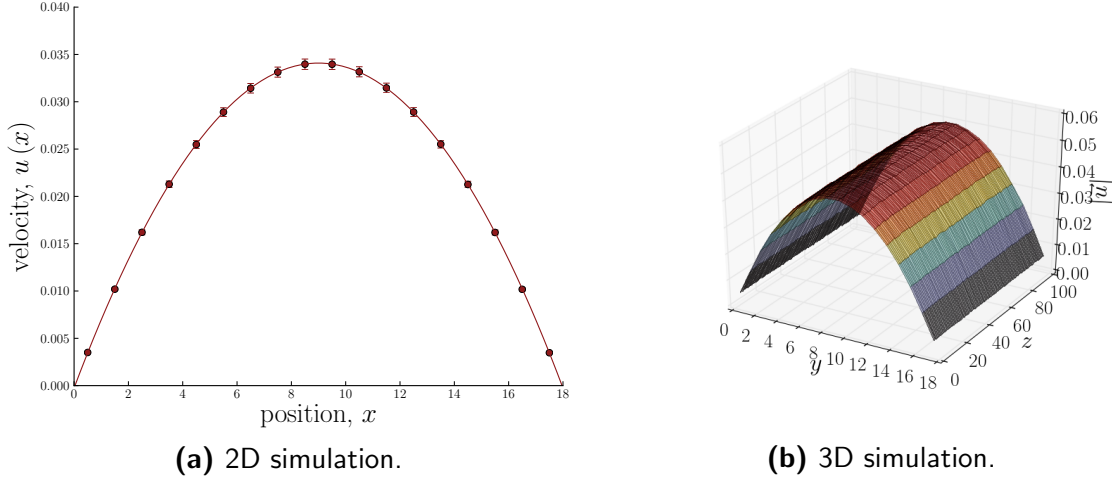


Figure 3.4.2: Flow profile produced by Anderson-MPCD simulations of 2D and 3D Poiseuille flow.

applied (§ 3.1). The resulting parabolic fit to the flow profile produced by the 2D simulation is quite accurate (Figure 3.4.2a). The 3D simulation demonstrates that utilizing periodic boundary conditions causes this flow profile to be maintained across $\hat{z}$ (Figure 3.4.2b).

3D Rectangular Channels In this thesis, two complications to Poiseuille flow are relevant in the context of field-flow fractionation (FFF). The first is flow through a 3D rectangular channel. Theories accounting for the elution of solutes through FFF channels commonly assume a 2D system and, correspondingly, Poiseuille flow (§ 5). However, a series solution for the 3D flow profile exists [93]. The solution depends on the nondimensionalized position within the channel and the aspect ratio $\tilde{w} = w/h$. The fluid velocity profile in a rectangular channel is then given by the Fourier sum

$$u(\tilde{y}, \tilde{z}; \tilde{w}) = [U] \sum_{n=1}^{\infty'} \frac{48}{(n\pi)^3} \left[1 - \frac{\cosh(n\pi\tilde{y})}{\cosh\left(\frac{n\pi\tilde{w}}{2}\right)} \right] \sin(n\pi\tilde{z}), \quad (3.29)$$

where $\sum'$ represents summation over odd terms only and the characteristic velocity is defined to be the average velocity of Poiseuille flow (Equation (3.28)), *i.e.* $[U] = h^2 (\partial P / \partial y) / (12\eta)$. Only a few terms are adequate to arrive at satisfactorily accurate solutions [94]. The cross-sectional averaged mean solvent velocity is found from Equation (3.29) to be [93]

$$\langle u \rangle = [U] \left[1 - \sum_{n=1}^{\infty'} \frac{192}{(n\pi)^5} \frac{\tanh\left(\frac{n\pi\tilde{w}}{2}\right)}{\tilde{w}} \right]. \quad (3.30)$$

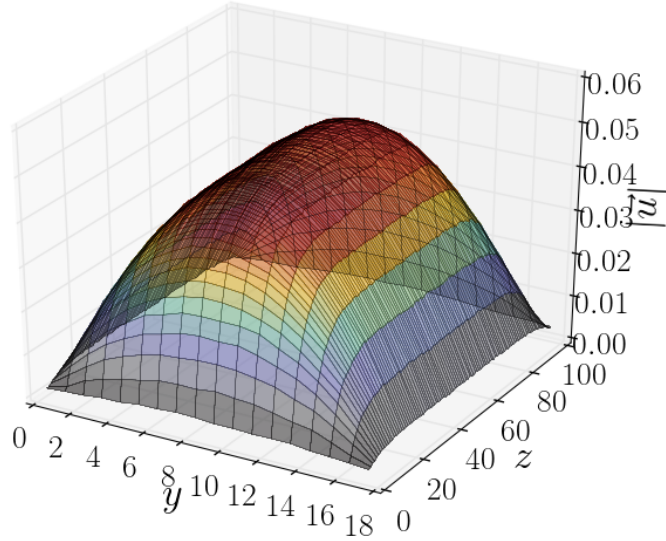


Figure 3.4.3: Flow profile produced by 3D Anderson-MPCD simulation of flow through a rectangular channel of aspect ratio $\tilde{w} = 9/50$.

This flow profile can be accurately reproduced by the Anderson-MPCD algorithm. An example aspect ratio of $\tilde{w} = 9/50$ is shown in Figure 3.4.3. The simulation uses the same parameters as the channel flow shown in Figure 3.4.2b except that the lateral periodic boundary conditions are replaced with no-slip walls. The flow profile is quite similar to Poiseuille flow far from the $\hat{x}\hat{y}$ -lateral walls. It is only when considering the regions that are within $z \approx h$ of the lateral walls that the velocity is significantly reduced from the value predicted by the 2D Poiseuille equation (Equation (3.27)).

Non-zero Slip Fractionation of solutes in an FFF channel that has arbitrary slip [95–97] at either wall will be considered in § 7.3. Slip can inadvertently arise or be induced in several ways including flow over rough surfaces, supplementation by electro-osmotic flow and nano-structured surfaces. The flow profile $u(y)$ of a pressure driven flow between two plates separated by a distance h is parabolic with distance $\tilde{y} = y/h$ from the “bottom” wall. Slip does not change this; instead, it shifts the velocity from zero to a finite value at the walls and in doing so moves the position of the maximum from the centre of the channel. A slip

length $\tilde{b}$ produces a boundary condition

$$u(\tilde{y} = 0) = \tilde{b} \left. \frac{\partial u}{\partial \tilde{y}} \right|_{\tilde{y}=0} \quad (3.31a)$$

at the bottom wall and a slip length $\tilde{t}$ makes the boundary condition at the top wall

$$u(\tilde{y} = 1) = -\tilde{t} \left. \frac{\partial u}{\partial \tilde{y}} \right|_{\tilde{y}=1}. \quad (3.31b)$$

Solving the Navier-Stokes equation (Equation (2.59)) with these boundary conditions predicts the fluid velocity profile to be

$$u(\tilde{y}, \tilde{b}, \tilde{t}) = 6[U] \left[\left(\frac{1 + 2\tilde{t}}{1 + \tilde{b} + \tilde{t}} \right) (\tilde{y} + \tilde{b}) - \tilde{y}^2 \right], \quad (3.32)$$

where, once again, the solution is stated in terms of the no-slip average fluid velocity $[U] = \int_0^1 u(\tilde{y}, 0, 0) d\tilde{y} = h^2 (\partial P / \partial y) / 12\eta$ for the same pressure gradient (Equation (3.28)). This equation reduces Equation (3.27) in the no-slip limit. When the pressure difference is kept constant, slip moves the maximum velocity away from the centre position $\tilde{y} = 1/2$ (unless $\tilde{b} = \tilde{t}$), and increases the average flow velocity

$$\langle u(\tilde{b}, \tilde{t}) \rangle = [U] \left[\left(\frac{1 + 2\tilde{t}}{1 + \tilde{b} + \tilde{t}} \right) (6\tilde{b} + 3) - 2 \right]. \quad (3.33)$$

Several flow profiles are shown in Figure 3.4.4. The equal slip such as $u(\tilde{y}, 1/4, 1/4)$ shifts the $u(\tilde{y}, 0, 0)$ parabolic profile up. The $u(\tilde{y}, 0, \infty)$ curve shows a continuous increase from zero at the no-slip wall to $6[U]$ at the perfect slip wall.

As discussed in § 3.3.2, if phantom MPCD particles are not included in cells that are intersected by a surface plane then an inviscid film forms on the surface and slip occurs. Figure 3.4.5 shows the resulting flow profile. It should be compared to Figure 3.4.2a for which phantom MPCD particles were included. The comparison reveals that the slip length is quite minute (only $[0.081 \pm 0.004]a$) but finite. Detailed studies of this effect exist in the literature [82, 84, 85]. In this work, phantom MPCD particles are always included for no-slip conditions.

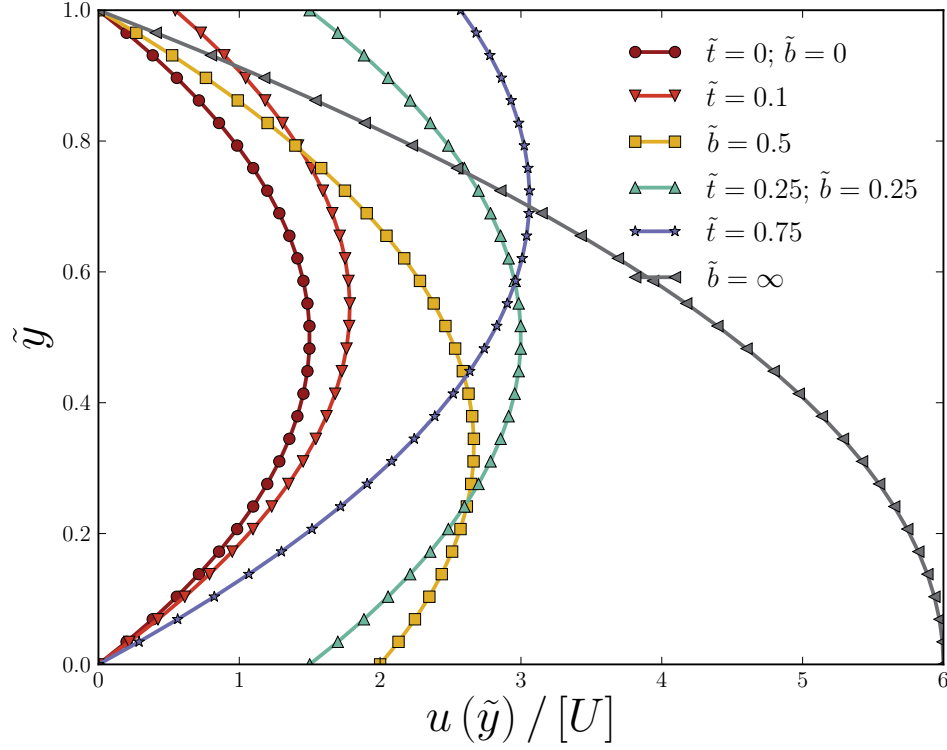


Figure 3.4.4: Fluid flow profiles for a variety of slip lengths $\tilde{b}$ and $\tilde{t}$, for the accumulation (or bottom) and depletion (or top) wall respectively. Fluid velocity normalized by no slip $[U] = h^2 (\partial P / \partial y) / 12\eta$.

Flow Past Obstacles

Immobile obstacles can be placed in the channel to simulate more complicated microfluidic geometries. The external force applied is kept at $10^{-3}ma/\delta t^2$ and two parallel no-slip boundary conditions form the channel walls, but they are now separated by a distance of $40a$ (*i.e.* 40 MPCD cells) in the $\hat{y}$ -direction. The simulation control volume has a length of $200a$, but periodic boundary conditions close the system, making it effectively infinite in the $\hat{x}$ -direction. Notice that the large aspect ratio of the system in the direction of the flow ($h/L = 1/5$) causes the representations in Figure 3.4.6 to appear compressed in the $\hat{x}$ -direction.

Placing a squirle against one of the channel walls produces a barrier (Figure 3.4.6a). As is to be expected, the velocity increases substantially within the constriction. Far from the barrier, the flow profile returns to Poiseuille flow. Likewise, a cylindrical obstacle fixed as a solid barrier in the centre of the channel (Figure 3.4.6b) causes the flow to accelerate on either side as the fluid passes. A slit can be produced by affixing squirles to opposite walls (Figure 3.4.6c). As with each of these situations, a substantial increase in velocity is

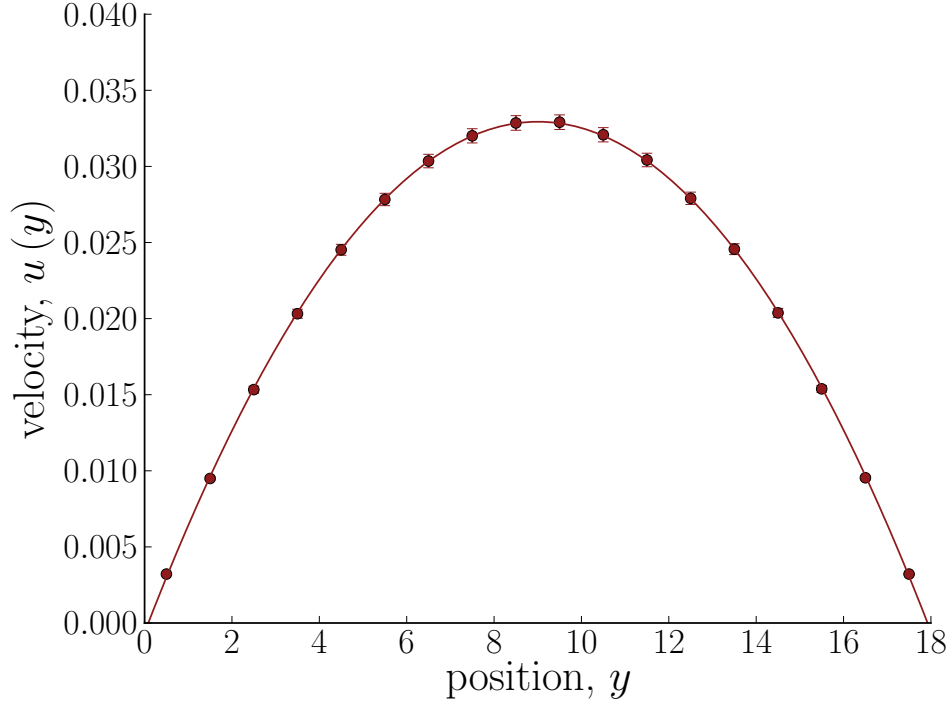


Figure 3.4.5: Same as Figure 3.4.2a but without including phantom MPCD particles. Minute slip is observable at the limits.

observed in the constriction.

Another variation on the channel geometry is the Craighead device [98]. Craighead channels are common in microfluidic devices and have potential in terms of separation science since the mobility of different solutes eluting through such devices can vary as a function of solute conformation. The Anderson-MPCD algorithm with a series of squircles forming the raised portion simulates the flow profile. The fluid velocity drops above the cell and the streamlines curve downward into it (Figure 3.4.6d).

The other relevant simulation setup is flow past obstacles in free solution [99]. In § 3.5 mobile solutes that are free to diffuse and be carried by flows are discussed, but for now consider the flow past some simple, fixed bodies. As discussed in § 3.3.1, the implemented algorithm can include spheres (Figure 3.4.7a), ellipsoids (Figure 3.4.7b and Figure 3.4.7c) and approximate squares (Figure 3.4.7d). In the flow profiles shown in Figure 3.4.7, the flow-producing external force is still $10^{-3}ma/\delta t^2$ but the system size is increased to a $300a \times 300a$ area enclosed by four periodic boundary conditions. Each obstacle has a “radius” of 10. For the circular obstacle this value is indeed truly a radius of $r = 10$ (Figure 3.4.7a). For the shown ellipsoids, the ratio of major to minor axis is 4. For the oblate ellipsoid (Figure 3.4.7b) the boundary surface is defined by Equation (3.20) and takes the specific form $\mathcal{S}(\vec{x}) =$

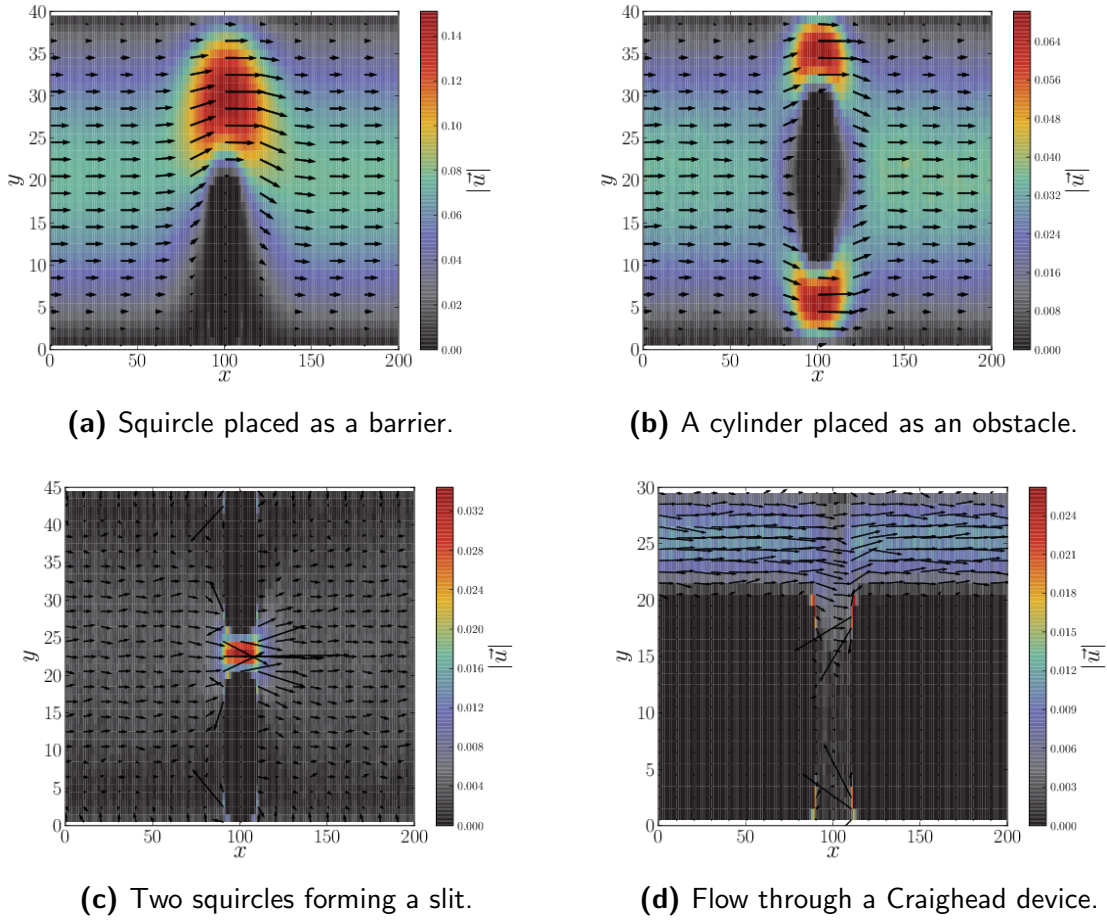


Figure 3.4.6: Flow profiles of various complications within a no-slip channel produced by Anderson-MPCD simulations of 2D flow.

$(x - 150)^2 + (y - 150)^2 / 16 - 100$. Likewise, for the prolate ellipsoid, $\mathcal{S}(\vec{x}) = (x - 150)^2 / 16 + (y - 150)^2 - 10^2$ (Figure 3.4.7c). The square obstacle is approximated by a squircle of power 6 such that the bounding surface function is $\mathcal{S}(\vec{x}) = (x - 150)^6 + (y - 150)^6 - 10^6$.

Capillary Flows

By inverting a cylindrical rod boundary condition, a capillary tube geometry can be simulated. Doing so allows flow through a cylindrical tube of diameter d_H to be simulated using the MPCD algorithm ($d_H = 20a$ in Figure 3.4.8a). The simulated flow profile agrees with

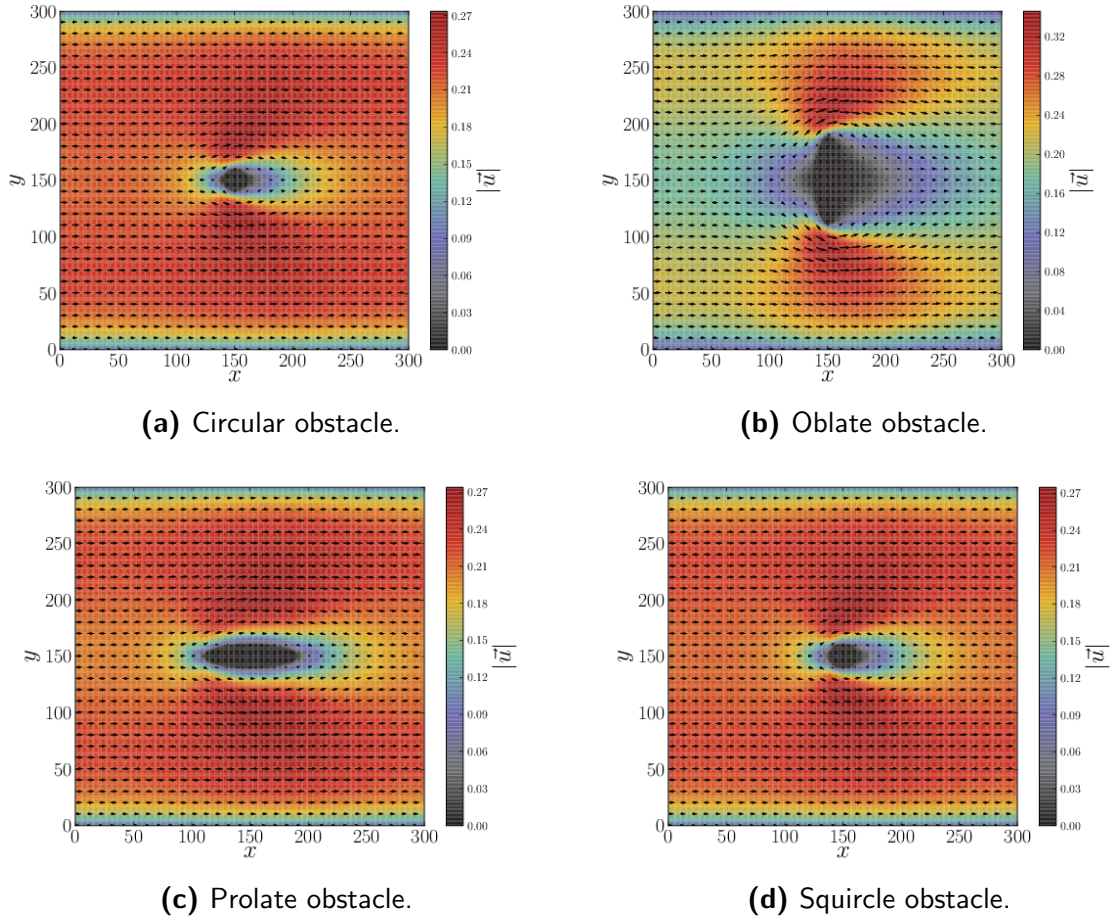


Figure 3.4.7: Flow profiles past various fixed, no-slip obstacles in free-solution produced by Anderson-MPCD simulations of 2D flow.

the theoretical solution

$$u = 2 \langle u \rangle \left[1 - \left(2 \frac{x}{d_H} \right)^2 \right], \quad (3.34)$$

which is called the Hagen-Poiseuille equation. As is done with channel flow, the Hagen-Poiseuille equation is written in terms of the average velocity $\langle u \rangle = d_H^2 (dP/dx) / (32\eta)$.

More complicated cylindrical geometries can easily be created, such as the example flow shown in Figure 3.4.8b. In Figure 3.4.8b a no-slip, impermeable rod of radius 10 is fixed in the center of a tube of diameter $d_H = 40$. The flow occurs between these concentric cylinders.

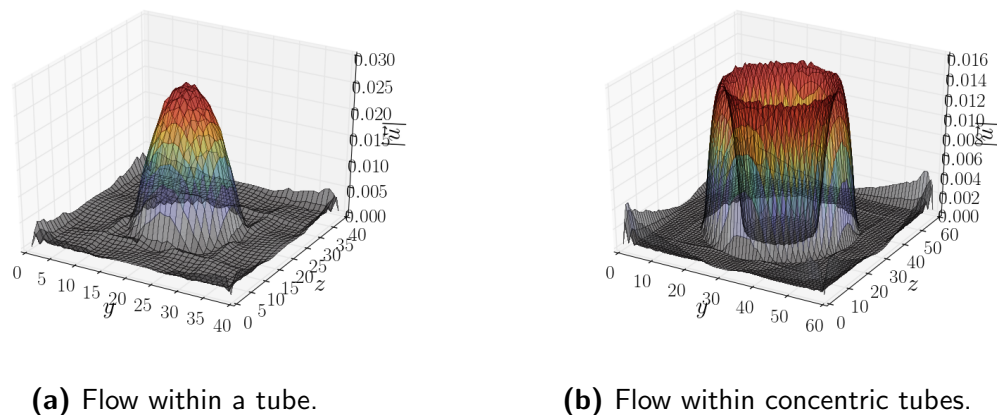


Figure 3.4.8: Flow profiles in a capillary and in a concentric tube geometry.

3.5 Simulating Solutes

The final computational component required to perform simulations of separation phenomena in microfluidic devices is a mechanism for simulating the solutes themselves. In the previous section (§ 3.4.3), immobile bodies were considered but if MPCD is used, the fluid itself is not of interest. In this thesis, the behaviour of mobile solutes is the primary focus. Multiple methods exist to couple solutes to the fluid and three have been implemented here. This section explains the three implemented options. Large colloids can be modelled as mobile, spherical boundary conditions, while smaller objects, which interact through more complicated pair potentials (such as the monomers constituting polymers and polyelectrolytes), can be modelled as Molecular Dynamics beads.

3.5.1 Hard Colloids

If a boundary condition object is not fixed in space but rather acts as a mobile hard colloid whose surface is defined by Equation (3.20), it can simulate a hard colloidal body. However, to interact with the solvent such a *BC-solute* must exchange momentum with MPCD fluid particles. Although simple in concept, properly exchanging momentum can be computationally exhaustive for arbitrary solute shapes. Because many computational operations are required, it is not always advisable to properly conserve both momentum and energy in MPCD-BC collision events.

Since mobile BC-solutes may collide with one another as well as MPCD point particles, consider the collision of two hard, arbitrarily shaped bodies (labelled A and B). This general

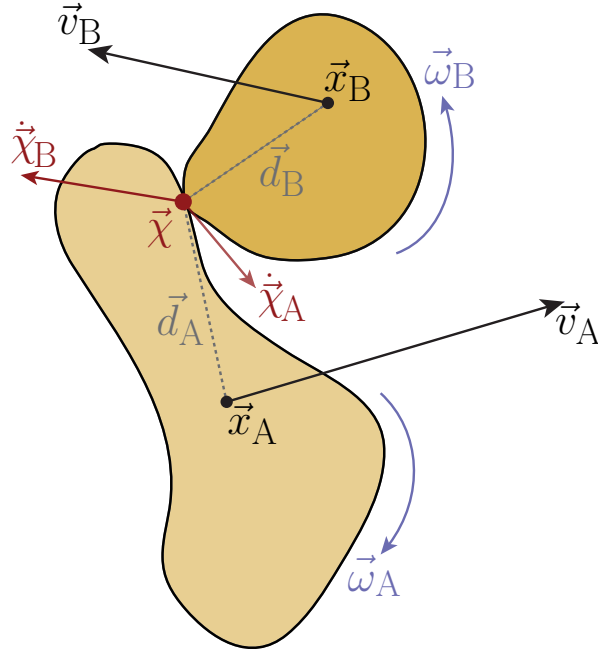


Figure 3.5.1: Two arbitrarily shaped bodies (with centre of mass positions $\vec{x}_A$ and $\vec{x}_B$) collide at a contact point $\vec{\chi}$. Immediately prior to the collision they have velocities $\vec{v}_A$ and $\vec{v}_B$ and angular velocities $\vec{\omega}_A$ and $\vec{\omega}_B$. At the contact point, the velocity of the surface of one body was $\dot{\vec{\chi}}_A$, while the other was $\dot{\vec{\chi}}_B$.

situation can be reduced to the collision between an MPCD fluid particle and a BC-solute after the general theory has been established. Let the centre of mass positions be $\vec{x}_A$ and $\vec{x}_B$ and the centre of mass velocities be $\vec{v}_A$ and $\vec{v}_B$. The collision only occurs at a single point (since the bodies are assumed to be hard and incompressible). This point is called the *contact point* $\vec{\chi}$. If one of the bodies (say, body A) is an MPCD fluid particle, then $\vec{\chi} = \vec{x}_A$.

Since the BC-solutes have mathematically sharp boundaries, it is best to utilize *impulse-based contact analysis* rather than forces to characterize collision events [100]. Let the impulse $\vec{\mathcal{J}} = \mathcal{J}\hat{n}$ be in the direction $\hat{n}$. By conservation of momentum, the post-collision linear velocities are

$$\vec{v}'_A = \vec{v}_A + \frac{\mathcal{J}\hat{n}}{m_A} \quad \text{and} \quad \vec{v}'_B = \vec{v}_B - \frac{\mathcal{J}\hat{n}}{m_B}. \quad (3.35a)$$

Generally speaking, the direction of the impulse $\hat{n}$ need not be necessarily normal to the surfaces at the contact point. It must be known *a priori*. In practice, the direction of the impulse $\hat{n}$ is dictated by the boundary condition rules (§ 3.3.2).

For arbitrary bodies, the application of an impulse at the contact point $\vec{\chi}$ results in a

torque $\mathcal{N}_A = (\vec{\chi} - \vec{x}_A) \times \vec{\mathcal{J}}$ whenever the contact point does not correspond with the centre of mass. The post-collision angular velocities are thus

$$\vec{\omega}'_A = \vec{\omega}_A + \hat{\mathbb{I}}_A^{-1} \cdot (\vec{d}_A \times \mathcal{J}\hat{n}) \quad \text{and} \quad \vec{\omega}'_B = \vec{\omega}_B - \hat{\mathbb{I}}_B^{-1} \cdot (\vec{d}_B \times \mathcal{J}\hat{n}) \quad (3.35b)$$

where $\vec{\omega}_i$ is the initial angular velocity, m_i the mass and $\hat{\mathbb{I}}_i$ the momentum of inertia tensor of body i . The vector from the centre of mass of the body to the contact point has been denoted $\vec{d}_i = \vec{\chi}_i - \vec{x}_i$ for convenience.

Disregarding Energy Conservation

Consider the bounce-back BCs introduced in § 3.3.2 applied to an MPCD fluid particle. The impulse on the fluid particle that collides with the BC-solute at a point $\vec{\chi} = \vec{x}$ is $\vec{\mathcal{J}} = -2\vec{v}m$. The linear and angular velocity of the BC-solute can be found by inserting these into Equation (3.35a) and Equation (3.35b). There is no torque on the MPCD fluid particle since it is a point particle; however, there is torque on the BC-solute.

Although momentum and angular momentum are conserved, implementing bounce-back collision rules in this manner does not conserve energy. This may seem like a deal-breaker; however, it is not as grievous as it seems. Many implementations of the MPCD algorithm include a thermostat (either directly incorporated into the collision operator [§ 3.2.2 and § 3.2.4] or superimposed [§ 3.4.2]). When a thermostat is applied, energy generation or destruction that occurs during collision events is negated by the virtual reservoir. The entire purpose of the thermostat is to judiciously controvert energy conservation, and so this scheme is often quite acceptable. However, difficulties may persist if the applied thermostat acts indiscreetly to the entire system such that inhomogeneous temperature fields may arise with hotter regions of fluid near boundary surfaces if the kinetic energies involved are large.

The benefit of implementing bounce-back BCs in this manner is that they are computationally inexpensive as very few operations are involved. Disregarding energy conservation is always recommended when utilizing thermal-wall BCs since they act as a local thermostat, not concerning themselves with energy conservation.

Proper Energy Conservation

Properly conserving momentum and energy for a collision between two arbitrarily shaped objects requires a longer operation. Prior to the collision, the velocity of the surface at the

collision point $\vec{\chi}_i$ of body i in terms of the centre of mass velocity and the angular velocity is

$$\dot{\vec{\chi}}_i = \vec{v}_i + \vec{\omega}_i \times \vec{d}_i. \quad (3.36a)$$

After the collision, the velocity of the surface at the contact point is

$$\begin{aligned} \dot{\vec{\chi}}'_i &= \vec{v}'_i + \vec{\omega}'_i \times \vec{d}_i \\ &= \dot{\vec{\chi}}_i \pm \mathcal{J} \left\{ \frac{\hat{n}}{m_i} + \left[\hat{\mathbb{I}}_i^{-1} \cdot \left(\vec{d}_i \times \hat{n} \right) \right] \times \vec{d}_i \right\}, \end{aligned} \quad (3.36b)$$

where Equation (3.35a) and Equation (3.35b) are used and (+) is appropriate for body A, while (−) is appropriate for body B. The pre- and post-collision velocities of the point of contact on each surface are related by appealing to *Newton's law of restitution*.

Newton's law of restitution is a phenomenological linear law in the spirit of those presented in § 2.1.2 (specifically Equation (2.27)) that relates the relative separation speed of two bodies ($\vec{v}'_{\text{rel}} = \dot{\vec{\chi}}'_B - \dot{\vec{\chi}}'_A$) to their relative speed of approach ($\vec{v}_{\text{rel}} = \dot{\vec{\chi}}_B - \dot{\vec{\chi}}_A$) by a *coefficient of restitution*:

$$\vec{v}'_{\text{rel}} = -\varepsilon \vec{v}_{\text{rel}}. \quad (3.37)$$

Collisions for which $\varepsilon = 1$ are elastic, while $\varepsilon = 0$ signals a perfectly inelastic collision. Inserting the velocities of the surface points and performing a bit of algebra allows one to write the magnitude of the impulse as

$$\mathcal{J} = - \frac{(1 + \varepsilon) \hat{n} \cdot \left(\vec{v}_A - \vec{v}_B + \vec{\omega}_A \times \vec{d}_A - \vec{\omega}_B \times \vec{d}_B \right)}{m_A^{-1} + m_B^{-1} + \left[\hat{\mathbb{I}}_A^{-1} \cdot \left(\vec{d}_A \times \hat{n} \right) \right] \cdot \left(\vec{d}_A \times \hat{n} \right) + \left[\hat{\mathbb{I}}_B^{-1} \cdot \left(\vec{d}_B \times \hat{n} \right) \right] \cdot \left(\vec{d}_B \times \hat{n} \right)}, \quad (3.38)$$

where the triple product rule was used for the angular velocity terms in the denominator. Since this is a lengthy relation (which is precisely why it is computationally costly), let it be written more concisely (but less explicitly) as

$$\mathcal{J} = \frac{-(1 + \varepsilon) \hat{n} \cdot \vec{v}_{\text{rel}}}{m_A^{-1} + m_B^{-1} + c_A + c_B}, \quad (3.39)$$

where $c_i \equiv \left[\hat{\mathbb{I}}_i^{-1} \cdot \left(\vec{d}_i \times \hat{n} \right) \right] \cdot \left(\vec{d}_i \times \hat{n} \right)$ is defined for convenience.

Although Equation (3.38) is a non-trivial function, all of the terms are known except the

coefficient of restitution (which must be given) and the direction of the impulse, $\hat{n}$. The direction must be specified by the BC rules from § 3.3.2. Calculating the magnitude of the impulse using Equation (3.38) and applying it in the direction appropriate for bounce-back or reflective BC rules is the quickest method to deal with a collision event between two arbitrarily shaped bodies, while respecting conservation of momentum, angular momentum and energy.

Two reduced forms of Equation (3.38) are quite common:

- If the i^{th} body is immobile and fixed in place then $m_i^{-1} = 0$ and $\hat{\mathbb{I}}_i^{-1} = \hat{0}$.
- If the i^{th} body is a point particle then $\vec{d}_i = \vec{0}$, $\hat{\mathbb{I}}_i^{-1} = \hat{0}$ and $\vec{\omega}_i = \vec{0}$.

Friction

One further complication to collision events is in order. Newton's law of restitution relates only the normals of the speed of approach and speed of separation. The tangential component is dictated by a *frictional force*, which resists any slipping or sliding between two colliding bodies. The resistive friction force is directly related to the normal force by a coefficient of (kinetic) friction $f_{\text{fric}} = \zeta_k |f_{\hat{n}}|$. By multiplying the force by the (unknown but unneeded) time of the collision, this can be written as

$$\mathcal{J}_s = \zeta_k \mathcal{J}, \quad (3.40)$$

where $\mathcal{J}$ is a *compression impulse* and $\mathcal{J}_s$ is a *slipping impulse* [100]. The slipping impulse is in the direction $\hat{t} = \dot{\vec{\chi}}_B - \dot{\vec{\chi}}_A - \hat{n} \cdot (\dot{\vec{\chi}}_B - \dot{\vec{\chi}}_A)$. By separating the total impulse into components $\hat{n}$ and $\hat{t}$ the equations of motion are

$$\vec{v}'_A = \vec{v}_A + \mathcal{J} \frac{\hat{n}}{m_A} + \mathcal{J}_s \frac{\hat{t}}{m_A} \quad ; \quad \vec{\omega}'_A = \vec{\omega}_A + \hat{\mathbb{I}}_A^{-1} \cdot \left[\vec{d}_A \times (\mathcal{J} \hat{n} + \mathcal{J}_s \hat{t}) \right] \quad (3.41a)$$

$$\vec{v}'_B = \vec{v}_B - \mathcal{J} \frac{\hat{n}}{m_B} - \mathcal{J}_s \frac{\hat{t}}{m_B} \quad ; \quad \vec{\omega}'_B = \vec{\omega}_B - \hat{\mathbb{I}}_B^{-1} \cdot \left[\vec{d}_B \times (\mathcal{J} \hat{n} + \mathcal{J}_s \hat{t}) \right]. \quad (3.41b)$$

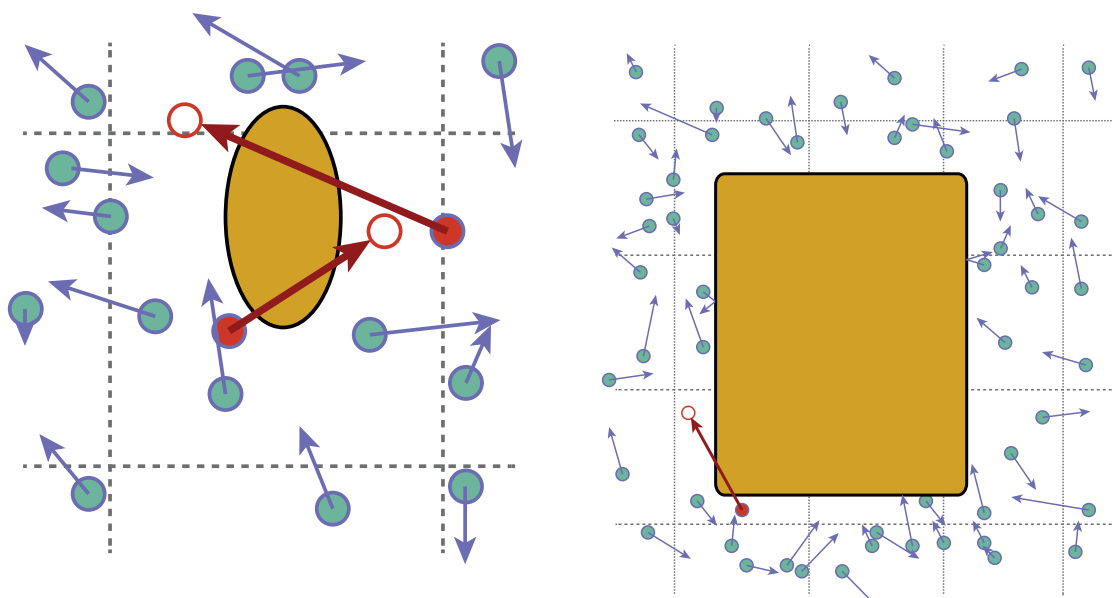
The exact same calculation as was performed for the fully conserved case can be repeated to find

$$\mathcal{J} = \frac{-(1 + \varepsilon) \hat{n} \cdot \vec{v}_{\text{rel}}}{m_A^{-1} + m_B^{-1} + c_A + c_B + \zeta_k \left\{ \left[\hat{\mathbb{I}}_A^{-1} \cdot (\vec{d}_A \times \hat{t}) \right] \cdot (\vec{d}_A \times \hat{n}) + \left[\hat{\mathbb{I}}_B^{-1} \cdot (\vec{d}_B \times \hat{t}) \right] \cdot (\vec{d}_B \times \hat{n}) \right\}}, \quad (3.42)$$

which is written in its compact form but is conveniently comparable to Equation (3.39). This gives the magnitude of the impulse along the direction of compression $\hat{n}$ and Equation (3.40) can be used to find the impulse due to friction. The equation for the compression impulse is long but hardly more difficult to compute than Equation (3.38). One need only specify friction and restitution coefficients to realistically model collisions between hard, arbitrarily shaped bodies.

A few comments about such BC-solutes are in order:

- As has already been discussed, the more realistic collision operators are included but rarely utilized, since the coarse-grained, mesoscopic nature of the MPCD solvent and the detailed considerations of Equation (3.38) (or Equation (3.42)) are at odds. Unless otherwise noted, any results involving BC-solutes presented in this thesis disregard energy conservation and utilize the Anderson-MPCD algorithm with bounce-back BCs and phantom particles.
- BC-solutes may possess angular momentum as discussed above. However, rigid-body rotations have not been implemented. Therefore, squircles, ellipsoids and other bodies without spherical symmetry should not be used as mobile BC-solutes. They will diffusely translate but will not rotate (despite possessing an angular velocity). Only spherical BC-solutes can be safely implemented as mobile solutes due to their symmetry. It is recommended that rigid-body rotations be implemented by future researchers.
- The MPCD fluid particles are point particles so ideally there should be no depletion attraction between BC-solutes. Depletion forces are entropic attractions due to sterically excluded states that is discussed in detail in § 8. When the surfaces of two BC-solutes are close, an individual MPCD fluid particle may bounce between them. If the surfaces are very close, multiple collisions may be required within a single time-step δt . The implemented MPCD algorithm checks for such multi-collision events and resolves them in continuous time — one collision at a time. Because it is possible that many multi-collisions occur, a large cut-off number is included. If the number of collisions for a single MPCD fluid particle exceeds this value in a single time-step then the MPCD particle is randomly replaced in the volume. Thus a small, residual depletion force may exist between BC-solutes if they remain in direct contact for extended periods of time.
- BC-solutes may only be used to represent relatively large colloids. If a BC-solute is comparable to the mean-free path of the MPCD fluid (λ) then the fluid particles may



(a) If a BC-solute is much smaller than the mean-free path of MPCD fluid particles then particles may commonly pass through the body without undergoing a collision event.

(b) MPCD fluid particles may pass through the edges of sharp bodies without colliding.

Figure 3.5.2: Situations in which MPCD fluid particles may erroneously pass through BCs without colliding.

commonly pass through the colloid in a single time-step δt (Figure 3.5.2a). In fact, not only should the size of the BC-solute be large compared to the mean-free path but also the radius of curvature so that it is unlikely that MPCD particles pass through the edges (Figure 3.5.2b).

3.5.2 Polymer Chains

BC-solutes are appropriate for modelling colloids that are much larger than the intrinsic size-scale of an MPCD cell. A different method must be employed for solutes with a dimension that is small or comparable to an MPCD cell. Polymer chains for instance, although quite long, are composed of units (monomers) that may be small compared to MPCD cells. In order to treat such objects, a *Molecular Dynamics* (MD) code is superimposed over the MPCD fluid. This is a standard MD program, except that the monomers are coupled to the MPCD fluid.

Molecular Dynamics

Molecular Dynamics numerically integrates the classical equations of motion for a large number of particles that interact via a set of pair potentials. It is a common approach to simulating polymer dynamics [3]. At any given time each MD particle lies in a potential well created by all its neighbouring MD particles. The net force on the i^{th} particle can be calculated from this potential and Newton's second law provides the particle's acceleration g_i . The velocity-Verlet algorithm [101–103] gives the particle's velocity and position at the next time step

$$\vec{x}_i(t + \delta t) = \vec{x}_i(t) + \delta t \vec{v}_i(t) + \frac{\delta t^2}{2} \vec{g}_i(t) \quad (3.43a)$$

$$\vec{v}_i(t + \delta t) = \vec{v}_i(t) + \frac{\delta t}{2} [\vec{g}_i(t) + \vec{g}_i(t + \delta t)]. \quad (3.43b)$$

By numerically integrating for each particle, a new configuration at time $t + \delta t$ is generated.

Other than numerically integrating the trajectories, the MD algorithm consists of calculating the interaction force from the potential as $\vec{f} = -\vec{\nabla}U(\rho_{ij})$ for pairs of particles that are separated by a distance ρ_{ij} . The MD algorithm used here includes the most common pair potentials for simulating polymers.

Lennard-Jones (LJ) Potential, U_{LJ} : The interaction between two free, uncharged molecules (respectively labelled i and j) has two nearly universal components:

1. A steep, short-ranged repulsion preventing overlap is required. Molecules cannot occupy the same space. Other than this qualitative statement, this term is not specified and is rather arbitrary. It can be an exponential rise or more commonly increase as $U_{\text{LJ}} \sim \rho_{ij}^{-12}$.
2. There is a long-range attraction arising from weak interactions due to induced dipole effects (dispersion forces). These van der Waals forces can be Keesom forces, Debye forces or London forces. The exact origin is inconsequential; they all go as $U_{\text{LJ}} \sim -\rho_{ij}^{-6}$.

The *Lennard-Jones potential* models these generic, near-universal effects:

$$U_{\text{LJ}}(\rho_{ij}) = 4\varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{\rho_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{\rho_{ij}} \right)^6 \right], \quad (3.44)$$

where ρ_{ij} is the separation, ε_{ij} is the well depth and σ_{ij} is the effective diameter of the pair of MD particles.

Weeks-Chandler-Andersen (WCA) Potential, U_{WCA} : A purely repulsive, truncated form of the LJ potential called the *Weeks-Chandler-Andersen* potential is commonly used as a coarse-grained interaction. The LJ potential is shifted and truncated at its minimum value so that it ends smoothly at a distance ρ_c . The WCA potential can be generally written as

$$U_{\text{WCA}}(\rho_{ij}) = \begin{cases} 4\varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{\rho_{ij} - \Delta_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{\rho_{ij} - \Delta_{ij}} \right)^6 \right] + \varepsilon_{ij} & \text{for } \rho_{ij} < \rho_c \\ 0 & \text{for } \rho_{ij} \geq \rho_c. \end{cases} \quad (3.45)$$

where Δ_{ij} is an effective shifting of the potential. If the interacting monomers have different sizes then there are two ways to implement the WCA potential:

1. The *shifted-WCA* (sWCA) model for hard spheres consists of setting

$$\sigma_{ij} = \sigma \quad \forall ij \quad ; \quad \Delta_{ij} = \frac{\sigma'_i + \sigma'_j}{2} - \sigma \quad ; \quad \rho_c = 2^{1/6}\sigma + \Delta_{ij} \quad (3.46)$$

where σ'_i and σ'_j are the sizes of particles i and j , respectively. The resulting repulsion of $U_{\text{sWCA}}(\rho_{ij})$ rises rapidly from zero at ρ_c and crosses ε at $\rho_{ij} = (\sigma'_i + \sigma'_j)/2$.

2. On the other hand, the *combinatorial-WCA* (cWCA) model does not shift the WCA model but rather sets an effective size between two MD particles

$$\sigma_{ij} = \frac{\sigma'_i + \sigma'_j}{2} \quad ; \quad \Delta_{ij} = 0 \quad \forall ij \quad ; \quad \rho_c = 2^{1/6}\sigma_{ij}. \quad (3.47)$$

The resulting $U_{\text{cWCA}}(\rho_{ij})$ is hard — the repulsion still rises rapidly as $\sim \rho_{ij}^{-12}$ — but is slightly softer than U_{sWCA} . This difference will play a role in the discussion of depletion-induced polymer collapse (§ 8).

Coulombic Potential, U_{coul} : The bare electrostatic interaction between pairs of particles is given by the *Coulombic potential*

$$U_{\text{coul}}(\rho_{ij}) = \frac{1}{4\pi\varepsilon_0\varepsilon_r} \frac{Q_i Q_j}{\rho_{ij}}; \quad (3.48)$$

where Q_i and Q_j are the **effective** charges on each particle after charge reduction due

to counterion condensation. For mesoscopic simulations, $\varepsilon_0\varepsilon_r$ is an effective dielectric constant that includes screening effects due to the medium. Condensation effects can be encapsulated in the *Bjerrum length*

$$\lambda_B = \frac{e^2}{4\pi\varepsilon_0\varepsilon_r k_B T}. \quad (3.49)$$

The Bjerrum length is the distance beyond which thermal energy is greater than electrostatic energy. In terms of the Bjerrum length, Coulombic interactions can be written

$$U_{\text{coul}}(\rho_{ij}) = k_B T \frac{Q_i Q_j}{e^2} \frac{\lambda_B}{\rho_{ij}}.$$

The Coulombic interactions are long-range scaling like $\sim \rho_{ij}^{-1}$ just like unscreened hydrodynamic interactions (as seen in § 2.3.4). The long-range contributions are important and for this reason, inclusion of electrostatic effects can be computationally expensive. However, as discussed in § 2.4.1 in a solvent electrostatic interactions are screened by the electric double layer and so it is not always necessary to use U_{coul} . Often a much shorter-ranged electrostatic interaction will suffice.

Debye-Hückel Potential, U_{DH} : The electrostatic interactions are typically screened. In § 2.4.1, Equation (2.100) was found to be the Debye-Hückel approximation for the electrostatic potential of a charged sphere. This electric potential was given in terms of the zeta-potential. Since MD monomers do not conveniently have a zeta-potential, Equation (2.102) can be used to replace the zeta-potential with the effective charge on the i^{th} monomer (of radius $r = \sigma/2$) *i.e.* $Q_i^{\text{eff}} = 4\pi r^2 \sigma_{\text{el}\delta} = 4\pi\varepsilon_0\varepsilon_r r \zeta (1 + r/\lambda_D)$. Then by Equation (2.100), the potential between two spheres of effective charge Q_i^{eff} and Q_j^{eff} becomes

$$\begin{aligned} U_{\text{DH}}(\rho_{ij}) &= Q_i^{\text{eff}} \Psi_j(\rho_{ij}) \\ &= k_B T \left(\frac{Q_i Q_j}{e^2} \right) \left(1 + \frac{\sigma_{ij}}{2\lambda_D} \right)^{-1} \frac{\lambda_B}{x} \exp \left(-\frac{x - \sigma_{ij}/2}{\lambda_D} \right). \end{aligned} \quad (3.50)$$

In the Hückel limit of $\sigma_{ij}/\lambda_D \ll 1$ (§ 2.4.3), this becomes the *Debye-Hückel potential*

$$U_{\text{DH}}(\rho_{ij}) \approx k_B T \left(\frac{Q_i Q_j}{e^2} \right) \frac{\lambda_B}{x} \exp \left(-\frac{x}{\lambda_D} \right). \quad (3.51)$$

It is worth noting that if the concentration of counterions around two charged monomers is large then an osmotic pressure due to two overlapping diffuse layers generates a fur-

ther repulsion. Since monomers are small and not highly charged, this diffuse layer effect is neglected.

Finitely Extensible Bonds, U_{FENE} : In any coarse-grained model of a polymer, adjacent MD beads must be connected together to form a chain of monomers. The use of harmonic potentials can lead to unphysical bond stretching effects. For this reason, a finitely extensible force with a hard limit on the bond length is utilized. The most common choice is the *finitely extensible nonlinear elastic potential* (FENE):

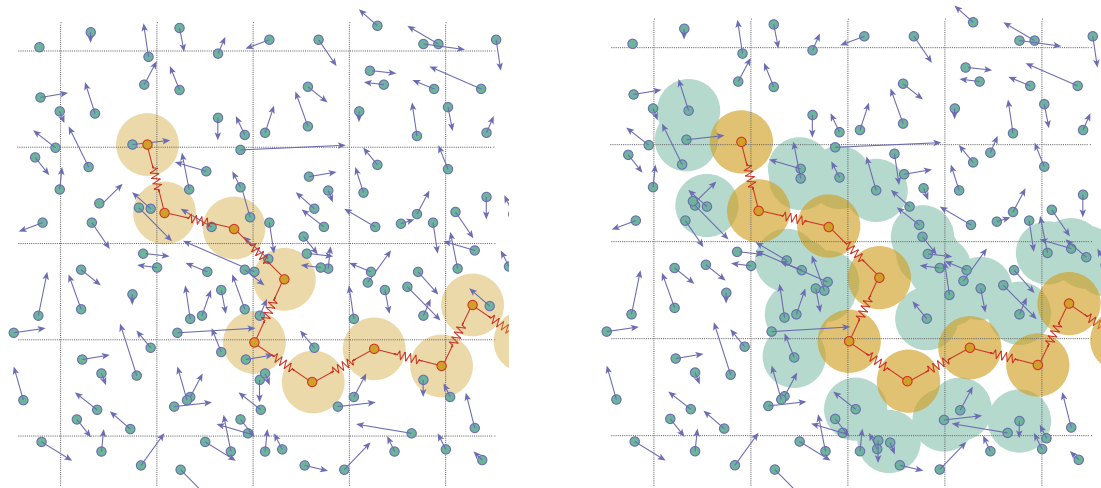
$$U_{\text{FENE}}(\rho_{ij}) = -\frac{k\rho_0}{2} \ln \left(1 - \frac{\rho_{ij}}{\rho_0} \right) \quad (3.52)$$

where k is a spring constant representing the stiffness of the bond and ρ_0 is the maximum bond length. While using the FENE potential, “bond crossing” events are rare and self-avoiding chain behaviour is produced.

Bond Bending Interactions: In MD simulations, potentials controlling bond angles can be quite useful. Since the simulations are coarse-grained these are not intended to represent chemical bending potentials but can give polymer chains microscopic characteristics such as correlations along the back-bone (a non-zero *persistence length*). Bond bending potentials are not implemented in this work so all the polymers are *freely-jointed chains*.

These are, by no means, the only possible MD pair potentials but these are the ones that have been implemented here.

Molecular dynamics simulations allow one to study macromolecules from a coarse-grained, mechanistic perspective. Neutral chains, polyelectrolytes, polymer brushes can all be studied using the potentials introduced above. The polymers of interest to this work reside in an MPCD solvent medium. Therefore, their dynamics must be coupled to the fluid. Coupling can be done in two ways. Either the MD beads can be included in the MPCD algorithm or the MPCD particles can be included in the MD calculations. In a sense, both coupling schemes can be referred to as *multi-scale simulations* since a more computationally expensive algorithm is used for the macromolecule, while the more coarse-grained MPCD algorithm is simulating the fluid.



(a) The MD-in-MPCD coupling scheme calculates MD-MD interactions, while including MD monomers as point particles in MPCD collision steps.

(b) The MPCD-in-MD coupling scheme calculates MD-MD and MD-MPCD interactions.

Figure 3.5.3: MD/MPCD coupling schemes.

MD-in-MPCD Coupling

The MD monomers can be included in the MPCD collision operations. This scheme is referred to as *MD-in-MPCD coupling*. In the MD-in-MPCD scheme the MD algorithm integrates the MD monomer trajectories over some number of MD time-steps N_{MD} . In a sense, during this period the MD simulation occurs in a vacuum. After this the program exits the MD portion of the algorithm and enters the MPCD side. The MD beads are ported into the MPCD algorithm as point particles and are included in the collision operation. In this way, the MD beads exchange momentum with the fluid. The MPCD particles perform the streaming step (the MD particles do not) before the MD algorithm is reinitiated for another N_{MD} MD time-steps.

Values of N_{MD} are typically large (on the order of 10 although smaller values have been used in the literature). This separation of time scales ensures that the momentum transferred to the polymer during the collision step is well distributed throughout the chain [104]. MD-in-MPCD coupling is considered the more mesoscopic of the two coupling techniques.

MPCD-in-MD Coupling

The second coupling scheme is more accurately described as a hybrid MPCD/MD approach. In the *MPCD-in-MD coupling* scheme MPCD solvent particles are included in the MD

integrator. In the MD portion of the program the monomers interact with one another (monomer-monomer interactions) and also with the solvent fluid particles (monomer-solvent interactions) but the solvent particles do not interact with each other (no solvent-solvent interactions). In this implementation the MPCD fluid particles are cast as MD beads with the same properties as the monomers but this is not necessary. If desired, the program could be altered such that the MPCD particles would be cast as MD beads with different pair potentials than the monomers. The position and velocity of the all MPCD fluid particles is updated in time by the Verlet algorithm (Equation (3.43)). After N_{MD} MD time-steps, the MD integrator is exited and the MPCD portion of the program is performed. MPCD fluid particles are binned into cells and exchange momentum by the MPCD collision operator, $\mathbf{R}$. Since their positions are updated in the MD portion of the program, they do not stream.

The number of MD steps between an MPCD step can be much smaller than in the MD-in-MPCD scheme. In this work, the number of MD steps per MPCD step is $N_{\text{MD}} = 5$ for the MPCD-in-MD coupling scheme.

In both coupling schemes, the MD particles are typically given a mass m_{MD} that is larger than the MPCD particles' mass m in order to achieve efficient coupling [52]. In this work, $m_{\text{MD}} = 3m$, $\langle N_c \rangle = 5$ and $N_{\text{MD}} = 5$ for all simulations presented.

Summary and Implementation Map

The core of the MPCD algorithm is simply the streaming (Equation (3.1)) and collision (Equation (3.2)) operations. The collision operator can be any stochastic operation that conserves local momentum. Variants are the stochastic rotation dynamics (Equation (3.3)), Anderson-MPCD (Equation (3.12)) and Langevin-MPCD (Equation (3.18)) algorithms. Boundary conditions ensure that all MPCD fluid particles remain within the control volume and act appropriately at solid surfaces (Equation (3.20)). Achieving no-slip boundary conditions requires the use of phantom particles to counteract decreased viscosity at boundaries (Equation (3.22)). These boundary objects can be allowed to be mobile in order to simulate large colloids. Simple bounce-back collision rules do not conserve energy but thermostat algorithms ensure that this is not a problem, although impulse-based contact analysis makes it possible to conserve energy, momentum and angular momentum (Equation (3.38)) or even account for friction during collisions. Smaller solutes and polymer chains can be simulated through standard MD simulations that are coupled to the MPCD algorithm.

Actually implementing the MPCD algorithm into a fully functional simulation program requires many further essential actions. The current implementation is a serial program that is documented in the following schematic (Figure 3.5.4).

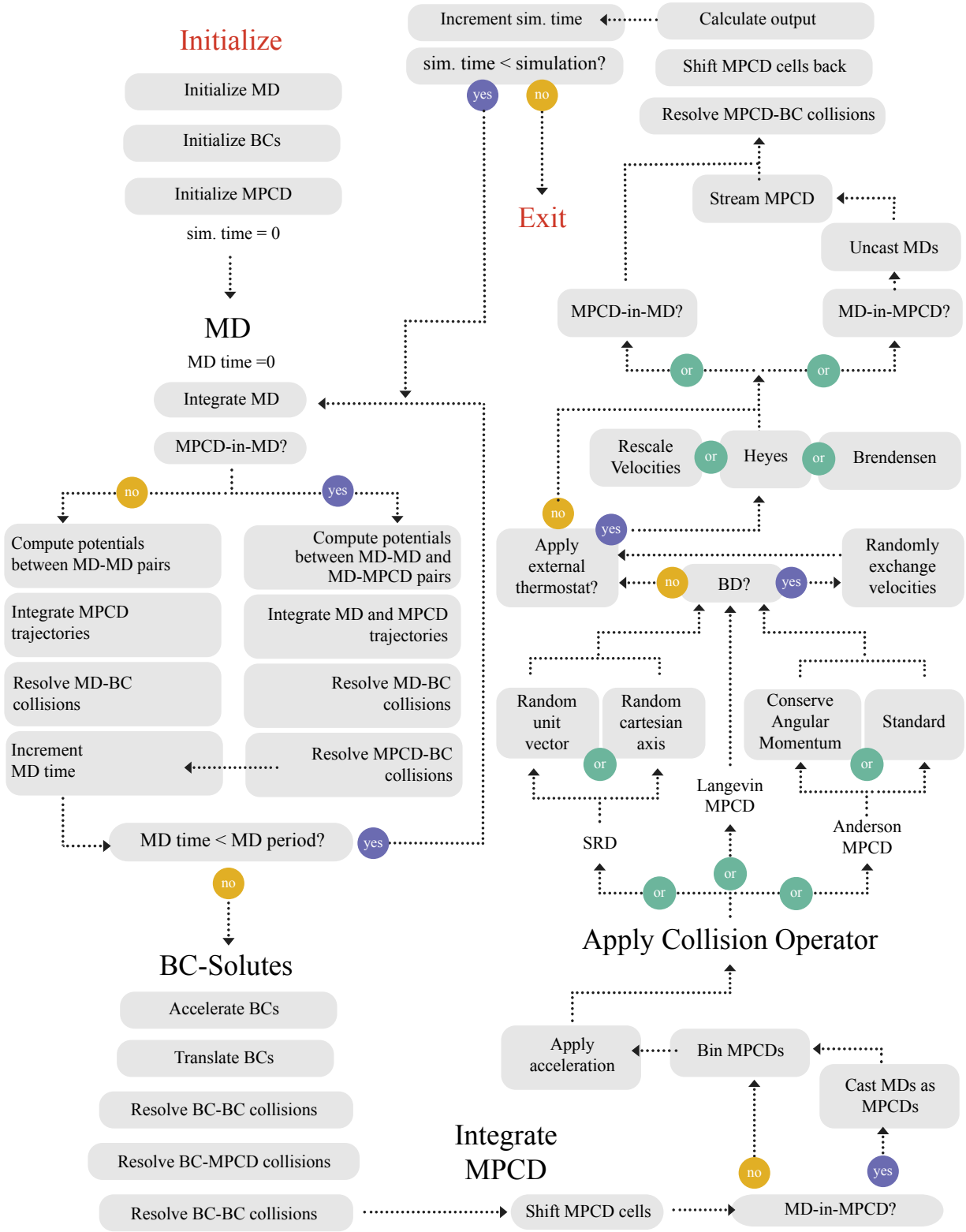


Figure 3.5.4: Flowchart mapping the implementation of MPCD.

*By the time you have ten experimental swords in
there, no theory is going to survive.*

Tom McLeish [105]

4

Electrophoresis: When Hydrodynamics Matter

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The study of charged macromolecules (*polyelectrolyte* chains) is of utmost importance to the development of many techniques used for the characterization and analysis of biologically relevant polymer molecules, such as DNA, RNA and proteins. Since such bio-molecules are quite often charged entities, utilizing an electric field to drive convection is a natural notion. The separation of polyelectrolytes, particularly nucleic acids, by *electrophoresis* has long been a topic of interest due to its practical importance. Electrophoretic separation based on *contour length* is a key step in traditional DNA sequencing technologies.

In this chapter, the well-known fact that polyelectrolyte coils possess free-solution electrophoretic mobilities that are independent of their molecular weight is introduced [106]. Clearly, this is a barrier to separation based on contour length. Currently, differences in electrophoretic mobility as a function of length are achieved by performing electrophoresis in polymer matrices (or *gels*). Electrophoresing polyelectrolytes must “snake” their way through a jungle of effective tubes that block their path. This motion of a polymer through a matrix of cross-linked polymers is called *reptation* and because of it some length-dependent migration rates can lead to separation.

Electrophoresis in gels has a long history, is widely utilized and is well understood in a mean-field sense. However, gels have a variety of drawbacks as electrophoretic sieving media. For instance, cross-linking is random and pores are disordered with a wide distribution of sizes, which is detrimental to separation because it acts as a source of randomness and leads to dispersion. Gel electrophoresis is effective for DNA sequencing, though the desire for affordable, high-throughput and automatic fractionation has driven the recent advances in electrophoresis technology.

The ability to utilize gels as components in lab-on-a-chip devices could be highly valuable but incorporating gels into microfluidic technology holds many difficulties including:

1. Loading gels into small spaces (such as microfluidic channels).
2. Degradation of gels over time.
3. Characterizing or verifying the state of gels within closed systems.

Thus, on-chip gels are unlikely to find widespread utility. However, the ability to engineer nano-structures within devices is flourishing. This allows the development of *artificial gels*, manufactured and ordered obstacles, which act as sieving matrices with which the macromolecules interact [107, 108]. Arrays of micro-fabricated posts [109–112] belong to this

family of electrophoretic techniques that depend on the coordinated action of electric fields and geometrical constraints.

By engineering nanofluidic separation systems greater control over the phenomena leading to separation is achieved. However, if this increased ability is to be properly leveraged, greater understanding of the physical mechanisms must go hand-in-hand with developments in nanofabrication. A greater level of detail must be accurately accounted for. This chapter presents a novel mesoscopic simulation method for simulating the electro-hydrodynamics of polyelectrolytes with finite Debye layers. To do so, this *mean-field MPCD-MD Debye-Hückel* algorithm utilizes the multi-particle collision dynamics (MPCD) algorithm from § 3 and the Debye-Hückel approximation introduced in § 2.4.1.

The algorithm is applied to situations in which the finite range of electro-hydrodynamic interactions play an important role in establishing the electrophoretic behaviour of charged macromolecules. The first situation considered is the free-solution electrophoresis of short, charged chains. The mean-field MPCD-MD Debye-Hückel algorithm captures the experimentally observed rise in mobility with chain length. By including the effects of charge condensation, the algorithm reproduces the non-monotonicity of the electrophoretic mobility curve.

As will be seen, the free-solution mobility increases as a function of chain length because of overlapping Debye layers. For this same reason, the total electrophoretic mobility depends on the Debye length and simulations will be presented which show this relation. Furthermore, it is possible for net neutral polyampholytes to possess a non-zero mobility, which is also demonstrated by the MPCD-MD Debye-Hückel algorithm. This section is based on the publication reporting the algorithm that is included in § E.

The mesoscopic algorithm was applied to the electrophoresis of confined polyelectrolytes. Though electrophoresis in nanofluidic devices holds great potential as a replacement for the current generation of gel electrophoresis separation devices [113, 114], the motion of polyelectrolytes in nanochannels raises a number of fundamental questions that are not fully understood. This work uses the MPCD-MD Debye-Hückel algorithm to tackle a single issue:

- Do the conformational deformations that accompany confinement leave the chain as a free-draining object with an unaltered electrophoretic mobility?

This is a difficult question to answer directly by experimental investigations, since the confinement necessarily involves the presence of impermeable, likely charged, no-slip channel

walls. On the other hand, the simulations performed here are highly simplified. An axially symmetric, radial potential that acts only on the polyelectrolyte and not on the fluid particles deforms the polymer coil, making it highly asymmetric. It will be explicitly seen that in this situation, the polyelectrolyte remains free-draining but surprisingly, when the polymer is highly confined the simulated electrophoretic mobility rises above the chain's free-solution value.

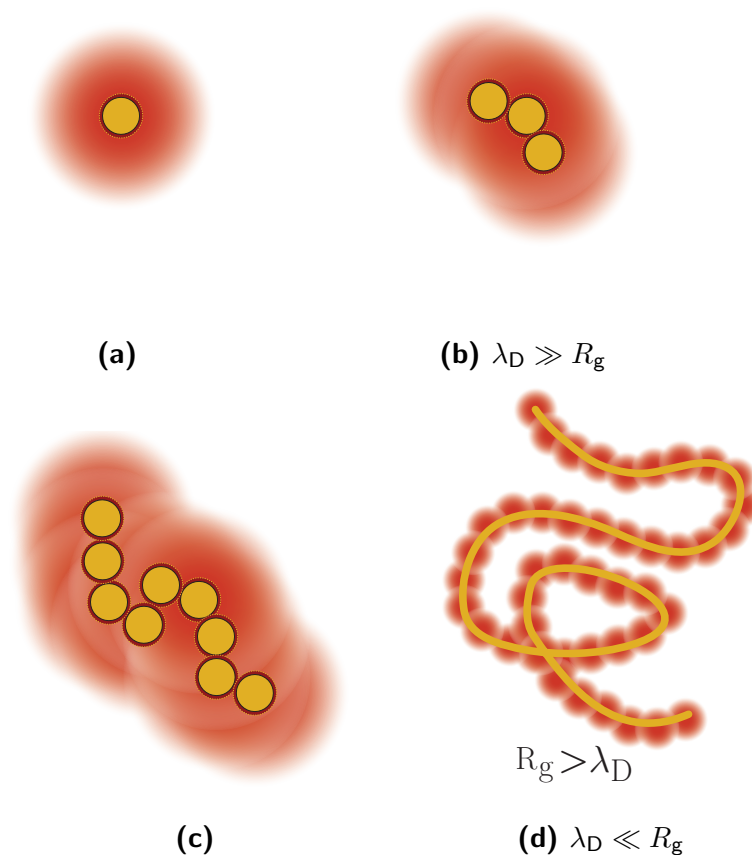


Figure 4.1.1: Schematic representation of Debye layer overlap in charged oligomers and polyelectrolytes.

4.1 Free-Draining Mobility

Electro-hydrodynamics are introduced in § 2.4, where the counterion distribution surrounding simple geometric shapes and corresponding electro-osmotic flow fields are considered. In particular, the electrophoretic mobility of spherical particles that are calculated in § 2.4.3. These particles are either smaller (the Hückel limit) or larger (the Smoluchowski limit) than their associated Debye layers. These concepts are now extended to charged polymer chains. Once the pertinent physical mechanisms are understood, a mesoscopic model can be constructed to simulate the electrophoresis of polyelectrolytes.

4.1.1 Oligomers

The electrophoretic mobility of a single charged bead is considered in § 2.4.3. Now consider a chain of charged beads (or monomers) of size σ . The monomers are coarse-grained models of a small segment of a chain and are assumed to be small compared to the Debye length ($\lambda_D > \sigma$). Since $\lambda_D > \sigma$, a single polyelectrolyte monomer ($N = 1$; Figure 4.1.1a) is in the Hückel limit. Thus, if a second monomer is bonded to it, their diffuse layers overlap (Figure 4.1.1b). Because the shearing of the fluid happens over the length scale λ_D (the viscous drag depends on the size of the ion sheath), the drag of the dimer does not double (see § 2.3.5). However, the charge Q^{eff} does double. Since the charge increases substantially more than the drag does, the electrophoretic mobility $\mu_{\text{el}} = Q^{\text{eff}}/\zeta$ of the two connected beads is much greater than that of the isolated monomer. As more monomers are added, the dimer becomes an *oligomer*. In a good solvent, sufficiently long oligomers adopt a self-avoiding coil configuration with a characteristic size $R_g(N)$, the *radius of gyration*. If $R_g < \lambda_D$ then the entire oligomer is in the Hückel limit.

As R_g becomes greater than λ_D , however, portions of the chain are separated by distances greater than λ_D (Figure 4.1.1d). These segments are both electrostatically and hydrodynamically screened: Motion of one segment does not affect the other. Therefore, the total friction coefficient of these distant segments is the sum of the friction coefficients of the local segments. The viscous drag on the polymer now grows linearly with further increase of the contour length. Since both the drag and the charge growing linearly with N , the electrophoretic mobility saturates [115, 116]. This is called the *free-solution electrophoretic mobility* $\mu_{\text{el}0}$.

4.1.2 Free-Draining Behaviour

Sufficiently long DNA molecules migrate with the same electrophoretic mobility regardless of their contour length L . This well-known effect precludes the use of free-solution electrophoresis as a separation method for most polydisperse DNA samples. The key feature responsible for this effect is the screening of the hydrodynamic interactions between the DNA monomers. As a result, the DNA chain friction coefficient is simply N times a characteristic local monomer friction coefficient:

$$\boxed{\zeta = N\zeta_0}. \quad (4.1)$$

As is now shown, this so-called *free-draining* behaviour is due to the complementary response of charged monomers and their associated counterions to an electric field.

Just as is discussed in § 2.4.4 (Figure 2.4.4), when a moderate field is applied, the force on the monomer is equal and opposite to the force on its double layer. To first order, the momentum flux on the double layer is viscously transferred to the monomer and vice versa; the field pulls the monomer one way, and the counterions in the opposite way, and the viscous forces on the fluid from each cancel, as is shown schematically in Figure 2.4.4.

For a thick Debye layer, the hydrodynamic flow field decays exponentially [117], whereas for thin Debye layers the residual hydrodynamic field goes as ρ^{-3} [45]. In either limit, the effective hydrodynamic interactions decay rapidly with distance. Notice how this differs from the response to non-electric forces, such as applied mechanical forces acting on specific monomers or body forces such as gravity that act on all monomers. For these forces there is a net stress on the fluid and flows decay as ρ^{-1} [118]. The resulting motion of all chain segments is coupled together, leading to collective and cooperative action, and the chain appears as a consolidated object opaque to fluid flow [119]. Thus, the friction on the polymer depends explicitly on its conformation and scales as its hydrodynamic radius $R_H \sim R_g$. Interestingly, Brownian thermal forces on the monomers also generate long-range hydrodynamic interactions between the monomers of a polyelectrolyte, even in the presence of an electric field. Thus, the motion of a polyelectrolyte during electrophoresis is a superposition of the convective flow of a free-draining polymer superimposed with diffusive motion governed by a Stokes-like diffusion coefficient that scales like $1/R_H$ [115]. As a consequence, one cannot use a Nernst-Einstein equation (Equation (2.33b)) to link the electrophoretic mobility μ_{el} to the diffusion coefficient $\mathcal{D}$ of a polyelectrolyte subjected to an electric field.

4.1.3 Counterion Condensation

It is interesting to note what would happen if hydrodynamic interactions were ignored since both hydrodynamics and electrostatics are necessary to explain the physics leading to free-draining.

In the absence of hydrodynamic interactions, the drag on the monomers is generated locally and not over the extent of the ion sheath. In this case, overlapping diffuse layers are of no consequence, and the drag increases with N right from the start. Simulations that neglect hydrodynamic interactions completely fail to capture cooperative shearing and so the

electrophoretic mobility decreases monotonically [120, 121]. The reason for this somewhat surprising decrease in mobility is due to *charge renormalization effects*. As the contour length $L = N\sigma$ increases the electrostatic potential energy within the Debye layer increases, causing counterions to condense out of the diffuse counterion cloud into what is effectively the Stern layer of the polymer chain. This is called *Manning condensation* and results in a reduced effective charge of each monomer [122–124]. Reducing the effective charge reduces the electrophoretic mobility of the polyelectrolyte chain. Recent simulations confirm that counterion condensation increases with chain length [120, 121].

The competition between the Manning condensation effects that decreases the net charge and cooperative shearing effects within overlapping Debye layers that decrease local friction produces the observed non-monotonic dependence of μ_{el} on N [120, 121]. The electrophoretic mobility is non-monotonic. It rises to a maximum for intermediate sizes before decreasing slightly towards the asymptotic free-solution mobility $\mu_{\text{el}0} \equiv \mu_{\text{el}}(N \rightarrow \infty)$. Few simulations have been able to reproduce this maximum in electrophoretic mobility of charged oligomers.

4.2 Finite Debye Length Simulation Algorithm

The free-draining effect described in § 4.1 suggests that hydrodynamics are mostly screened during electrophoresis and, therefore, early simulations of polyelectrolyte electrophoresis generally neglected long-range hydrodynamic interactions. Such a simplification was generally adequate because early simulations focused on understanding traditional gel electrophoresis through polymer matrices, in which the matrix screens hydrodynamic interactions. However, this is a perilous strategy since it is not always obvious when and to what extent electrohydrodynamic interactions will be screened. The double layer thickness of polyelectrolytes cannot always be assumed to be negligibly thin [125]. Accounting for hydrodynamic interactions has become even more pertinent since new microfluidic methods have emerged to conduct electrophoresis, in which hydrodynamic interactions are only partially screened.

Algorithms that explicitly take these effects into account while simulating electrophoresis are thus necessary. There are a wide variety of methods in the literature to simulate electrohydrodynamics [126]. Often the fluid medium is simulated mesoscopically, while the electrostatics are calculated explicitly as in molecular dynamics simulations with either lattice-Boltzmann [120, 127] or MPCD fluids [121, 128]. These simulations reproduce the full free-solution electrophoretic behaviour but the **computational cost** is quite high since long-range electrostatic interactions between many salt ions must be explicitly calculated. This limits the size of the systems and the time scales that can be explored. The cost becomes prohibitively large for short Debye lengths, where a large number of additional positive and negative salt ions are present.

Several methods to eliminate these costly electrostatic calculations use an *infinitely thin Debye layer approximation*, in which there is a Smoluchowski slip (Equation (2.105)) between the monomers and the surrounding fluid. Such methods have been coupled to lattice-Boltzmann fluids [129], dissipative particle dynamics simulations [130, 131] and the Navier-Stokes equation [132] to study electrophoresis and electro-osmotic flows.

None of these methods reproduce the fluid velocity profile within the Debye layer. Therefore, they are limited to situations in which the Debye length is significantly smaller than all other relevant length scales.

In this section, a simulation technique using Multi-Particle Collision Dynamics (MPCD) is developed which is valid in the limit of finite Debye lengths. This is not the first attempt to create mesoscopic simulations for finite Debye lengths. There have been several approaches

based on Brownian dynamics methods [133, 134]. None of these approaches require thin Debye layers nor use costly electrostatic calculations. However, they suffer the inherent difficulties of Brownian dynamics simulations, namely:

- nontrivial boundary conditions
- poor scaling of the computational cost with system size [135].

The novel MPCD-based algorithm will naturally inherit the versatility demonstrated in § 3.4.3 and will have the computational efficiency associated with short-range interactions arising from the local nature of the MPCD collision operators.

4.2.1 MPCD and MD Mesoscopic Simulations

The general method employed by this new mean-field MPCD-MD Debye-Hückel algorithm is to reject an explicit simulation of the many counterions and their long-range electrostatic interactions and replace it with the Debye-Hückel approximation for the distribution of counterions surrounding a charge. This is done by assigning effective charges to the MPCD fluid particles within a few Debye lengths of each charged monomer. This effectively approximates the counterion sheath without resorting to simulating the explicit behaviour of individual counterions and salt ions. The computation time is significantly reduced and the algorithm remains local so that simulation times scale linearly with system size and does not depend strongly on the implicit salt content. In order to account for Manning condensation, a simple charge cutoff scheme is utilized that effectively neutralizes a fraction of the monomer charge.

The polymers are simulated via the molecular dynamics scheme described in § 3.5.2. In particular, the polymers are composed of purely repulsive combinatorial-WCA beads (Equation (3.45) with Equation (3.47)). The length scale of the WCA interactions is set to half of an MPCD collision cell ($\sigma = a/2$) and the energy scale ε is set to $k_B T$ as is often done in coarse grained simulations [3]. The N monomers in the polymer are linked together by finitely extensible non-linear elastic (FENE) bonds (Equation (3.52)), which have a maximum extension of $\rho_0 = 1.5\sigma$ and a spring constant $k = 30\varepsilon/\sigma^2$ controlling the strength of the bond. Additionally, the Debye-Hückel potential (Equation (3.51)) describes the screened repulsion between charged beads. Every monomer is given a charge of Q and the Bjerrum length is set to $\lambda_B = 3\sigma$, an appropriate value for reproducing experimental

conditions [120]. The cutoff beyond which the Debye-Hückel potential is no longer calculated is $r_{\text{cut}} = 5\lambda_{\text{D}}$.

The polymer is embedded in the coarse-grained MPCD fluid model described in § 3. The Anderson-MPCD variant (§ 3.2.2) is chosen and a density of $n_{\text{MPCD}} = 5a^{-3}$ is set for all simulations. The boundaries are periodic (§ 3.3.2). Unless otherwise stated the MD-in-MPCD coupling scheme of including monomers in the MPCD collision-step is employed (§ 3.5.2).

4.2.2 Implicit Counterions

The key to the mean-field MPCD-MD Debye-Hückel algorithm is to coarse-grain the electrostatics within this standard mesoscopic simulation setup. In order to add the effect of electrostatics to the MPCD algorithm, fluid particles neighbouring MD monomers are given an effective, fractional charge. The counterion density is assumed to follow the Debye-Hückle approximation (Equation (2.101)). In order to improve efficiency, a cutoff is applied to the distance ρ . This cutoff ρ_c is kept at $5\lambda_{\text{D}}$ and is used such that the number density distribution of counterions ϱ_{el} within the simulation is explicitly

$$\varrho_{\text{el}} \propto \begin{cases} \frac{\exp(-\rho/\lambda_{\text{D}})}{\rho} & \rho < \rho_c \\ 0 & \rho > \rho_c \end{cases}. \quad (4.2)$$

The proportionality symbol is used because the density of counterions is not to be explicitly calculated. Instead, an effective charge is assigned to MPCD fluid particles that is proportional to the Debye-Hückle potential at the given distance ρ . The total charge shared by the MPCD fluid particles located around the monomer is set to the MD monomer's charge Q . When the electric field is applied, a force of magnitude QE acts on the MD beads and an opposing force of $Q_i E$ acts on each charged MPCD particle. By substituting $\rho = 5\lambda_{\text{D}}$ in Equation (4.2), it can be seen that the cutoff does not significantly affect the distribution of implicit counterions since $e^{(-\rho/\lambda_{\text{D}})}/\rho \approx 0.001$. The algorithm is shown schematically in Figure 4.2.1.

This method of assigning the counter force to the fluid ensures both local momentum conservation and a reasonable distribution of the force on the surrounding fluid. The electrostatic portion of the algorithm is independent of box size (since salt ions are not explicitly treated). This offers an improvement over explicit ion simulations whose computational

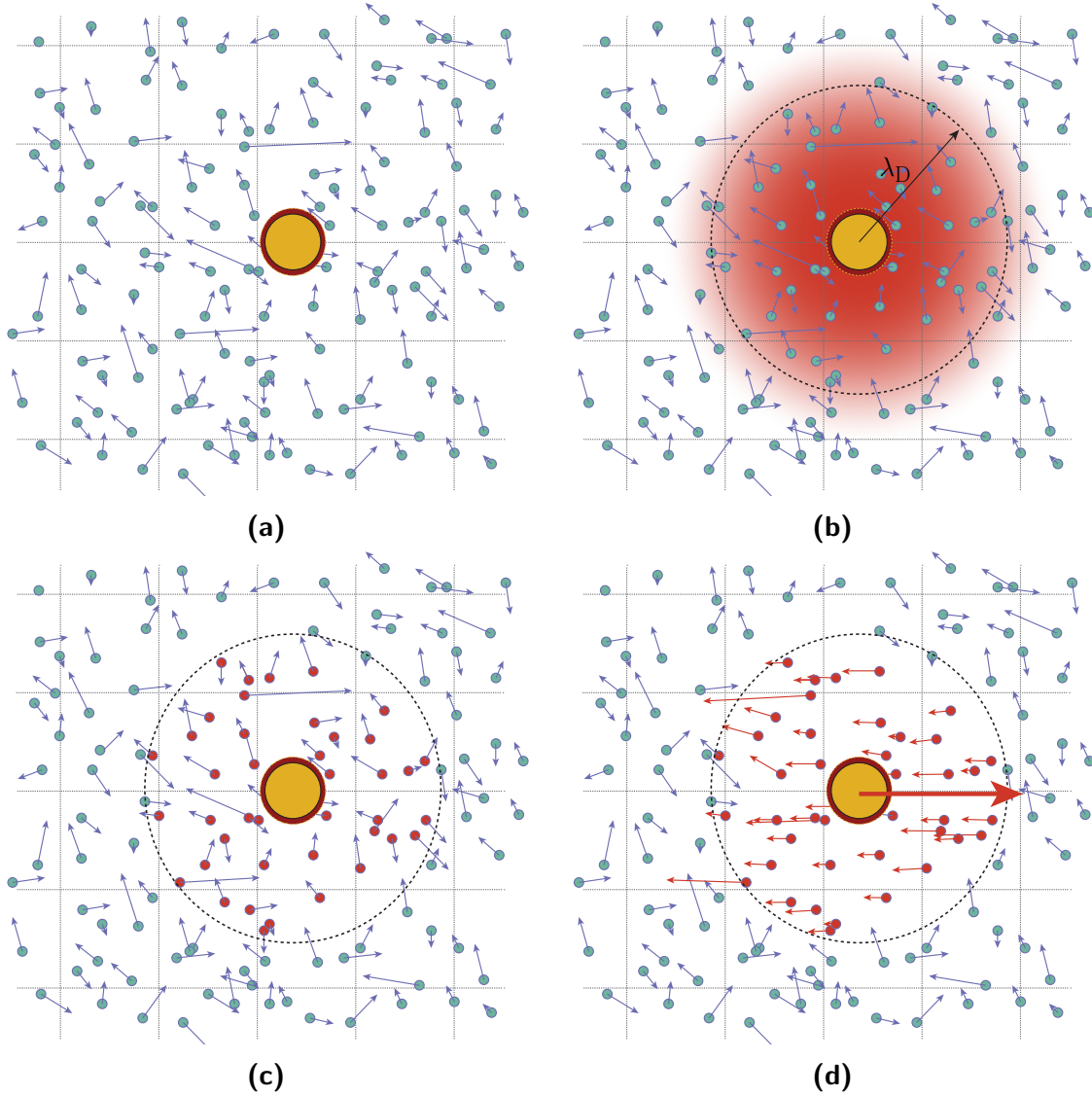


Figure 4.2.1: Schematic representation of the mean-field MPCD-MD Debye-Hückel algorithm. (a) A charged MD bead is surrounded by point-like MPCD fluid particles. (b) In reality the charged bead would possess a counterion cloud of size λ_D . (c) In the MPCD-MD Debye-Hückel algorithm, MPCD fluid particles are given charges based on their position. In this way, including explicit counterions is avoided. The charges assigned to each MPCD particle are proportional to the local counterion distribution that is predicted by the Debye-Hückel approximation and are normalized such that the sum of all partial charges is $1Q$. (d) The electric field applies a force to both the MD bead and the MPCD particles.

time increases as the box length cubed, at best. Thus, the algorithm is ideal for the migration of individual (or dilute solutions of) polyelectrolytes in intricate micro- and nanofluidic structures, where experimental systems are increasingly complex (see § 4.4).

4.2.3 Implicit Charge Condensation

Finally, the effect of charge condensation can be included in this model. It is included by considering an MPCD counterion to be condensed if the charge on the particle exceeds some threshold value, Q_T^{eff} . The threshold value is chosen to reproduce a reasonable net effective charge for long polymers. This has been previously estimated to be $\sim \rho_0/\lambda_D \approx 0.3$ where ρ_0 is the bond length [120]. When a counterion is condensed, the charge on the MPCD particle is set to zero and that fractional charge is subtracted from the corresponding MD beads from which it originally received its charge. The force $Q_i E$ only operates on the MD and MPCD particles after the effective (post-condensation) charge is set. This heuristic approach to including counterion condensation is appropriate for such a mesoscopic technique. The next section shows that this model provides good results while also being computationally inexpensive.

4.3 Simulations of Free-Solution Electrophoresis

The mean-field MPCD-MD Debye-Hückel algorithm is now applied to the free-solution electrophoresis of charged polymers. If the mesoscopic algorithm faithfully captures all of the relevant physics then the effects described in § 4.1 should be reproduced. Namely,

1. Electrophoretic mobility should rise steeply as a function of the *degree of polymerization* for short oligomers.
2. Mobility should plateau to the free-solution value for sufficiently long chains.
3. Counterion condensation should produce a maximum at intermediate lengths.

Simulations presented in this chapter ran on SHARCNET [136] utilizing approximately 30 serial CPUs for 7 days each.

4.3.1 Free-Solution Electrophoresis of Charged Oligomers

Consider a charged oligomer subject to an electric field in free-solution. This situation is discussed in § 4.1 to detail the competing effects that lead to free-draining during electrophoresis. If the MPCD-MD Debye-Hückel algorithm can reproduce the electrophoretic mobility of charged oligomers as a function of their length then it successfully models the electro-hydrodynamics of polyelectrolytes at a coarse-grained level.

As the degree of polymerization, N , is increased from $N = 1$ there is an initial rise in the electrophoretic mobility, followed by a small maximum, before decreasing to the free-draining limit [120, 125] as discussed in § 4.1. Figure 4.3.1 shows the mobility as a function of the degree of polymerization N for a variety of simulation setups

The yellow diamonds show simulation results when monomers have their velocity reduced by $-\mu_{\text{el}0}E$ prior to the collision step. This is essentially the infinitely thin Debye layer limit [129]. Reducing the velocity by $-\mu_{\text{el}0}E$ prior to coupling the beads to the fluid causes their steady-state velocities to be $-\mu_{\text{el}0}E$ greater than the local fluid velocity instead of equal to it. As expected for a thin Debye layer method, the electrophoretic mobility is independent of N . Since each monomer collides independently with the fluid, both the effective charge and the effective friction grow linearly with N resulting in a mobility independent of length. While this captures the correct behaviour for large objects, when the length scale of the

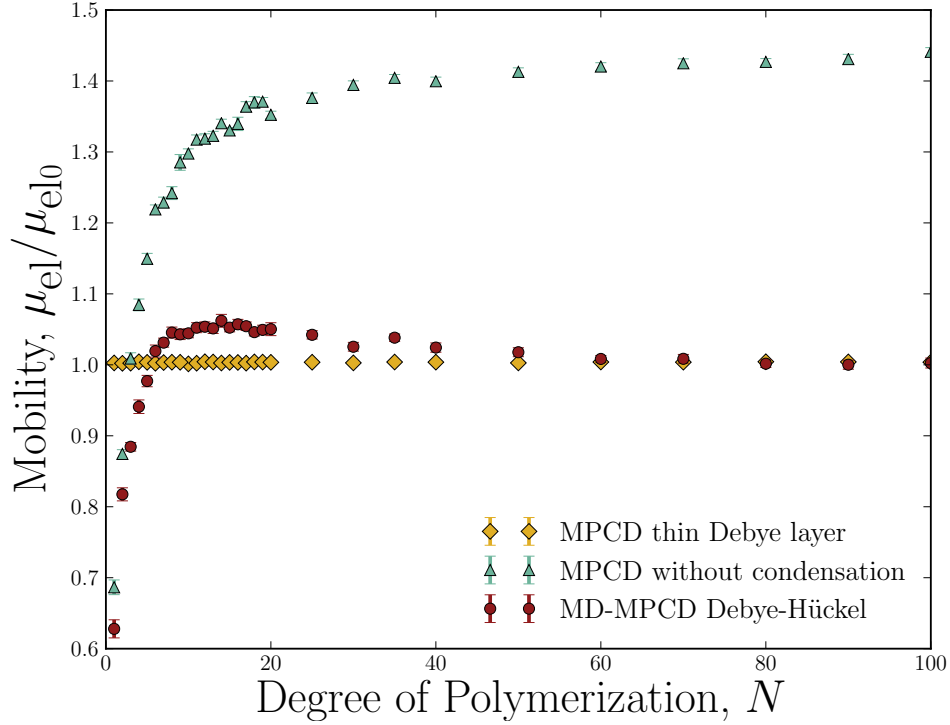


Figure 4.3.1: The electrophoretic mobility of polyelectrolytes as a function of the degree of polymerization N normalized by the free-draining value μ_{el0} . Thin Debye layer simulations (diamonds) produce the free-draining mobility for all contour lengths, while MPCD-MD Debye-Hückel simulations without charge condensation (triangles) capture the rise due to hydrodynamic interactions within finite Debye layers. The full results of hybrid simulations with counterion condensation (circles) capture the non-monotonic form of the electrophoretic mobility curve.

object is comparable to the Debye length, complex behaviour cannot be reproduced (*i.e.* when N is small).

The triangles show the result when the Debye-Hückel approximation is used in the algorithm to apply a counter force without attempting to take into account charge condensation. In this case, there is a sharp rise of the mobility for small values of N followed by a plateau. While the initial rise is qualitatively correct, these simulations fail to reproduce the experimental data in two respects. First, the rise in mobility continues for larger values of N than in the experiment. Second, these simulations cannot reproduce the experimentally observed non-monotonicity around $N \approx 10$ because condensation is neglected (Figure 4.3.2).

The open circles in Figure 4.3.1 show the full MPCD-MD Debye-Hückel algorithm when charge condensation is included. This curve has all of the sought-after characteristics: It rises rapidly to a maximum then relaxes down to the free-draining value. The iterations of the MPCD-MD algorithm are compared to simulations with explicit ions as well as experi-

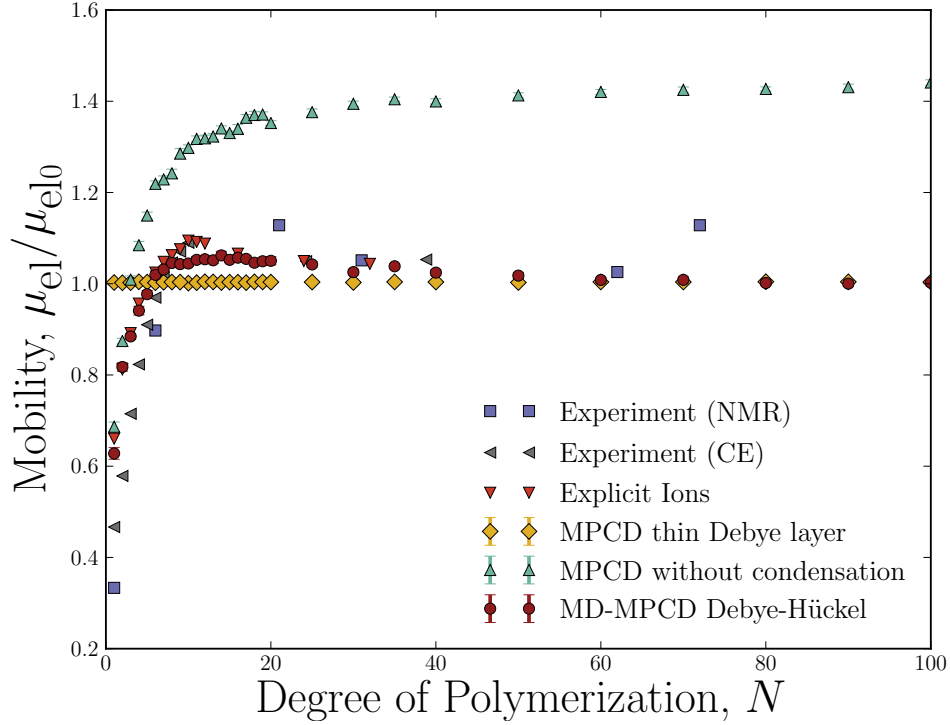


Figure 4.3.2: The electrophoretic mobility of polyelectrolytes as a function of the degree of polymerization N normalized by the free-draining value μ_{el0} . The results of hybrid simulations with counterion condensation (circles) compare well to both the experimental results (squares and left-facing triangles [120]) and simulations using explicit ions (upward triangles [120]).

mental results in Figure 4.3.2. The results of the MPCD-MD Debye-Hückel algorithm with counterion condensation agree well with experiments (squares and left-facing triangles) and explicit ion simulations (downward triangles) [120]. The sharp initial rise at small N , the plateau to the free-draining electrophoretic mobility at large N , and the slight maximum at intermediate N are all present.

Since the condensation is controlled in these simulations, the effective charge Q^{eff} on each monomer is known and so the effective friction coefficient of each monomer as a function of segment position can be calculated. The effective charge and effective friction ζ^{eff} values from the mean-field MPCD-MD Debye-Hückel approach proposed here are compared to those from simulations using explicit ions [120] in Figure 4.3.3. The average effective charge as a function of N from the mesoscopic simulations agree well with the simulations that used explicit ions. The effective friction also shows very similar behaviour between this approach and the explicit ion simulations. Notice that the effective friction coefficient for the MPCD algorithm with charge condensation is considerably lower than when there is no charge condensation. This is because the charges that adsorb to the polymer are those that

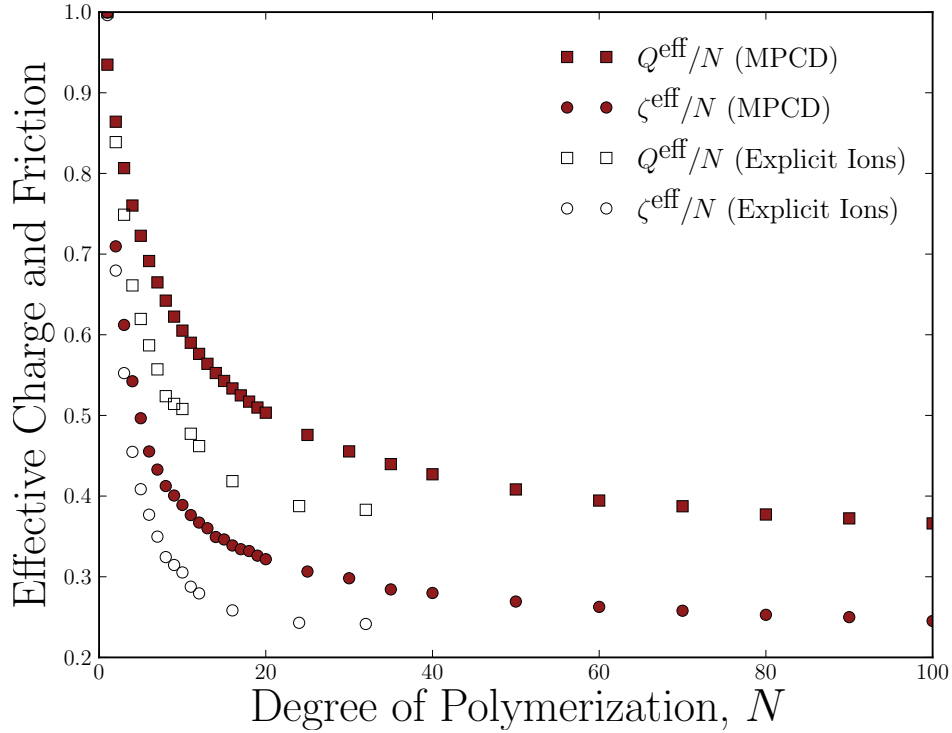


Figure 4.3.3: The effective friction ζ^{eff} (circles) and effective charge Q^{eff} (squares) as a function of the degree of polymerization N . The filled symbols are the hybrid simulation results, while the open symbols are simulations using explicit ions reproduced with permission from [120]. The diamonds show the effective friction when there is no charge condensation.

are closest to the backbone and are, thus, the most strongly hydrodynamically coupled to it.

Despite using a simple approach based on the Debye-Hückel approximation, the proposed mesoscopic method displays not only the right effective charge as a function of N but also a remarkably accurate distribution of effective charges as a function of monomer number as seen in Figure 4.3.4. The increased charge on the ends produces slightly higher electrophoretic mobility. These charge distributions agree well with explicit ion simulations [129, 137]. The fact that both the average effective charge as well as its distribution along the chain agree with results using explicit ions indicates that the simplistic approach taken to model charge condensation is reasonable.

4.3.2 Mobility as Function of Debye Length

Since the MPCD-MD Debye-Hückel algorithm can properly simulate finite Debye lengths, the impact of λ_D on electrophoretic mobility can be examined. This is done for chains of

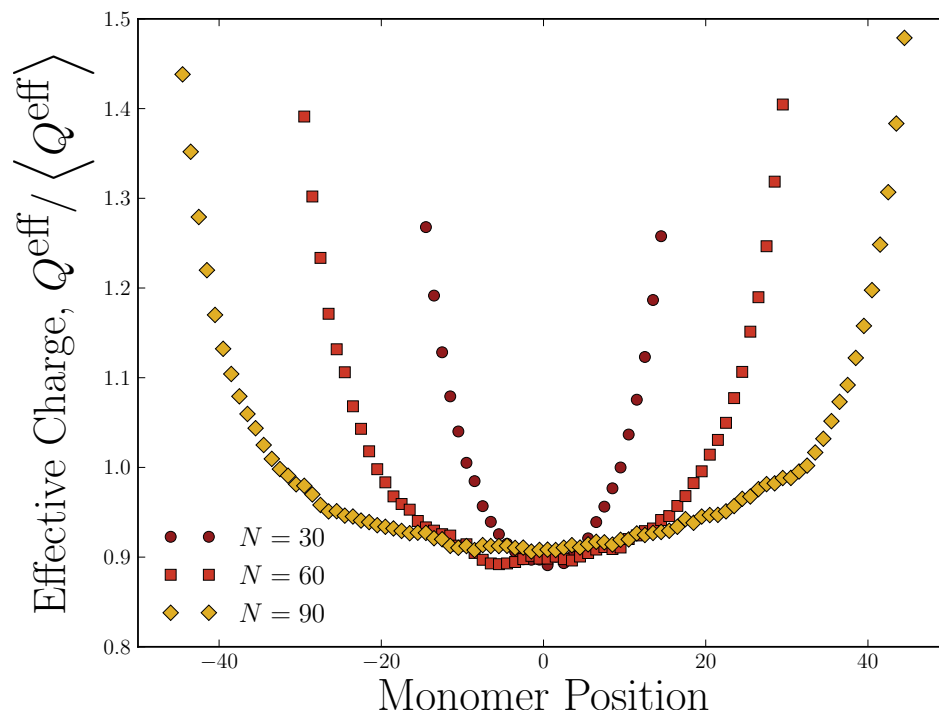


Figure 4.3.4: The effective charge as a function of monomer position normalized by the average effective charge on all monomers. The apparent asymmetry is a result of the method used to enumerate monomer position.

length $N = 20$ in Figure 4.3.5. The electrophoretic mobility increases with λ_D as is expected since cooperative shearing within the Debye layer occurs over a greater distance and hence includes more monomers (§ 4.1.2). The mobility begins to saturate as the Debye length becomes large. When the Debye length becomes larger than the radius of gyration, the polyelectrolyte will enter the Hückel limit.

4.3.3 Free-Solution Electrophoresis of Polyampholytes

Another topic that can be explored only with simulation techniques that account for finite Debye lengths and screened hydrodynamics is polyelectrolytes with complex charge distributions. In 1998, Long *et. al.* demonstrated that the electrophoretic mobility of *polyampholytes* depends strongly on the charge distribution [137]. In fact, they predicted that polyampholytes with the same charge but different distributions can have electrophoretic mobilities with opposite signs (*i.e.* move in different directions) and that net neutral polyampholytes can have non-zero mobilities.

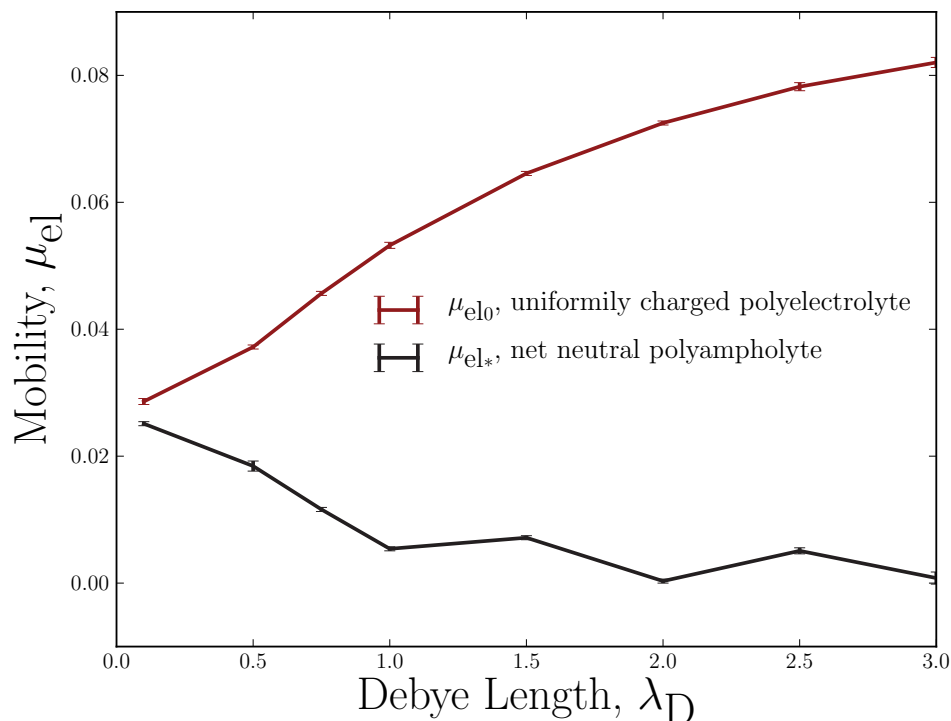


Figure 4.3.5: Electrophoretic mobility of a uniformly charged polymer, μ_{el0} , as a function of Debye length λ_D . Also mobility of a net neutral block copolymer, μ_{el*} , rescaled by a constant factor.

The maximum electrophoretic mobility of net-neutral polyampholytes occurs for a *symmetric block co-polyampholytes* with equally long ends (*i.e.* $N_{\text{cut}} = N/4$) [129]. The maximum mobility of this polymer, μ_{el*} as a function of λ_D is shown in Figure 4.3.5 along side the mobility of a uniformly charged polyelectrolyte. The fact that the uniformly charged polymer and block polyampholyte copolymer show opposing trends in electrophoretic mobility with respect to λ_D highlights the different physical mechanisms involved in the two cases:

- μ_{el0} , the electrophoretic mobility of a uniformly charged chain, increases with λ_D because λ_D acts as the hydrodynamic interaction layer within which increased cooperative shearing occurs
- μ_{el*} , the electrophoretic mobility of a symmetric polyampholyte decreases because the non-zero mobility of the polyampholyte is the result of locally generated electro-osmotic flow, which becomes less local as the Debye length increases.

Next, consider a net neutral diblock ring polymer cut to create a net neutral linear triblock polyampholyte with a negatively charged centre block and positively charged ends of different

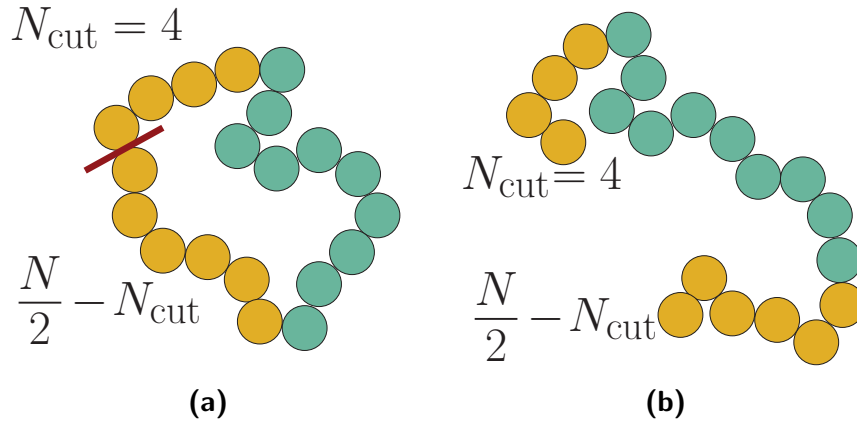


Figure 4.3.6: Schematic explaining the polyampholyte structure. A di-block (one positive block and one negative block) ring polymer, of net neutral charge is cut. The number of monomers away from the junction at which point the cut is made is N_{cut} . A tri-block linear polymer results that has positive ends of length N_{cut} and $N/2 - N_{\text{cut}}$.

lengths (Figure 4.3.6). If the ring polymer is cut at the positive-negative junction ($N_{\text{cut}} = 0$) creating a diblock polyampholyte, the electrophoretic mobility is zero. However, if the cut position is at any other point ($N_{\text{cut}} \neq 0$) as schematically drawn in Figure 4.3.6, then the net neutral complex has a non-zero mobility [137, 138]. In Figure 4.3.7 the importance of finite Debye lengths on this phenomenon is apparent: The smaller of the two Debye lengths simulated ($\lambda_D = 0.5$) produces a non-negligible mobility while the larger value ($\lambda_D = 3$) results in a significantly reduced electrophoretic mobility. Non-zero mobility requires that λ_D be smaller than the scale of the charge heterogeneity of the polyelectrolyte complex. This is because the mobility arises from locally generated electro-osmotic flows along the backbone of the chain. For the $\lambda_D = 3$ case, this is never true (regardless of cut position) and so the electrophoretic mobility is zero.

Significant opportunity exists for the MPCD-MD Debye-Hückel algorithm to be applied to biologically relevant macromolecules. Here only a simple tri-block linear polymer was considered by studying the electrophoretic mobility of chains with more complicated charge distributions would be interesting. Here only a simple tri-block linear polymer was considered but studying the electrophoretic mobility of chains with more complicated charge distributions would be interesting. Such mesoscopic polyampholytes with finite Debye layers and charged domains might be useful models of electrophoresing proteins. Further work in this direction is encouraged.

In this section, multiple electrophoretic situations have been considered but so far they have all been limited to electrophoresis in free solution. In the next section, a simple con-

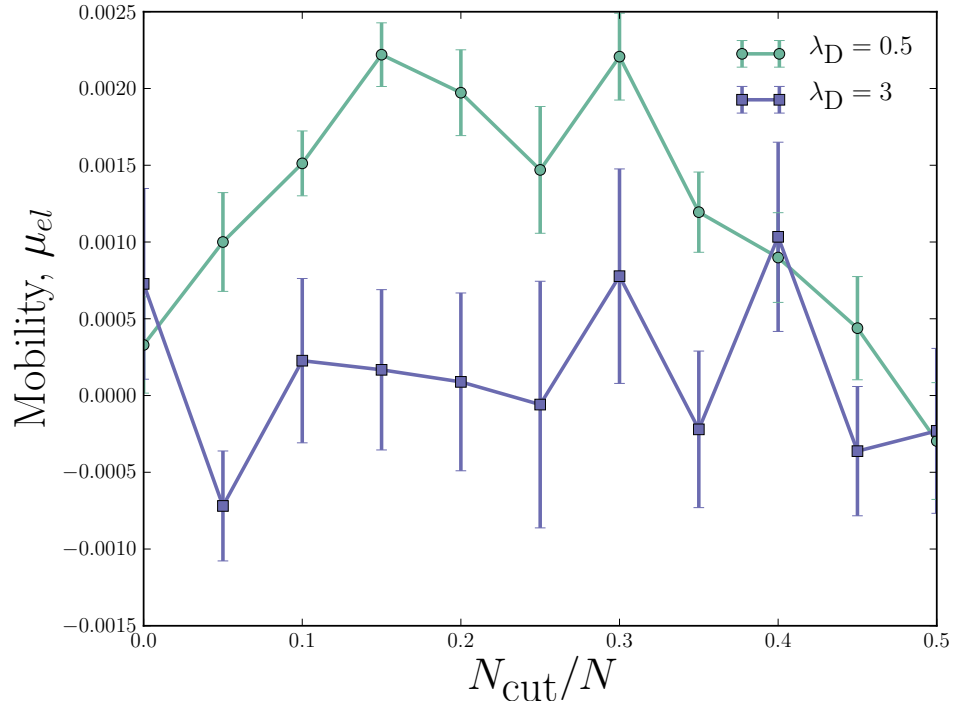


Figure 4.3.7: Electrophoretic mobility of a net neutral block copolymer ($N = 20$) as a function of cutting position N_{cut} for two values of λ_D .

finement will be used to force a conformational change in the translating chains.

4.4 Electrophoresis in Confined Geometries

The previous section presented the mean-field MPCD-MD Debye-Hückel algorithm. One of the major advantages of this mesoscopic method is the ability to simulate charged macromolecules in the geometry of a given microfluidic device. As described in the introduction of this chapter, microfluidic devices have great potential as the next generation of DNA fractionation technology. Often, they can separate chains because the device enacts mechanical forces on the polyelectrolytes, which breaks the electro-hydrodynamic screening. This section will demonstrate that mobility can change within confining geometries even in cases when the electro-hydrodynamic screening is not broken. It will be seen that confinement within microfluidic devices has fundamental effects that lead to electrophoretic mobility variation.

4.4.1 Electro-hydrodynamic Equivalence Principle

Free-solution electrophoretic fractionation of long DNA fragments is shown to be impossible in § 4.3. However, if electric and non-electric forces act in concert, the picture is different. An electric force acts on both the polyelectrolyte and the surrounding ions, but a non-electric force $\vec{f}$ can be chosen to act only on the monomers. Under the simultaneous action of the two forces, the resulting motion is, in the linear regime, a superposition of the two responses [139]. The steady state drift velocity $\vec{V}$ is achieved when the drag force $\vec{f}_\zeta = \vec{V} \cdot \hat{\zeta}$ balances the electric force $\vec{f}_E = \hat{\zeta} \cdot (\hat{\mu}_{\text{el}} \cdot \vec{E})$ and the non-electric force $\vec{f}$

$$0 = \vec{f} - \hat{\zeta} \cdot (\vec{V} - \hat{\mu}_{\text{el}} \cdot \vec{E}) \quad (4.3)$$

where $\hat{\zeta}$ and $\hat{\mu}_{\text{el}}$ are the effective hydrodynamic friction and electrophoretic mobility tensors. Since the counterions are unaffected by the mechanical $\vec{f}$, the total force on the ion sheath and on the chain no longer cancel and a long ranged hydrodynamic flow field ($\sim 1/\rho$) appears, which generates collective responses in the chain. The transport coefficients $\hat{\zeta}$ and $\hat{\mu}_{\text{el}}$ are then explicitly dependent on the polyelectrolyte conformation and in principle have a tensorial character. In particular, the drift velocity $\vec{V}$ is, in general, a function of the polyelectrolyte's size N , in contrast to the universal plateau electrophoretic mobility $\mu_{\text{el}0}$ observed in free-solution. In other words, the polyelectrolyte is no longer free-draining [118] and size fractionation is in principle now possible.

The result of Equation (4.3), first proposed for polymers in the seminal work of Long *et al.*,

suggests an *electro-hydrodynamic equivalence principle* [139]. The term $\widehat{\mu}_{\text{el}} \cdot \vec{E}$ can be thought of as an effective local velocity $\vec{V}_s$. In full, the equivalence principle states

- when polyelectrolytes with small Debye lengths are acted on by a low electric field $E \ll \zeta/\lambda_D$ and simultaneously by a mechanical force f , one can replace a consideration of the full electrostatics and hydrodynamics with an effective local flow, which is simply the Smoluchowski slip velocity (Equation (2.105)).

An elegant way of considering the electro-hydrodynamic equivalence principle is to envision the chain as moving through an effective local electro-osmotic flow field $\widehat{\mu}_{\text{el}} \cdot \vec{E}$ (generated by the surrounding ions) with a drift velocity $\vec{V}$ [138–140].

One oft-given example of the electro-hydrodynamic equivalence principle is the electrophoretic motion of a deformed polyelectrolyte through a nanofluidic channel [141]. It is argued that confinement increases the probability of interaction between monomers and the molecules of the channel walls, thereby inducing a non-electric force during electrophoresis due to added friction of the monomers in transient contact with the wall. This non-electric frictional force is, presumably, an extensive quantity that increases linearly with the number of monomers in contact with the wall and their electrophoretic speed. By inputting such a resistive, mechanical force into the electro-hydrodynamic equivalence principle (Equation (4.3)) and assuming that the chain remains free-draining [33–35]), the mobility can be calculated. The resistive force has been modelled as arising from planar surfaces composed of a uniform distribution of adhesive sites. A version of this argument has been previously proposed for large gap sizes [141]. Even in large fields, λ -DNA appears to move through nanoslits in a stick-slip manner [142], supporting this argument. This suggests that the mean friction ζ_w may be field-dependent and a result of entropic or dielectrophoretic trapping that reduces the electrophoretic mobility [141]. However, conflicting experimental results have also been reported in which mobility is observed to increase with confinement [107, 143]. What physical mechanism allows for mobility to increase in microfluidic devices?

4.4.2 Wall-less Confinement

These arguments posit that the change in electrophoretic mobility of such confined polymers is due to forces applied to the monomers at the channel walls. But is that the only significant effect? This section examines the situation in which the polyelectrolyte's conformation is

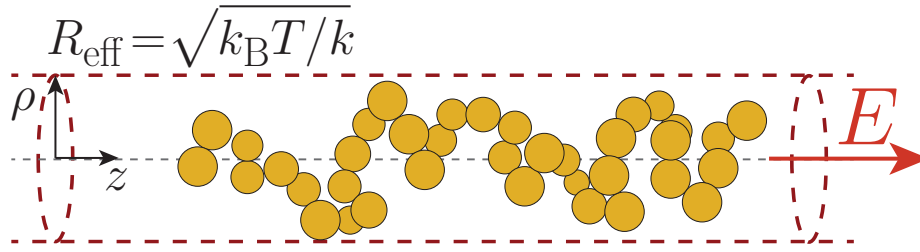


Figure 4.4.1: A polyelectrolyte chain of unperturbed radius of gyration R_g undergoing electrophoretic motion under the action of an electric field E . A harmonic potential with a cylindrical geometry confines the chain radially.

deformed by a radial potential that acts transverse to the electric field (Figure 4.4.1). The validity of the free-draining assumption is explicitly studied and it is questioned whether the electrophoretic mobility remains strictly conformation-independent while being squeezed by a net-zero mechanical force transverse to the electric field $E\hat{z}$ in the absence of walls. In a manner, this is an acid-test of the ideas proposed at the end of the previous section. A harmonic potential $k\rho^2/2$ instead of solid walls is used to constrain the conformation (where k is the confinement strength and ρ is the radial distance from the $\hat{z}$ -axis). Although wall-less confinements have been experimentally demonstrated by “aqueous virtual pores” [144], it is dubious that they could reach the strong confinements considered here at the present time. This study does not seek to reproduce an achievable experimental system but rather to perform a nonphysical *in-silico* experiment that will shed light on the nature of electrophoresis of polyelectrolytes in confined environments. Impermeable walls add complication by introducing shear stresses, modifying friction coefficients and screening hydrodynamic interactions, all of which directly affect the dynamics of the polymer chain [145–147]. This study does away with these complications and leaves only the effects of confinement on polymer conformation. The radial potential forms an effective tube of radius $R_{\text{eff}} \equiv \sqrt{k_B T / k}$ that imposes lateral constraints on the polymer conformation but does not act on the fluid. Substituting the mechanical radial force into the electro-hydrodynamic equivalence principle does not produce a prediction of the mobility, since the force acts perpendicular to the electric field, which causes axial electrophoretic mobility μ_{el} to cancel throughout.

The change in conformation due to confinement in the harmonic potential can be discussed in terms of the *asymmetry ratio* φ , which is defined as the ratio of the radius of gyration along the axis $R_{g,z}$ to the radial element of the radius of gyration tensor $R_{g,\rho}$. While the harmonic potential is weak ($R_{\text{eff}} \gg R_g$), the chain is relatively symmetric and $\varphi = 1$. This unperturbed regime can be seen at a large R_{eff} for all contour lengths in Figure 4.4.2. When $R_{\text{eff}} < R_g$,

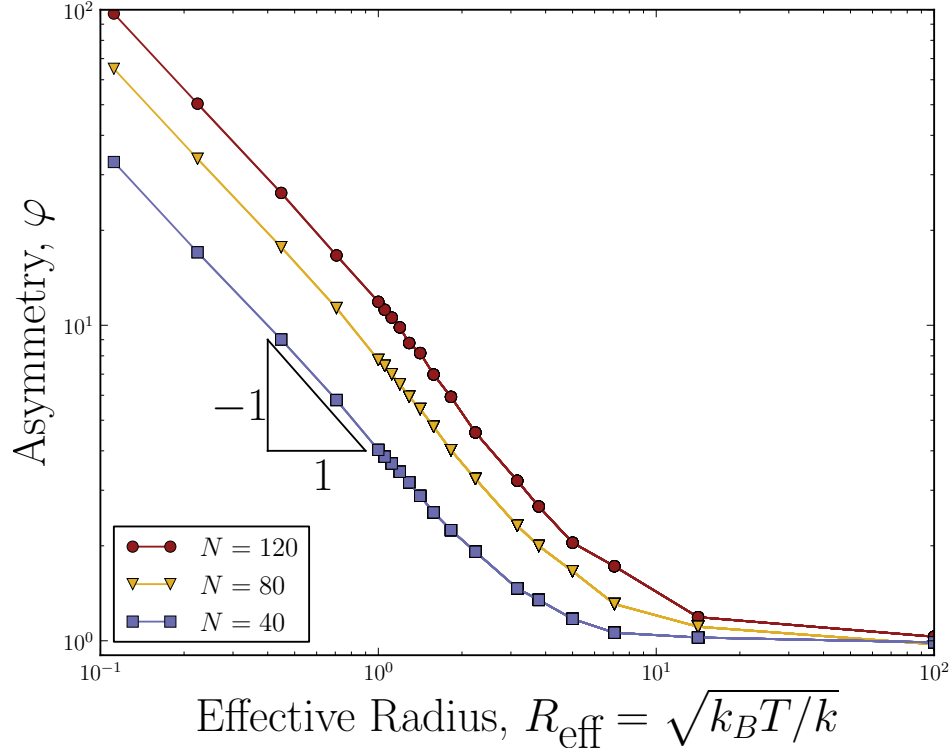


Figure 4.4.2: Asymmetry ratio $\varphi = R_{g,z}/R_{g,\rho}$ as a function of effective confinement radius of the harmonic well $R_{\text{eff}} = \sqrt{k_B T/k}$.

the confinement stretches the polymer into a string of correlation blobs. This situation is similar to the de Gennes regime [148] of polymers in hard cylindrical tubes, in which the blobs are of size R_{eff} and the asymmetry ratio scales as $\varphi = R_{g,z}/R_{\text{eff}} \sim N(\sigma/R_{\text{eff}})^{5/3}$. In soft tubes, the perturbation is expected to scale less strongly because excluded-volume swelling and Brownian motion can compete against the soft potential [149, 150]. Figure 4.4.2 shows that the asymmetry ratio of a polyelectrolyte radially confined by a harmonic potential is $\varphi \sim R_{\text{eff}}^{-1}$.

For weak confinements, the mean-field MPCD-MD Debye-Hückel simulations verify that the mobility is independent of effective tube diameter and chain length as expected by the free-draining nature of the polyelectrolytes (Figure 4.4.3). Simulations performed with effective confinement radii larger than shown in Figure 4.4.3 ($R_{\text{eff}} = \{14, 100\}$) show a slow, continuous approach to μ_{el0} . In weak confinements, the mobility remains near the free-solution value even as the asymmetry ratio (Figure 4.4.2) begins to climb. However, at strong confinements the electrophoretic mobility rises above the free-solution value (Figure 4.4.3).

The electrophoretic mobility increases quite sharply for confinements with effective radii

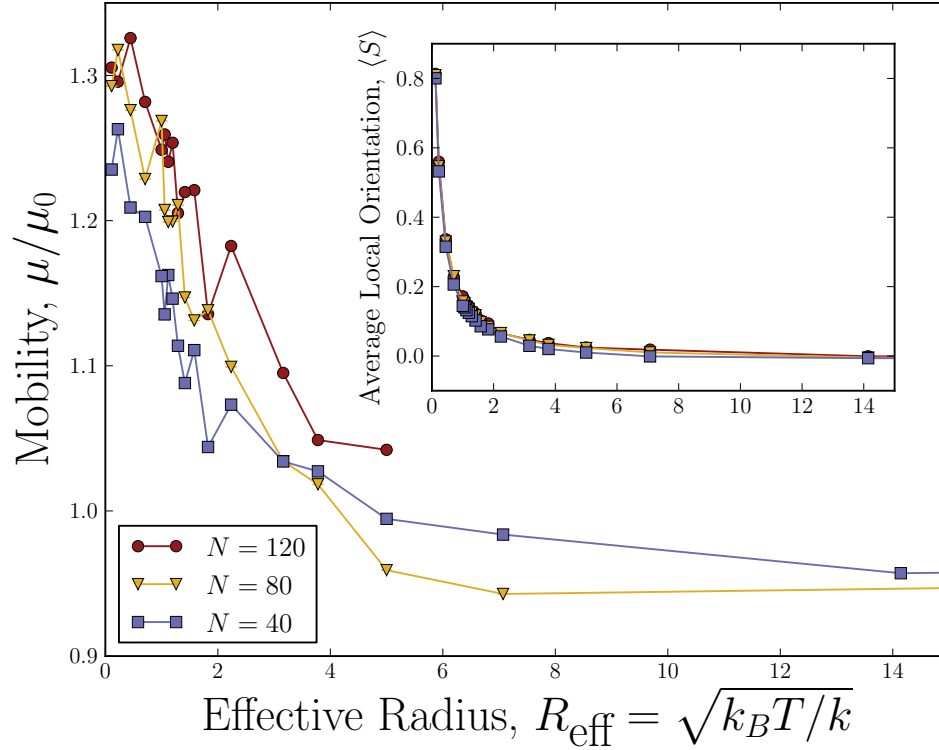


Figure 4.4.3: The electrophoretic mobility of confined polyelectrolytes as a function of effective confinement radius of the harmonic well $R_{\text{eff}} = \sqrt{k_B T / k}$ normalized by the free-solution value $\mu_{\text{el}0}$ for various degrees of polymerization N . The inset shows the average local order parameter of the bonds between monomers as a function of the effective confinement radius R_{eff} .

$R_{\text{eff}} \lesssim 3$. The mobility rises to $\mu_{\text{el}} \approx 1.3\mu_{\text{el}0}$ for all chain lengths. There is no apparent length dependence, which means that, though the electrophoretic mobility changes, the chain remains free-draining. If the polyelectrolyte were not free-draining, long-range hydrodynamic interactions would couple distant segments of the chain and the global resistance to electrophoretic motion would depend strongly on the contour length. The polyelectrolyte would electrophorese as a *Zimmian chain*: An asymmetric object **opaque** to flow that entrains fluid along with it.

The entrainment of fluid within the polyelectrolyte coil can be explicitly recorded (Figure 4.4.4). Even in the absence of any confinement (Figure 4.4.4.a), fluid within the coil of the chain is observed to possess an electro-osmotic speed that is a small fraction of the free-solution electrophoretic speed of the chain, as is to be expected since the Debye length is finite. However, this flow field is local and only observed in the core of the chain. As is appropriate for a free-draining chain, there are no far-field perturbations to the surrounding fluid.

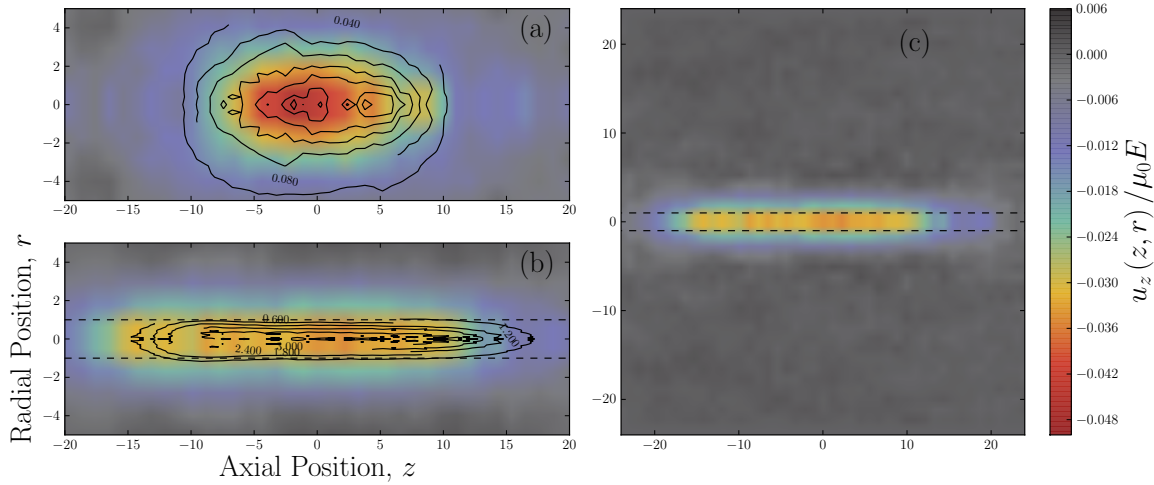


Figure 4.4.4: Speed of the fluid about the polyelectrolyte’s centre of mass normalized by the electrophoretic speed of the chain. The degree of polymerization is $N = 200$, the electric field is $E = 2$ and the polymer is coupled to the fluid via the MPCD-in-MD scheme. (a) shows the fluid speed field generated by the electrophoresis of an unconfined, free-solution polyelectrolyte. (b) shows the field over the same range but for a charged chain that is confined by an axially symmetric harmonic potential with an effective radius of $R_{\text{eff}} = 1$. (c) presents the far-field fluid speeds for the same situation as shown (b). The speed of the surround fluid is zero immediately outside of the polymer coil.

The flow-field produced by the present MPCD-MD Debye-Hückel simulations of an electrophoresing chain confined to an effective tube of $R_{\text{eff}} = 1$ are quite similar (Figure 4.4.4.b). If free-draining were thwarted by confinement, then the entrained fluid would be expected to move with a speed comparable to the electrophoretic speed of the chain and long-range perturbations akin to a those produced by the motion of a no-slip, impermeable body or stokeslet that decay as $\sim \rho^{-1}$ would be expected. However, this is not observed. Rather, the entrained fluid speed remains significantly less than that of the translating chain (Figure 4.4.4.b). Furthermore, both the unconfined and confined coils have the same free-draining quality. This is emphasized in Figure 4.4.4.c, which explicitly shows that that far from the confined polyelectrolyte the flow field is zero.

Therefore, the polyelectrolyte must be described in terms of effective local properties. In this framework, the total friction coefficient is the sum of the friction of each segment ($\zeta \equiv N\zeta_D$). The ambiguous term “**local**” is more precisely a subsection of the chain that has a local radius of gyration that is less than the Debye length λ_D . On this scale, neither electrostatic not hydrodynamic interactions are screened between monomers and subsegments are coupled. The electrophoretic mobility of this subsection (μ_D) is the effective charge of the segment Q_D^{eff} divided by its effective friction coefficient ζ_D parallel to the direction of



Figure 4.4.5: The local order parameter is, on average, $\langle S_\theta \rangle = 0$ for an unconfined chain but if the chain is strongly confined then the bonds between monomers are more likely to be aligned.

motion $\mu_D = Q_D^{\text{eff}}/\zeta_D$.

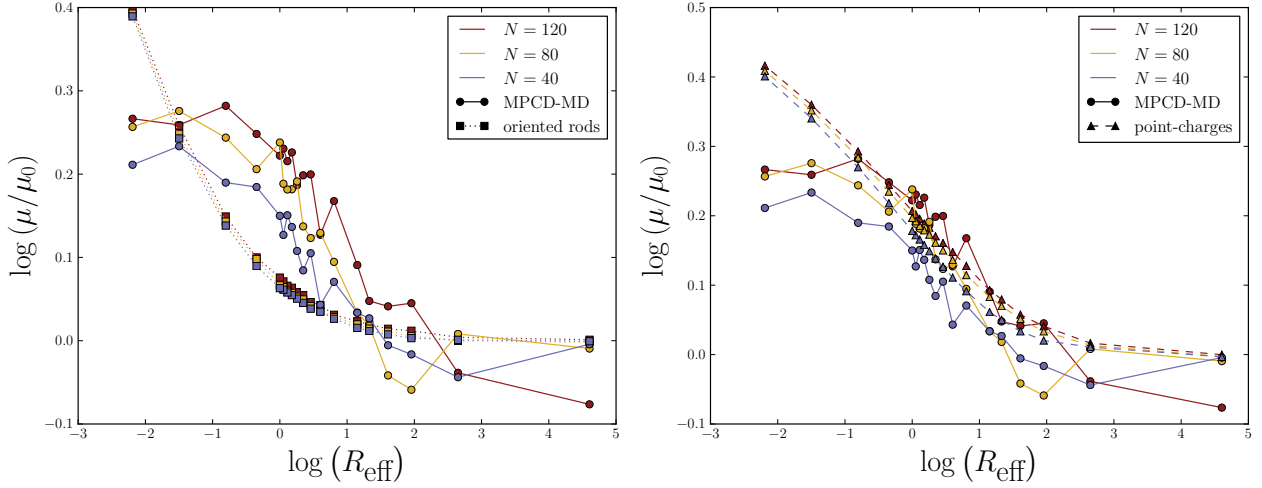
Because electrostatic repulsion is not fully screened within the Debye layer, the local segment is roughly a rigid rod of length λ_D . In free-solution, these rod-like local segments randomly sample all orientations. However, when the chain is axially confined, the rod-like segments orient. The orientation of local segments can be assessed from the MPCD-MD Debye-Hückel simulations by calculating the *orientation order parameter*,

$$S_\theta = \left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle, \quad (4.4)$$

of the bonds between monomers. The average local order parameter for all segments $\langle S_\theta \rangle$ (Figure 4.4.3; inset) is highly correlated to the mobility (Figure 4.4.3). Like the mobility, the local order parameter is flat for large effective tube sizes and suddenly rises once the confinement strength is large enough. Figure 4.4.5 schematically illustrates how confinement impacts local orientation between segments.

Though the MD monomers are spherically symmetric, the segment is rod-like within the Debye layer (Figure 4.1.1c) and so the friction coefficient is approximated as the friction coefficient appropriate for a rod. Essentially, the friction coefficient of a rod aligned perpendicular to its motion $\zeta_\perp$ is nearly twice that of a rod aligned parallel to its motion $\zeta_\parallel$ [151]. Since the friction tensor of a rod with its major axis pointing along $\hat{n}$ can be written using Equation (2.82d) (*i.e.* $\hat{\zeta} = \zeta_\parallel \hat{n}\hat{n} + \zeta_\perp [\hat{1} - \hat{n}\hat{n}]$), the effective friction coefficient of the segment parallel to the electrophoretic motion is related to the order parameter of the rod S_θ as

$$\zeta_D \approx \zeta_\parallel \left(\frac{5 - 2S_\theta}{3} \right). \quad (4.5)$$



(a) Dotted lines model the rise in mobility by approximating the mean local segment friction coefficients as oriented rods.

(b) Dashed lines model the polyelectrolyte as an ensemble of charged point forces at the instantaneous position of the monomers from simulations as calculated by the Long-Ajdari screened hydrodynamic interaction tensor.

Figure 4.4.6: The electrophoretic mobility of confined polyelectrolytes as a function of the effective confinement radius of the harmonic well $R_{\text{eff}} = \sqrt{k_B T/k}$ normalized by the free-solution value $\mu_{\text{el}0}$ for various degrees of polymerization N . Solid lines represent mobility as measured from the simulated speed of confined polyelectrolytes.

Notice that when $S_\theta = 0$, the effective friction coefficient parallel to the motion is $5\zeta_{\parallel}/3$ and describes the resistance to motion in the $\hat{z}$ -direction. This value is slightly different than the random-orientation value of $3\zeta_{\parallel}/2$ [152], which is related to **diffusive** rather than **directed** translation. Thus, local orientation can decrease the friction of segments of the polyelectrolyte at length scales smaller than λ_D and cause the chain's electrophoretic mobility to increase as $\sim \langle 5 - 2S_\theta \rangle^{-1}$ (Figure 4.4.6a). The model captures the magnitude of the rise quite well though it is seen that the modelled electrophoretic mobility is systematically lower than simulation values. This suggests that the local friction ζ_D may be over-predicted.

The above model does not rest on the connectivity and rod-like nature of the local segments; rather, it rests on the fact that the Debye layer functions as the hydrodynamic interactions layer. Consider a lone point charge in solution and surrounded by a finite-sized counterion sheath. The far-field hydrodynamic flow that arises due to the force on the fluid by the motion of such a point charge is described by the Long-Ajdari hydrodynamic interaction tensor (§ 2.4.4). Besides terms that decay exponentially, the Long-Ajdari tensor possesses a residual geometric term that goes $\sim \rho^{-3}$, which decays significantly faster than the $\sim \rho^{-1}$ of unscreened hydrodynamic interactions although not technically short-ranged [35]. The elec-

trophoretic mobility of the ensemble of charged point forces at the instantaneous positions of the simulated monomers can be calculated via the Long-Ajdari tensor (Equation (2.114)). The result is shown in Figure 4.4.6b. In this way, physical connectivity is omitted and only screened electro-hydrodynamic interactions are accounted for.

The electrophoretic mobility of the point charges (and associated Debye layers) follows the simulated mobility (Figure 4.4.6b). This result demonstrates that the essential fact is that the local electrophoretic mobility of a segment changes with orientation regardless of how the subsegments are modelled. Modelling subsegments as oriented rods (as in Equation (4.5)) or as ensembles of point charges (as is assumed by the Long-Ajdari interaction tensor) both produce total electrophoretic mobilities that are constant at large effective confinement radii and rise rapidly at strong confinements.

This section considers a simplified system of a polyelectrolyte electrophoresing while radially confined by a potential rather than an impermeable wall. In this way, the independence of electrophoretic mobility on conformation and free-draining was tested. Yet, mean-field MPCD-MD Debye-Hückel simulations demonstrate that the electrophoretic mobility increases radically for strong confinements. The change in mobility arises not from the global conformation but from the conformation within the hydrodynamic interaction layers. The local orientation leads to a change in local electrophoretic mobility, which can be modelled equally well by treating the local segment as a rod-like object or an ensemble of point particles. The fundamental finding is that although free-draining ensures that global conformation does not determine total electrophoretic mobility, conformation can alter mobility if it alters local friction coefficients at length scales less than the Debye length.

Understanding the fundamental behaviour of confined polyelectrolytes is vital for the design of next generation electrophoretic separation techniques. Nanofluidic systems may someday be engineered to replace gel media, but before that can happen a fundamental understanding of DNA mobility within such confining geometries must be established. Since many competing factors act on the chains, dividing theoretical studies into simplified questions allow us to construct a full picture of electrophoresis within confining channels.

Summary

In this chapter, a novel algorithm has been proposed to simulate the electrophoresis of polyelectrolyte chains with finite Debye layers. This mean-field MPCD-MD Debye-Hückel algorithm models the polymer as an MD chain within an MPCD fluid but does not explicitly

include counterions or the calculation of computationally costly electrostatic interactions. Rather it replaces the explicit salt ions with the Debye-Hückel approximation, by giving MPCD fluid particles an effective charge based on their position and the mean-field counterion number density distribution. Counterion condensation effects are included heuristically by negating a portion of monomers' charge if surrounding fluid particles are given a charge greater than a cutoff value.

This mesoscopic algorithm is able to reproduce mobility effects that arise from the finite nature of the Debye layer. In particular, the electrophoretic mobility as a function of chain length is seen to be non-monotonic, rising from small values for oligomers, through a maximum that results from condensation effects and saturates at the free-solution electrophoretic mobility for long chains.

Furthermore, this novel algorithm is used to study the impact of Debye length on mobility and finds that the electrophoretic mobility of homogeneously charged chains increases with Debye length, since cooperative shearing occurs over greater portions of the chain. On the other hand, non-zero mobilities of net neutral polyampholytes decreases as the Debye length is increased, since this mobility arises from locally generated electro-osmotic flows.

Finally, the mobility of polyelectrolytes that are radially confined by a harmonic potential is considered. Mobility is known to change in confined nanofluidic devices, but this is often attributed to interactions with channel walls that invoke the electro-hydrodynamic equivalence principle. The work presented here demonstrates that confining geometries can result in changes to the electrophoretic mobility without the presence of impermeable, friction-generating walls. The rise in mobility at strong confinements is a result of local orientation within Debye layers that modify the mobility of local segments of the chain. Thus, despite the observed change in mobility, there is no change in the free-draining nature of the polymer and indeed, the resulting flow due to the electrophoretic motion of the chains is seen to be small and to not extend outside of the coil.

*Very wrong ideas can be “obviously right” for
very long times. Don’t take anything on trust.*

Tom McLeish [153]

5

Unified Field-Flow Fractionation

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The previous chapter (§ 4) discussed electrophoresis in confining microfluidic geometries and observed that changes in mobility are a direct consequence of the confinement. However, the research concluded that though mobility varies with confinement it does not vary as a function of chain length, and so is not suitable for separation by segment size. This chapter also considers the application of a traditional, macroscopic technique (called *field-flow fractionation* [FFF]) in a microfluidic chip. Once again, it will be seen that confinement facilitates mobility differences, however, microfluidic confinement exacerbates non-monotonic solute elution speeds.

Through a careful consideration of the elution metric called the retention ratio, a single **unified, ideal retention theory** that can predict a wide range of separation behaviours is developed in this chapter. The retention theories for hydrodynamic chromatography, normal-mode FFF and steric-mode FFF are unified into a single theory by explicitly accounting for solute size. Analysis of the predicted elution order as a function of particle size quantitatively predicts the transitions between the different operational modes of FFF. By extending this analysis to include variation of stress over particle surfaces, an unreported regime called Faxén-mode FFF is predicted. This mode arises when particle sizes approach the channel height, as can readily occur when microfluidic devices are utilized for FFF.

Previously, only the transition between normal- and steric-mode FFF (called the steric-inversion point) had been reported in the literature. Although, this steric-inversion is the transition that is most relevant to macroscopic apparatus, in microfluidic channels solute size may span the entire range of allowable sizes, from nanoscopic specks to colloids that barely fit in the channel. Although, the ideal theory technically extends to such large sizes, it will be seen in § 7 that unavoidable hydrodynamic interactions with the microchannel walls complicate matters.

The operational-modes maps that result from this ideal theory show each of the regimes and the transitions between them. These may guide future microfluidic device design. Rather than limiting the applicability, these effects allow the microfluidic channel size to be tuned to ensure high selectivity.

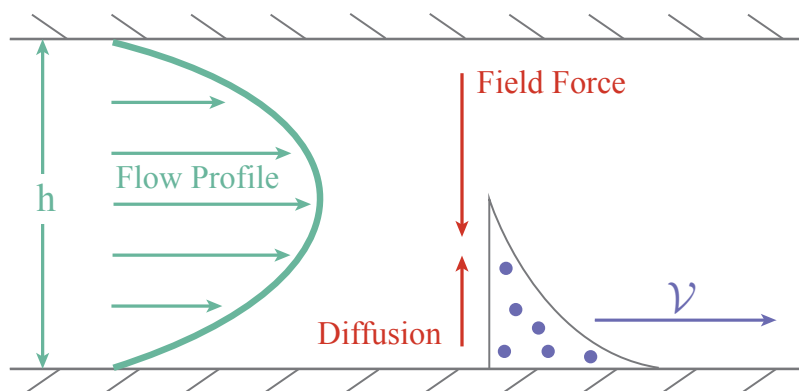


Figure 5.1.1: Schematic of normal-mode FFF. An external force, perpendicular to the flow profile pushes the solute against the accumulation plate. The competition between the force and the diffusivity establishes a concentration gradient. The mean velocity of the solute is a function of the solute concentration and solvent velocity profiles.

5.1 Introduction to Field-Flow Fractionation

Field-Flow Fractionation (FFF) is an elegant separation technique based on a simple concept: Separation of a mixture of different species can occur when solute particles, carried through a channel by a non-uniform, laminar flow profile, are acted on by an external field applied perpendicular to the flow profile (Figure 5.1.1). The perpendicular field pushes the ensemble of solutes against the *accumulation wall* but diffusion disperses the solute and resists the inhomogeneity. An exponential concentration distribution results in equilibrium. In particular, each species of solute may have a different concentration profile if one chooses the field appropriately. Since solute particles near the channel wall are subject to slower flowing fluid than particles near the centre, samples with a mean height close to the accumulation wall are carried along with a slower average velocity than samples with a larger mean height.

5.1.1 Literature Review

The simplicity of FFF disguises the flexibility of the technique. FFF has been used to separate particles as small as 1 nm [154–157] and as large as 100 μm [158, 159]. This makes it a versatile size-separation method that has been used to analyze a wide variety of analytes including macromolecules [160–164] such as starches [165] and tannins [166], microorganisms such as bacteria [167], erythroleukemia cells [168–171] and viral structures [172], environmental particulates [173], nanoparticles [174] and a host of other colloids [173, 175–177].

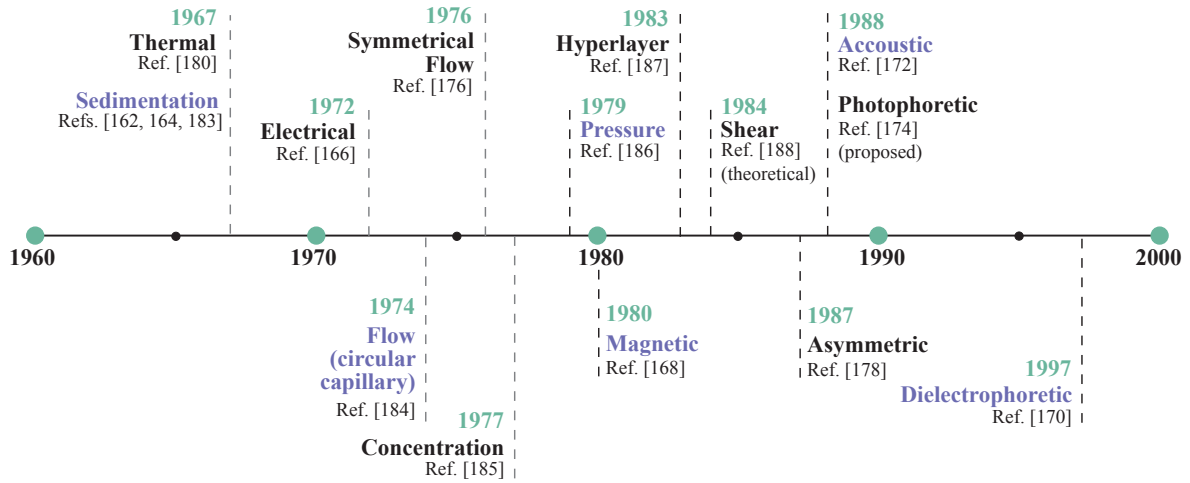


Figure 5.1.2: Historical development of the family of FFF subtechniques. The subtechniques of thermal [196], sedimentation [178, 180, 199], electrical [182], flow (circular capillary) [200], symmetrical flow [192], concentration [201], pressure [202], magnetic [184], hyperlayer [203], shear [204], asymmetric flow [194], acoustic [188], photophoretic [190] and dielectrophoretic [186] FFF are included. Those whose first papers involved Calvin Giddings are denoted by bold black text. Please note that shear FFF [204] has not been experimentally demonstrated and that photophoretic FFF was proposed in 1988 by Giddings [190] but was not experimentally demonstrated until 1997 by Kononenko *et al.* [191]. After Cölfen and Antonietti [197].

The flexibility of FFF results from the large assortment of transverse external fields that can be used. Gravitational [178, 179], centrifugal sedimentation [180, 181], electrical [182, 183], magnetic [184, 185], dielectrophoretic [186, 187], acoustic [188, 189], photophoretic [190, 191], cross flow (both symmetrical [192, 193] and asymmetrical [194, 195]) and thermal [163, 196] fields have all been used to generate non-uniform solute concentration distributions. Sedimentation, cross flows (both symmetrical and asymmetrical), and thermal gradients are most commonly used in macroscopic-scale devices, while thermal gradients and electric potentials are often preferable in microfluidic devices. However, electrical, pressure, magnetic, dielectrophoretic, acoustic and photophoretic fields have been demonstrated as acceptable [197, 198].

The family of FFF methods was proposed [205] and pioneered by J. Calvin Giddings [8, 29, 158, 159, 182, 190–192, 194, 196, 201, 203, 204, 206–219] and early work occurred primarily at the University of Utah, although the discovery of the technique appears to have occurred simultaneously elsewhere [178, 180, 199]. The four most commonly used subtechniques were also the first to be developed:

- In 1967, Giddings' first experimental demonstration of FFF utilized a thermal gradient

(thermal-FFF) to separate polystyrene particles (Figure 5.1.2) [196].

- In the same year, sedimentation-FFF was performed on *E. coli* bacteriophages (Figure 5.1.2) [178, 180, 199].
- Five years later in 1972, electrical-FFF was first demonstrated to fractionate populations of proteins [182].
- The first flow-FFF experiments were reported in 1974 on dextran, diluted human plasma and CuSO_4 colloids [200]. This work utilized a cylindrical capillary as an elution channel. In 1976, flow-FFF was performed and characterized in a rectangular channel configuration [192], which is the standard configuration in modern apparatus.

The experimental demonstration of other fields came later, though they have found less widespread utility. The timeline showing the chronological development of various subtechniques within the FFF family is shown in Figure 5.1.2. Field-flow fractionation has been excellently reviewed many, many times in the literature [156, 157, 160, 162, 177, 197, 198, 214, 220–228] and is the sole subject of entire books [229–231]. The work presented in this thesis focuses on the consequences of performing FFF in microfluidic chips.

The typical dimensions for traditional, macroscopic field-flow fractionation channels are a length of $L \sim 25 - 100$ cm, a breadth of $w \sim 2$ cm and a channel height (or *spacer height*) of $h \sim 100 - 1000$ μm [156, 198]. Sub-millimeter spacer heights are quite common and the further miniaturization of FFF techniques for wide-spread use in microfluidic devices appears tenable. Some consequences of using microfluidic channels as FFF devices have been discussed in the literature [215, 224, 232]. Initial discussions centred on the role of channel dimensions in FFF [215]. In ideal situations, efficiency and speed are independent of channel height, while selectivity is reduced with decreased channel height. At the time of these initial discussions, there seemed to be a consensus that there was no immediate advantage to decreasing channel size.

Work on miniaturization of FFF halted for a half decade until research by Bruce Gale led to its renewal [183, 224, 233–238]. It was pointed out that a subset of FFF techniques do in fact benefit from miniaturization. Electrical- [233, 236], dielectrophoretic- [186, 239], and thermal- [235, 240] FFF appear particularly amenable to micro- and nanofluidic devices [224, 238]. In particular, the resolution of electrical-FFF is increased as channel height is decreased, allowing for shorter analysis times in microchannels [234]. Improvements to both the resolution and the separation efficiency have been quantitatively demonstrated [237]. It

is now recognized that in practice miniaturization entails both a decrease in channel height and also length, which can result in higher resolution [237]. Furthermore, the reduction in channel volume automatically causes low sample consumption, and shorter elution channels lead to shorter analysis times resulting in lower analysis costs [157].

However, miniaturization must be approached with caution. Effects due to miniaturization can be subtle and easily overlooked.

Experimentalists seeking to reap the benefits of using microfluidic channels in FFF apparatus must recognize that, depending on sample and chosen channel height, the size of solute particles may vary from relatively small to large and confined. Although this does not necessarily preclude analytic interpretation of resulting chromatograms, it does require a unified retention theory that can predict the fractionation for samples of everything from the tiniest tracer particles eluting in the *hydrodynamic chromatography* (HC) limit of FFF to the largest beads that fit snugly in the channel. In practice, a microscopic system size is needed for these two limits to be achieved in a single device.

5.1.2 1D Elution Model

As is emphasized in § 2.2, the convection-diffusion transport equation (Equation (2.48)) generally describes all chromatographic separation. In preparation for a FFF retention theory, it will now be reduced to a form appropriate for the elution of a distribution of solutes through a long channel. This section will show how the full convection-diffusion transport equation reduces to a more straightforward model that is effectively 1D.

Consider a localized distribution of solutes. Because the solutes are not evenly distributed the system is not in equilibrium (§ 2.1.1), but in the absence of a strong flow or large concentration gradient, the ensemble of solutes is likely in **quasi-equilibrium** (§ 2.1.2). Quasi-equilibrium is an acceptable assumption if the following conditions are met:

1. A moving reference frame can be found in which the concentration distribution $n(\vec{x}, t)$ appears to have no convective motion. The **reference frame that moves with the solute zone** is $x' = x - \langle \mathcal{V} \rangle t$ where $\langle \mathcal{V} \rangle$ is the average velocity of solutes through a plane

$$\langle \mathcal{V} \rangle = \frac{\langle nu \rangle}{\langle n \rangle}, \quad (5.1)$$

which is in the axial direction $\hat{x}$ and where $\langle \cdot \rangle$ denotes the average over the channel height. There may still be a residual flow field $u'(y) = u(y) - \mathcal{V}$ but its mean is zero by construction.

2. The **concentration varies gradually** in the direction of the flow ($\hat{x}$ -direction) such that the axial diffusion is insignificant compared to the convection of the whole zone (this is a large Péclet assumption in the axial direction [§ 2.2.3]). If the concentration gradients are small then the concentration evolves slowly in the moving reference frame and at any given instant the distribution appears to be in an appropriate steady-state ($\partial n / \partial t \approx 0$). Under this assumption, the $\hat{x}$ -component of the convection-diffusion becomes

$$\mathcal{D} \frac{\partial^2 n}{\partial x^2} \approx 0. \quad (5.2)$$

Technically, the concentration distribution $n(\vec{x}, t)$ is a function of all dimensions and time, but these assumptions about the behaviour in the axial direction lead to a significant simplification.

In order to demonstrate the point, first consider elution in the absence of an external field. In the moving reference frame and in the absence of an external force, the full convection-diffusion equation (Equation (2.48)) is reduced to simply

$$\frac{\partial^2 n}{\partial y^2} \approx \frac{u'}{\mathcal{D}} \frac{\partial n}{\partial x'}.$$

This equation can become useful if the gradient of the concentration $\partial n / \partial x'$ can be approximated. All of the flows of interest to this work can be expressible as a dimensionless polynomial of order m ($u' = \langle u \rangle P_m(\tilde{y}, \mathbf{a})$) where $\mathbf{a}$ is the set of coefficients belonging to the polynomial. In fact, they are all quadratic $m = 2$ but writing them in this general form is meant to emphasize the universality of the following conclusion and that the values of the coefficients $\mathbf{a}$ are not important to the point being made. Using this form, the concentration profile can be evaluated and expressed in terms of the average concentration at some point along the elution channel

$$n = \langle n \rangle + \left(\frac{\langle u \rangle h^2}{\mathcal{D}} \frac{\partial n}{\partial x'} \right) P'_{m+2}(\tilde{y}, \mathbf{b}).$$

The values of the new set of coefficients $\mathbf{b}$ in the new polynomial P'_{m+2} are unimportant

because, regardless of the polynomial, this form for the concentration immediately demonstrates that

$$\begin{aligned}\frac{\partial n}{\partial x'} &= \frac{\partial}{\partial x'} \left[\langle n \rangle + \left(\frac{\langle u \rangle h^2}{\mathcal{D}} \frac{\partial n}{\partial x'} \right) P'_{m+2}(\tilde{y}, \mathbf{b}) \right] \\ &\approx \frac{\partial \langle n \rangle}{\partial x'}\end{aligned}\tag{5.3}$$

since the curvature of the concentration is already assumed to be small (Equation (5.2)). So what has been demonstrated? Simply that at any point along the elution channel, the slope of the concentration is approximately the average over all points on the plane. This rather unassuming result underpins retention theory in chromatographic separation techniques because it essentially reduces a complicated multi-dimensional problem to a tractable, 1D problem. It allows the *average flux* through the plane to be determined to be

$$\langle j_{\langle n \rangle} \rangle = \frac{1}{h} \int_0^h n u' dy = -\frac{1}{n_o} \left(\frac{\langle u \rangle^2 h^2}{\mathcal{D}} \right) \frac{\partial \langle n \rangle}{\partial x'}.\tag{5.4}$$

The average flux depends on the average divergence and a dimensionless constant of order $n_o \sim 50$ that depends only on the specific flow profile.

Recognizable within Equation (5.4) is a Péclet number:

$$\langle j_{\langle n \rangle} \rangle = \left(\mathcal{D} \frac{\text{Pé}_{\parallel}^2}{n_o} \right) \frac{\partial \langle n \rangle}{\partial x'}.\tag{5.5}$$

This form reveals that the average flux takes the same form as the flux did in § 2.2.1 (Equation (2.40)) but has an **effective** dispersion coefficient $\mathcal{D}_{\text{eff}} \equiv \mathcal{D} \text{Pé}_{\parallel}^2 / n_o$ due to convective transport. When the molecular flux is non-negligible then the effective dispersion coefficient should be a superposition of both molecular and transport-caused dispersion *i.e.*

$$\mathcal{D}_{\text{eff}} \equiv \mathcal{D} \left(1 + \text{Pé}_{\parallel}^2 / n_o \right).\tag{5.6}$$

This is the **Taylor-Aris dispersion coefficient** which has been derived for many specific flow profiles [26, 241]. It can be interpreted to mean that in a reference frame that moves with the solutes, the average elution looks just as it would for a 1D flux, except with an enhanced diffusion coefficient due to shear.

What should be concluded from this discussion is that a transport theory for quasi-equilibrium elution processes need not worry about the full convection-diffusion equation

(Equation (2.48)) but rather, a retention theory about the mean elution of the solutes can be constructed. To return to the laboratory frame of reference from the moving reference frame, the material derivative of the averaged concentration must balance the divergence of the average flux:

$$\frac{\partial \langle n \rangle}{\partial t} + \langle \mathcal{V} \rangle \frac{\partial \langle n \rangle}{\partial x} = \mathcal{D}_{\text{eff}} \frac{\partial^2 \langle n \rangle}{\partial x^2}. \quad (5.7)$$

Thus the elution of a distribution of solutes has been reduced to 1D transport with an effective solute velocity and dispersion coefficient [242].

Since experimental conditions can vary, this average solute velocity $\langle \mathcal{V} \rangle$ (Equation (5.1)) should be compared to the average velocity of the solute carrier fluid, $\langle u \rangle$. The *retention ratio* R is defined as the ratio of these two mean velocities. Since both elute through the same length of channel, the ratio of the mean time it takes the solute to elute (called the *retention time*) and the time it takes the solvent to elute (called the *void time*) is equivalent to the ratio of mean velocities as

$$R = \frac{t_o}{t_R} = \frac{\langle \mathcal{V} \rangle}{\langle u \rangle} = \frac{\langle nu \rangle}{\langle n \rangle \langle u \rangle}. \quad (5.8)$$

In a very real sense the retention ratio is the linear transport coefficient (§ 2.1.2) relating the resulting elution velocity of an ensemble of solutes to the applied driving velocity. As such, the remainder of much of this chapter and the next will be spent developing accurate theories for R in microfluidic systems and engineering strategies to control the retention ratio.

This analysis did not include a transverse, external field. However, analyses have been performed proceeding in the same manner as above that form the basis of non-equilibrium assessments of band broadening in FFF [210, 242–244]. The conclusions are qualitatively the same but the effective dispersion coefficient differs slightly in value from the Taylor-Aris dispersion coefficient for the same profile [244].

5.1.3 Normal-Mode FFF

From Equation (5.7) the mean transport or elution of solutes through a channel is given by $\langle \mathcal{V} \rangle$, the average solute velocity that is better discussed in terms of the retention ratio R (Equation (5.8)). The retention ratio is set by a convolution of the velocity profile of the solvent and of the concentration distribution of the solutes. When the solutes are assumed to

be point-like particles subject to a strong transverse field, the regime of FFF (or *operational-mode*) is called *normal-mode FFF*.

Consider a distribution $n(\vec{x}, t)$ of point-like particles in a channel of height h . Some constant external force $\vec{f} = f\hat{y}$ acts on the solutes, pushing them against the accumulation wall (away from the *depletion wall*). Hereafter $\tilde{\cdot}$ denotes scaling by channel height, h such that the normalized distance from the accumulation wall is written $\tilde{y} = y/h$ and particle radii is $\tilde{r} = r/h$, etc. .

Concentration Distribution

The ensemble of solutes in quasi-equilibrium has been argued to elute in an essentially 1D manner in the $\hat{x}$ -direction (§ 5.1.2). However, the perpendicular external force $f\hat{y}$ creates a non-uniform concentration distribution in the yz -plane. In the moving reference frame, the elution zone (or band) is approximately in a steady-state. This means that the $\hat{y}$ -component of the convection-diffusion equation is quite nearly the equilibrium equation. Therefore, in each translating plane of the moving reference frame, the solution of the convection-diffusion equation is the Boltzmann distribution:

$$n(\vec{x}, t) \approx n_0(x) \exp\left(\frac{fy}{k_B T}\right) = n_0(x) \exp\left(-\frac{\tilde{y}}{\lambda}\right), \quad (5.9)$$

where $n_0(x)$ is the concentration of solute at the accumulation wall, $\tilde{y} = 0$, at any point x along the length of the channel. The *retention parameter* λ is strictly defined as the ratio between the thermal and potential energy scales

$$\lambda \equiv \frac{k_B T}{fh} = \frac{1}{\text{Pé}_\perp}. \quad (5.10)$$

This (inverse) lateral Péclet has special importance in FFF processes since it controls the average distance of solutes from the accumulation wall, which in turn determines the elution speed.

Velocity Profile

In a microfluidic device, the Reynolds number is small ($\text{Re} \ll 1$) so the flow is described by the Stokes equation (Equation (2.64)) rather than the full Navier-Stokes equation (Equation (2.59)). If the channel width is large compared to the height (as is commonly the case in

FFF apparatus) then the Stokes equation is solved in 2D with no-slip boundary conditions to find the Poiseuille equation (Equation (3.27))

$$u(\tilde{y}) = 6 \langle u \rangle (\tilde{y} - \tilde{y}^2),$$

which is written in terms of the average velocity of the solvent $\langle u \rangle$ and the normalized distance from the accumulation wall $\tilde{y} = y/h$. Because of its shape, the Poiseuille flow is often called parabolic flow.

Complications can occur that perturb the flow profile from this ideal form. The external perpendicular field used to establish the concentration distribution (Equation (5.9)) can distort the flow (as occurs when the thermal-gradient in thermal-FFF causes the viscosity of the carrier fluid to change, and so tends to skew the velocity profile [197, 245, 246]). Slip at the walls is discussed in § 7.3. Another complication is the finite breadth of the channel, which is discussed in § 7.4.

Retention Theory

Because a 1D model is utilized, the mean concentration height for point particles can be calculated using Equation (5.9):

$$\langle \tilde{y} \rangle = \frac{\langle \tilde{y}n \rangle}{\langle n \rangle} = \lambda + \frac{1}{1 - \exp(-1/\lambda)}. \quad (5.11a)$$

The limits of the mean height of the solutes from the accumulation wall (Equation (5.11a)) can be found:

- In the strong force limit, the potential energy drop dominates over the thermal energy and so the retention parameter λ (Equation (5.10)) becomes small and the mean height (Equation (5.11a)) reduces to

$$\langle \tilde{y} \rangle \simeq \lambda - \exp(-1/\lambda) \simeq \lambda. \quad (5.11b)$$

In this limit, λ should be interpreted as the dimensionless length scale of the concentration distribution. Qualitatively, the solutes can be envisioned to reside within a thin layer $\langle y \rangle = h\lambda$ from the accumulation wall. As the force increases, this layer decreases and the solutes reside closer to the wall (Figure 5.1.3). They are therefore subject to slower moving carrier fluid than if the layer extended further out into the channel.

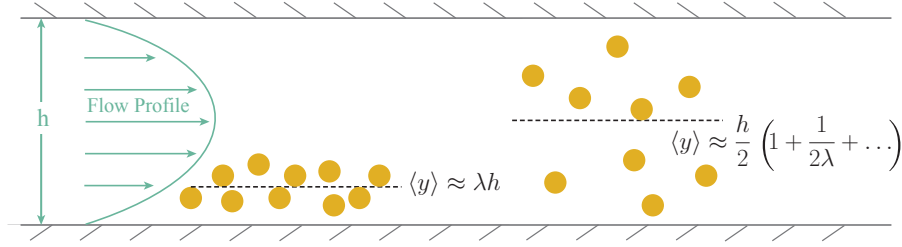


Figure 5.1.3: The two limiting behaviours of the concentration distribution. In strong forces, the solutes are qualitatively confined to a film of thickness $\langle y \rangle = \lambda h$ (Equation (5.11b)). In weak fields, the distribution is perturbed from being homogeneous according to Equation (5.11c).

- In the negligible force limit, λ is large and expanding the exponential in Equation (5.11a) to third order leads to

$$\langle \tilde{y} \rangle \simeq \frac{1}{2} \left[1 - \frac{1}{2\lambda} + \frac{1}{4\lambda^2} + \dots \right] \simeq \frac{1}{2}. \quad (5.11c)$$

This is expected for a uniform concentration in which the solutes easily diffuse across the entire height of the channel (Figure 5.1.3).

In much the same way as was done for $\langle \tilde{y} \rangle$, an analytic expression for the retention ratio is found by evaluating the averages in Equation (5.8). The resulting retention ratio is, by construction, independent of flow rate:

$$R(\lambda) = 6\lambda \mathcal{L} \left(\frac{1}{2\lambda} \right) \quad (5.12)$$

where $\mathcal{L}(x) = \coth(x) - 1/x$ is the *Langevin function*. Equation (5.12) is very versatile for parameterizing retention [197, 198] and characterizes what is called **normal-mode FFF**.

Different populations of point-like solutes have different retention parameters λ and so elute with different retention times $t_R = t_o/R$ (Equation (5.8)). When the force is strong, the retention parameter is small $\lambda \ll 1$ and the Langevin function approaches unity. In this limit the retention ratio for normal-mode FFF of point-like particles in a strong field reduces to the commonly encountered [198] form

$$R \approx 6\lambda. \quad (5.13)$$

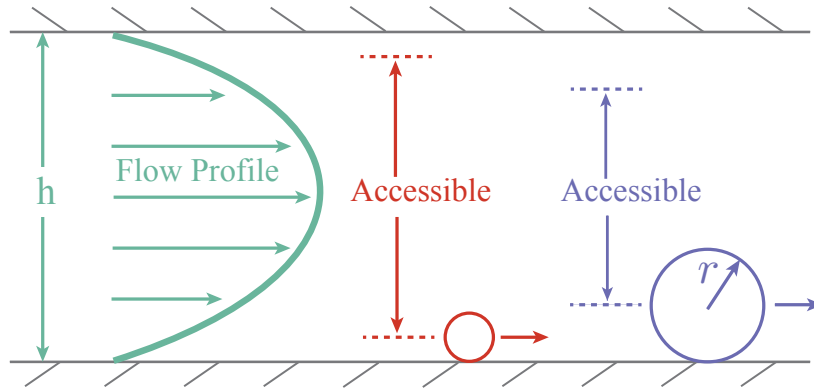


Figure 5.1.4: Schematic representation of steric-mode FFF. When hard, finite-sized solutes undergo elution, they are excluded from a region near the wall where the fluid velocity is low. As a result, they tend to elute earlier than smaller particles.

5.1.4 Steric-Mode FFF

In microfluidic devices the assumption that solutes are point-like is suspect. The primary effect of finite sizes is that steric interactions with the channel walls must be accounted for.

The steric interaction of finite-size solutes with the accumulation wall creates an *excluded region* (Figure 5.1.4). Only $1 - 2\tilde{r}$ of the channel is accessible to the centre of spherical particles. To include the effects of steric repulsion from the wall, the integration limits on the profiles used to determine the retention ratio must be altered. Steric effects are handled by stating that $n(\tilde{y})$ is the concentration profile of the centre of masses of the spherical particles. It is assumed that:

- The solutes are ideally infinitely hard, spherical colloids that do not have friction interactions with the accumulation wall or have any hydrodynamic complications.
- The friction coefficients of the colloids are assumed to be well approximated by Stokes' law despite the fact that they move near channel walls and not through an infinite, free solution. This assumption is improved upon in § 7.1.
- The flow field is assumed to be slow (low particle Reynolds number and insignificant inertial contributions) [218, 247, 248]. This assumption is addressed in § 7.2.
- The flow is relatively unperturbed by the presence of the solute (infinitely wide channels and dilute solutions) [249, 250]. Although theoretical results in this work will always

assume that the flow profile is unperturbed and infinitely dilute samples, finite channel width is accounted for in § 7.4.

- For the time being, the velocity of the solutes is assumed to be the velocity of the fluid at the centre of mass of the particle. This assumption is rejected in § 5.3.

The variable n_0 in the plane of the zone is redefined as the concentration at the lowest point, *i.e.* a distance $\tilde{r}$ from the accumulation wall, so that the concentration profile becomes

$$n(\tilde{y}) = \begin{cases} n_0 \exp [-(\tilde{y} - \tilde{r}) / \lambda] & \text{for } \tilde{r} < \tilde{y} < 1 - \tilde{r} \\ 0 & \text{otherwise.} \end{cases} \quad (5.14)$$

The concentration distribution controls the mean height

$$\langle \tilde{y} \rangle = \lambda + \frac{1}{1 - e^{(1-2\tilde{r})/\lambda}} + \tilde{r} \coth \left(\frac{1 - 2\tilde{r}}{2\lambda} \right), \quad (5.15a)$$

which is similar to Equation (5.11a) except that there is a correction term due to the finite size of the solutes. The two limits are now given by

$$\langle \tilde{y} \rangle \simeq \begin{cases} \tilde{r} + \lambda - \exp [-(1 - 2\tilde{r}) / \lambda] & \lambda \ll 1 \\ \frac{1}{2} - \left(\frac{1 - 2\tilde{r}}{4\lambda} \right) \left(1 - \frac{2\tilde{r}}{3} \right) \simeq \frac{1}{2} & \lambda \gg 1. \end{cases} \quad (5.15b)$$

$$(5.15c)$$

First note that Equation (5.15c) agrees with Equation (5.11c) when $\tilde{r} \rightarrow 0$, as it should. For the very strongest forces (the small retention parameter λ case of Equation (5.15b)), solute particles are pushed right against the wall and so the mean concentration height is expected to grow linearly with the particle size $\tilde{r}$. This linear relationship is evident in the mean concentration height for fixed, small values of λ (Figure 5.1.5). For relatively strong fields, the average remains linear but an intercept develops:

$$\langle \tilde{y} \rangle \simeq \tilde{r} + \lambda. \quad (5.16)$$

For larger values of λ (weak forces) the solute particles can to diffuse further from the accumulation wall and the non-linear terms in Equation (5.15a) become apparent.

As a result of steric-interactions shifting the mean particle height $\langle \tilde{y} \rangle$ towards the centre of the channel, the mechanism for separation changes to that of *steric-mode FFF* and the

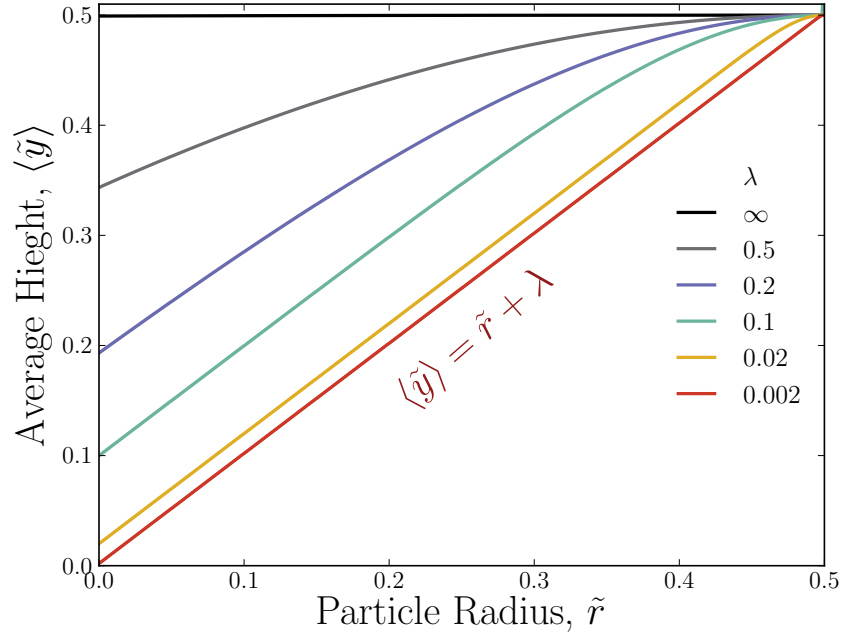


Figure 5.1.5: Mean concentration height for a variety of retention parameters varying from no force ($\lambda = \infty$) to a relatively large forces ($\lambda = 0.002$). The mean concentration height grows with particle size $\tilde{r}$ when $\lambda \ll 1$.

retention ratio becomes

$$R(\tilde{r}, \lambda) = 6\lambda[1 - 2\tilde{r}] \mathcal{L}\left(\frac{1 - 2\tilde{r}}{2\lambda}\right) + \mathcal{F}_p, \quad (5.17)$$

which is strikingly similar to the form for normal-mode FFF (Equation (5.12)). The fraction of the channel accessible to the particle, $1 - 2\tilde{r}$, appears in Equation (5.17) while in Equation (5.12) it is unity. As expected, steric interactions effectively reduce the channel size. However, they do so without altering the flow profile, so beyond this effective reduction of channel height steric effects lead to additional changes to the behaviour of the retention ratio. For later convenience, these effects are encapsulated into the new term

$$\mathcal{F}_p(\tilde{r}) = 6\tilde{r}(1 - \tilde{r}). \quad (5.18)$$

This result is in agreement with the commonly accepted form [158, 211, 251]. Retention curves as a function of particle size are given for various retention parameters λ in Figure 5.1.6. The point particle retention ratio (Equation (5.12)) is the $\tilde{r} \rightarrow 0$ limit of Equation (5.17) and so gives the points on the R -intercept of Figure 5.1.6.

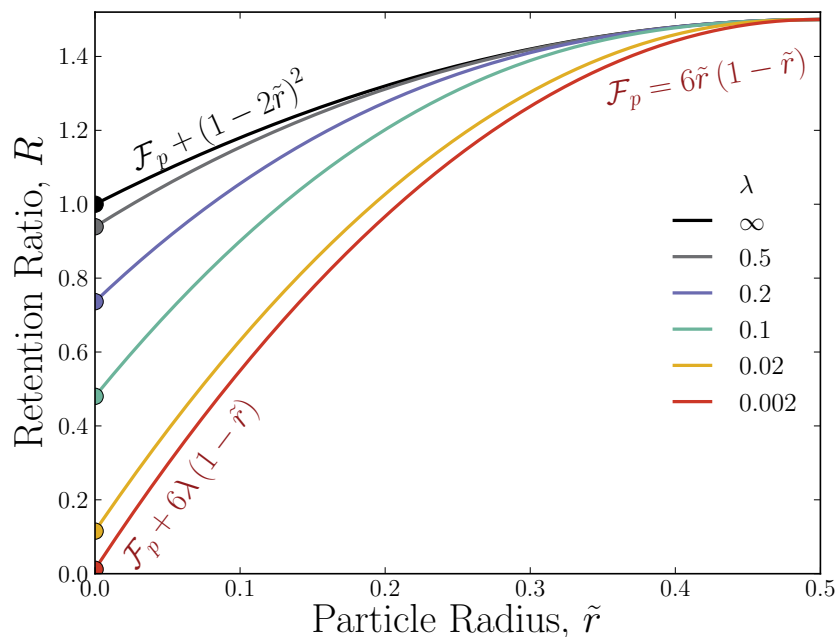


Figure 5.1.6: Retention ratio for a variety of retention parameters varying from no force ($\lambda = \infty$) to relatively large forces ($\lambda = 0.002$). Solid lines denote Equation (5.17) and closed circles on the R -axis represent the values predicted by Equation (5.12).

Each line in Figure 5.1.6 corresponds to a different λ , held fixed regardless of particle size (the explicit size dependence of λ is treated in § 5.2). Even if λ is held fixed, with no size dependence, the steric-mode retention ratio (Equation (5.17)) demonstrates that particles of different sizes can be separated. Historically [211], λ is kept fixed and only the explicit size effects in the retention ratio (Equation (5.17)) are included, as is done in Figure 5.1.6.

At first it may appear odd that the retention ratio can be larger than unity, since this indicates that the dispersed solutes are actually eluting faster on average than the carrier fluid itself is flowing. This is possible specifically because the solutes' excluded region is the near-wall region where the flow is slowest *i.e.* the accessible region is the region of fastest flowing fluid. In the absence of an external force ($\lambda \rightarrow \infty$) the retention ratio is always greater than unity for a finite sized particle.

5.1.5 Hydrodynamic Chromatography Limit

The present size dependence of Equation (5.17) has an interesting consequence for the **zero force** limit of $\lambda \rightarrow \infty$. Even in the absence of a field, steric interactions allow fractionation

as seen by the large λ curves in Figure 5.1.6. The $\lambda \gg 1$ limit represents vanishingly small forces and corresponds to the *hydrodynamic chromatography limit of FFF* [197, 212, 252] in which the steric mode of separation results in retention even when the field is zero. Since there is no force towards the accumulation wall, the particles have a uniform concentration distribution across the channel (outside of their exclusion region) with a mean height of $\langle \tilde{y} \rangle = 1/2$. Hydrodynamic chromatography separates particles by excluding them from regions of slow moving solvent near the wall.

In the limit $\lambda \rightarrow \infty$, the retention ratio (Equation (5.17)) reduces to

$$R = \mathcal{F}_p + (1 - 2\tilde{r})^2 = 1 + 2\tilde{r} - 2\tilde{r}^2. \quad (5.19)$$

This form agrees with what is commonly written in the hydrodynamic chromatography literature [253, 254]. In terms of the language used here, the hydrodynamic chromatography community writes

$$R_{\text{HC}} = 1 + b_{\text{HC}}\tilde{r} - c_{\text{HC}}\tilde{r}^2. \quad (5.20)$$

The value of b_{HC} is ideally expected to be 2 for HC in both channels and capillaries [255, 256], which agrees with Equation (5.19). Various values of c_{HC} have been proposed for hydrodynamic chromatography in different systems (primarily capillary tube [253]) and different solutes, such as polymers and colloids. In hydrodynamic chromatography in microfluidic rectangular channels [257–259], the constant is expected to be $c_{\text{HC}} \approx 2.72$ for polymers and $c_{\text{HC}} = 4$ for hard colloids [255, 258]. The simple form of Equation (5.20) is close to these values, though $c_{\text{HC}} = 2$ is lower than the hard colloid value of 4. Improvements are made to Equation (5.20) in § 5.3 and § 7.4.

In Figure 5.1.6, cases of $\lambda \geq 1$ well approximate the hydrodynamic chromatography limit (Equation (5.20)). However, the hydrodynamic chromatography limit is only one of four specific limits of Equation (5.17). The four possible limits are:

$$R \simeq \begin{cases} 6\lambda\mathcal{L}\left(\frac{1}{2\lambda}\right) & \tilde{r} \rightarrow 0 \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \tilde{r} \rightarrow \frac{1}{2} \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \lambda \gg 1 \\ \mathcal{F}_p + 6\lambda(1 - 2\tilde{r}) - 12\lambda^2 & \lambda \ll 1. \end{cases} \quad \begin{array}{l} (5.21a) \\ (5.21b) \\ (5.21c) \\ (5.21d) \end{array}$$

In the limit $\tilde{r} \rightarrow 0$, Equation (5.17) reduces to Equation (5.12), the point particle prediction.

Interestingly, the hydrodynamic chromatography result appears when $\lambda \gg 1$, as was found before, but is now also seen when $\tilde{r} \rightarrow 1/2$. This reflects the fact that as the particle size approaches the channel height steric effects will always dominate over the external field. The fourth limit is the strong force limit. The steric function $\mathcal{F}_p$ is present, but so is λ — neither can be neglected in the finite $\tilde{r}$ and large λ limit. Each of these limits is marked in Figure 5.1.6. The point particle limit agrees with the $\tilde{r}$ -intercept and is marked by closed circles, while the equations for hydrodynamic chromatography and strong forces are noted in Figure 5.1.6.

5.2 Unified, Ideal Retention Theory

The previous section introduced a zoo of field-flow fractionation regimes or operational modes:

- **normal-mode FFF** (Equation (5.12)) for point particles,
- **steric-mode FFF** (Equation (5.17)) for finite sized solutes and
- **hydrodynamic chromatography** (Equation (5.20)) for solutes in the absence of an external field.

In normal-mode FFF, smaller solutes elute before larger solutes. In steric-mode FFF and hydrodynamic chromatography the order is inverted with large solutes eluting more quickly. While the steric-mode and hydrodynamic chromatography retention ratios have explicit size dependence, the normal-mode R does not. So how are solutes separated in normal-mode FFF? The answer is, of course, that the retention parameter can have an implicit size dependence through the force.

The following section **unifies** all three operational modes by writing a retention theory that has no implicit size dependence. The resulting unified ideal retention theory is able to quantitatively predict transitions and generate operational-mode diagrams. This is necessary for microfluidic FFF apparatus because mixed samples are more likely to span multiple operational modes. They, therefore, may have differing elution orders, which leads to non-monotonicity that confuses separation unless retention theory can adequately account for it. However, this section is not explicitly concerned with micro- and nano-scopic channel heights. Rather, it extends the traditional theory of Giddings [158, 206, 211] into operation regimes that at one time were quixotic but in today's age of microfluidics and nanotechnology are within reach. The same simplistic view of the FFF process is maintained as in the previous section. The theory is **ideal** in the sense that the flow field is assumed to be slow and unperturbed and particles' interactions with the wall are assumed to be solely steric, without frictional or hydrodynamic interactions. Similarly, the theory neglects changes to the friction coefficients of the particles caused by the motion of nearby solutes (see § 2.3.5). This requires that the concentration is quite dilute since hydrodynamic interactions are long-range (though the channel walls certainly screen interactions on length scales comparable to the distance between the walls). Nonideal concentration effects have been considered previously in the literature [249]. Furthermore, complicating effects such as particle slip, non-parabolic solvent flow and lift are intently ignored in this chapter [260].

FFF-Method	f	α	Λ
Sedimentation	$\frac{4\pi}{3}G\Delta\rho r^3$	3	$\frac{3}{4\pi}\frac{k_B T}{G\Delta\rho h^4}$
Flow	$6\pi\eta\frac{hV_c}{V_0}r$	1	$\frac{k_B T}{6\pi\eta h^3}\frac{V_0}{V_c}$
Thermal	$6\pi\eta\frac{\mathcal{D}_T\Delta T}{h}r$	1	$\frac{k_B T}{6\pi\eta\mathcal{D}_T\Delta T}$
Magnetic	$\frac{4\pi}{3}\varrho\chi_m H_m\Delta H_m r^3$	3	$\frac{3}{4\pi}\frac{k_B T}{h^4\varrho\chi_m H_m\Delta H_m}$
Electrical	$6\pi\eta\mu_{el}E\mathcal{D}$	1	$\frac{k_B T}{6\pi\eta\mu_{el}Eh^2}$

Table 5.2.1: External forces f (with corresponding scaling exponents α and device retention parameters Λ) for some FFF techniques. Variable names are the same as used by Cölfen and Antonietti in Table 1 of Ref. [197] with the notable exceptions that here the particle size is r , the centrifugal acceleration is G and the Stokes-Einstein equation $\mathcal{D} = k_B T / 6\pi\eta r$ (Equation (2.91)) is used.

5.2.1 Device Retention Parameter

Although the theory presented so far has treated the retention ratio as a function of particle size and retention parameter, the retention parameter is implicitly size dependent. For instance, in sedimentation-FFF it is generally appropriate to assume a constant solute mass density such that the gravitational force f goes as the volume $\sim r^3$ of the spherical particle or $\lambda \sim f^{-1} \sim r^{-3}$. To consider this in a general manner, let the force scale as the particle radius r to some power α such that the retention parameter can be rewritten

$$\lambda = \frac{k_B T}{c_f r^\alpha h} = \frac{k_B T}{c_f h^{1+\alpha}} \tilde{r}^{-\alpha} \equiv \Lambda \tilde{r}^{-\alpha}, \quad (5.22)$$

where the normalized size dependence $\tilde{r}^\alpha$ is extracted from both the force ($f \equiv c_f r^\alpha$) and the retention parameter λ . The resulting dimensionless number, Λ , characterizes the FFF apparatus itself (for this reason let it be called the *device retention parameter*). The device retention parameters and powers α for a few example external forces are given in Table 5.2.1.

In § 5.1.3, the normal-mode retention ratio had only implicit size dependence. By inserting $\lambda = \Lambda \tilde{r}^{-\alpha}$ into the retention ratio (Equation (5.12)) the point particle theory of normal-mode FFF predicts that small particles elute before large particles do (Figure 5.2.1). The monotonic decrease in elution time with particle size remains true for all device retention parameters Λ and all α -scaling. The magnitude of the slope increases as Λ is reduced (Figure 5.2.1), which is indicative of why the resolution increases with external force.

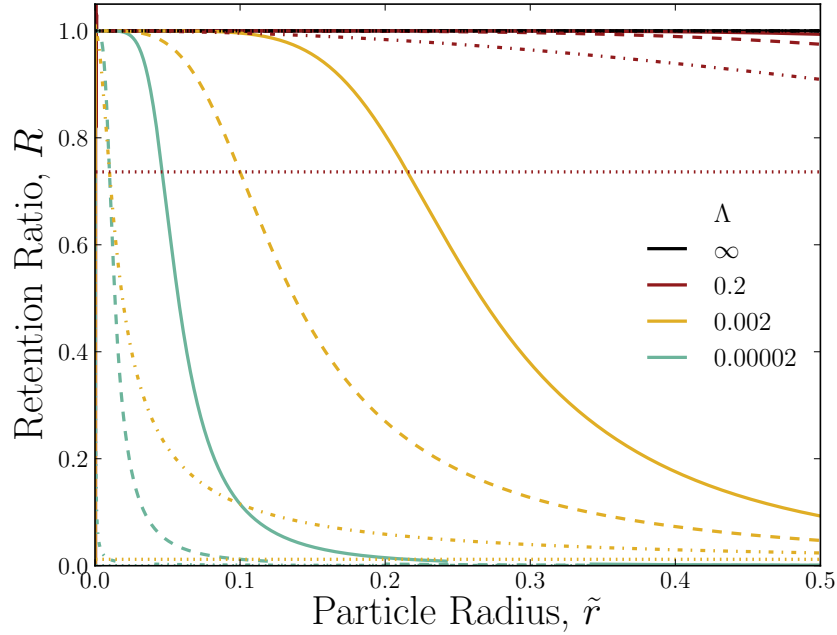


Figure 5.2.1: Retention ratio $R(\tilde{r})$ as predicted by the standard point particle theory for normal-mode FFF (Equation (5.12)). The retention parameter λ is assumed to vary like $\lambda = \Lambda \tilde{r}^{-\alpha}$ but the particle size is otherwise not taken into account. Solid lines denote $\alpha = 3$, dashed $\alpha = 2$, dash-dot $\alpha = 1$ and dotted lines denote $\alpha = 0$ (all of which are horizontal). Small particles elute first except when $\alpha = 0$ where retention time is not a function of particle size.

5.2.2 Unified Ideal Retention Ratio

The retention theory that includes steric interactions but is for a fixed retention parameter λ is reviewed in § 5.1.4. This is the manner in which the situation is generally framed in the literature [158]; however, as is broached in § 5.2.1, it is far more reasonable to allow the force to change with particle size. Now both steric effects and size dependent forces are incorporated into a unified description by substituting $\lambda = \Lambda \tilde{r}^{-\alpha}$ (Equation (5.22)) into the retention ratio that accounts for finite-sized solutes (the results of § 5.1.4). This produces the concentration profile

$$n(\tilde{y}) = \begin{cases} n_0 \exp\left(-\frac{[\tilde{y}-\tilde{r}]\tilde{r}^\alpha}{\Lambda}\right) & \text{for } \tilde{r} < \tilde{y} < 1 - \tilde{r} \\ 0 & \text{otherwise,} \end{cases} \quad (5.23)$$

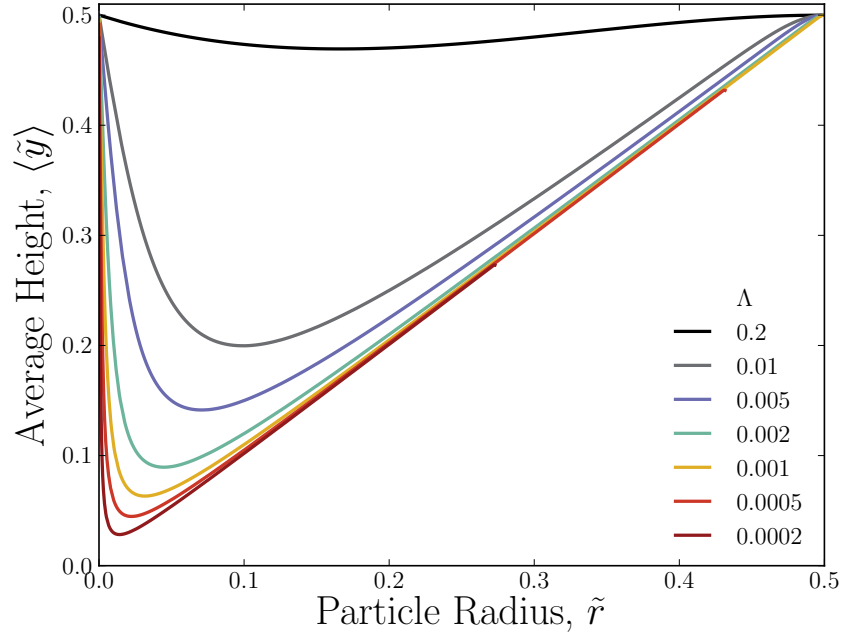


Figure 5.2.2: Average height of solutes from the accumulation wall for $\alpha = 1$. As Λ decreases, the mean concentration height's minimum occurs at smaller and smaller particle sizes.

the average height

$$\langle \tilde{y} \rangle = \Lambda \tilde{r}^{-\alpha} + \frac{1}{1 - e^{(1-2\tilde{r})\tilde{r}^\alpha/\Lambda}} + \tilde{r} \coth \left(\frac{[1 - 2\tilde{r}] \tilde{r}^\alpha}{2\Lambda} \right), \quad (5.24)$$

and the retention ratio

$$R(\tilde{r}, \Lambda) = \frac{6\Lambda}{\tilde{r}^\alpha} [1 - 2\tilde{r}] \mathcal{L} \left(\frac{[1 - 2\tilde{r}] \tilde{r}^\alpha}{2\Lambda} \right) + \mathcal{F}_p(\tilde{r}), \quad (5.25)$$

explicitly in terms of particle size $\tilde{r}$. The steric function $\mathcal{F}_p$ remains unchanged from Equation (5.18), *i.e.* $\mathcal{F}_p = 6\tilde{r}(1 - \tilde{r})$. In these terms, $\alpha = 0$ represents a hypothetical force that is not a function of particle size (as was used in Figure 5.1.6). When $\alpha = 0$, the unified retention theory (Equation (5.25)) reduces to the traditional steric-mode version (Equation (5.17)).

The average height of the solutes from the accumulation wall shows non-monotonicity with a global minimum for all values of Λ (Figure 5.2.3). When Λ is small, the vast majority of particle sizes are above this minimum where the average concentration height increases fairly linearly, $\langle \tilde{y}(\tilde{r}) \rangle \simeq \tilde{r}$. Indeed, in this strong field limit the external force completely

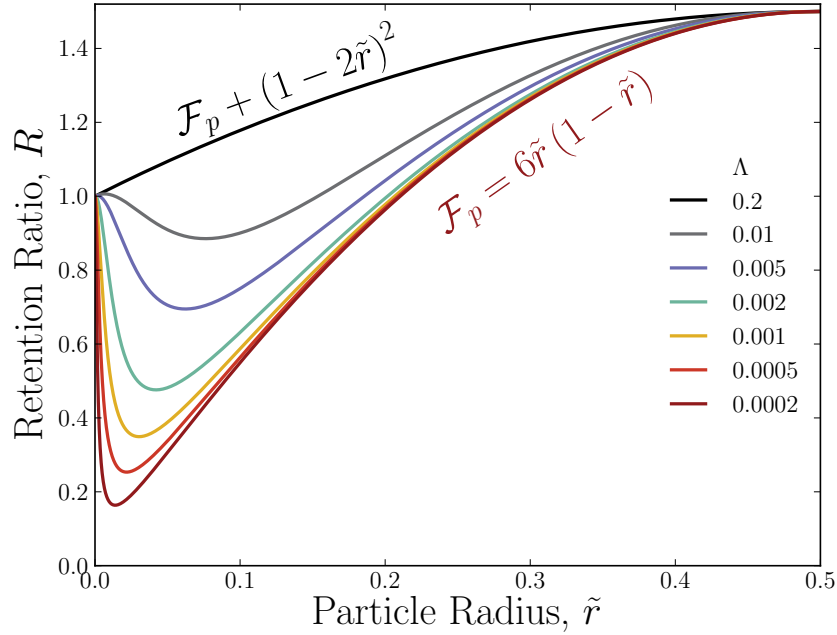


Figure 5.2.3: Retention ratio for $\alpha = 1$. When the force varies as $\tilde{r}^\alpha$, the retention ratio no longer rises in a simple monotonic manner, as it did in Figure 5.1.6 (which was essentially equivalent to $\alpha = 0$). Below the critical device retention parameter $\Lambda_c = 1.90 \times 10^{-2}$, the retention ratio becomes non-monotonic.

dominates over thermal motion and solute particles are pinned to the accumulation wall. The hard steric repulsion ensures that the mean height of the concentration profile grows linearly with $\tilde{r}$, as it does in Figure 5.1.6. The distribution of particle sizes below the minimum results not from the steric exclusion but from the competition between the force and the thermal noise.

The retention ratios for a range of Λ when $\alpha = 1$ are shown in Figure 5.2.3, which is the appropriate scaling for many examples of FFF techniques (most importantly flow-FFF) (Table 5.2.1). Consider the extreme particle sizes in Figure 5.2.3:

- The smallest particle sizes all have a retention ratio approaching $R \rightarrow 1$ regardless of Λ because then Brownian motion dominates over the applied force. Small $\tilde{r}$ solutes constitute *tracer particles* that are able to diffuse across the entire channel and sample the entire solvent velocity profile, resulting in $\langle \tilde{y} \rangle = 1/2$ and $R = 1$ for all Λ . The tracer particles move with the carrier fluid regardless of the strength of the external field applied because the force must go to zero as $\tilde{r} \rightarrow 0$. This behaviour is far more

physical than the small radius behaviour observed in the traditional steric-mode FFF plots (Figure 5.1.6).

- Next, consider the largest particle sizes. Figure 5.2.2 shows that once again the mean height has become $\langle \tilde{y} \rangle = 1/2$ for all Λ because the largest particles fit snugly into the channel, causing the concentration distribution to be sharply distributed about $\tilde{y} = 1/2$. Practically speaking, such large particles will inevitably clog the microfluidic device but ideally this is allowable. As is discussed further in § 5.3, the solvent velocity at the centre point is $v(1/2) = 3\langle v \rangle/2$, which causes the retention ratio to approach $R = 3/2$ (Figure 5.2.3). However, $R = 3/2$ is physically nonsensical even in an ideal sense (disregarding all friction and clogging effects). Even ideally, the speed of such large particles is not well represented. This point is the topic of § 7.1.

5.2.3 Regimes and Transitions

The unified retention theory of § 5.2.2 allows all the operational modes of FFF to be encapsulated into a single equation (Equation (5.25)). Determining the transition between operational modes sets the range over which researchers can be confident that the elution order has not inversed and there is 1 : 1 correspondence between a given retention time and solute size. This is a particularly important task in microfluidic FFF since the ratio of solute size to channel height can easily range from near-zero to near-unity. The transitions can be determined by numerically finding the roots of the derivative of Equation (5.25). Although unwieldy to write, the derivative is

$$\begin{aligned} \frac{\partial R}{\partial \tilde{r}} = & -\alpha \frac{6\Lambda}{\tilde{r}^{1+\alpha}} \left[(1 - 2\tilde{r}) \coth \left((1 - 2\tilde{r}) \frac{\tilde{r}^\alpha}{2\Lambda} \right) - \frac{2\Lambda}{\tilde{r}^\alpha} + (1 - \tilde{r}) \frac{\tilde{r}^{1+\alpha}}{\Lambda} \right] \\ & + \frac{6\Lambda}{\tilde{r}^\alpha} \left[(1 - 2\tilde{r}) \left\{ 1 - \coth^2 \left((1 - 2\tilde{r}) \frac{\tilde{r}^\alpha}{2\Lambda} \right) \right\} \left\{ -\frac{\tilde{r}^\alpha}{\Lambda} + (1 - 2\tilde{r}) \frac{\alpha \tilde{r}^{\alpha-1}}{2\Lambda} \right\} \right. \\ & \left. - 2 \coth \left((1 - 2\tilde{r}) \frac{\tilde{r}^\alpha}{2\Lambda} \right) + 2 \frac{\alpha \Lambda}{\tilde{r}^{\alpha+1}} + (1 + \alpha) (1 - \tilde{r}) \frac{\tilde{r}^\alpha}{\Lambda} - \frac{\tilde{r}^{1+\alpha}}{\Lambda} \right]. \quad (5.26) \end{aligned}$$

A simple bisection method numerically determines the roots, which is enough to determine the transitions.

Positive portions of the slope (Figure 5.2.4) indicate the hydrodynamic chromatography and steric-mode elution order (larger particles eluting before smaller particles), while the negative portion of the slope represents normal mode (for which smaller particles elute before larger particles). A more thorough discussion relating the slope of the retention ratio

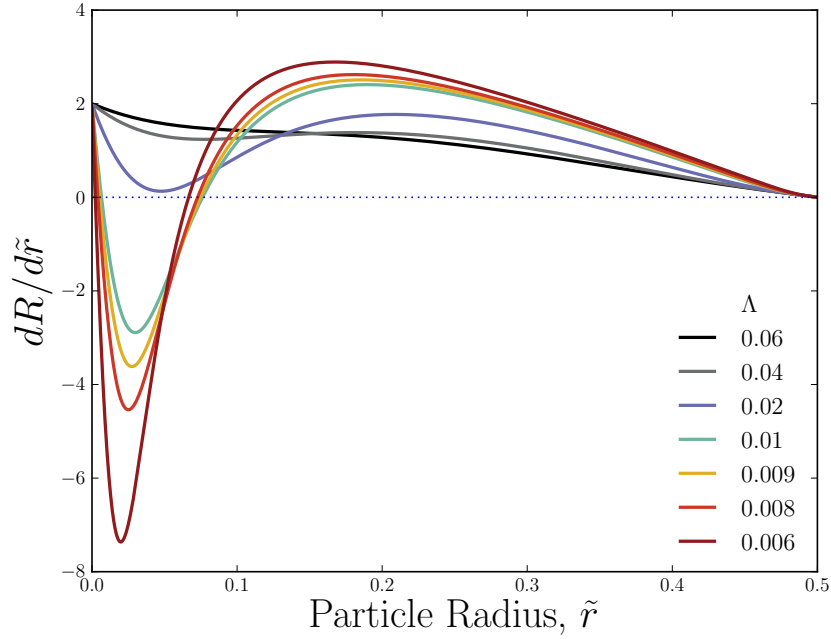


Figure 5.2.4: Derivative of retention ratio with respect to particle size (Equation (5.26)) for $\alpha = 1$.

to a device's ability to separate similar samples is found in § 6 but qualitatively speaking, it is intuitive that portions of the curve with larger differences in retention ratio (larger magnitudes of the slope) possess greater ability to separate solutes.

Steric-Inversion Point

The most immediately striking property of the unified ideal retention curves in Figure 5.2.3 is that the retention ratio R is not necessarily a monotonic function of particle size as it was in traditional theories (Figure 5.1.6). For large device retention parameters Λ , the retention ratio remains monotonic (a perturbation about the hydrodynamic chromatography limit), but when Λ decreases below some *critical device retention parameter* Λ_c the retention ratio becomes non-monotonic with a minimum at some particle size $\tilde{r}_{\text{NS}}$ (Figure 5.2.3). The derivatives shown in Figure 5.2.4 are for device retention parameters about the critical value of $\Lambda_c = 1.90 \times 10^{-2}$ (for $\alpha = 1$). Below Λ_c , the behaviour changes on either side of $\tilde{r}_{\text{NS}}$:

- Above the minimum $\tilde{r}_{\text{NS}}$, the retention ratio in Figure 5.2.3 grows in a manner similar to its growth in Figure 5.1.6.

- However, the behaviour of $R(\tilde{r})$ for small particle sizes in the regime $\tilde{r} < \tilde{r}_{\text{NS}}$ is much different. At small sizes, as the particle size increases the retention ratio falls drastically. This is the same behaviour as in Figure 5.2.1 and it is caused by the same mechanisms. As always, the concentration distribution results from the competition between the external field, thermal energy and the hard steric interaction with the wall, but at these sizes particles are too small for steric effects to dominate. Instead, in this range the behaviour is the same as that predicted by normal-mode FFF and shown in Figure 5.2.1.

It is this difference in mechanism that suggests the importance of $\tilde{r}_{\text{NS}}$. It is a very generally stated rule in the field of FFF that smaller particles elute before larger particles in normal-mode FFF. On the other hand, in steric-mode FFF larger particles elute before smaller particles [197]. Both modes of operation are considered monotonic and from the definition of $R \propto t_R^{-1}$ in Equation (5.8), this rule means that the slope $\partial R / \partial \tilde{r}$ is negative for normal-mode FFF and positive for steric-mode FFF.

Before the retention theory was unified, it had been intuitively obvious that there must be a transition between the two modes of operation. The transition between normal- and steric-mode FFF is sometimes called the *steric-inversion point*. By extracting $\tilde{r}^{-\alpha}$ from λ , as is done here, the transition $\tilde{r}_{\text{NS}}$ between the two operational modes can be quantitatively predicted. In fact, the particle size for which this transition occurs is a function of Λ and can be determined by numerically finding the minima in Figure 5.2.3 (the roots of $\partial R / \partial \tilde{r}$). The subscript NS was chosen in retrospect to signify that $\tilde{r}_{\text{NS}}$ represents the normal-to-steric transition (the steric-inversion point). Although this is called the steric-inversion point in the literature, it is now clear that it is not so much a point but a curve that depends on the device retention parameter Λ . The transition size $\tilde{r}_{\text{NS}}$ is traced in Figure 5.2.5, creating an operational-mode diagram for $\alpha = [1, 2, 3]$.

A surprising result of Figure 5.2.5 is just how far normal-mode operation can extend along $\tilde{r}$ when the force is a strong function of the particle size. When $\alpha = 1$, the normal mode has a maximum $\tilde{r}_{\text{NS}}$ of 0.078. When $\alpha = 3$, normal-mode FFF can be achieved for particle radii as large as 17% the channel height. This rather surprising result indicates that some methods of FFF (notably sedimentation-FFF [Table 5.2.1]) have normal-mode regimes that extend to much larger radii than others (such as flow- or thermal-FFF [Table 5.2.1]).

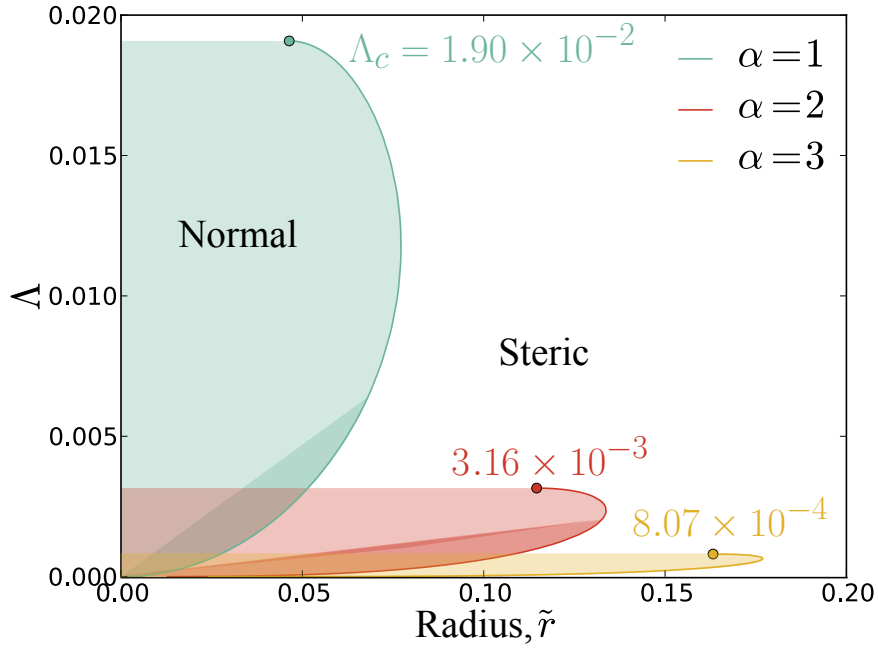


Figure 5.2.5: A tentative operational-mode diagram for FFF (see also Figure 5.2.7 and Figure 5.3.4). The dividing line between normal-mode FFF and large particle steric-mode FFF is the numerically determined critical particle size $\tilde{r}_{\text{NS}}$. Normal-mode FFF is represented by the shaded regions and does not exist above a critical device retention parameter Λ_c ; the critical device retention parameter values ($\Lambda_c = 1.90 \times 10^{-2}$ for $\alpha = 1$; $\Lambda_c = 3.16 \times 10^{-3}$ for $\alpha = 2$; and $\Lambda_c = 8.07 \times 10^{-4}$ for $\alpha = 3$) are marked by a closed circle.

A transition from normal- to steric-mode FFF exists only below a critical Λ_c . Figure 5.2.5 marks these values. They are numerically found to be

$$\Lambda_c \simeq \begin{cases} 1.90 \times 10^{-2} & \text{for } \alpha = 1 \\ 3.16 \times 10^{-3} & \text{for } \alpha = 2 \\ 8.07 \times 10^{-4} & \text{for } \alpha = 3. \end{cases} \quad (5.27a)$$

$$(5.27b)$$

$$(5.27c)$$

Above Λ_c , there simply is no normal-mode FFF and the retention ratios shown in Figure 5.2.3 monotonically increase with particle size. In a sense, for $\Lambda > \Lambda_c$ the force on a solute particle is not significant and the retention behaviour is approximately hydrodynamic chromatography for all particle sizes. For $\alpha = 3$, a much smaller Λ (stronger external field) is required in order to reach normal-mode FFF.

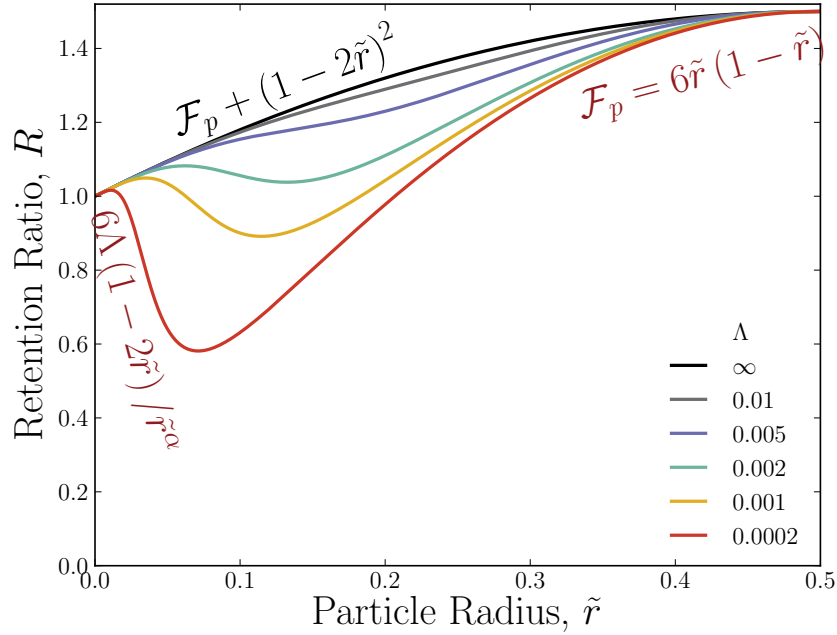


Figure 5.2.6: Retention ratios for $\alpha = 2$. The retention ratio is monotonic above the critical device retention parameter $\Lambda_c = 3.16 \times 10^{-3}$. As discussed in the text, there are three modes of operation when $\Lambda < \Lambda_c$: For the smallest particles the force is negligible, the slope is positive and the mode of operation is hydrodynamic chromatography. For moderately small particles the force dominates, the slope is negative and the mode of operation is normal-mode FFF. For the largest particles the effects of sterically excluded regions are most important; the slope is once again positive and the mode of operation is steric-mode FFF.

Hydrodynamic Chromatography to Normal-Mode Transition

A careful investigation of very small solute sizes ($\tilde{r}$ values) when Λ is near Λ_c (*e.g.* see the $\Lambda = 0.01$ curve) in Figure 5.2.3 reveals that there is a small region starting at $\tilde{r} = 0$ for which larger particles elute before smaller particles. To investigate this further, consider the retention ratio R for the same set of device retention parameters Λ but with $\alpha = 2$ (Figure 5.2.6).

The steric-inversion point for the transition between normal- and steric-mode FFF (at $\tilde{r} = \tilde{r}_{\text{NS}}$) remains (Figure 5.2.6); however, a local maximum at $\tilde{r} = \tilde{r}_{\text{HN}}$ (with $\tilde{r}_{\text{HN}} < \tilde{r}_{\text{NS}}$) is clearly evident for small Λ values. Since $\partial R / \partial \tilde{r}$ is positive in the region $\tilde{r} < \tilde{r}_{\text{HN}}$, the mode of operation has the same elution order as steric-mode FFF. However, since the thermal forces dominate over the external field in this region, this is actually a small-particle hydrodynamic chromatography regime (hence the subscript HN for this transition between hydrodynamic chromatography and normal-mode FFF).

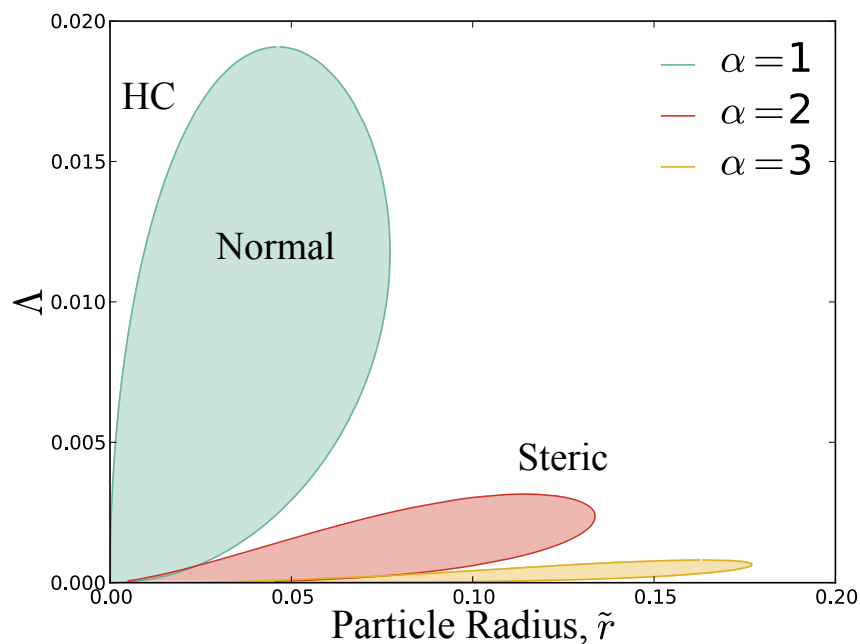


Figure 5.2.7: Enclosed regions are the areas of normal-mode FFF in which smaller particles elute before larger particles. Outer areas are steric-mode and hydrodynamic chromatography limit regions of operation in which larger particles elute before smaller particles. Solid lines are the numerically determined roots of $\partial R/\partial \tilde{r}$ ($\tilde{r}_{\text{HN}}$ is a local maximum of R and so forms the left border while $\tilde{r}_{\text{NS}}$ is a local minimum and forms the right border).

Normal-mode operation only exists over a range of particle sizes above which steric effects dominate because of the large size of the particle and below which the external force is insignificant due to the solute particles' small sizes. This is seen explicitly in Figure 5.2.7. The normal mode of operation exists as a lobe when $\Lambda < \Lambda_c$ (Figure 5.2.7). Figure 5.2.7 is an improvement on Figure 5.2.5. Above Λ_c , this new small particle hydrodynamic chromatography regime seamlessly transitions directly to steric-mode FFF, but below Λ_c normal-mode FFF exists between the hydrodynamic chromatography and steric-mode FFF regimes. While it has long been recognized that hydrodynamic chromatography is the limit of low field FFF, it does not seem to be widely appreciated that even at the strongest fields, normal-mode FFF exists as a finite lobe bracketed by hydrodynamic chromatography from below and steric-mode FFF from above.

5.2.4 Limits

Now that the transition between operational modes has been considered, it is once again valuable to evaluate the limits of the retention ratio (Equation (5.25)) in order to get a sense of the behaviour in each regime. The four limits are

$$R \simeq \begin{cases} \mathcal{F}_p + (1 - 2\tilde{r})^2 & \tilde{r} \rightarrow 0 & (5.28a) \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \tilde{r} \rightarrow \frac{1}{2} & (5.28b) \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \Lambda \gg 1 & (5.28c) \\ \mathcal{F}_p + \frac{6\Lambda}{\tilde{r}^\alpha} (1 - 2\tilde{r}) - \frac{12\Lambda^2}{\tilde{r}^{2\alpha}} & \Lambda \ll 1. & (5.28d) \end{cases}$$

The hydrodynamic chromatography operational mode was seen previously for small fields and for large particle sizes. In both these cases steric-exclusion dominates the behaviour. However, now that explicit solute size has been considered, the same behaviour is seen as $\tilde{r} \rightarrow 0$. This is fundamentally different from what was seen previously (Equation (5.21)), because it is now physically impossible to have both extremely small particles and extremely large forces. Thus, three of four limits take the form $R \simeq \mathcal{F}_p + (1 - 2\tilde{r})^2 = 1 + 2\tilde{r} - 2\tilde{r}^2$, as is clear in Figure 5.2.6.

The abnormal limit is the strong field case ($\Lambda \ll 1$). In fact, inspecting Figure 5.2.3 reveals that this limit itself has two sub-limits:

1. When the particle radius is large, the first term in Equation (5.28d) is most important such that $R \approx \mathcal{F}_p = 6\tilde{r}(1 - \tilde{r})$. As the particle size becomes large, all the retention ratios grow in the same manner, even at the strongest fields and smallest Λ . The behaviour may seem fairly universal at this point in the discussion but this is improved upon in § 5.3.
2. When the particle radius is small but the field is extremely strong (*i.e.* $\Lambda \ll \tilde{r}^\alpha$ or equivalently $\lambda \ll 1$) then the second term in Equation (5.28d) dominates, meaning that $R \approx 6\Lambda(1 - \tilde{r})/\tilde{r}^\alpha \approx 6\lambda$ and that the strong force limit is recovered.

5.3 Faxén-Mode Field-Flow Fractionation

The finite size of the particles is included as a steric exclusion from the wall, but it has a secondary effect as well. Until now, the velocity of particles whose centres of mass are at some height $\tilde{y}$ were assumed to have a velocity $\mathcal{V}(\tilde{y}) = u(\tilde{y})$ (given by Equation (3.27)). However, this is not strictly true. In a sense, $\mathcal{V} \neq u$ is a non-ideality (non-ideal effects are considered in § 7). However, this section shows that the most important correction to $\mathcal{V} \neq u$ can be treated in a very ideal manner and result in a retention theory that is no more complicated than that of § 5.2.

There are multiple hydrodynamic reasons for this assumption to break down. This section considers the consequences of the parabolic velocity profile that is not symmetric about the centre of mass, except at $\tilde{y} = 1/2$ [218, 261].

The first hints of this shortcoming were already observed in the non-physical behaviour at large particle sizes that is discussed in § 5.2.2. As the particle diameter approaches the channel height in Figure 5.2.3 and Figure 5.2.6, steric exclusion dominates such that the centre of mass is relegated to the centre of the channel ($\tilde{y} = 1/2$) and $n(\tilde{y})$ approaches a Dirac-delta function, regardless of the strength of the perpendicular field represented by Λ . The erroneous assumption that even in this limit Equation (5.1) does an adequate job approximating particle velocity means that by Equation (5.8) the retention ratio becomes $R(\tilde{r} = 1/2, \Lambda) = 3/2$, which indicates that the elution time of the largest particles will be significantly shorter than the void time. Intuitively, such large, ideal particles see the entire flow profile and so should be expected to move with the same average velocity as the solvent, not faster. That is to say, one should expect (in the absence of any experimental complications) that $R(\tilde{r} = 1/2, \Lambda) = 1$. Although it is true that steric effects do dictate the retention ratio regardless of Λ in the large particle limit, continuing to estimate $\lim_{\tilde{r} \rightarrow 1/2} \langle \mathcal{V} \rangle$ by its point-particle value of $u(\tilde{y} = 1/2) = 3 \langle u \rangle / 2$ becomes increasingly inaccurate.

5.3.1 Retention Theory: Faxén's Law

At large sizes, a significant portion of the particle's surface is subject to a much different velocity than $u(\tilde{y})$. Rather than using Equation (5.1), a particle's velocity at the position $\tilde{y}$ is better estimated by

$$\langle \mathcal{V} \rangle = \frac{\langle \mathcal{V} n \rangle}{\langle n \rangle}, \quad (5.29)$$

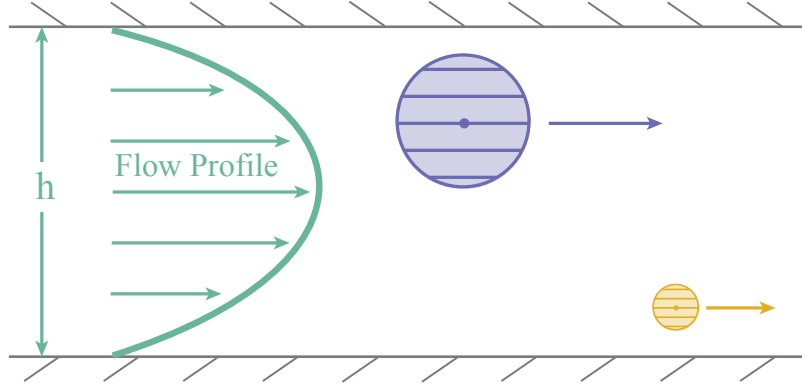


Figure 5.3.1: Schematic of area cross sectional integration of the particle's velocity

where the velocity of the large spherical particles in the presence of the confining walls is no longer estimated to be what the fluid speed would have been at the centre of mass of the solute *i.e.* $\mathcal{V}(\tilde{y}) \neq u(\tilde{y})$. In order to correct for the curvature of the fluid velocity due to the no-slip condition at the walls, the stress on the sphere must be integrated over its surface area as schematically shown in Figure 5.3.1. This results in *Faxén's Law* and predicts that the velocity of a spherical particle in steady-state is

$$\mathcal{V}(\tilde{y}) = \left(1 + \frac{r^2}{6} \nabla^2\right) u(\tilde{y}). \quad (5.30)$$

With this alteration, the retention ratio can be re-calculated by substituting Faxén's Law (Equation (5.30)), the concentration distribution (Equation (5.14)) and Poiseuille flow (Equation (3.27)) into Equation (5.8) to find

$$R(\tilde{r}, \Lambda) = \frac{6\Lambda}{\tilde{r}^\alpha} [1 - 2\tilde{r}] \mathcal{L}\left(\frac{[1 - 2\tilde{r}] \tilde{r}^\alpha}{2\Lambda}\right) + \mathcal{F}_f(\tilde{r}), \quad (5.31)$$

which is identical to Equation (5.25) but with a steric function $\mathcal{F}_f$ that is appropriate for finite sized particles. This new steric function $\mathcal{F}_f$ differs trivially from $\mathcal{F}_p$ (Equation (5.18)) and is

$$\mathcal{F}_f(\tilde{r}) = 6\tilde{r} \left(1 - \frac{4}{3}\tilde{r}\right). \quad (5.32)$$

The tiny change from $\mathcal{F}_p$ to $\mathcal{F}_f$ is enough to pull the retention ratio for large particles down from $R(\tilde{r} = 1/2, \Lambda) = 3/2$ to $R(\tilde{r} = 1/2, \Lambda) = 1$ (Figure 5.3.2) as is more physically

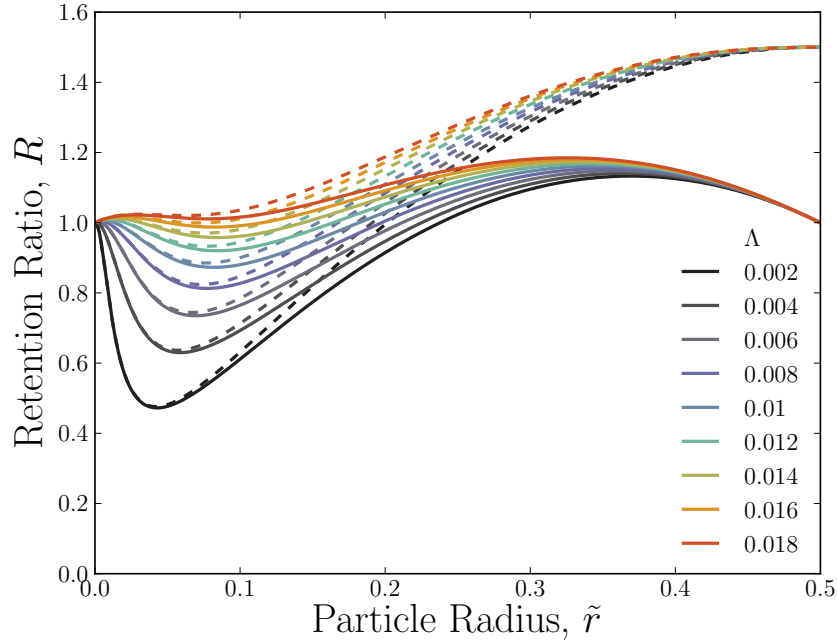


Figure 5.3.2: The retention ratio for $\alpha = 1$, taking into account both Faxén’s Law compared to simply assuming the centre of mass velocity, as done previously. Dashed lines correspond to Equation (5.25) while solid lines are from Equation (5.31) and Equation (5.32).

intuitive. As seen in Figure 5.3.2, this produces a region of $\partial R / \partial \tilde{r} < 0$, which exists for all values of Λ . This constitutes a fourth ideal operational mode. This new regime shall be called *Faxén-mode field-flow fractionation*.

Since the form of Equation (5.31) is the same as Equation (5.25), even the limits on the retention ratio given by Equation (5.28) remain robust by simply replacing the steric term $\mathcal{F}_p$ with $\mathcal{F}_f$. However, it is interesting to make one note about the hydrodynamic chromatography limit: Without including Faxén’s law the hydrodynamic chromatography limit was given by Equation (5.20) with $b_{\text{HC}} = 2$ and $c_{\text{HC}} = 2$, which was reasonable except that c_{HC} was only half the commonly expected value of $c_{\text{HC}} = 4$ [255, 256, 258]. By inserting the new steric function (Equation (5.32)) into the hydrodynamic chromatography limit (Equation (5.28c)) the retention ratio becomes

$$R = 1 + b_{\text{HC}}\tilde{r} - c_{\text{HC}}\tilde{r}^2, \quad (5.33)$$

with $b_{\text{HC}} = 2$ and $c_{\text{HC}} = 4$.

As is done in § 5.2.3, it is possible to take the derivative of Equation (5.31) and find the

slope of the retention ratio (which is the equivalent of Equation (5.26)) to be

$$\begin{aligned}
 R' = & -\alpha \frac{6\Lambda}{\tilde{r}^{1+\alpha}} \left[(1-2\tilde{r}) \coth \left((1-2\tilde{r}) \frac{\tilde{r}^\alpha}{2\Lambda} \right) - \frac{2\Lambda}{\tilde{r}^\alpha} + \left(1 - \frac{4}{3}\tilde{r} \right) \frac{\tilde{r}^{1+\alpha}}{\Lambda} \right] \\
 & + \frac{6\Lambda}{\tilde{r}^\alpha} \left[(1-2\tilde{r}) \left\{ 1 - \coth^2 \left((1-2\tilde{r}) \frac{\tilde{r}^\alpha}{2\Lambda} \right) \right\} \left\{ -\frac{\tilde{r}^\alpha}{\Lambda} + (1-2\tilde{r}) \frac{\alpha \tilde{r}^{\alpha-1}}{2\Lambda} \right\} \right. \\
 & \left. - 2 \coth \left((1-2\tilde{r}) \frac{\tilde{r}^\alpha}{2\Lambda} \right) + 2 \frac{\alpha \Lambda}{\tilde{r}^{\alpha+1}} + (1+\alpha) \left(1 - \frac{4}{3}\tilde{r} \right) \frac{\tilde{r}^\alpha}{\Lambda} - \frac{4}{3} \frac{\tilde{r}^{1+\alpha}}{\Lambda} \right]. \quad (5.34)
 \end{aligned}$$

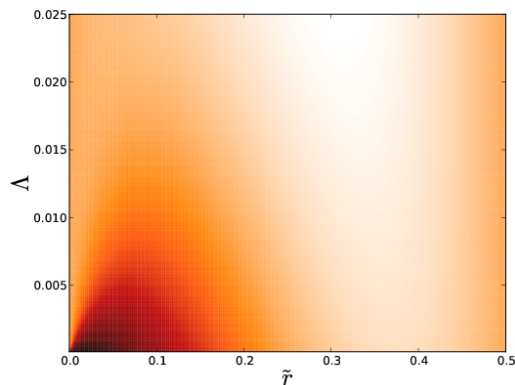
Finding the roots of this equation reveal any changes to the operational-mode diagram, that result from taking into account Faxén's law.

5.3.2 Regimes and Transitions

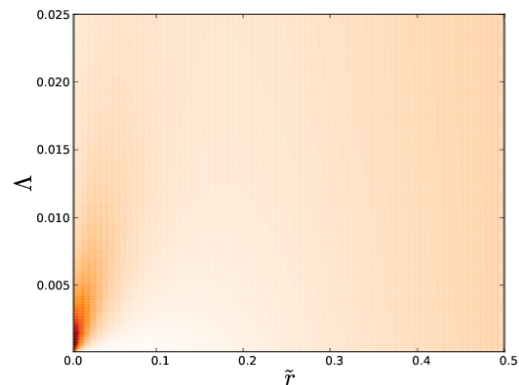
Utilizing Faxén's Law results in a unified, ideal retention theory that is more physically satisfying than the one presented in § 5.2.2; however, the forms of the retention ratio as a function of device retention parameter and particle size (Equation (5.31)), and its derivative with respect to particle size (Equation (5.34)), are identical to the forms of the previous versions. Therefore, much of what can be said about them has been already said.

The borders between all four ideal operational modes can be traced out for a given α . The process is shown in Figure 5.3.3: From the retention ratio (Equation (5.31) and Figure 5.3.3a) the derivative is calculated (Equation (5.34) and Figure 5.3.3b). Although the roots are not visible in the phase-space diagram (Figure 5.3.3b), they can be found (Figure 5.3.3c) and numerically calculated to create an operational-mode diagram for FFF (Figure 5.3.3d). This is done for $\alpha = 1$ in Figure 5.3.3. The enclosed, shaded regions represent the normal-mode elution order (smaller particles eluting first), while the unshaded, outer regions operate in the steric-mode elution order (larger particles eluting first). An entirely new region at large $\tilde{r} > \tilde{r}_{\text{SF}}$ with normal-mode elution order exists. The transition from steric- to Faxén-mode FFF is labelled with the subscript SF. Faxén-mode FFF exists for all Λ . $\tilde{r}_{\text{SF}} \rightarrow 0.25$ at the largest values of Λ , which means that the transition occurs when the particle radius is a quarter of the channel height. At smaller Λ , the concentration is biased towards the accumulation wall and the transition doesn't occur until $\tilde{r}_{\text{SF}} = 0.375$.

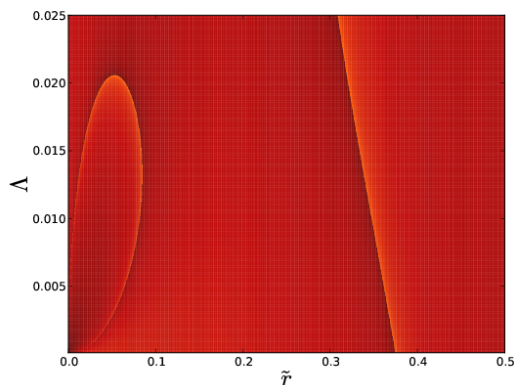
Thus, the operational-mode diagram for $\alpha = 1$ when Faxén's law is included is similar to the predictions that neglect it (Figure 5.2.7) at small solute sizes, but has an entirely novel regime at larger sizes. Much the same can be said about $\alpha = 2$ but when $\alpha = 3$



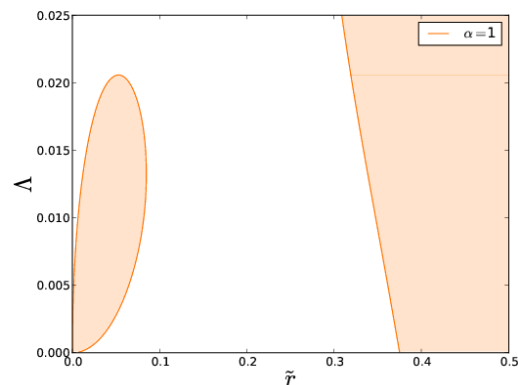
(a) The retention ratio explicitly depends on the particle size $\tilde{r}$ and the device retention parameter Λ (Equation (5.31)). The greatest magnitude is found in the strong fields/small particle size region.



(b) The derivative of Figure 5.3.3a (Equation (5.34)). It is large only in the steep normal-mode region.



(c) Though the roots of the derivative give the transitions between operational modes, they are not clearly visible in Figure 5.3.3b. In order to see them more clearly, the logarithm of the magnitude is taken.



(d) The roots of the derivative Figure 5.3.3b give the transitions between operational modes for $\alpha = 1$. Enclosed regions are the areas of normal- and Faxén-mode FFF. The outer area includes the steric-mode FFF and hydrodynamic chromatography regions of operation.

Figure 5.3.3: The generation of the operational-mode phase space for $\alpha = 1$ from the retention ratio. Scales are not shown here in order to emphasize the procedure for determining the transitions between operational modes.

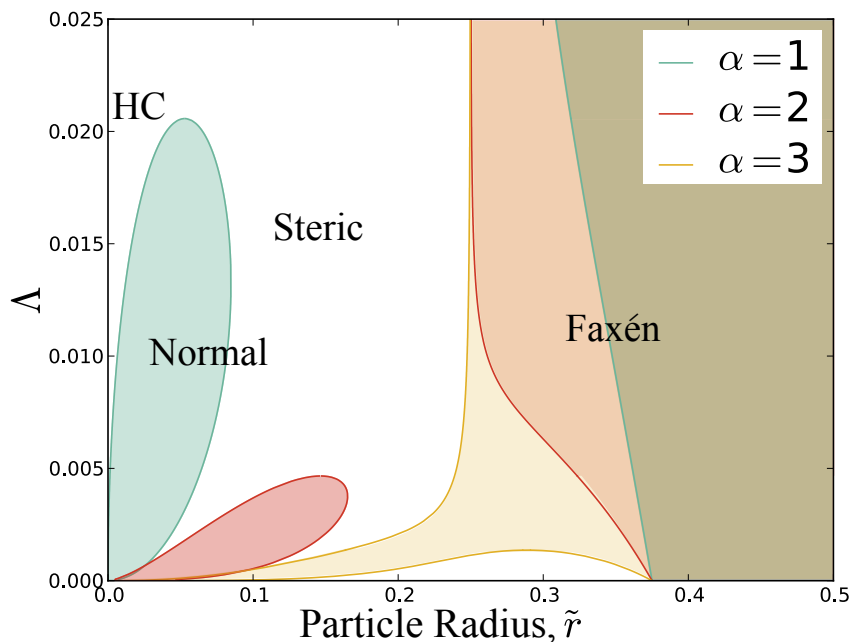


Figure 5.3.4: Ideal FFF-modes map. The numerically determined roots of $\partial R/\partial \tilde{r}$ give the critical sizes marking the transitions between the four different modes of operation $\tilde{r}_{\text{HN}}$, $\tilde{r}_{\text{NS}}$ and $\tilde{r}_{\text{SF}}$.

the operational-mode diagram becomes strikingly different (Figure 5.3.4). For $\alpha = 1, 2$ the normal- and Faxén-mode areas form disconnected regions while hydrodynamic chromatography and steric-mode FFF are a connected space. On the other hand, when $\alpha = 3$ the border between the normal and steric modes ($\tilde{r}_{\text{NS}}$) extends to such large particle sizes that it passes $\tilde{r}_{\text{SF}}$ and so normal-mode FFF interconnects with the Faxén-mode regime. In this case, it is the hydrodynamic chromatography limit of FFF and steric-mode FFF that are disconnected regions (Figure 5.3.4).

5.3.3 Approximations

Although the retention ratio is given analytically by Equation (5.31), the transition sizes $\tilde{r}_{\text{HN}}$, $\tilde{r}_{\text{NS}}$ and $\tilde{r}_{\text{SF}}$ must be found numerically by solving the roots of $\partial R/\partial \tilde{r} = 0$ (Equation (5.34)), as done in Figure 5.3.3. This can be inconvenient at times. Luckily, in some experimentally relevant limits, the transitions can be well approximated by simple forms.

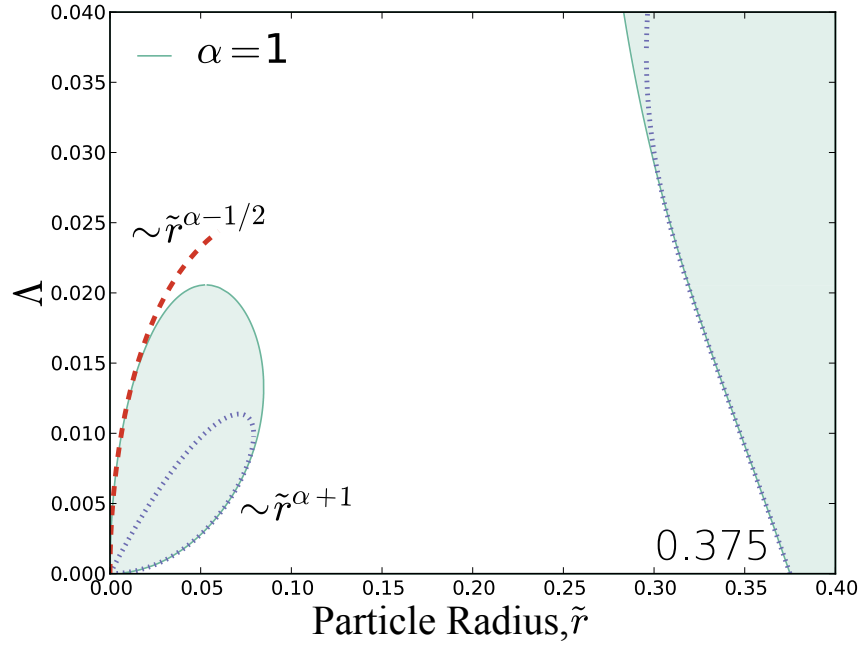


Figure 5.3.5: Approximations to $\tilde{r}_{\text{SF}}$ and $\tilde{r}_{\text{NS}}$ work well for small device retention parameters Λ as shown by the dotted lines from Equation (5.35) for the example of $\alpha = 1$. The dashed line from Equation (5.37) shows the small $\tilde{r}$ approximation for the $\tilde{r}_{\text{HN}}$ transition.

Steric-Inversion and Steric-Faxén Transition

The domain of the normal-mode region is estimated by taking the small Λ limit of $\partial R / \partial \tilde{r}$, such that the hyperbolic cotangent terms approach unity ($\coth(x) \approx 1$). This results in the approximation

$$\frac{\partial R}{\partial \tilde{r}} \approx 6 [a + b\Lambda + c\Lambda^2] = 0, \quad (5.35)$$

where

$$a \equiv 1 - \frac{8}{3}\tilde{r}, \quad b \equiv \frac{2\alpha\tilde{r} - 2\tilde{r} - \alpha}{\tilde{r}^{\alpha+1}}, \quad c \equiv \frac{4\alpha}{\tilde{r}^{2\alpha+1}}.$$

This has two simple analytic roots, which are plotted in Figure 5.3.5 as dotted blue lines. Because it is the small Λ limit of $\partial R / \partial \tilde{r}$, Equation (5.35) captures the steric-inversion curve ($\tilde{r}_{\text{NS}}(\Lambda)$) and steric- to Faxén-mode transition ($\tilde{r}_{\text{SF}}(\Lambda)$) borders quite well.

To first order, Equation (5.35) demonstrates that the Λ -value for which the transition

occurs is $\Lambda \approx a/b$, which scales as

$$\Lambda \sim \tilde{r}^{\alpha+1}. \quad (5.36)$$

While this small Λ approximation does a poor job estimating the total area of the normal-mode regime (Figure 5.3.5), the first root does accurately estimate $\tilde{r}_{\text{NS}}$ at small Λ . The second root predicts $\tilde{r}_{\text{SF}}$ and agrees exceedingly well in the infinite force limit of $\lim_{\Lambda \rightarrow 0} \tilde{r}_{\text{SF}} = 3/8 = 0.375$, but cannot approximate the hydrodynamic chromatography limit of $\lim_{\Lambda \rightarrow \infty} \tilde{r}_{\text{SF}} = 0.25$ because calculating it depends on a small- Λ assumption.

Transition between Hydrodynamic Chromatography and Normal-Mode FFF

The opposite approximation corresponds to the small particle sizes for which the expansion is $\coth(x) \approx x^{-1} + x/3 + x^3/45 + \dots$. To order $2\alpha + 1$, the rate of change of the retention ratio with particle size is

$$\frac{\partial R}{\partial \tilde{r}} \approx A + \frac{B}{\Lambda^2} = 0, \quad (5.37)$$

where

$$A \equiv 2 - 8\tilde{r},$$

$$B \equiv \frac{1}{5} \left[-\frac{\alpha}{6} \tilde{r}^{2\alpha-1} + \frac{2(2\alpha+1)}{3} \tilde{r}^{2\alpha} - 4(\alpha+1) \tilde{r}^{2\alpha+1} \right].$$

Again, the roots to the approximation are simple and have been plotted in Figure 5.3.5 as a dashed line. Despite being a small $\tilde{r}$ -approximation, Equation (5.37) fails as Λ approaches the critical value Λ_c .

To order $2\alpha + 1$, the Λ -value for which the transition occurs is $\Lambda \approx \sqrt{A/B}$, which scales as

$$\Lambda \sim \tilde{r}^{\alpha-1/2}. \quad (5.38)$$

Interestingly, this scaling means that the concavity of $\tilde{r}_{\text{HN}}$ changes with α . When $\alpha = 1$ the normal-mode regime is a “petal,” as in Figure 5.3.4, but when $\alpha > 1$ the concavity flips and the normal-mode regime is more “tear-drop” shaped.

Summary

The equations governing the FFF elution time of hard spherical particles in low Reynolds number flow are well known. Normal-mode FFF is governed by Equation (5.12) (though it is often simply approximated as Equation (5.13)), while Equation (5.17) is used for steric-mode FFF. By explicitly including the external force's dependence on particle size, the ideal retention theory is unified (Equation (5.25)). This unified theory is able to quantitatively predict the transition between these two modes of operation. There exists a critical device retention parameter, Λ_c , below which the transition between normal- and steric-mode FFF exists but above which normal-mode operation is not possible and hydrodynamic chromatography and steric-mode FFF become indistinguishable.

Furthermore, a mode of operation exists at the smallest particle sizes with the same elution order as steric-mode FFF. The force pushing the particles towards the accumulation wall is size dependent and is insignificant compared to the steric force in this size regime. This is the remnant of hydrodynamic chromatography that exists at the smallest particle sizes even in the strongest fields. The transition between this small-particle hydrodynamic chromatography limit of FFF and normal-mode FFF is quantitatively traced and it is found that the domain of normal-mode operation only exists as a closed area on the $\Lambda - \tilde{r}$ mode-diagram below Λ_c .

By integrating the fluid stress over the surface area of the particles, the predictions of ideal retention theory for the case of small channel heights (microfluidic devices) is further improved (Equation (5.31)). The steric function $\mathcal{F}_f$ in the retention ratio is altered but is no more complicated than when this improvement was ignored. The improved expression for the ideal retention ratio predicts a new mode of operation for particles with diameters that approach the channel height. The elution order of this new ideal Faxén-mode FFF is the same as normal-mode FFF and exists for all Λ . When the external force on the particles scales as $\alpha = 3$ (as is true for sedimentation FFF) the normal- and Faxén-mode FFF regimes intersect and form a continuous space.

Accurately predicting the transition points between the four regions can be done through two quadratic equations for the approximate rate of change of the retention ratio with respect to particle size (Equation (5.35) and Equation (5.37)) in the limiting cases of strong fields or small particle sizes.

Field-flow fractionation can act as a valuable characterization tool in microfluidic devices but the elution time is not necessarily a one-to-one function of particle size. Non-monotonicity

is more likely to be encountered in microfluidic systems because solute sizes are more likely to span the entire range from minute tracer particles to colloids that are comparable in size to the micro-scale channel height. Although this complicates the interpretation of retention profiles, four operational modes exist, each of which can be utilized to separate samples. By utilizing Figure 5.3.4, one may choose to construct microfluidic channels designed to specifically function in one operational mode or another for given samples. Doing so would avoid transitions and maintain the retention ratio as a monotonic function of particle size. On the other hand, by using the non-monotonic form of the unified ideal retention ratio (Equation (5.31)), a microfluidic device need not be limited to a single operational mode but can be employed to measure all possible particle samples, from relatively tiny tracer particles to particles as large as the microfluidic channel itself.

As a poor man, I'm averaging what I have.
Alexander Grosberg [262]

6

Selectivity and Separation Power

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In the previous chapters, various separation methods are discussed. Fractionation methods of all sorts seek to separate mixed ensembles of different populations of solutes based on some *characteristic-of-interest* (COI). Separation science is concerned with particulate matter, ions, macromolecules, cells and generally all forms of dispersed solutes. The primary purposes of fractionation are to detect the presence of specific species, to measure their properties or to purify and extract species into resolved volumes. Electrophoretic mobility and retention ratios are considered in § 4 and § 5 respectively, but not whether the differences between the mobilities of two species is sufficient to successfully fractionate the mixture into its constituents. What metrics should be used to assess the ability of a microfluidic device to separate solutes?

Traditionally, the field-flow fractionation community uses the metric of *selectivity*. However, this chapter will demonstrate that traditional selectivity can be misleading in some situations because it is built around relative changes in an analyte's COI. While dealing with relative differences may be appropriate for many situations, in others it is the absolute difference that is the cardinal concern. These thoughts lead to the proposal of a new device selectivity that preserves many of the advantages of the traditional definition but avoids many of its pitfalls by assessing absolute rather than relative differences.

For concreteness, this chapter focuses on elution-based methods that fractionate via some COI, $z = z(r)$, that is assumed to vary smoothly with particle size r . Indeed, the characteristic-of-interest is often size itself ($z = r$), though it could be chain length, charge, *etc.* The present discussion focuses on FFF, although it would also be straightforward to treat methods like electrophoresis (§ 4) using the same approach.

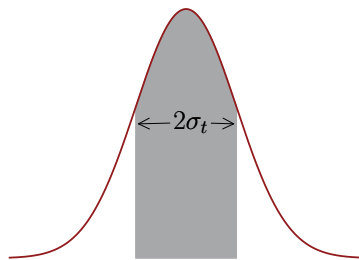


Figure 6.1.1: Colloquially, the width of a zone is twice the standard deviation of a Gaussian peak.

6.1 Resolution

In previous chapters, only the mean elution time is considered, with only a brief mention that elution times have an effective dispersion coefficient in § 5.1.2. Inevitably, both sample loading processes and various sources of dispersion yield retention time distributions (colloquially called *zones* or *bands* [230]). These variations can arise due to factors such as Brownian diffusion, adsorption-desorption events, Taylor-Aris dispersion (§ 5.1.2) and system inhomogeneities (such as pore size distributions in gels). Whatever the root cause, the fact remains that retention time is distributed about the value predicted by ideal theory. The Central Limit Theorem suggests that this distribution should become normal after long times, but in reality such perfectly symmetric and predictable peaks are rare [263, 264].

6.1.1 Ideal Peaks

As two bands representing two populations of species elute through a microfluidic separation device, they translate with different mean elution/retention times $\bar{t}_i \equiv \overline{t(\mathbf{z}_i)}$ and the peak spacing increases with total elution time. The difference in the time required for the two zones to arrive at a given elution point is $\Delta\bar{t} = \bar{t}_2 - \bar{t}_1$. Concurrently, dispersion occurs and band widths increase. The width of each band is taken to be twice its standard deviation $2\sigma_{t_i} = 2\sqrt{\overline{t_i^2} - \bar{t}_i^2}$ (Figure 6.1.1). The factor of 2 is a rather arbitrary choice defining the zone width to encompass 68% of the presumed Gaussian distribution, but it is conventionally adopted [265].

Thus, the ability to discern between various species i undergoing separation depends fully on their peak-to-peak separation and their band widths. It is not simply the peak spacing that determines the ability to discern between species (subscript 1 and 2), but rather the

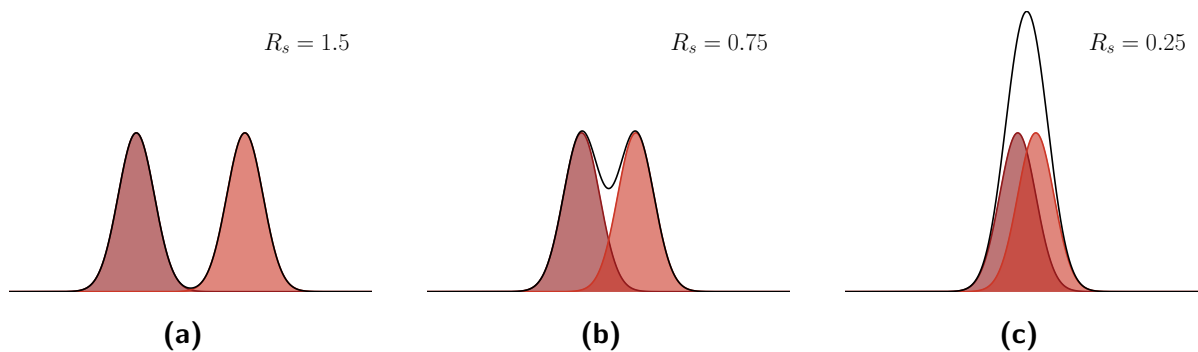


Figure 6.1.2: Three examples of resolution R_s between two identical Gaussian peaks. Figure 6.1.2a shows a highly resolved pair of Gaussian peaks, while Figure 6.1.2c shows a poorly resolved pair. Figure 6.1.2b shows the intermediate, near-unity case.

peak spacing relative to the total broadening, $4 \langle \sigma_t \rangle \equiv 2\sigma_{t_2} + 2\sigma_{t_1}$ (in this chapter care must be taken to delineate between $\langle x \rangle$, which is the average of the two values for populations 1 and 2, and $\bar{x}_i$, which is the mean of a single solitary distribution). If it is assumed that the species' zones are essentially Gaussian and of roughly equal total population, then it is the ratio of separation to broadening that defines the *resolution factor*:

$$R_s \equiv \frac{\bar{t}_2 - \bar{t}_1}{2\sigma_{t_2} + 2\sigma_{t_1}} = \frac{\Delta \bar{t}}{4 \langle \sigma_t \rangle}. \quad (6.1)$$

The competition between zone separation (through $\Delta \bar{t}$) and zone broadening (through $\langle \sigma_t \rangle$) is encapsulated in the dimensionless parameter R_s . In practice, the choice of a detection tool and of an analytical goal will dictate the value of R_s required to obtain satisfactory fractionation but the precise definition roughly follows the common usage as can be seen from the examples in Figure 6.1.2.

6.1.2 Resolution and Statistical Certainty

The previous section states that the resolution factor R_s is a suitable metric for assessing the quality of a separation between two peaks. However, it **assumes** that the two peaks are confidently identified and are individual Gaussian distributions that have adequately similar numbers of events and widths, as in Figure 6.1.2. Are such assumptions required to define the resolution? And if so, why?

When the standard deviations of two peaks differ greatly or the concentrations of the two populations are dissimilar (Figure 6.1.3) (as can be the case when considering environmen-

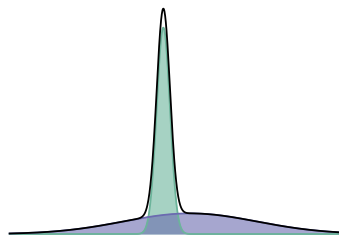


Figure 6.1.3: Two Gaussian peaks with very different standard deviations (2 and 20). In such cases, resolution is not an adequate metric of the ability to discern between peaks.

tal contaminants [177] or different protein concentrations [227]), then the resolution factor R_s **does not** adequately represent the confidence that one should have in identifying two peaks as significantly different. Instead, the statistical confidence that the means of two populations are different should be considered. When the standard deviations (σ_{t_i}) and the number of counts (n_i) are substantially different, then the *two sample t-statistic* (Welch's generalization [266])

$$t_{\text{test}} = \frac{\bar{t}_1 - \bar{t}_2}{\sqrt{\frac{\sigma_{t_1}^2}{n_1} + \frac{\sigma_{t_2}^2}{n_2}}} \quad (6.2)$$

is the proper representation of the statistical confidence that the two population means ($\bar{t}_1$ and $\bar{t}_2$) differ.

In the case that the number of counts are comparable ($n_2 \approx n_1 = n$) and the standard deviations are similar ($\sigma_{t_2} \approx \sigma_{t_1} = \sigma_t$), the resolution factor is proportional to the t-statistic normalized by $\sqrt{n}$. In that limiting case, the resolution factor becomes a suitable metric of the statistical confidence. This can be seen by defining

$$n_2 = n_1 + \delta n \equiv n + \delta n \quad (6.3a)$$

$$\sigma_{t_2} = \sigma_{t_1} + \delta \sigma \equiv \sigma_t + \delta \sigma. \quad (6.3b)$$

In the limit of small δn and $\delta \sigma$, the t-statistic to first order becomes

$$\lim_{\substack{\delta n \ll n \\ \delta \sigma \ll \sigma_t}} t_{\text{test}} \approx \frac{\Delta \bar{t}}{\sigma_t} \sqrt{\frac{n}{2}} \left[1 - \frac{1}{2} \frac{\delta \sigma}{\sigma_t} + \frac{1}{4} \frac{\delta n}{n} + \dots \right]. \quad (6.4a)$$

Under the same conditions, the resolution factor is

$$\lim_{\delta\sigma \ll \sigma_t} R_s = \lim_{\delta\sigma \ll \sigma_t} \frac{\Delta \bar{t}}{4 \langle \sigma_t \rangle} \approx \frac{\Delta \bar{t}}{4\sigma_t} \left[1 - \frac{1}{2} \frac{\delta\sigma}{\sigma_t} + \dots \right]. \quad (6.4b)$$

This means that in the limit that the two distributions have **the same widths and heights**, the t-statistic is related to the resolution factor as

$$\lim_{\substack{\delta n \ll n \\ \delta\sigma \ll \sigma_t}} t_{\text{test}} \approx R_s \sqrt{8n} \left[1 + \frac{1}{4} \frac{\delta n}{n} + \dots \right]. \quad (6.4c)$$

This suggests that in the limit that the number of counts and the widths of the peaks are comparable, the resolution factor is a **short-hand** for describing the normalized t-statistic. When either of these conditions are not respected, then Equation (6.1) and Equation (6.2) are not compatible and the t-test should be preferred over the resolution factor.

Therefore, in order to address the metrics commonly utilized by the chromatography community (including the FFF literature), the traditional assumptions that

1. the peaks are Gaussian
2. the areas under the peaks are roughly equal, and
3. the widths are similar

must be accepted in this chapter. Under these restrictions, the resolution factor is a valuable metric of the ability to discern between two peaks.

For similar peaks the resolution factor is the key parameter for separation. However, it is convenient (and indeed conventional) to split metrics of the ability of an apparatus to resolve different values of the COI into two dimensionless terms that embody

1. the ability to separate mean peak positions (called the *selectivity*), and
2. zone **broadening**.

The remainder of this chapter assumes that the conditions required for resolution to be useful are met and focuses on selectivity, since it is the term that explicitly includes the COI itself. It will be demonstrated that the traditional definition of selectivity can be specious and so an alternative selectivity is proposed. Several selected examples show that the new definition is either equivalent to the traditional definition or superior.

6.2 Fractionating Power and Selectivity

What the resolution factor R_s does not say is whether or not there is a large or a small difference in the COI, $\Delta z = z_2 - z_1$. That is to say, it does not make a statement about the finesse or ability to resolve between two similar samples. For that, a metric called the *fractionating power* is required. The fractionating power could, for example, be given by the ratio:

$$F_{\text{pow}} = \frac{R_s}{\Delta z}. \quad (6.5a)$$

However, the fractionating power as defined above has dimensions ($\sim 1/z$). In practice, this is often undesirable, especially if one wants to compare the performance of different instruments. Therefore, the definition requires an additional factor that shares the same dimensions as the COI itself. In principle, one can choose this normalizing factor using parameters related to the analytes (the average value of the COI is traditionally used — as will be seen in § 6.2.1), to the separation device or to the experimental conditions (§ 6.2.2), or even a combination thereof. This choice of a normalization factor seems mundane at a certain level but in actuality the choice that is made has an impact on the potential usage of the resulting metrics.

It is interesting to note that the dimensions can actually be practical if one considers instead

$$F_{\text{pow}}^{-1} = \frac{\Delta z}{R_s}. \quad (6.5b)$$

The metric F_{pow}^{-1} can be interpreted as the smallest increment of COI that a separation apparatus can resolve. As such, this *size resolution factor* can be a very useful tool in separation science [267].

6.2.1 Traditional, Relative Selectivity

In order to recover a dimensionless metric, Giddings and co-workers defined the fractionating power F_R as the resolution factor with respect to the relative change in COI [268–270]

$$F_R \equiv \frac{R_s}{(\Delta z / \langle z \rangle)}. \quad (6.6)$$

The denominator in Equation (6.6) is the **fractional difference** in the COI; therefore, F_R has been defined to provide information on relative differences but not on absolute differences.

In order to better understand the performance of a device, it is useful to break the fractionating power into a dimensionless factor representing zone separation and a dimensionless factor for zone broadening. Historically, this is done by writing the traditional fractionating power as

$$F_R \equiv \frac{\sqrt{N_R}}{4} S_R. \quad (6.7a)$$

Here, the *number of theoretical plates* [231, 271]

$$N_R \equiv \left(\frac{\langle \bar{t} \rangle}{\langle \sigma_t \rangle} \right)^2 \quad (6.7b)$$

describes peak quality (i.e., the **efficiency** of the transport process). A high value of N_R signifies that the peaks remain narrow over long elution processes. The separation between two distinct zone centres is given by the *selectivity* [209, 272, 273]

$$S_R \equiv \frac{\Delta \bar{t} \langle \dot{z} \rangle}{\langle \bar{t} \rangle \Delta \dot{z}}. \quad (6.7c)$$

While S_R does not provide any information about peak widths, N_R is essentially a peak property. In fact, the selectivity S_R describes how the COI impacts transport, while the N_R encompasses the non-ideal factors that broaden the zones. Because only the selectivity includes explicit information about the COI, it will be the focus of this discussion. For reasons that will become clear in § 6.3, the subscript R refers to these definitions as **relative** fractionating power, **relative** number of plates and **relative** selectivity.

Similar Peaks

The above discussion is solely concerned with pairs of distinct species each with its own zone centre $\bar{t}_i$ and band width $2\sigma_{t_i}$. One can consider two peaks that are very close (as is the primary concern of separation scientists) and ask what the consequence of varying the COI by an infinitesimal amount would be on the elution behaviour. If one takes the infinitesimal

limits $\langle x \rangle \rightarrow x$ and $\Delta x \rightarrow dx$, the **continuous** forms become

$$F_R = \left(\frac{\bar{t}}{4\sigma_t} \right) \left(\frac{z d\bar{t}}{\bar{t} d\bar{z}} \right) \quad (6.8a)$$

$$N_R = \left(\frac{\bar{t}}{\sigma_t} \right)^2 \quad (6.8b)$$

$$S_R = \frac{z d\bar{t}}{\bar{t} d\bar{z}}. \quad (6.8c)$$

There is a subtle point in shifting to continuous forms:

- Even if the peaks are infinitesimally close, it is conceivable that the zone widths or even the values of the COI could be quite different. This would be the case, for example, if one were performing sedimentation FFF to measure the size of particulates of significantly different density, since they may then have similar radii but significantly different masses. In this thesis, it is assumed that all physically relevant properties are smooth functions of the COI (§ 6.1).

Notable Forms of the Traditional Relative Selectivity

In the literature, S_R is often written in slightly different forms than Equation (6.8c):

1. One encounters S_R written as

$$S_R \equiv \frac{d \log(\bar{t})}{d \log(z)}. \quad (6.9)$$

This is the traditionally used form of selectivity [203, 209, 231, 272, 273] and is fully equivalent to Equation (6.8c).

2. FFF researchers discuss the retention ratio $R = t_o/\bar{t}$ rather than retention time. Indeed, § 5 uses only R , without discussing the retention time $\bar{t}$ or void time t_o (the time required for the carrier fluid to transverse the length of the channel). In terms of $R(z)$, the relative selectivity is

$$S_R = -\frac{z dR}{R d\bar{z}} = -\frac{d \log(R)}{d \log(z)}. \quad (6.10)$$

3. The absolute value $|S_R|$ is commonly used. Dropping the sign of S_R disregards information about elution order, which is of interest if $\overline{t(z)}$ is a non-monotonic function of the COI. The unified retention theory is seen in § 5.2 to be highly non-monotonic. Yet, taking the absolute value can be necessary when plotting the selectivity on a log-log scale, which can be tempting when thinking of S_R in terms of Equation (6.9) or Equation (6.10) [215].

6.2.2 Device Selectivity

One is often interested in the **absolute** difference in COI between adjacent peaks. A modified metric of selectivity may be appropriate for such situations. Since the percent change for some absolute difference itself depends on particle size, the traditional definition of selectivity has an implicit particle size dependence, which the proposed definition will not.

Non-dimensionalizing the fractionating power by a constant factor z_o that is related to the experimental conditions and/or the apparatus (rather than the analytes) leads to analogous but different definitions for the number of theoretical plates and the selectivity. A practical choice of z_o for FFF and hydrodynamic chromatography apparatus is made in § 6.3, but for now a more general framework is maintained. In general, the novel fractionating power between two separate peaks becomes

$$\begin{aligned}
 F_D &\equiv \frac{R_s}{\Delta z / z_o} \\
 &= \left(\frac{t_o}{4 \langle \sigma_t \rangle} \right) \left(\frac{z_o \Delta \bar{t}}{t_o \Delta z} \right) \rightarrow \left(\frac{t_o}{4 \sigma_t} \right) \left(\frac{z_o d\bar{t}}{t_o dz} \right) \\
 &\equiv \frac{\sqrt{N_D}}{4} S_D,
 \end{aligned} \tag{6.11a}$$

where now

$$N_D \equiv \left(\frac{t_o}{\langle \sigma_t \rangle} \right)^2 \rightarrow \left(\frac{t_o}{\sigma_t} \right)^2 \tag{6.11b}$$

$$S_D \equiv \frac{z_o \Delta \bar{t}}{t_o \Delta z} \rightarrow \frac{z_o d\bar{t}}{t_o dz}. \tag{6.11c}$$

Notice that these definitions are similar in form to those given by Giddings *et. al.* [231, 268–270, 273]. Previously, the change in COI was normalized by the present value of the COI (z) and the retention time was normalized by the retention time ($\bar{t}$) (Equation (6.8c), *etc.*

in § 6.2.1). Now the normalization procedure uses parameters that are not related to the analytes (*i.e.* z_o and t_o). For this reason, the traditional definitions are referred to as **relative** (subscript _R) metrics and the modified forms as **device** (subscript _D) metrics. In terms of the retention ratio, the *device selectivity* can be expressed as

$$S_D = -\frac{z_o}{R^2} \frac{dR}{dz}. \quad (6.12)$$

While this particular rewriting lacks the form traditionally expected for selectivity, it has clear benefits as shall now be seen through a series of pedagogical examples specific to the ideal, unified FFF retention theory of § 5.

6.3 Pedagogical Examples

To demonstrate that the traditional definition of relative selectivity can be an odd and at times misleading quantity, a few instructive examples are now given. An interpretation of S_R and S_D will follow naturally from these examples.

In order to be concrete, this section's considerations are limited to cases of FFF techniques in which the external force acting on the sample, pushing it towards the FFF accumulation wall, is linear with respect to analyte size, *i.e.* $\alpha = 1$. Thermal-gradient [163, 274] and cross-flow [275–277] based FFF techniques are examples of such methods (see Table 5.2.1) but this choice is made in order to keep the discussion clear and does not affect the resulting conclusions. Since analyte size directly controls separation in these techniques, the COI is size itself, $z = r$. For simplicity, complications such as non-parabolic flow [217], non-dilute concentration effects [249], slip (§ 7.3), hydrodynamic/lift interactions (§ 7.2) or increased drag (§ 7.1) will be neglected and the ideal, unified retention theory will be utilized for analysis (Equation (5.25)).

For techniques that separate on the basis of particle size such that $z = r$, the non-dimensionalizing factor z_o must be a length scale. Several relevant length scales related to the apparatus exist for FFF methods. For instance, there is the length L of the channel and the channel height h . The channel length is a reasonable enough option (length controls the elution time), but the channel height is more natural since it is also the maximum size of an analyte for the given FFF device. Indeed, throughout § 5 discussing FFF theory h is used to re-scale particle sizes; therefore, $z_o = h$ is chosen.

6.3.1 Normal-Mode FFF

As a first example, consider normal-mode FFF when the external field is strong and $\tilde{r} \ll 1$. Normal-mode FFF is introduced in § 5.1.3. Figure 6.3.1 shows the retention time and retention ratio as a function of $\tilde{r}$ for a typical case in which the device retention ratio is $\Lambda = 10^{-6}$. The normal-mode regime is the narrow region at small particle sizes over which the retention ratio R falls rapidly with increased particle size (Equation (5.13)), or equivalently over which the retention time is approximately

$$\bar{t}(r) \approx ar, \quad (6.13)$$

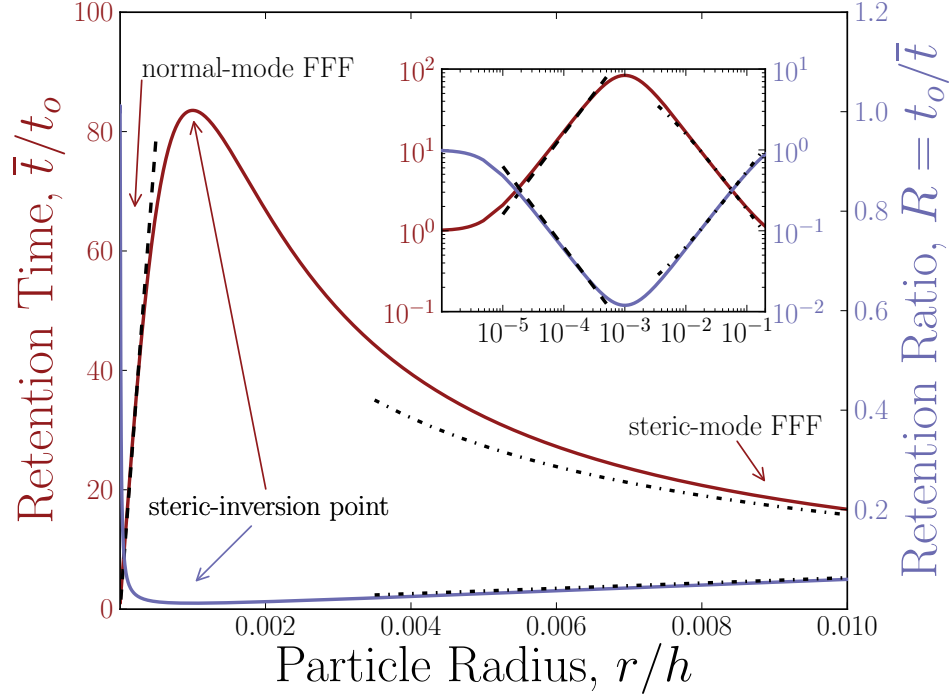


Figure 6.3.1: An example of a retention time (normalized by void time, t_o) and retention ratio as a function of scaled particle size $\tilde{r}$, computed using Equation (5.25) with $\Lambda = 10^{-6}$. The inset shows the same ideal retention curve on a log-log scale.

where $a = t_o/(6\Lambda h)$ is a positive constant and any intercept is negligible [198, 203]. For experimentally relevant external field strengths, the range of normal-mode FFF over which the approximation of a linear retention time as a function of particle size is accurate can be quite small. This is seen in Figure 5.2.7 and can explicitly be seen for this value of Λ by considering the black dashed line in Figure 6.3.1. In this normal-mode range, the traditional relative selectivity is ideally given by Equation (6.8c) and is

$$S_R = \frac{r}{\bar{t}} \frac{d\bar{t}}{dr} = \frac{1}{a} \frac{d\bar{t}}{dr} = 1. \quad (6.14a)$$

The ability to separate two distinct peaks depends only on the difference in size and so the selectivity is **constant** regardless of r . In fact, S_R is simply proportional to $d\bar{t}/dr$ when Equation (6.13) is a good approximation.

Similarly, the device selectivity (Equation (6.11c)) for this case of $z = r$ and $\bar{t} \approx ar$ is also constant:

$$S_D = \frac{h}{t_o} \frac{d\bar{t}}{dr} = \frac{h}{t_o} a. \quad (6.14b)$$

Although it is also size-independent, S_D has an interesting dependence on $a = t_o / (6\Lambda h)$. This dependence correctly signifies that increasing the slope, a , increases the distance between peaks at elution. Notice that this important fact is not reflected in the traditional definition of relative selectivity S_R .

6.3.2 Linear Retention Ratio

Although Equation (6.13) is the customary form used for normal-mode FFF, tiny tracer particles must move with the same velocity as the carrier fluid and so the intercept of the retention ratio must approach the void time t_o , not zero (as is the approximation of Equation (6.13)). This in turn means that the scaled retention time $\bar{t}/t_o$ must have an intercept of unity, which is generally insignificant for the large slopes that are relevant when performing normal-mode FFF. Consider a case when the intercept is constant that is not assumed to be insignificant such that the retention time is given by

$$\bar{t}(r) \approx ar + t_o. \quad (6.15)$$

Because selectivity is to represent the growing time between peaks and since the retention time still changes linearly with particle size, it is expected that the selectivity remains constant, just as it did for the previous example. However, the traditional relative selectivity is only constant significantly far from the intercept:

$$S_R = \frac{r}{\bar{t}} \frac{d\bar{t}}{dr} = \left[1 + \frac{t_o}{ar} \right]^{-1} \approx 1 + \frac{t_o}{ar} + \dots \quad (6.16a)$$

On the other hand, when an intercept is included, the proposed device selectivity remains strictly constant

$$S_D = \frac{h}{t_o} \frac{d\bar{t}}{dr} = \frac{h}{t_o} a. \quad (6.16b)$$

This value is identical to Equation (6.14b), properly reflecting the fact that only differences in elution times (not absolute values) matter for peak separation.

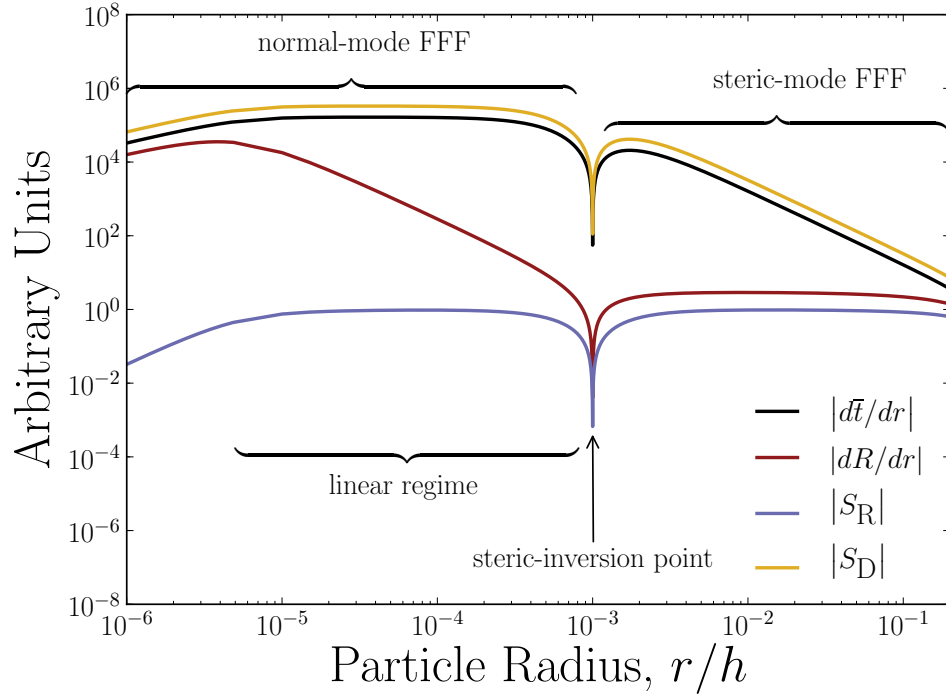


Figure 6.3.2: Rate of change of the retention time ($d\bar{t}/dr$), rate of change of the retention ratio (dR/dr), relative selectivity (S_R) and device selectivity (S_D) as a function of scaled particle size $\tilde{r} = r/h$ for the retention curve shown in Figure 6.3.1.

6.3.3 Steric-Mode FFF

Next, consider steric-mode FFF, which was introduced in § 5.1.4. As seen in Figure 6.3.1, a sizable portion of the retention ratio curve at particle sizes larger than the steric-inversion point is adequately represented by a simple linear approximation

$$R \approx Ar, \quad (6.17)$$

where $A \approx 6/h$ (Equation (5.28)) is a positive constant [215, 278, 279]. This approximation is shown as a dash-dot line in Figure 6.3.1.

Recalling the commonly written form of Equation (6.10) and quickly glancing at the retention ratio plotted on a log-log scale (inset of Figure 6.3.1) may lead one to believe that the abilities of normal- and steric-mode FFF to resolve differences in size are nearly identical, since the rate of change in logarithmic-scales are comparable. Indeed, applying Equation (6.10) to the approximation in Equation (6.17) leads to a relative selectivity

$$S_R = -1, \quad (6.18a)$$

that is exactly the same (absolute) value as is found for normal-mode FFF (Equation (6.14a)).

The relative selectivity as a function of particle size is shown in Figure 6.3.2 for both normal- and steric-mode FFF. The linear regime of normal-mode FFF discussed in § 6.3.1 can be seen as the flat portion in the region $3 \times 10^{-6} \lesssim \tilde{r} \lesssim 3 \times 10^{-4}$ for this example ($\Lambda = 10^{-6}$). As predicted by Equation (6.18a), the traditionally defined selectivity possesses a second plateau between $2 \times 10^{-3} \lesssim \tilde{r} \lesssim 2 \times 10^{-1}$. The plateaus have the same magnitude in both normal- and steric-mode FFF as the log-log scale retention time suggests they would (inset Figure 6.3.1). The steric-inversion point is the dip between the two regimes (the selectivity passes through zero).

This is, however, a misleading representation of the situation. As seen in Figure 6.3.1, the slope of the retention time in the steric-mode regime is always less than in the normal-mode FFF regime. This observation that the rate of change of the retention time with variation of COI is less in steric-mode FFF, which would have been missed if one had only considered the log-log representation (inset Figure 6.3.1), communicates the fact that the peak separation is never predicted to be as great as in normal-mode FFF. If the selectivity is to communicate the change in retention time relative to changes in COI, then S_R fails to do so here. What remains the same (and what S_R represents) is that steric-mode FFF has the same ability to resolve **fractional changes** in COI, not absolute changes.

Furthermore, Equation (6.18a) predicts S_R to be constant in this regime; however, glancing at either Figure 6.3.1 or Figure 6.3.2 demonstrates that the rate of the change of retention time decreases with $\tilde{r}$. The traditional relative selectivity does not capture the decreasing separation between peaks as the particle size increases.

The device selectivity for ideal steric-mode FFF retention is also shown in Figure 6.3.2. As discussed in § 6.3.1, both the relative and the device selectivity were constant in normal-mode FFF, but one can see that the proposed device selectivity behaves quite differently for steric-mode FFF. While the traditional definition follows the derivative of the retention ratio and so is constant, the device selectivity follows the derivative of the retention time. It is never as large as in the normal-mode regime, and from Equation (6.12) drops as

$$S_D \approx -\frac{h}{Ar^2}. \quad (6.18b)$$

Device selectivity is never constant in the steric-mode regime, reflecting the fact that it measures the absolute difference between peaks. From Equation (6.18b) or Figure 6.3.2, the steric-mode device selectivity is seen to vary as $S_D \sim r^{-2}$.

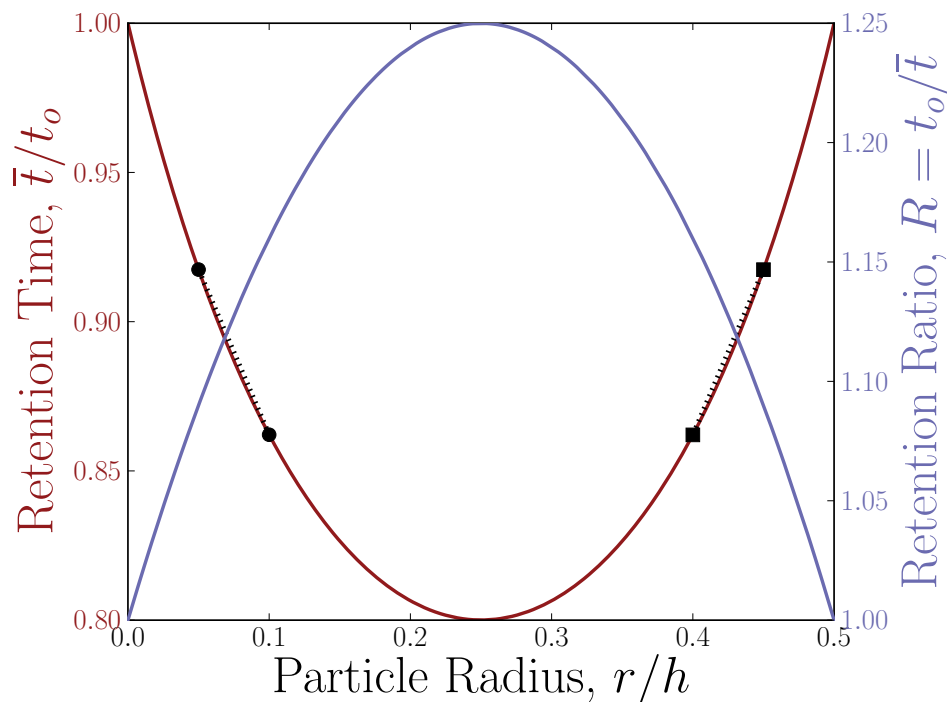


Figure 6.3.3: Retention time $\bar{t}$ (normalized by the void time t_o) and its inverse (the retention ratio R) as a function of scaled particle size $\tilde{r}$ in the hydrodynamic chromatography limit.

6.3.4 Hydrodynamic Chromatography

Another important example is the HC limit of FFF. It is shown (for the first time) in § 5.1.5 that hydrodynamic chromatography is always theoretically present at the tiniest species sizes (not shown in Figure 6.3.2), but its existence is rarely observable or significant unless the external field strength approaches zero. In absence of a field, the ideal, theoretically predicted retention ratio is parabolic (Equation (5.33); repeated here for convenience)

$$R = 1 + 2\frac{r}{h} - 4\left(\frac{r}{h}\right)^2,$$

which is symmetric about $r = h/4$ as seen in Figure 6.3.3. This is a considerable idealization as drag effects due to hydrodynamic interactions with the wall decrease $R(r)$ at large $\tilde{r} = r/h$ values, breaking the symmetry (as is discussed in detail in § 7.1). However, the ideal curve is still useful to show the differences between the traditional definition of relative selectivity and the proposed device selectivity.

The slope of $R(\tilde{r})$ is clearly symmetric, as seen in Figure 6.3.3, as is the rate of change of $\bar{t}$. Therefore, the separation between the two species represented as closed circles and the separation between the two closed squares in Figure 6.3.3, are expected to be equal.

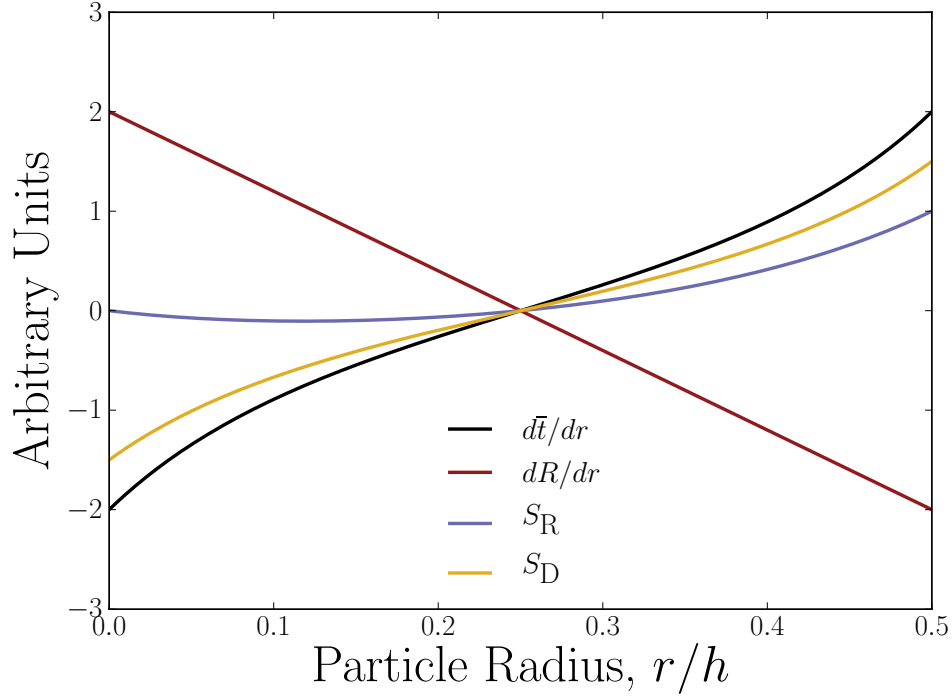


Figure 6.3.4: Rate of change of the retention time ($d\bar{t}/dr$), rate of change of the retention ratio (dR/dr), relative selectivity (S_R) and device selectivity (S_D) as a function of scaled particle size r/h for hydrodynamic chromatography. The traditionally defined relative selectivity S_R is highly non-symmetric.

However, since the traditional definition is relative, S_R will be larger for the squares, while S_D will not be.

Indeed, one can use Equation (5.33) to once again calculate both selectivities as a function of size (from Equation (6.10) and Equation (6.12), respectively) to be

$$S_R = \frac{1}{2R} \tilde{r} \left(\tilde{r} - \frac{1}{4} \right); \quad (6.19)$$

$$S_D = \frac{8}{R^2} \left(\tilde{r} - \frac{1}{4} \right), \quad (6.20)$$

where $R(\tilde{r})$ is given by Equation (5.33). Although the retention ratio $R(\tilde{r})$ is symmetric, the relative selectivity (Equation (6.19)) is non-symmetric about $r = h/4$. This is seen in Figure 6.3.4. In this situation (just as in steric-mode FFF), the magnitude of S_R favours larger solutes.

On the other hand, the device selectivity given by Equation (6.20) is symmetric about $r = h/4$. This is seen explicitly in Figure 6.3.4. As always, S_D follows $d\bar{t}/dr$ and so in this case, too, S_D cleanly represents the separation between peaks.

In a sense, this situation is the opposite of what was seen while discussing FFF; in FFF the relative selectivity's bias to larger particle sizes creates a symmetry between normal- and steric-mode regimes (captured in the log-log scale of Figure 6.3.1 and the fact that both regimes had $|S_R| = 1$), despite having different rates of change of retention time. On the other hand, in the case of HC, the relative selectivity does not capture the obvious symmetry — the device selectivity does. Therefore, the relative selectivity is once again seen to favour larger sizes and is valuable to describe relative differences in the COI but is not an appropriate metric for the absolute separation between peaks, which is better represented by the device selectivity.

6.4 Number of Theoretical Plates

Prior to this point, the focus has been on selectivity, but the other factor in the fractionating power, namely the number of theoretical plates (N_R from Equation (6.8b) and N_D from Equation (6.11b)), warrants a brief comment. By construction, if selectivity is to represent the power to separate peak centres, then the number of theoretical plates is to represent a dimensionless *zone spreading index* [230, 231]. Ideally this is done in some orthogonal manner to selectivity. The number of theoretical plates being **inversely** proportional to the dispersion/variance of the zones, larger values represent less broadening for a given elution time.

The traditional definition of N_R uses $\bar{t}$ as a normalization time scale, while the proposed alternative devices number of plates N_D uses t_o instead. This is analogous to the choice of h over r discussed previously in the context of selectivity. The relative number of theoretical plates thus mixes elution (through $\bar{t}$ in the numerator) and dispersion (through σ_t in the denominator). On the other hand, the device selectivity N_D focuses only on peak width and so is more orthogonal to separation between peaks (*i.e.* the selectivity). Even independently of the benefits of the device selectivity, this might prove a better tool to use in order to characterize a given FFF system.

Summary

Based upon the pedagogical examples presented, it appears that relative selectivity as defined by Equation (6.8c) works well for describing the distance between peaks when the retention time as a function of particle size is assumed to be linear with an intercept that is approximately zero. In other words, it works well for a first order approximation of normal-mode FFF in a relatively strong field. However, the interpretation of traditional relative selectivity for more complicated situations can be less clear. This stems from the choice of using $\Delta z / \langle z \rangle$ in the fractionating power (Equation (6.6)) as a dimensionless difference in characteristic-of-interest (COI). Because of this choice, relative selectivity is a good measure of the precision of the separation, but it is not a direct metric of the separation between peaks. For example, the relative selectivity highlights the fact that being able to resolve differences of a single nanometer for micron-sized species is far more precise than resolving between a 1 nm molecule and a 2 nm molecule. This is indeed what led to the asymmetry discussed with respect to HC (Figure 6.3.4) and to a constant, high selectivity in the steric-mode regime of FFF (Figure 6.3.2), despite a varying and small rate of change of retention time.

If one seeks to assess the ability of an FFF apparatus to fractionate differences in COI, then the device selectivity proposed in this chapter (Equation (6.11c)) may be more appropriate. The device selectivity makes a statement not about relative difference in COI but rather absolute differences. Thus, it is a direct, continuous measure of the separation between peaks that does not introduce extraneous species size-dependence. It can be simply interpreted as directly proportional to the slope of the elution time as a function of COI or equivalently it is the slope of the **normalized** elution time as a function of **normalized** COI:

$$S_D = \frac{z_o}{t_o} \frac{d\bar{t}}{dz} = \frac{d(\bar{t}/t_o)}{d(z/z_o)}. \quad (6.21)$$

It is important to stress that neither metric is **more correct** than the other, but one must carefully interpret the meaning of these values. In fact, it is clear that there is a trivial transformation relating the two:

$$S_D = \left(\frac{\bar{t}/t_o}{z/z_o} \right) S_R. \quad (6.22)$$

With this transformation, one can quickly convert between the two and understand any traditional, relative selectivity recorded in the literature in terms of the device selectivity.

Adoption of the device selectivity requires that the number of theoretical plates be modified similarly to

$$N_D = \left(\frac{t_o}{\bar{t}} \right)^2 N_R. \quad (6.23)$$

Either form of discussing selectivity may be more relevant for accurately describing a given situation, but it is essential to bear in mind that the traditional definition does not act as a direct measure of the separation between peaks, while the device selectivity does.

In the chapters that follow, the focus will remain on directly calculating retention ratios. This is because the information in the selectivity metric is always intrinsic to the retention curves and can be accessed by applying Equation (6.12) to the retention ratio. At times, the rate of change of retention ratio with solute size will be discussed to give a more intuitive sense of a system's ability to separate peaks, but strictly speaking, the device (or relative) selectivity should be worked out.

*When problems get really bad, they get good
again and this one is so horrible that it's easy.*

Tom McLeish [280]

7

Non-ideal Field-Flow Fractionation: Complications and Opportunities

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In § 5.3 the unified **ideal** retention theory for field-flow fractionation in microfluidic devices was presented; however, the situation is more complicated in reality. Microfluidic systems include non-ideal factors that can complicate the retention behaviour. The theory of § 5 is ideal in the sense that the flow field is assumed to be slow and unperturbed from Poiseuille flow, and particle interactions with the wall are assumed to be solely steric, without frictional or hydrodynamic interactions. Complications to the situation could drive experimentally measured retention ratios away from the ideal prediction (Equation (5.31)).

In this chapter, four complications are tackled. Paramount amongst non-ideal effects are hydrodynamic interactions that lead to wall-induced increased drag (wall drag), which are discussed in § 7.1. It is seen that the ideal retention theory agrees well with literature data for small solutes about the steric-inversion point, but simulations show that the ideal retention theory fails at much large solute sizes due to hydrodynamic interactions with the channel walls. However, multi-particle collision dynamics simulations confirm the more exotic transitions predicted in § 5, while highlighting the fact that increased drag due to hydrodynamic interactions with the microchannel walls (wall drag) significantly decreases the retention ratio at relatively large particle sizes. In order to make quantitative predictions across the entire range of possible solute sizes, models for wall drag are included in the theory. The retention ratio calculated using the results from a multipole expansion method find the highest agreement with the simulation data and with experimental data. These findings suggest that particle velocimetry methods must account for the wall-induced lag when determining flow rates in highly confining systems.

Hydrodynamic interactions are both resistively anti-parallel to the solutes' motion and also perpendicular to the channel walls. Such inertial lift forces arise from a combination of symmetry, breaking of the wake by the wall, and the shear-gradient generated force. They are complicated in form and the subject of ongoing fluid dynamic research *i.e.* their form is not the subject of this research. However, including their effects in retention theories is valuable since so-called hyperlayer FFF utilizes inertial forces to achieve separation. Therefore, this chapter presents a simple framework in which such effects can be incorporated into FFF retention theory.

Since walls play such an essential role, can their properties be modified in such a way as to improve the FFF retention ratio? Although breakdown of the no-slip condition (§ 3.4.3) may appear to be a complication, it is seen here that under certain conditions a novel operational-mode for FFF can be engineered if large slip-lengths can be constructed on the channel walls of microfluidic FFF apparatus.

Following this, the chapter's attention will turn to the fact that microfluidic channels are actually 3D rectangular channels and not 2D infinite channels. It turns out that lateral walls affect the retention ratio less significantly than wall drag. Although this is in a sense a null result, it is interesting since it can be unavoidable or even desirable at times to design rectangular elution channels with near-unity aspect ratios in microfluidic chips. Rather than limiting devices' ability to resolve samples, the derived series solution for the retention ratio in finite aspect ratio rectangular channels predicts that normal-mode FFF is well approximated by the infinite plate solution derived in § 5.3 and that steric-mode FFF and HC are slightly improved for small solutes. In each of these cases, a single complication to the ideal theory is tackled one at a time. Any further complications are still intently ignored.

7.1 Wall-Induced Lag

In this section, the ideal retention ratio is compared to multi-particle dynamics (MPCD) simulations (§ 3) and experimental data. Mesoscopic MPCD simulations quite naturally encompass complications due to the confinement of mobile colloids in a flowing, thermal fluid, yet are ideal in the sense that they do not possess the experimental issues mentioned above. These simulations will demonstrate that the ideal retention theory over-predicts the retention ratio of solutes that are comparable in size to the microchannel’s height because hydrodynamic interactions with the channel walls effectively increase the friction coefficient of the solutes (wall drag). Various models of wall drag can be incorporated into the retention theory to account for these interactions. Many are found to be inadequate. Only a slip function based on the multipole expansion method (§ 2.3.4) is found to be successful at all solute sizes.

7.1.1 Steric-Inversion Point

Experiments utilizing FFF can only avoid ambiguity in determining solute size due to the non-monotonicity of Equation (5.31) by working in only one of the four operational modes discussed in § 5.1 (usually normal-mode FFF for its high selectivity). In the literature, the **only** transition observed experimentally is the steric-inversion point between normal-mode FFF and steric-mode FFF [7–11, 216, 281].

Retention ratio measurements near the steric-inversion point have been reported for both gravitational- and sedimentation-FFF (Figure 7.1.1) in the literature. In both of these non-microfluidic subtechniques the force scaling exponent is $\alpha = 3$ (Table 5.2.1). A least-square fitting procedure that varies the device retention parameter and the void time are used to determine the best fits of Equation (5.31) for the gravitational, $\alpha = 3$ data in Figure 7.1.1. The unified ideal retention theory fits the transition from normal-mode FFF to steric-mode FFF quite well, regardless of Faxén’s law — dotted lines neglect Faxén’s law, while dashed lines include it. For the given range of normalized sizes, the two forms of $\mathcal{F}(\tilde{r})$ are essentially **indistinguishable**.

Likewise, the fit to the transition from normal- to steric-mode FFF when $\alpha = 3$ is also quite accurate (Figure 7.1.1). In such non-microfluidic situations, the correction due to Faxén’s law is trivial to include but of negligible importance. Both ideal retention theories (Equation (5.25) and Equation (5.25)) fit the data in Figure 7.1.1 well because the particles

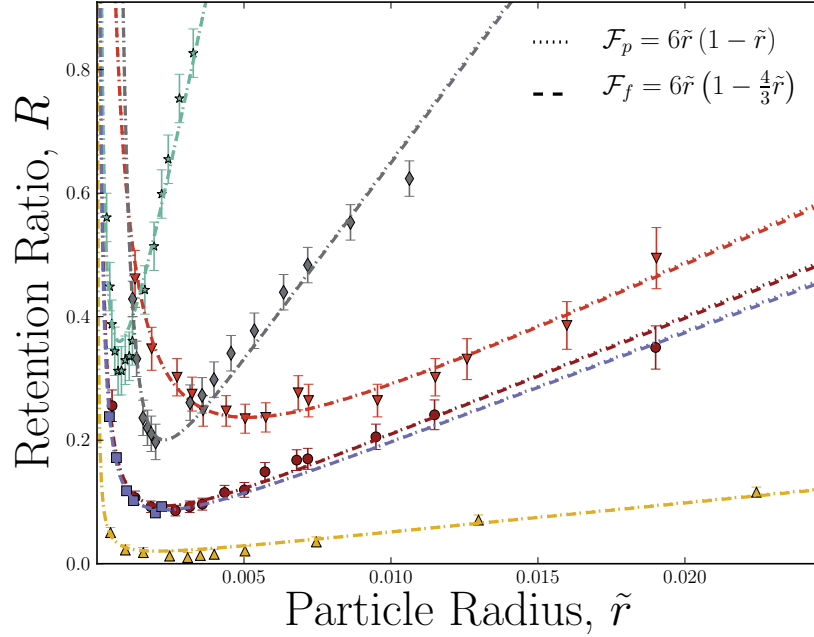


Figure 7.1.1: Fit of Equation (5.31) to a variety of FFF systems. Dotted lines ignore Faxén’s law, while dashed lines account for it. In this range, the dotted and dashed lines overlap perfectly. Thermal FFF utilizes a temperature gradient and $\alpha = 1$ [11]. The data are for $\Delta T = 40$ K ($\bullet$) and $\Delta T = 5$ K ($\blacktriangledown$). Symmetric ($\blacktriangle$ [7]), asymmetric ($\blacksquare$ [10]), and hollow-fiber ($\star$ [9]) FFF use a cross flow and have $\alpha = 1$. For GrFFF, $\alpha = 3$ ($\blacklozenge$ [8]).

are small relative to the channel height ($\tilde{r} < 0.025$ in all cases). When the particle sizes are larger, the theories differ greatly for the different forms of $\mathcal{F}(\tilde{r})$ (Equation (5.18) and Equation (5.32)). In order to explore these more exotic regimes of FFF, MPCD simulations of eluting colloids were performed.

7.1.2 MPCD Simulations of FFF

Simulations of spherical colloids embedded in a coarse-grained multi-particle collision dynamics fluid were performed as described in § 3.5.1. The colloids were modelled as mobile, BC-solutes (§ 3.5.1) and simple bounce-back rules govern collisions between MPCD particles and walls (both channel walls and colloid surfaces), although phantom fluid particles must be included in the collision operator of cells intersecting walls to ensure that artificial viscous thinning does not occur and that the no-slip boundary conditions were respected (§ 3.3.2). Energy conservation during collision events is neglected since the Anderson-MPCD collision operator is utilized (§ 3.2.2) with a number density of $n_{\text{MPCD}} = 5a^{-3}$ and dynamic viscosity

Dimensions			Flow		External Force	
h [a]	w [a]	L [a]	$g\hat{x}$ [$\delta t^{-2}a$]	Re	$G\hat{y}$ [$\delta t^{-2}a$]	Λ
210	1050	2100	1.0×10^{-4}	14	0	∞
					2.81×10^{-9}	1.87×10^{-4}
					2.81×10^{-8}	1.87×10^{-5}
					2.81×10^{-6}	1.87×10^{-7}
90	450	900	5.44×10^{-4}	10	0	∞
					8.32×10^{-8}	1.87×10^{-4}
					8.32×10^{-7}	1.87×10^{-5}
					8.32×10^{-5}	1.87×10^{-7}
26	130	260	6.54×10^{-3}	7	0	∞
					1.19×10^{-5}	1.87×10^{-4}
					1.19×10^{-4}	1.87×10^{-5}
					0.0119	1.87×10^{-7}
15	75	150	1.96×10^{-2}	4	0	∞
					1.08×10^{-4}	1.87×10^{-4}
					1.08×10^{-3}	1.87×10^{-5}
					0.108	1.87×10^{-7}

Table 7.1.1: Simulation parameters. The channel dimensions are height:width:length ($h : w : L$). The flow is driven by an acceleration g , which produces a Reynolds number Re. An external force of acceleration G pushes the solutes against the accumulation wall. The force simulates gravity and so by Table 5.2.1 $\alpha = 3$ and the device retention ratio is $\Lambda = (3/4\pi) (k_B T / G \Delta \rho h^4)$.

of $\eta = 1.08179 [m a^{-1} \delta t^{-1}]$ (§ 3.2.2). The system is periodic in the $\hat{x}$ - and $\hat{z}$ -directions with the mean fluid flow in the $\hat{x}$ -direction. Planar channel walls were in the xz -plane. Contact events between the channel walls and the hard solutes are treated as elastic collisions with a coefficient of restitution $\varepsilon = 1$ (see § 3.5.1). In all cases, the length of the system varies as $10 \times r$ and the breadth as $5 \times r$. Four channel heights $h = \{210, 90, 26, 15\} a$ were used (Table 7.1.1). Simulations presented in this section ran on SHARCNET [136] utilizing approximately 10 processors for 7 days each.

Flow is driven by an external acceleration $g\hat{x}$, which is equivalent to a pressure gradient

$$\vec{\nabla} P = -n_{\text{MPCD}} m \vec{g}, \quad (7.1)$$

as discussed in § 2.3.1 and § 3.4.3. Changing the channel height changes the Reynolds number (Equation (2.62)) unless a different fluid acceleration g is used. Fluid accelerations are chosen to keep the Reynolds numbers small ($\text{Re} \lesssim 100$; Table 7.1.1). This is two orders of magnitude lower than necessary to keep the flow laminar. Keeping the particle Reynolds number $\text{Re}_P \equiv \mathcal{V}r/\eta \approx (r/h) \text{Re}$ low ensures that inertial forces (lift) remain insignificant. Lift forces pushing the solutes toward the centre of the channel would needlessly complicate the situation [282] and a simplified model for hyperlayer FFF near the steric-inversion point

is discussed in § 7.2.

Simulations are of a single eluting colloid, which correspond to the infinitely dilute limit assumed in this thesis (§ 5; page 172). Quantitatively, the dilute limit occurs when solutes rarely interact and since hydrodynamic interactions are long range (§ 2.3.4) this requires that the average distance between solutes is larger than the hydrodynamic screening length. Since the screen length is the distance between channel walls $\sim h$ (§ 2.3.5), this dilute limit corresponds to a colloid concentration of $n \lesssim h^{-3}$. The colloid density is $\varrho = 750m/(\pi a^3)$ and subjected to an external force in the $\hat{y}$ -direction to simulate gravitational acceleration G . Since gravitational-FFF (see Table 5.2.1) is being simulated, $\alpha = 3$ and the device retention parameter is

$$\Lambda = \left(\frac{3}{4\pi} \right) \left(\frac{k_B T}{G \Delta \varrho h^4} \right) \quad (7.2)$$

In order to efficiently simulate each operational mode of FFF, the box size is varied, requiring that G and g are varied to keep Λ strictly constant and the particle Reynolds number Re_p roughly constant (Table 7.1.1). Four different limits of applied external field are considered:

Zero field $\Lambda = \infty$.

Weak field $\Lambda = 1.87 \times 10^{-4}$.

Intermediate field $\Lambda = 1.87 \times 10^{-5}$.

Strong field $\Lambda = 1.87 \times 10^{-7}$.

It was found that for the strongest forces used, the BC-solute could become pinned to the wall with no fluid between the particle surface and the plane. When this occurred, the colloid did not translate for the extent of the simulation because bounce-back conditions ensure no-slip between the two surfaces. This unphysical situation is due to the discrete particle nature of MPCD and demonstrates a limitation of the method. For this reason, large beads in strong fields that had a null average velocity were rejected from the analysis.

Strong Field Simulations

The simulation data for strong fields (Figure 7.1.2) agree relatively well with the ideal retention theory in the normal-mode regime during which the retention ratio drops rapidly with

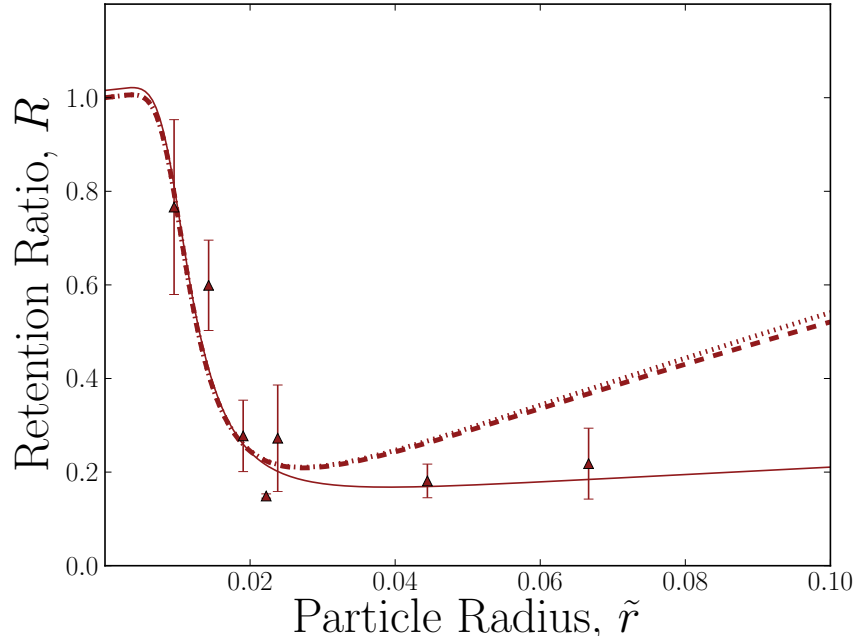


Figure 7.1.2: MPCD simulation results for $\alpha = 3$ and $\Lambda = 1.87 \times 10^{-7}$. This is a relatively strong external field. The dotted lines show the retention curve that neglects Faxén’s law (Equation (5.25) with Equation (5.18)), while the dashed line shows the unified ideal retention theory that accounts for Faxén’s law (Equation (5.31) with Equation (5.32)). Solid lines show the theory that accounts for increased drag [5].

particle size. Because large particles subject to this strong field become pinned to the wall, particles larger than $\tilde{r} \gtrsim 0.07$ could not be simulated as explained above.

As was true with the experimental data (§ 7.1.1), the unified ideal theory with (dashed line) and without (dotted line) accounting for Faxén’s law agree in this regime. However, available MPCD simulation data points are systematically lower than both ideal theories predict (Figure 7.1.2). Therefore, the MPCD data suggest that the ideal theory over-predicts the retention ratio R of large solutes in microfluidic devices, regardless of Faxén’s law.

Zero Field Simulations

Next consider the opposite limit of no applied field $\Lambda = \infty$, which is the hydrodynamic chromatography limit (Figure 7.1.3). The retention behaviour differs significantly from the strong field case (Figure 7.1.2). While the retention ratio dropped rapidly from unity in the strong field case (Figure 7.1.2), these simulations of neutrally buoyant colloids demonstrate

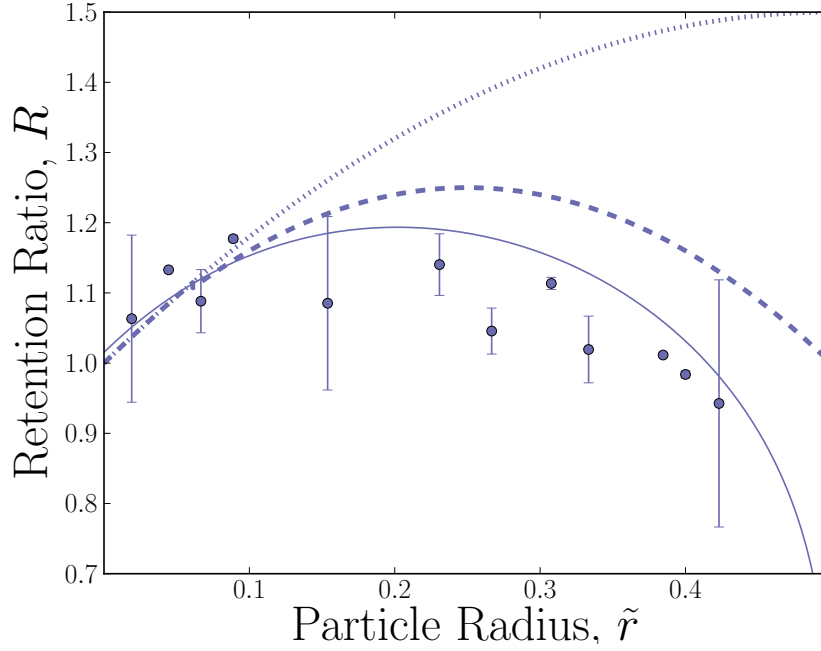


Figure 7.1.3: MPCD simulation results for $\alpha = 3$ and $\Lambda = 0$. This is the HC limit with no external field. The dotted lines show the retention curve that neglects Faxén’s law (Equation (5.20)), while the dashed line shows the unified ideal retention theory that accounts for Faxén’s law (Equation (5.33)). Solid lines show the theory that accounts for increased drag [5].

that the retention ratio is greater than unity for the vast majority of particles. It is qualitatively observed that neither normal-mode FFF nor steric-mode FFF exist (Figure 7.1.3). Simulations show two regions: For small particles, R increases, while for large particles, R decreases.

The simulated retention ratio rises slightly to a global maximum and then decreases slowly. This is qualitatively what is predicted by the retention theory that includes Faxén’s law (Equation (5.33)), which parabolically rises to a maximum of $R = 1.25$ at $\tilde{r}_{\text{SF}} = 0.25$ (dashed line in Figure 7.1.3). The ideal theory that neglects Faxén’s law (Equation (5.20)), on the other hand, rises to a global maximum of $R = 1.5$ at the largest particle sizes (dotted lines in Figure 7.1.3). Therefore, the MPCD simulations **qualitatively verify** the predicted Faxén-mode FFF operational mode. However, the ideal, theoretical curves (dashed lines) fail to predict the simulated retention ratios in any quantitative sense beyond $\tilde{r} \gtrsim 0.15$. Just as in strong fields (Figure 7.1.2), at all but the smallest particle sizes, the ideal theory overpredicts the retention ratio. Interestingly, recall from § 7.1.1 that Equation (5.31) represents the literature data for FFF channels with larger channel heights extremely well (Figure 7.1.1) but fails here for large colloids eluting through microfluidic channels. The same is observed

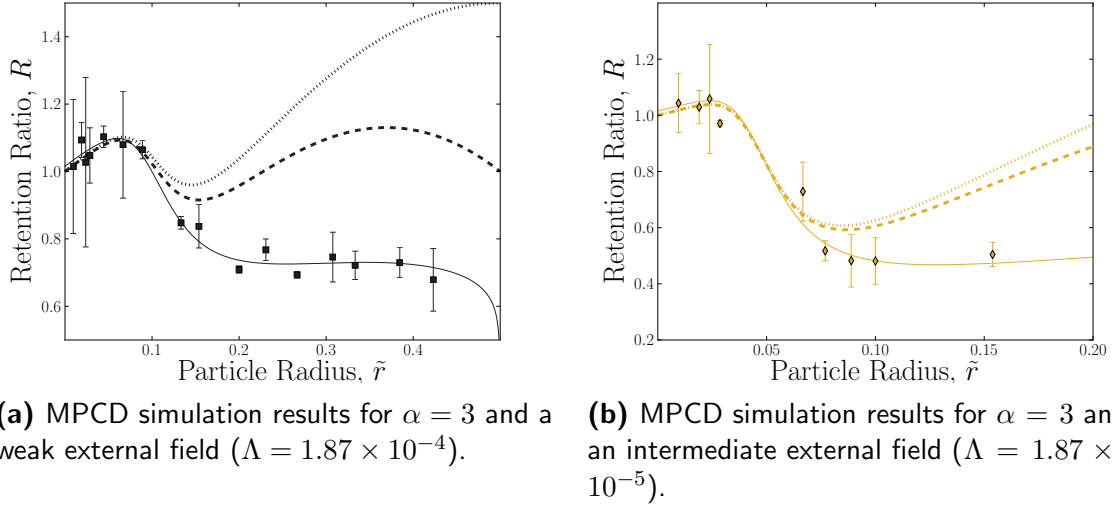


Figure 7.1.4: The dotted lines show the retention curve that neglects Faxén’s law (Equation (5.25) with Equation (5.18) or Equation (5.20)), while the dashed line shows the unified ideal retention theory that accounts for Faxén’s law (Equation (5.31) with Equation (5.32) or Equation (5.33)). Solid lines show the theory that accounts for increased drag [5].

here. Accounting for such effects is considered in detail in § 7.1.3.

Weak and Intermediate Fields

The MPCD data for elution in a weak external field ($\Lambda = 1.87 \times 10^{-4}$) in Figure 7.1.4a extend across the entire range of possible particle sizes and, although agreement between ideal theories and simulations is once again observed at small solute sizes ($\tilde{r} \lesssim 0.1$), the observed retention curve is quite different from the ideal theory, using either form for $\mathcal{F}(\tilde{r})$ (dotted and dashed lines), especially in the case of large colloids. The normal-mode descent continues to larger particle sizes than predicted and past $\tilde{r}_{\text{NS}}$ the steric-inversion point of the simulations, the retention ratio is significantly less than predicted by Equation (5.31) (dashed lines). In fact, the retention ratio remains relatively constant such that it is almost impossible to discern steric-mode FFF, and Faxén-mode FFF is not observed.

The simulations demonstrate that even under simplified prototypical conditions, steric-mode FFF and Faxén-mode FFF are **significantly suppressed** in microfluidic channels, leaving an extended range of particle sizes over which the retention ratio does not rise. There is little selectivity or resolution between particles larger than $\tilde{r} \gtrsim 0.2$ when $\Lambda = 1.87 \times 10^{-5}$ (Figure 7.1.4a). Although the theory that neglects Faxén’s law over-predicts the retention

ratio more greatly than the theory that accounts for it, neither model accurately predicts the retention curve. As is discussed in § 7.1.3, this is due to wall-induced hydrodynamic effects.

When the field is intermediate ($\Lambda = 1.87 \times 10^{-5}$), the retention curves possess a normal-mode FFF region of steep descent after a brief hydrodynamic chromatography climb (Figure 7.1.4b). The simulation data show a steric-inversion point $\tilde{r}_{\text{NS}}$ at a lower retention ratio and larger particle size than predicted by the ideal theory. For strong fields and large colloids, the discrete particle nature of MPCD once again cannot adequately model a continuous fluid, and simulations do not span the entire range of sizes.

Although these simulations reveal the inaccuracy of the unified ideal retention theory at large colloid sizes, they also demonstrate its strength. The MPCD data initially rise through a hydrodynamic chromatography regime at the smallest particle sizes, pass through a maximum $\tilde{r} = \tilde{r}_{\text{HN}}$ then drop for normal-mode FFF. Thus, this is an observation of the theoretical transition from hydrodynamic chromatography and normal-mode FFF, which was first predicted by the unified ideal retention theory developed in § 5.2.3 (Figure 5.2.7).

7.1.3 Increased Friction Coefficient

The MPCD simulations of field-flow fractionation in a microfluidic channel are not well-predicted by the ideal retention theory encapsulated in Equation (5.31) (Figures 7.1.2-7.1.4). The most significant complication omitted from ideal retention theory is hydrodynamic interactions with the microfluidic channel walls, leading to an increased effective friction coefficient. Hydrodynamic interactions can be divided into forces acting anti-parallel (*wall drag*) and forces acting perpendicular (*lift*) to the wall. Drag forces are generated because the no-slip condition on the surface of the mobile colloid and on the channel wall causes greater shearing of the fluid located between the wall and the particle and hence a greater effective friction coefficient of the particle than in the absence of boundaries. In the MPCD simulations, the particle Reynolds number was kept small, such that lift forces remained negligible in Figures 7.1.2-7.1.4. Increased drag forces, however, cannot be neglected as shall now be seen.

Research on the retardation of solute velocity due to wall drag remains ongoing despite being first considered in the 1920s by Faxén [283]. No-slip boundary conditions at both the channel wall and the particle surface ensure that fluid must be sheared more greatly between the wall and the solute than it would in free solution (Figure 7.1.5). The primary course

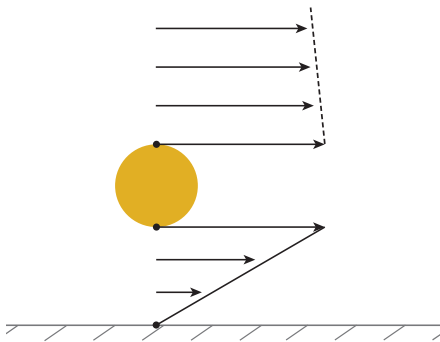


Figure 7.1.5: Schematic illustrating that no-slip boundary conditions at the solute surface and the channel walls demands higher shears than in free solution. This leads to larger drag forces and so a larger effective drag coefficient.

of action is to implement intensive numerical algorithms to determine the particle velocity at a given point in a system with walls. Direct computer simulations are popular [284, 285] but numerical methods are most often the tool of choice. Various types of boundary-integral methods have been shown to be very accurate [286–290], as have multipole expansion methods [291–295].

By binning and averaging the velocity of the BC-solutes at different heights from the wall, a measure of the solute velocity profile can be constructed (Figure 7.1.6). When the BC-solute is significantly smaller than the channel height, the near-wall solute velocity profile is nearly identical to the velocity profile predicted by Faxén’s law, which itself is only a minor perturbation from the carrier fluid velocity profile (Figure 7.1.6a). For such small radii, the small solutes behave as tracer particles. For relatively large BC-solutes, Faxén’s law predicts a significantly decreased solute velocity near the channel walls (which is precisely what lead to the lowered retention ratio and Faxén-mode FFF). However, the MPCD simulations produce solute velocity profiles that are even lower than predicted at all distances from the wall (Figure 7.1.6). The measured velocity distributions for many relative particle radii are shown in Figure 7.1.6. At any point, the solute velocity is lower than the solvent velocity profile (the parabolic curve is the theoretical Poiseuille flow [Equation (3.27)] based on the measured average fluid velocity) or the Faxén law profile. The solute velocity is not simply shifted down by an amount $-2\langle v \rangle \tilde{r}^2$ as predicted by Equation (5.30):

- For small particle sizes (Figure 7.1.6a), the measured velocity profile of the solutes corresponds rather well with the solvent velocity and also Faxén’s law in the near-wall region.

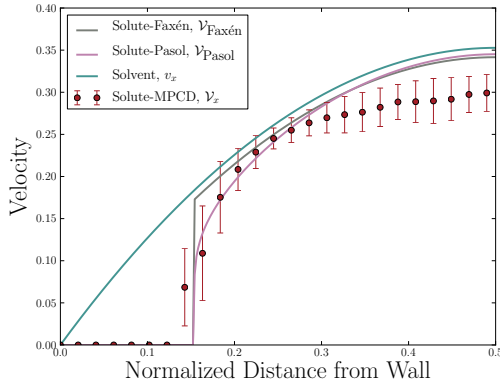
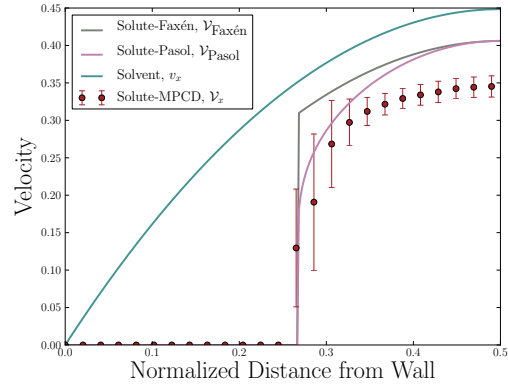
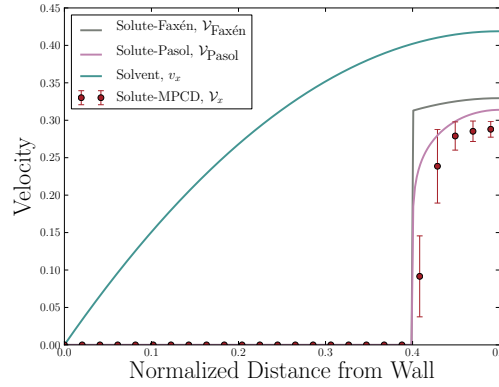
(a) Particle $r = 4a$ in channel $h = 26a$.(b) Particle $r = 4a$ in channel $h = 15a$.(c) Particle $r = 6a$ in channel $h = 15a$.

Figure 7.1.6: The simulated solute speed for a variety of solute sizes. The hard, steric repulsion from the channel walls is clearly visible in each, as is the fact that Faxén’s law shifts the ideal solute velocity lower than that of a tracer particle (solvent velocity v_x). The observed velocity is lower than the Faxén velocity profile. The multipole expansion value of Pasol *et. al.* describes the velocity of the solutes in the near-wall region more accurately [5].

- At larger sizes (Figure 7.1.6b-7.1.6c), the solute velocity becomes flatter near the centre and further and further from the Faxén’s law profile in the near-wall region.

Approximate Friction Coefficients

Many simple, approximate but analytic forms for the friction coefficient of a sphere moving near a planar surface exist in the literature. Most of these are only accurate for a narrow set of parameters. Such equations are most often encountered in terms of the force acting anti-parallel to the wall on a particle (Equation (2.78a)) but in this case the force is due to

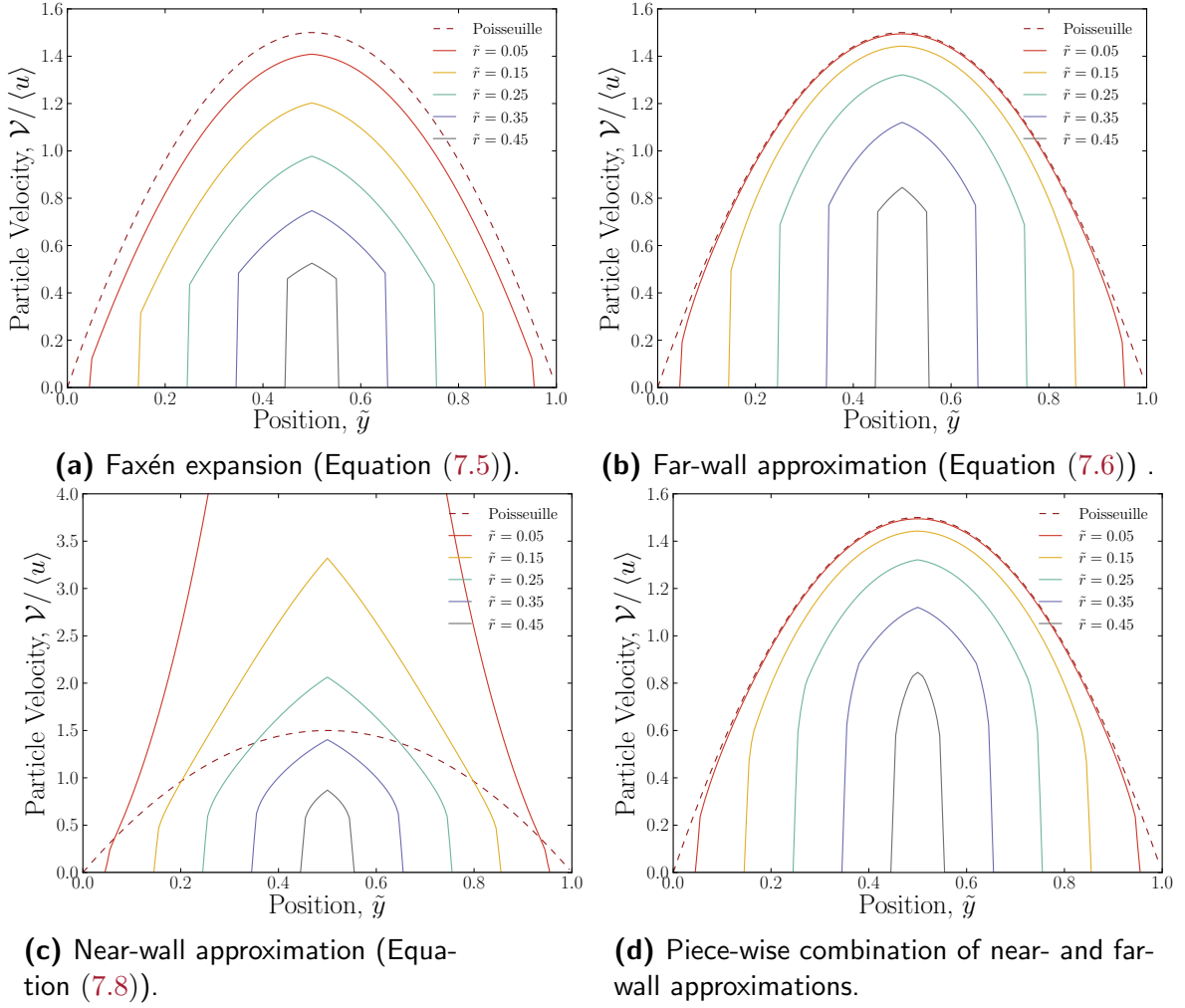


Figure 7.1.7: Solute velocity profiles for spherical particles between two walls in which the slip function is approximated as that for the nearest wall only. Example profiles for solutes of size $\tilde{r} = \{0.05, 0.15, 0.25, 0.35, 0.45\}$.

a flow incident on the solutes so the solute velocity is given by

$$\mathcal{V}(\vec{x}, t) = \frac{\zeta_0}{\zeta(\vec{x})} v(\vec{x}, t), \quad (7.3)$$

where $\zeta_0 = 6\pi\eta r$ is Stokes' friction coefficient for a sphere (Equation (2.80)). The ratio ζ_0/ζ is recognized from § 2.3.5 to be the inverse of the resistance or the slip function

$$K^{-1}(\vec{x}, t) = \frac{\zeta_0}{\zeta(\vec{x}, t)} = \frac{\mu(\vec{x}, t)}{\mu_0}. \quad (7.4)$$

Faxén Expansion Faxén first developed an analytic expression for $\zeta_0/\zeta(\vec{x})$ for a spherical particle moving near a single wall [283] and then later a form for a particle between two walls [30, 296]

$$K^{-1}(r, \ell) = \frac{\mathcal{V}(\vec{x}, t)}{v(\vec{x}, t)} = 1 - A\left(\frac{r}{\ell}\right) + B\left(\frac{r}{\ell}\right)^3 - C\left(\frac{r}{\ell}\right)^5 + \dots \quad (7.5)$$

where ℓ is the distance from the centre of the particle to the nearest wall and forms for A and B are long but can be found in the literature [30]. By numerically calculating A , the velocity distribution can be found (Figure 7.1.7a). The velocity between two plates can be naively approximated by applying the *Faxén expansion* (Equation (7.5)) to the nearest wall and neglecting the more distant wall. Some example velocity profiles show that the velocity instantaneously jumps from zero to a finite value when the particles are in contact with the wall ($\tilde{y} = \tilde{r}$) and that the slope of the velocity profile is discontinuous at the centre of the channel (Figure 7.1.7a).

Goldman-Cox-Brenner Far-Wall Approximation Goldman, Cox and Brenner performed the classic theoretical study on the motion of a sphere parallel to a single planar wall [297, 298]. They provided the approximate formula

$$K^{-1}(r, \ell) \approx 1 - \frac{5}{16} \left(\frac{r}{\ell}\right)^3 \quad (7.6)$$

as having good agreement with their numerical data when the distance between the surface of the particle and the wall is large. This form does not have a linear term like the Faxén approximation did (Equation (7.5)). Applying the *Goldman-Cox-Brenner far-wall approximation* (Equation (7.6)) to the nearest wall and neglecting the more distant wall produces the particle velocity profiles seen in Figure 7.1.7b. These profiles suffer the same criticisms as the Faxén one-wall expansion.

Goldman-Cox-Brenner Near-Wall Approximation In the same paper as when they developed the far-wall approximation (Equation (7.6)), Goldman, Cox and Brenner derived a *near-wall approximation* as well [298]. They estimated that

$$K^{-1} = \frac{0.6491 - 0.6802 \ln\left(\frac{\ell-r}{r}\right) - [0.1192 + 0.6293 \ln\left(\frac{\ell-r}{r}\right)] / [1 + (\frac{\ell-r}{r})]}{0.3181 - 0.6376 \ln\left(\frac{\ell-r}{r}\right) + 0.2 [\ln\left(\frac{\ell-r}{r}\right)]^2}, \quad (7.7)$$

where $\ell - r$ is the distance between the surface of the sphere and a single wall. The contact limit ($\ell/r \rightarrow 1$) of the slip function is [298, 299]

$$\lim_{\ell/r \rightarrow 1} K^{-1} \approx \frac{0.7431}{0.4950 - 0.2 \ln \left(\frac{\ell-r}{r} \right)}. \quad (7.8)$$

Empirical fits to experimental data share the same form as Equation (7.8), although they can be a bit more accurate [29]. The slip function of a solute moving between two walls is, once again, approximated as the slip function of the nearest wall only in Figure 7.1.7c. Whereas the previous approximations discontinuously jumped from zero velocity at the contact point $\ell = r$, the near-wall approximation rises continuously from zero (Figure 7.1.7c). That is to say that the predictions for the velocity of particles in the near-wall region appear reasonable. However, the near-wall approximation drastically over-predicts the velocity of particles even marginally far from the walls.

Near-Far Hybrid In order to achieve a slightly less unrealistic solute velocity profile, the Goldman, Cox and Brenner near- and far-wall approximations for a single wall can be combined by saying that the solute velocity is the minimum of Equation (7.6) and Equation (7.8) for the nearest wall. The resulting piece-wise function is shown in Figure 7.1.7d. The solute velocity profile is far from perfect but certainly the most realistic of the approximations considered so far.

Oseen Superposition Clearly, something essential is missing in the procedures attempted above. Fundamentally, they assume that the the slip function is dominated by the nearest wall. This is certainly not the case. In 1927, Oseen suggested that Faxén's result for the friction coefficient of a sphere moving parallel to a single plane [30, 283, 300]

$$\begin{aligned} K_1^{-1}(r, \ell) &= \frac{\zeta_0}{\zeta_1(\vec{x})} = 1 - \frac{9}{16} \left(\frac{r}{\ell} \right) + \frac{1}{8} \left(\frac{r}{\ell} \right)^3 - \frac{45}{25} \left(\frac{r}{\ell} \right)^4 - \frac{1}{16} \left(\frac{r}{\ell} \right)^5 + \dots \\ &\approx 1 - \frac{9}{16} \left(\frac{r}{\ell} \right) \end{aligned} \quad (7.9)$$

could be used to approximate the friction of a sphere moving between two planes via **superposition** [301, 302]. He approximated the linear combination

$$\zeta(r, y) = \zeta_0 + [\zeta_1(r, y) - \zeta_0] + [\zeta_1(r, h - y) - \zeta_0], \quad (7.10)$$

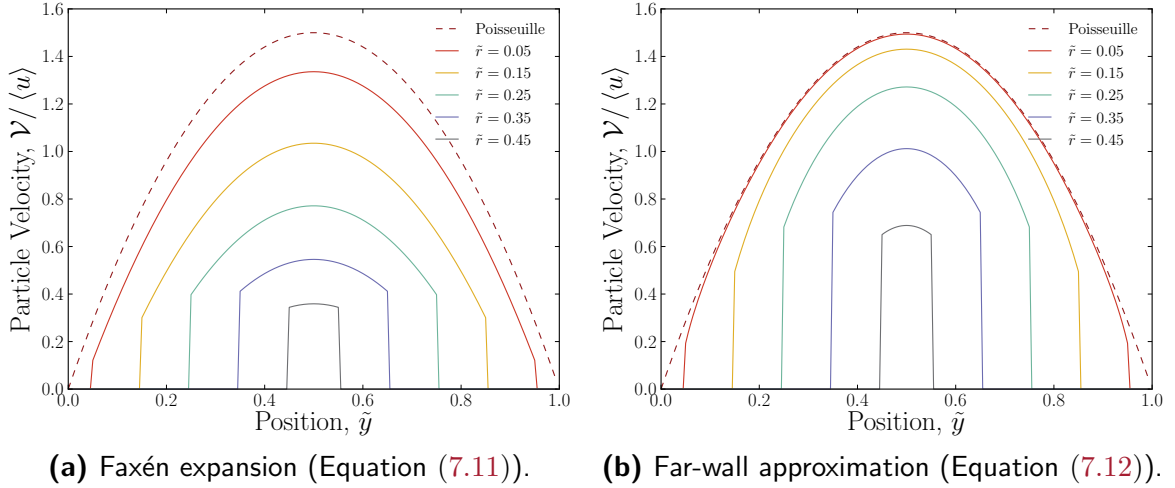


Figure 7.1.8: The Oseen superposition approximation for the velocity of a spherical particle moving between two parallel walls (Equation (7.10)) applied to the Faxén expansion and to Goldman, Cox and Brenner’s far-wall approximation. Example profiles for solutes of size $\tilde{r} = \{0.05, 0.15, 0.25, 0.35, 0.45\}$.

where ζ_1 is the friction coefficient of a sphere near a single planar wall. This produces a slip function

$$K^{-1}(r, y) = \left\{ 1 + \left[\frac{\zeta_1(r, y)}{\zeta_0} - 1 \right] + \left[\frac{\zeta_1(r, h - y)}{\zeta_0} - 1 \right] \right\}^{-1}. \quad (7.11)$$

Because it is a superposition of both walls, the resulting solute velocity profile is smooth at the centre of the channel (Figure 7.1.8a). This is an improvement over the previous estimates; however, the near-wall velocity is still discontinuous, which suggests that the *Oseen superposition approximation* will not accurately predict the retention ratios, since solutes are likely to be found quite near the accumulation wall.

Furthermore, a comparison of the Oseen approximation of superimposing Faxén’s expansion for a sphere near a single plane (Figure 7.1.8a) to the hybrid slip function (Figure 7.1.7d), suggests that Equation (7.11) under-predicts the velocity. As was discussed with respect to the Goldman-Cox-Brenner far-wall approximation (Figure 7.1.7b), the lowest order for the Faxén approximation is linear in r/ℓ but the Goldman-Cox-Brenner far-wall approximation begins $(r/\ell)^3$ and so does not predict as large a retardation. If Oseen’s idea of superimposing the solution for a sphere moving parallel to a single plane (Equation (7.11)) is performed but with the Goldman-Cox-Brenner far-wall approximation (Equation (7.6)) instead, then

the slip function reads

$$K^{-1}(r, y) = \left\{ 1 + \left[\frac{16}{5} \left(\frac{y}{r} \right)^{-3} - 1 \right]^{-1} + \left[\frac{16}{5} \left(\frac{h-y}{r} \right)^{-3} - 1 \right]^{-1} \right\}^{-1}, \quad (7.12)$$

which is seen to be smooth and not excessively slower than expected (Figure 7.1.8b).

Multipole Expansion Method It is explained in § 2.3.4 that stokeslets can be superimposed in the multipole expansion method. This was done first for spheres interacting with a single wall [287, 292–294, 303–306]. The multipole expansion method was applied to spherical particles eluting between two parallel surfaces by Pasol *et. al.* [5]. They performed numerical simulations using a multipole expansion algorithm that combines spherical and Cartesian representations of the flow [292–294]. The resulting numerical multipole expansion solution for all possible particle sizes at each point in the channel was fit to the function

$$K^{-1} = \frac{c_{yy}^{\text{rot}} f_{\text{pois}} + f_{xy}^{\text{rot}} c_{\text{pois}}}{c_{yy}^{\text{rot}} f_{xx}^{\text{trans}} - f_{xy}^{\text{rot}} c_{yx}^{\text{trans}}} \frac{1}{\tilde{y} - \tilde{y}^2}. \quad (7.13)$$

This equation accounts for the forces $\vec{f}$ and the torque $\vec{\mathcal{N}}$ on the solute. The fitted formula for the coefficients in Equation (7.13) are given by Pasol *et. al.* [5]. They are repeated here for completeness. In the following ς_i are the dimensionless gap sizes between the surface of the particle and each wall *i.e.* $\varsigma_1 = (y - r) / y$ and $\varsigma_2 = (h - y - r) / (h - y)$.

- The **translational** friction factor for a sphere **translating** with a velocity $\mathcal{V}_{\hat{x}}$

$$f_{xx}^{\text{trans}} = -\frac{f_{\hat{x}}}{6\pi r \eta \mathcal{V}_{\hat{x}}} \quad (7.14a)$$

$$\begin{aligned} &= 1.0051 - \frac{8}{15} (\log \varsigma_1 + \log \varsigma_2) - \frac{64}{375} (\varsigma_1 \log \varsigma_1 + \varsigma_2 \log \varsigma_2) \\ &\quad + 0.0190 \left(\tilde{y} - \frac{1}{2} \right)^2 - 0.613 \left(\tilde{y} - \frac{1}{2} \right)^4 \\ &\quad + \tilde{r} \left[-0.961 - 4.75 \left(\tilde{y} - \frac{1}{2} \right)^2 + 0.881 \left(\tilde{y} - \frac{1}{2} \right)^4 \right] \\ &\quad + \tilde{r}^2 \left[3.59 + 2.77 \left(\tilde{y} - \frac{1}{2} \right)^2 + 84.4 \left(\tilde{y} - \frac{1}{2} \right)^4 \right]. \end{aligned} \quad (7.14b)$$

- The **rotational** friction factor due to **translational** motion

$$c_{yx}^{\text{trans}} = \frac{\mathcal{N}_{\tilde{y}}}{8\pi r^2 \eta \mathcal{V}_{\hat{x}}} \quad (7.14c)$$

$$\begin{aligned} &= -\frac{1}{10} (\log \varsigma_1 - \log \varsigma_2) - \frac{43}{250} (\varsigma_1 \log \varsigma_1 - \varsigma_2 \log \varsigma_2) + 0.0209 (\varsigma_1 - \varsigma_2) \\ &\quad - 0.007 \left(\tilde{y} - \frac{1}{2} \right) + 0.037 \left(\tilde{y} - \frac{1}{2} \right)^3 \\ &\quad + \tilde{r} \left[0.097 \left(\tilde{y} - \frac{1}{2} \right) - 0.310 \left(\tilde{y} - \frac{1}{2} \right)^3 \right] \\ &\quad + \tilde{r}^2 \left[2.71 \left(\tilde{y} - \frac{1}{2} \right) - 7.21 \left(\tilde{y} - \frac{1}{2} \right)^3 \right]. \end{aligned} \quad (7.14d)$$

- The **translational** friction factor for a sphere **rotating** with an angular velocity $\omega_{\tilde{y}}$

$$f_{xy}^{\text{rot}} = \frac{f_{\hat{x}}}{6\pi r^2 \eta \omega_{\tilde{y}}} = \frac{4}{3} c_{yx}^{\text{trans}}. \quad (7.14e)$$

- The **rotational** friction factor due to **rotational** motion

$$c_{yy}^{\text{rot}} = -\frac{\mathcal{N}_{\tilde{y}}}{8\pi r^3 \eta \omega_{\tilde{y}}} \quad (7.14f)$$

$$\begin{aligned} &= -\frac{2}{5} (\log \varsigma_1 + \log \varsigma_2) - \frac{66}{125} (\varsigma_1 \log \varsigma_1 + \varsigma_2 \log \varsigma_2) + 0.1579 (\varsigma_1 + \varsigma_2 - 1) \\ &\quad - 0.206 - 0.0992 \left(\tilde{y} - \frac{1}{2} \right)^2 + 0.323 \left(\tilde{y} - \frac{1}{2} \right)^4 \\ &\quad + \tilde{r} \left[-0.101 - 1.70 \left(\tilde{y} - \frac{1}{2} \right)^2 + 16.83 \left(\tilde{y} - \frac{1}{2} \right)^4 \right] \\ &\quad + \tilde{r}^2 \left[0.307 + 15.4 \left(\tilde{y} - \frac{1}{2} \right)^2 - 728.7 \left(\tilde{y} - \frac{1}{2} \right)^4 \right]. \end{aligned} \quad (7.14g)$$

- The friction factor for a sphere fixed in Poiseuille flow [307]

$$4f^{\text{pois}} = \tilde{y}f_{xx}^{\text{shear}} - \tilde{y}^2f_{xx}^{\text{quad}} \quad (7.14h)$$

$$\begin{aligned} &= 1.014 - 4.0454 \left(\tilde{y} - \frac{1}{2} \right)^2 + 0.1580 \left(\tilde{y} - \frac{1}{2} \right)^4 \\ &\quad + \tilde{r} \left[1.939 + 0.456 \left(\tilde{y} - \frac{1}{2} \right)^2 + 11.07 \left(\tilde{y} - \frac{1}{2} \right)^4 \right] \\ &\quad + \tilde{r}^2 \left[3.53 - 14.34 \left(\tilde{y} - \frac{1}{2} \right)^2 + 63.88 \left(\tilde{y} - \frac{1}{2} \right)^4 \right] \\ &\quad + \tilde{r}^3 \left[-1.40 + 24.28 \left(\tilde{y} - \frac{1}{2} \right)^2 - 212.38 \left(\tilde{y} - \frac{1}{2} \right)^4 \right] \end{aligned} \quad (7.14i)$$

where

- the friction factor for a sphere held fixed in linear shear flow is f_{xx}^{shear} , and
- the friction factor for a sphere held fixed in quadratic flow is f_{xx}^{quad} .

- The rotational friction factor for a sphere fixed in Poiseuille flow [307]

$$c^{\text{pois}} = \frac{\tilde{r}}{2}c_{yx}^{\text{shear}} - \tilde{r}\tilde{y}c_{yx}^{\text{quad}} \quad (7.14j)$$

$$\begin{aligned} &= 0.0095 \left(\tilde{y} - \frac{1}{2} \right) - 0.0645 \left(\tilde{y} - \frac{1}{2} \right)^3 \\ &\quad + \tilde{r} \left[-4.382 \left(\tilde{y} - \frac{1}{2} \right) + 2.631 \left(\tilde{y} - \frac{1}{2} \right)^3 \right] \\ &\quad + \tilde{r}^2 \left[-0.328 \left(\tilde{y} - \frac{1}{2} \right) + 11.323 \left(\tilde{y} - \frac{1}{2} \right)^3 \right], \end{aligned} \quad (7.14k)$$

where

- the rotational friction factor for a sphere held fixed in linear shear flow is c_{yx}^{shear} , and
- the rotational friction factor for a sphere held fixed in quadratic flow is c_{yx}^{quad} .

Although unwieldy, the slip function provided by this fit to the multipole expansion method is well behaved at all particle sizes and all positions within the channel (Figure 7.1.9). It is smooth at the centre of the channel and also goes to zero continuously in the near-wall region.

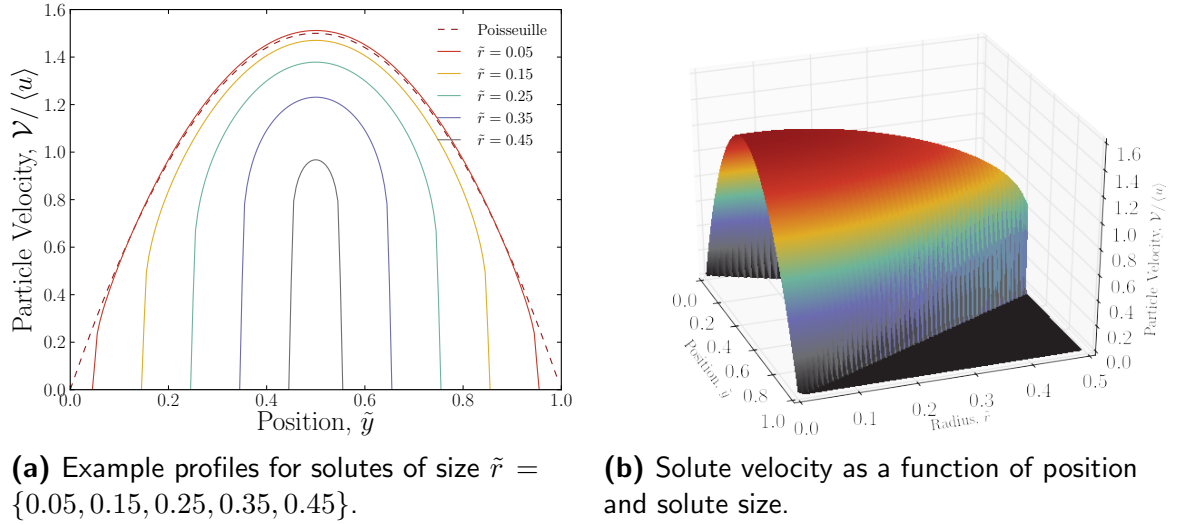


Figure 7.1.9: The fitting formula to the multipole expansion method of Pasol *et. al.* for the velocity of a spherical particle moving between two parallel walls (Equation (7.13)).

Comparison of Approximations and MPCD Simulations

The various approximations for the slip function can be used to numerically calculate the retarded retention ratio

$$R = \frac{\langle \mathcal{V} \rangle}{\langle v \rangle} = \frac{\langle n K^{-1} v \rangle}{\langle n \rangle \langle v \rangle} = \frac{1}{\langle v \rangle} \frac{\int_0^h n K^{-1} v \, dy}{\int_0^h n \, dy}. \quad (7.15)$$

Each slip function can be integrated over the entire channel weighted by the concentration at each height (Equation (5.8)) to determine the retarded retention ratio. Each of the retarded retention curves is less than the ideal retention theory (Figure 7.1.10). The Faxén expansion (Equation (7.5); grey line in Figure 7.1.10) and the Oseen superposition of Faxén’s single-plane solution (Equation (7.11); maroon line in Figure 7.1.10) reduce the retention ratio below the MPCD data, suggesting that the wall drag is over-predicted in these models. The hybrid of the near- and far-wall approximations (red line in Figure 7.1.10) agrees with the MPCD simulations for small solute sizes; however, at larger solutes the prediction does not saturate but rather continues to decrease roughly linearly.

The far-wall approximation of Goldman, Cox and Brenner is surprisingly accurate (violet line in Figure 7.1.10) and indeed this was the motivation for applying Oseen’s superposition to the far-wall approximation (light grey line in Figure 7.1.10). The resulting retention curve is accurate and the underlying solute velocity profiles are smooth (though discontinuous at the walls). The only retention curve that is as good or better than the Oseen superposition

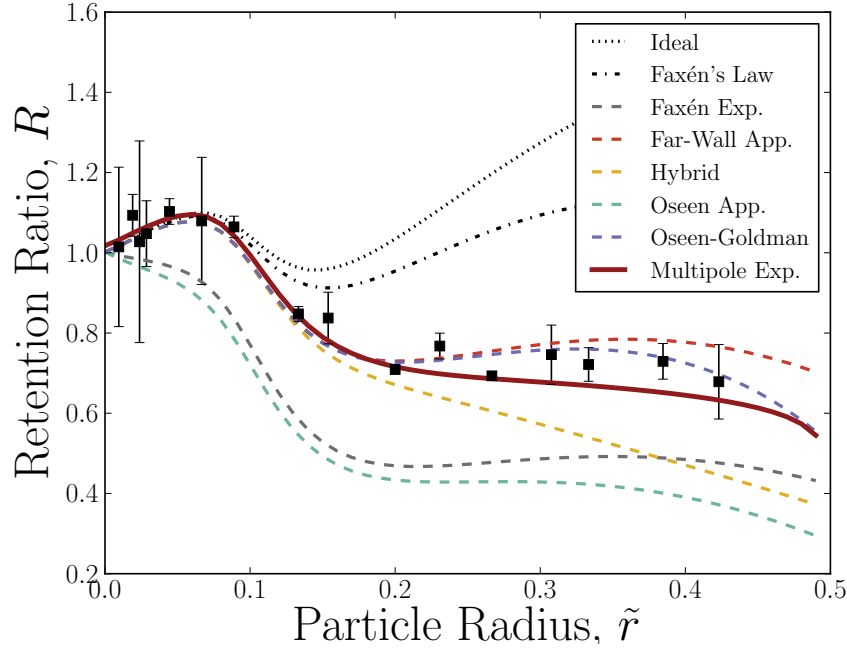


Figure 7.1.10: MPCD simulation results for $\alpha = 3$ and $\Lambda = 1.87 \times 10^{-4}$ (black squares [■]) are compared to the various approximations discussed in the text. The solid red line shows the predicted retention curve that numerically takes into account Faxén’s law and, more significantly, hydrodynamic interactions with both channel walls via the form given by the multipole expansion method [5].

of the far-wall approximations is the multipole expansion curve (purple line in Figure 7.1.10).

The form given by Pasol *et. al.* [5] that is shown in Figure 7.1.9 includes the effects of hydrodynamic interactions with both walls, as well as the effects of Faxén’s law, and so is accurate at large $\tilde{r}$ values. The numerically calculated retention ratios from these velocity profiles give agreement between theory and simulations and come out of physically reasonable particle velocity profiles. For all device retention parameters, Λ , the modified retention ratios numerically calculated using the friction coefficients found by Pasol *et. al.* **quantitatively agree** with the MPCD simulation data. In Figure 7.1.11, solid lines represent the numerically predicted retention ratios that take into account both Faxén’s law and, more importantly, effects via the fit to the multipole expansion (Equation (7.13)). Retention ratios for negligible fields are the least affected by frictional effects and remain relatively parabolic, as seen in the blue line in Figure 7.1.11 ($\Lambda = \infty$). Even in strong fields as in Figure 7.1.11 (red line; $\Lambda = 1.87 \times 10^{-7}$), HC is predicted to still exist for a brief size range and is relatively unaffected by wall-induced drag effects.

Likewise, qualitatively normal-mode FFF remains a region with a steep negative slope of the retention ratio. In weak and intermediate fields ($\Lambda = 1.87 \times 10^{-4}$ and $\Lambda = 1.87 \times 10^{-5}$ in

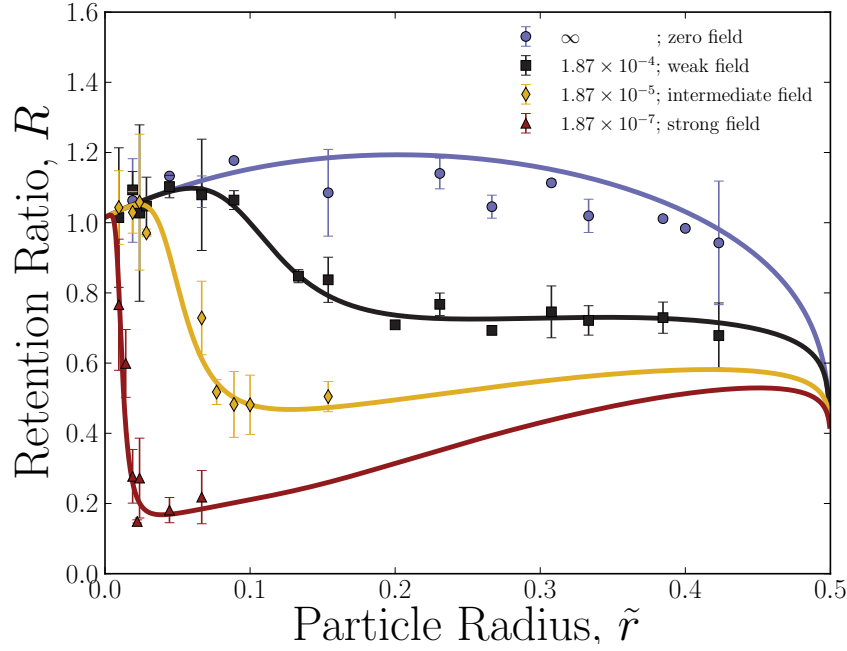


Figure 7.1.11: MPCD simulation results for $\alpha = 3$ and $\Lambda = \{\infty, 1.87 \times 10^4, 1.87 \times 10^5, 1.87 \times 10^7\}$ are shown as blue circles (●), black squares (■), yellow diamonds (◆) and red triangles (▲), respectively. The solid lines show the predicted retention curve for each Λ , numerically taking into account Faxén’s law and more significantly hydrodynamic interactions with both channel walls [5].

Figure 7.1.11), the slope is nearly zero for all larger colloids. In extremely high fields ($\Lambda = 1.87 \times 10^7$ in Figure 7.1.11), a slow climb remains such that steric-mode FFF is predicted to exist but with a significantly reduced slope compared to the ideal theory. Evidently, Faxén-mode FFF only exists for weak fields and is suppressed in larger fields. Thus, in microfluidic devices, frictional effects due to the walls are predicted to reduce resolution above the steric-inversion point compared to what would otherwise be predicted in the absence of increased drag. Finally, as $\tilde{r} \rightarrow 0.5$, the retention ratio drops suddenly, indicating that the effective friction coefficient suddenly increases. Accurately including the effect of wall drag in the retention theory allows the retention ratio to be quantitatively predicted across the entire range of particle sizes investigated for microfluidic separation devices.

Operational-Mode Diagram with Wall Drag

It is clear from Figure 7.1.10 that wall drag shifts the transition points between the operational modes of FFF. The ideal operational-mode diagrams are discussed in § 5.2.3 and

§ 5.3.2 and Figure 5.3.4 maps the ideal transitions. Figure 5.3.4 should be compared to Figure 7.1.12 which uses the Pasol *et. al.* [5] slip function to account for hindrance due to hydrodynamic interactions with the walls. Wall-induced lag causes the normal-mode regime to become larger in area (go to larger device retention parameter Λ values and larger solute sizes) when $\alpha = 1$. As expected from inspecting Figure 7.1.11, the transition out of the long, flat steric-regime to Faxén-mode FFF ($\tilde{r}_{\text{SF}}$) occurs at larger particle sizes than was ideally predicted. It is also noteworthy that the high Λ limit transition between hydrodynamic chromatography and the Faxén-mode regime has shifted to smaller particle sizes. While ideal, this transition occurs at the symmetric point of $\tilde{r}_{\text{SF}} = 0.25$. When wall drag is accounted for, this transition occurs at approximately $\tilde{r}_{\text{SF}} \approx 0.2$. Despite these comments, the wall drag has not qualitatively changed the operational-mode diagram for the important case of $\alpha = 1$.

When $\alpha = 2$, however, the connectivity between operational modes has completely changed compared to ideal expectations (Figure 5.3.4). One way to interpret this is to say that when $\alpha = 2$ the normal-mode regime grows (as it did for $\alpha = 1$) and in fact, the $\alpha = 2$ normal-mode regime grows so much that it intersects the Faxén-mode regime. The hydrodynamic chromatography and steric-mode FFF regimes become the disconnected spaces on the operational-mode diagram.

When $\alpha = 3$, the normal- and Faxén-mode regions remain connected as they were ideally. However, the steric-mode FFF regime becomes significantly smaller when wall drag is accounted for. The steric-mode regime is only just visible in Figure 7.1.12 at the very smallest device retention parameter values (in the strongest external fields). The multipole expansion slip function predicts that steric-mode FFF only exists for $\Lambda \lesssim 0.00025$ (Figure 7.1.12), which is easily an order of magnitude less than the ideal prediction (Figure 5.3.4).

7.1.4 Experimental Confirmation

MPCD simulations of eluting colloidal solutes have provided the motivation for including hydrodynamic interaction effects in the FFF retention theory. In actuality, the simulations were performed in concert with experiments performed by collaborators in Professor Michel Godin's laboratory.

By performing *video microscopy* of polystyrene colloids undergoing gravitational-FFF through a $h = 18 \pm 1 \mu\text{m}$ microfluidic channel ($100 \pm 10 \mu\text{m}$ wide), the Godin laboratory,

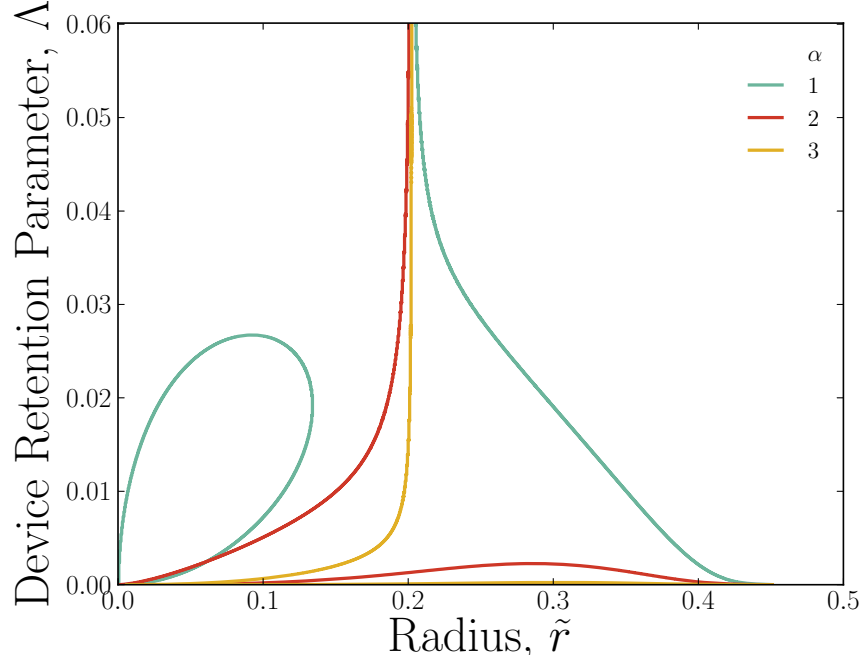


Figure 7.1.12: FFF-modes map. The roots of the retention ratio that numerically accounts for increased drag coefficients through the multipole expansion method results [5] are shown. The roots represent the transitions between the four different modes of operation $\tilde{r}_{\text{HN}}$, $\tilde{r}_{\text{NS}}$ and $\tilde{r}_{\text{SF}}$.

and in particular Radin Tahvildari, was able to experimentally verify the retarded retention theory. They were able to investigate two Λ values by adding glycerol to the carrier fluid. By decreasing the density difference between the colloid and the carrier fluid, they investigated a strong field case of $\Lambda = 1.6 \times 10^{-5}$ and low field case of $\Lambda = 10^{-3}$. Like the MPCD simulation results, the experimental retention ratio for a microfluidic channel is not well-approximated by the ideal retention theories (Equation (5.25) and Equation (5.31)). For solutes larger than roughly $\sim 5\%$ the channel height, increased friction due to hydrodynamic interactions with the walls (wall drag) must be accounted for. When wall-induced friction is included using the Pasol *et. al.* results [5] there is good agreement between experiments and theory. The article reporting these results can be found in § E or [6].

7.2 Hyperlayer and Adverse-mode FFF

Long-range hydrodynamic forces play an important role in retention in § 7.1. In the previous section, wall-induced drag is treated to account for the retention curves produced by MPCD simulations of field-flow fractionation in microfluidic channels. But other hydrodynamic forces can be important at higher elution velocities. Notably, **inertial lift forces** act perpendicular to the flow direction and can be non-negligible in FFF experiments. They complicate the interpretation of retention results but are also known to increase selectivity in the steric-mode operational regime. There is a need for an analytical retention theory that can simply model the essential features of lift effects in FFF without requiring substantial computational effort.

The task of this section is to construct just such a simplified model. Since lift acts as a force opposed to the external transverse FFF field, this is done by considering the application of multiple (potentially opposing) fields. The resulting model is expected to be accurate near the steric-inversion point but is likely too simplistic to account for complicated inertial effects far from this point. However, the model retention curve is interesting in its own right.

It will be seen that the retention curve that results from the application of two opposing forces that scale differently with particle size possesses a sharp peak beyond the steric-inversion point. While traditional FFF measures differences in retention times between solutes, a peak such as the one reported in this section could be utilized to measure solute size if the peak position is known. This novel method could be used in microfluidic devices that pit thermo-FFF against sedimentation-FFF to accurately measure solute size.

7.2.1 Inertial Lift Forces

The previous section considers hydrodynamic-induced lag in quite some detail. Another set of hydrodynamic forces that can play an important role in microfluidic channels are inertial lift forces. These forces arise from a combination of symmetry breaking of the wake by the wall and a shear-gradient generated force (Figure 7.2.1) [247, 284, 308, 309]. Since lift forces push solute particles across the flow's streamlines, the resulting motion is often referred to as *lateral migration*.

If the elution rate is not small enough, then such inertial forces are present in field-flow fractionation experiments. This is referred to as *hyperlayer-FFF* and occurs when

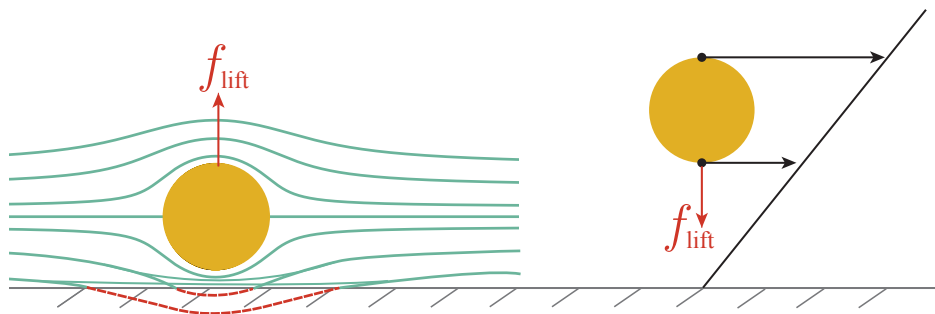


Figure 7.2.1: Inertial forces arise from two different mechanisms in microfluidic devices. The wall-caused lift breaks the symmetry of the flow and compresses streamlines between the particle and the wall generating a pressure away from the wall, while shear-gradient inertial forces tend to act in the opposite direction since they push solutes down shear-gradients.

working with large solutes that elute quickly. Primarily, the lift pushes solutes away from the accumulation wall (Figure 7.2.1) and so competes with the external transverse field. Effectively, multiple transverse forces act on the eluting solutes. Yet, despite the fact that the forces compete, it is well-known that hyperlayer has improved selectivity over steric-mode FFF [9, 203, 310, 311]. Why should the presence of a competing force increase selectivity? This will become intuitively clear in § 7.2.4.

The wall-caused lift pushes solutes away from microfluidic wall and towards the centre of the channel, while the shear-gradient lift acts down the shear-gradient, which is towards the walls for Poiseuille-like flows (Figure 7.2.1). These two lift forces thus compete and equilibrium positions (or *focusing points*) in channel flows arise [312–314]. In a tube or capillary, the equilibrium position for neutrally buoyant particulates is at 60% from the centreline of the tube to the tube wall for moderate Reynolds numbers [315]. Thus, the particles translate as an annular tube. This is the *tubular pinch effect* and such hydrodynamic focusing phenomena have recently been utilized for fractionation [316–320].

The exact form of these inertial forces is beyond the scope of this work [321]; however, the manner in which they scale as a function of solute size is quite pertinent. Theoretical predictions using asymptotic analysis have been performed, but their results can be captured by a simple scaling argument that the total lift force scales as the inertia of the fluid replaced by the particle ($\sim \rho r^3 [U]$) times the shear-gradient force acting on the particle ($\sim [U] r/h^2$),

which leads to

$$f_{\text{lift}} \sim \frac{\varrho [U]^2 r^4}{h^2}. \quad (7.16a)$$

Most commonly the characteristic velocity $[U]$ chosen for discussions of inertial forces is the maximum fluid velocity. The actual force is assumed to be the scaling result times a nondimensional lift coefficient $C_L(\tilde{y})$ that hides all the complications (principally how lift varies as a function of distance $\tilde{y}$ from the walls). Since the wall-induced lift acts toward the centreline in the near-wall region $C_L < 0$ is expected. On the other hand, $C_L > 0$ is expected near the centreline. So the lift is

$$f_{\text{lift}}(\tilde{y}) = \frac{\varrho [U]^2 r^4}{h^2} C_L(\tilde{y}) \quad (7.16b)$$

but the essential point for this discussion is that $f_{\text{lift}} \sim r^4$.

Modern experimental investigations in square microfluidic channels [261] demonstrate that the situation is more complicated:

- Near the centreline and far from the walls, the non-dimensionalization of

$$f_{\text{lift}}^{\text{near-wall}} = \frac{\varrho \langle u \rangle^2 r^3}{h} C_L \sim r^3 \quad (7.16c)$$

best collapses the data, with a C_L whose sign points toward the nearest channel wall. Field-flow fractionation experiments confirm this scaling [29, 218, 299]. **Unfortunately**, in disagreement with more modern results [261], this scaling was identified as the “*near-wall lift force*” in the FFF literature. A recent, ambitious theoretical study (which attempted to simultaneously account for both lift and lag forces) utilized this scaling [248].

- In the near-wall region, the better scaling is now suspected to be

$$f_{\text{lift}}^{\text{near-wall}} = \frac{\varrho \langle u \rangle^2 r^6}{h^4} C_L \sim r^6 \quad (7.16d)$$

with a C_L away from the wall (*i.e.* positive for the accumulation wall and negative for the depletion wall). Thus, the analytical scaling takes the middle ground between the far- and near-wall scalings.

Clearly, the situation is complicated and further work must be done by the microfluidics

community to resolve the discrepancies within the literature, but this thesis is not intent on addressing these issues. The current discussion limits itself to the simplest possible model applicable to field-flow fractionation:

- In this simplistic view, channel position dependence $\tilde{y}$ will be grossly neglected such that C_L will be treated as a **numerical constant**. Even the change of sign will be neglected — therefore, the analysis will be limited to solute particles that remain near the accumulation wall.
- **Both** scalings (Equation (7.16c) and Equation (7.16d)) will be presented and no statement about the correctness of either will be made.

If both the lift and the external transverse field scale as $\sim r^3$ then in this basic model Λ is simply decreased. However, none of the sub-techniques of FFF have a field that scales as $\alpha = 6$ (Table 5.2.1). Therefore, for this scaling of $\alpha = 6$ a fundamental modification *must* be made to the retention theory. In fact, what shall be done is in a sense quite general since the retention theory that shall be developed here treats the elution of solutes that are subject to multiple, constant forces that scale differently with solute size.

This is a simple model for hyperlayer FFF when one of the multiple forces is lift. It is likely that the model is too simplistic to reproduce hyperlayer FFF retention curves, though a systematic study comparing the results of this section to experimental results would be warranted.

Yet, it will become clear that even if inertial forces are too complicated to be treated by such a simplistic retention theory, FFF in the presence of multiple forces is valuable. While significant wall-induced lift makes multiple scalings inevitable in FFF, it can also be intentionally engineered. The first reported example of this occurred in 2012 when a microfluidic thermal-electric FFF device was demonstrated [322]. The retention theory for such a device is thus a specific case of the general framework developed here. In that work, the fields work in concert (which the authors refer to as *additive-mode*). Here, it shall be demonstrated that when two fields of different scaling oppose one another a sharp retention peak can result. This novel result will be referred to as *adverse-mode field-flow fractionation*.

7.2.2 Multi-Force Retention Theory

If the net force is the sum of many fields ($f = c_{f1}r^{\alpha_1} + c_{f2}r^{\alpha_2} + \dots = \sum c_{fi}r^{\alpha_i}$) then the retention parameter (Equation (5.22)) is

$$\lambda = \frac{k_B T}{h f} = \frac{k_B T}{h \sum c_{fi} r^{\alpha_i}}. \quad (7.17)$$

The field coefficients c_{fi} can be positive or negative. The concentration distribution (Equation (5.23)) immediately generalizes to

$$n(\tilde{y}) = \begin{cases} n_0 \exp(-[\tilde{y} - \tilde{r}] \Gamma) & \text{for } \tilde{r} < \tilde{y} < 1 - \tilde{r} \\ 0 & \text{otherwise,} \end{cases} \quad (7.18)$$

where $\Gamma \equiv \sum \Lambda_i^{-1} \tilde{r}^{\alpha_i}$ and $\Lambda_i \equiv k_B T / (h^{1+\alpha_i} c_{fi})$ have been defined. By now, the procedure for determining the retention ratio is standard: The averages across the height of the elution channel are performed to find that the average height above the channel is

$$\langle \tilde{y} \rangle = \frac{1}{\Gamma} + \frac{1}{1 - e^{(1-2\tilde{r})\Gamma}} + \tilde{r} \coth\left(\frac{[1 - 2\tilde{r}]\Gamma}{2}\right), \quad (7.19)$$

and the retention ratio (after applying Faxén's law [Equation (5.30)] to Poiseuille flow [Equation (3.27)]) is

$$R(\tilde{r}, \Lambda) = \frac{6[1 - 2\tilde{r}]}{\Gamma} \mathcal{L}\left(\frac{[1 - 2\tilde{r}]\Gamma}{2}\right) + \mathcal{F}_p, \quad (7.20)$$

where once again $\mathcal{F}_p = 6\tilde{r}(1 - 4\tilde{r}/3)$. Quite clearly, these have identical forms to the single force equations (Equation (5.24) and Equation (5.25) or Equation (5.31)) except that $\Gamma = \sum \Lambda_i^{-1} \tilde{r}^{\alpha_i}$ replaces $\Lambda^{-1} \tilde{r}^\alpha$. Again, the retention theory is written in a manner that understates the potential impact of the modification. It is possible to construct situations in which Equation (7.20) offers separation opportunities not available to traditional retention theory.

7.2.3 Hyperlayer FFF

Consider first the near-wall lift scaling of $f_{\text{lift}} \sim \tilde{r}^6$ (*i.e.* $\alpha_2 = 6$). If the lift competes with an external, transverse field that scales as $\alpha_1 = 1$ (which is appropriate for flow-, thermal- and electric-FFF [Table 5.2.1]) and has a moderate device retention parameter of $\Lambda_1 = 10^{-3}$ then

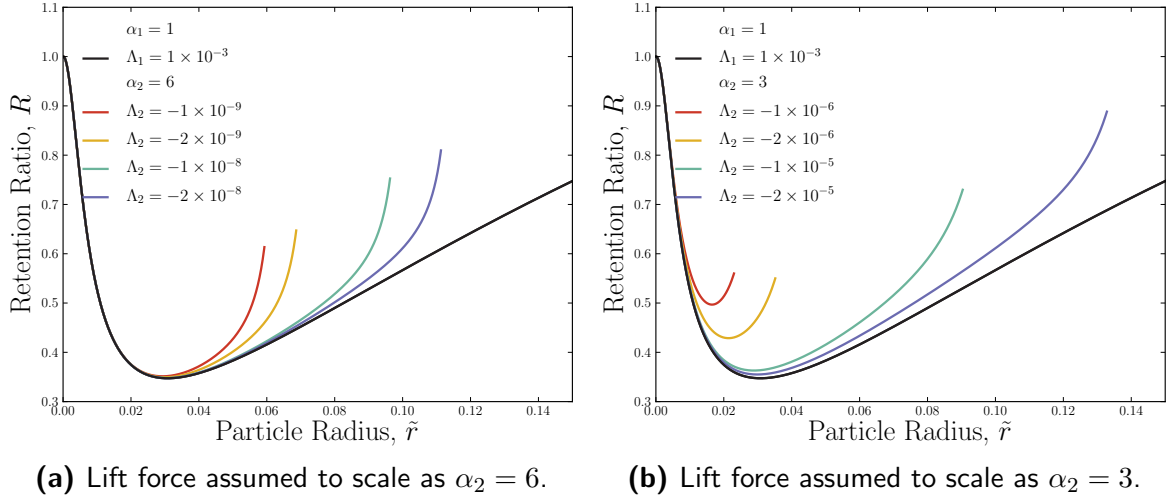


Figure 7.2.2: Retention ratio as a function of solute size for simple hyperlayer-mode FFF. The external force scales as $\alpha_1 = 1$ (which is appropriate for flow-, thermal- and electric-FFF [Table 5.2.1]) and the lift force scales as either $\alpha_2 = 6$ or 3. Retention curve truncated once R is 0.2 greater than the ideal retention ratio (the lower curve).

the resulting retention curves can be seen in Figure 7.2.2a. If the force is assumed to scale as $\alpha_2 = 3$ then the hyperlayer-mode FFF retention curve is approximated as Figure 7.2.2b. In either case, the retention curve is striking.

The retention ratio drops through normal-mode FFF while the α_1 external force dominates, but then rises up as the opposing lift pushes the particles away from the accumulation wall. Qualitatively speaking, a strong field generates normal-mode FFF at small solute size in which the retention ratio R drops rapidly as a function of particle size. At larger sizes, the retention ratio previously rose rather linearly away from the steric-inversion point, since steric-forces pushed the centre of the solute away from the accumulation wall. However, if the force is strong for normal-mode FFF but weakens for steric-mode FFF then the climb in retention ratio with particle size through the steric-mode regime will be steeper, since diffusion increases the average height from the wall above the radius of the particle r , which is acting as the steric-exclusion height.

In fact, the situation is even more dramatic. Consider a pair of opposing forces with different scaling. At small particle sizes the force with the lower α dominates and pushes the solute distribution against the accumulation wall, but as the size grows the opposing force becomes stronger and stronger. If the pair of the forces have the right magnitude then the opposing lift force will become greater than the original external force and the solutes will be pushed away from the accumulation wall. In a manner of speaking, this situation effectively generates two “normal-mode” regimes. Because a strong, constant opposing force

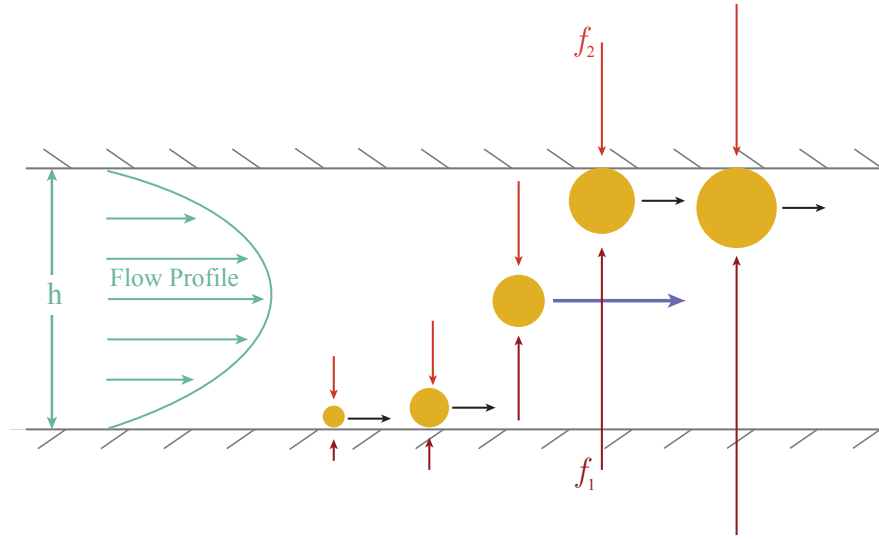


Figure 7.2.3: Schematic of the concept behind adverse-mode FFF. Two strong forces (f_1 and f_2), which scale differently with particle size, oppose one another. At small sizes, the force with the smaller scaling (say α_1) dominates and the solute undergoes steric-mode FFF at the accumulation wall. At the specific size $\tilde{r}_L$ the two forces are equal and the solute is effectively buoyant. At larger, sizes the adverse force dominates and the solute once again is pushed against the channel wall, only now it is what was previously the depletion wall.

would be unphysical for hyperlayer-mode FFF, the retention curves are truncated once the mean height of the particles rises substantially. In Figure 7.2.2, they are truncated once R is 0.2 greater than the ideal value. The assumption that C_L does not change substantially is only reasonable when the solutes are near the accumulation wall.

7.2.4 Benefit of Multiple Forces

However, imagine what would occur if the C_L coefficients were strictly constant and the “lift” force always pushed away from the accumulation wall.

If the forces act in opposite directions and have different scaling exponents, then Equation (7.20) can be leveraged to get increased selectivity in the steric-mode regime, even beyond what is seen in Figure 7.2.2. Why is this?

Consider Figure 7.2.3. If the lift coefficient is assumed to be a constant, the α_2 force simply keeps increasing even as the average position of the solutes passes over the centreline. For smaller sized solutes the distribution is pushed against the original accumulation wall, while

for larger solutes they are pushed against the opposite channel wall. In this situation, the force with the lower α dominates at smaller solute sizes and it pushes the solute distribution against the accumulation wall. However, the opposing force becomes stronger and stronger as larger sizes are considered. Eventually, it begins to dominate (as was seen in hyperlayer FFF). For lift, the retention curve was truncated but a truly constant force coefficient would be that at one special size the two forces would balance (the solute would be effectively buoyant) and its average height would be the centre of the channel. As the size (and therefore force) are increased further, the particle is pushed against the upper wall (formally the depletion wall) and the system has effectively flipped. For all larger sizes the adverse force dominates and steric-mode FFF occurs at what was originally the depletion wall.

That is to say, such a system would create an artificial hyperlayer FFF but one in which a peak in the retention ratio occurs where the opposing forces balance. These peaks can be seen in Figure 7.2.4a, which is the same as Figure 7.2.2b, except that the full retention curves are shown without being cutoff.

It is simple to predict the position of the peaks in Figure 7.2.4a. It is the point at which the forces balance ($\sum_i \Lambda_i^{-1} \tilde{r}_L^{\alpha_i} = 0$) and therefore for a pair of opposing forces it occurs at the particle size

$$\tilde{r}_L = \left(-\frac{\Lambda_1}{\Lambda_2} \right)^{1/(\alpha_1 - \alpha_2)}. \quad (7.21)$$

In Figure 7.2.4, Equation (7.21) is used to place the peaks near the steric-inversion point. Even when considering lift forces (for which the near-peak region is not believable), Equation (7.21) is valuable because it can be inverted to estimate the device retention parameter Λ_2 that one would desire to perform hyperlayer-mode FFF in the vicinity of the steric-inversion point.

Since inertial forces are not well-approximated by a constant field coefficient, what good is this discussion? There is an interesting realization to be made:

- Both scalings ($\alpha_1 = 1$ and $\alpha_2 = 3$) belong to experimentally demonstrated sub-techniques of FFF (Table 5.2.1).

So while Figure 7.2.4a represents the retention curve for an extremely simplified and inaccurate model of hyperlayer-mode FFF, it also represents a potentially achievable separation

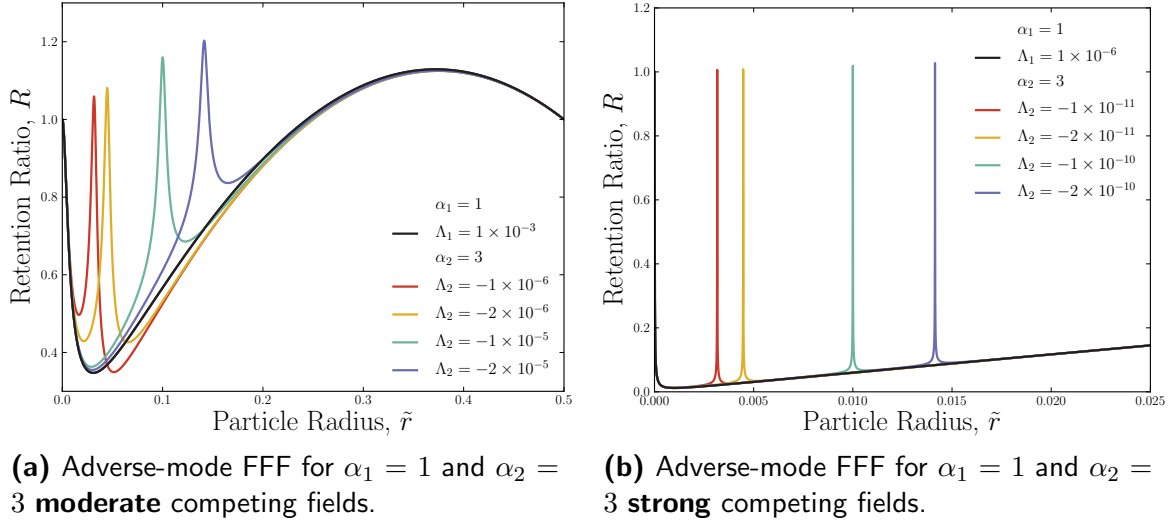


Figure 7.2.4: Retention ratio as a function of solute size for two, constant opposing fields. External transverse fields that scale as $\alpha_1 = 1$ and that compete against an opposing force that scales as $\alpha = 3$.

technology. One can construct an FFF apparatus that utilizes a strong, constant field that dominates at small solute sizes and an opposing field that dominates at larger solute sizes. The natural choice would be a pairing of opposing sedimentation- and thermal-FFF fields. In such a setup, the peaks in Figure 7.2.2b are expected to be accurate predictions of experimentally realizable retention curves.

It has been discussed how non-monotonicity in the retention ratio introduces uncertainty into the interpretation of solute size (§ 5.2). The peaks in Figure 7.2.4a are highly non-monotonic. So do multiple forces increase the complexity without technological benefit?

No.

It is proposed here that such *adverse-mode FFF* systems could be utilized to accurately measure sample solute sizes. By increasing the field strengths, peaks narrow significantly (Figure 7.2.4b). The change in R near the peak is large, while the steric-mode operation's variation that occurs as a “background” of sorts is small. Therefore, two samples of similar size elute with very similar retention times if they have sizes that both fall in the steric-mode background, but would have extremely large differences in retention times if one of the two samples has a size that corresponds to the peak position and the other does not. Then (and only then) the difference in R between them will be large and, consequentially, so will the resolution.

Resolving between two different samples would be possible, but the power of adverse-mode FFF does not lie in measuring the difference between solutes of different sizes. Rather, the

advantage lies in accurately measuring the size of a **single species**. All the FFF methods presented in this thesis rely on selectivity providing a difference in retention ratio, but if the peak position is known *a priori* then it can be matched to solute size to provide a non-comparative measure of size. In order to measure solute size, adverse-mode FFF would obey the following procedure:

- In order to measure the unknown size $\tilde{r}$ of a solute, an external field with $\alpha_1 = 1$ (such as thermal-FFF) would be applied. This field would have a small enough device retention parameter Λ_1 that elution occurs in the steric-mode regime of FFF. Λ_1 would be held fixed.
- A field with $\alpha_2 = 3$ (such as sedimentation-FFF) would also be applied to oppose the first field.
- Λ_2 would be incrementally decreased from an initial large value (the field would be increased). Incrementally decreasing Λ_2 shifts $\tilde{r}_L$ to smaller and smaller values.
- When $\tilde{r}_L(\Lambda_2) \approx \tilde{r}$ the retention ratio will abruptly and briefly increase (the retention time will plummet) as the sample would suddenly be buoyant.
- Equation (7.21) gives the size of the solute.
- Once $\tilde{r} = \tilde{r}_L$ has been determined through Equation (7.21), the pair of device retention ratios Λ_1 and Λ_2 can be decreased following Equation (7.21) to hone the peak and improve the measurement accuracy.

This section began with the intention of constructing a simple model for inertial effects in FFF. It succeeded in reproducing a steeper rise in retention ratio for solutes larger than the steric-inversion point, which qualitatively agrees with the behaviour of hyperlayer FFF. However, the simplifying assumptions required to create a tractable retention theory severely limit the model to the region immediately around the steric-inversion point. Although the retention curve above the steric-inversion point might be non-physical in terms of accounting for lift forces, it is quite interesting in terms of size identification. Experimentally demonstrated FFF sub-techniques could be combined to form adverse-mode FFF. Unlike additive-mode FFF, adverse-mode FFF would not simply increase the selectivity but would offer a procedure for measuring the size of a single solute species to arbitrary precision.

7.3 Slip-Walls

Traditionally, inventing a subtechnique of FFF is done by utilizing a different external field (Figure 5.1.2). However, it is also possible to design systems in which the elution is modified by a controllable flow profile. For instance, one way to potentially modify the performance of field-flow fractionation is to move the position of the maximum flow velocity away from the mid-point of the channel. This could be accomplished by using walls with non-zero slip lengths, for example. This section extends the ideal theory of FFF to include the effects of two **slip walls**. It demonstrates that while the hydrodynamic chromatography limit of FFF (weak fields) is not improved by engineering devices with slip-walls, the performance of normal-mode FFF can be enhanced by having slip at the depletion wall in moderate fields. Interestingly, a new regime is discovered (named slip-mode FFF) in which a large external field (typical of normal-mode FFF) and a large slip at the accumulation wall lead to sharp separations characterized by an elution order that is similar to that of hydrodynamic chromatography.

While exchanging external fields (using fields that depend differently on solute size — Table 5.2.1) is the subject of many investigations, it is generally taken for granted that the velocity of the carrier fluid obeys Poiseuille flow. Perhaps the exception to this is the difficulty associated with velocity skewing in thermal-FFF when thermal gradients are large enough to produce viscosity gradients [217, 246]. Yet, separation is completely dependent on the shape of the flow profile. In principle, it should be possible to change the elution times and improve the performance of FFF over some range of particle sizes by changing the flow profile.

Fractionation in an FFF channel that has arbitrary slip [95–97] at either (or both) wall will be considered here. Slip is of interest to FFF because:

1. Current flow-FFF channels are fitted with porous ceramic frits that allow the cross-flow to enter through the depletion wall (in symmetrical flow-FFF) and exit through the accumulation wall. Although it is well known that fluid slip can occur at porous surfaces [323–328], slip has not been investigated in flow-FFF apparatus.
2. Electro-osmotic flow with thin Debye layers is qualitatively similar to slip flow with a Smoluchowski slip velocity (§ 2.4.2). A point-particle retention theory for the linear combination of electroosmotic flow and Poiseuille flow exists for both neutral [219] and charged [329] analytes.

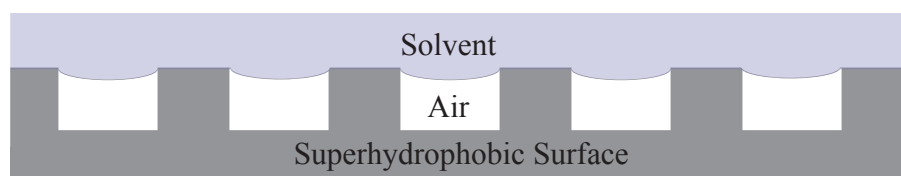


Figure 7.3.1: Schematic of a nano-structured, superhydrophobic surface at which a large slip length could be found.

3. The modern ability to engineer surfaces in microfluidic devices suggests that if slip is predicted to improve FFF in certain operational regimes, then channel walls can be constructed accordingly. This ability to nanoengineer patterned, ultrahydrophobic surfaces for drag reduction in microfluidic devices [330–337] is the primary motivation for the current theoretical study. The source of the non-zero slip lengths is theoretically inconsequential for this study. Generally speaking, *superhydrophobic* surfaces can be generated by engineering nano-structures on a plane that have length scales significantly smaller than that of the flow (Figure 7.3.1). For instance, slip occurs at rough surfaces, patterned organic surfaces, over polymer brushes and over surfaces that are patterned with nano-engineered arrays of structures (such as posts or grooves). Liquid flow in surface-nanostructured channels has been studied extensively by molecular dynamics simulation [338–340]. Many valuable reviews on the mechanisms leading to superhydrophobic surfaces are available [96, 341, 342].

7.3.1 Theory

It is described in § 3.4.3 that the flow profile between two planes $u(y)$ is always parabolic. Slip at either the accumulation wall (the “**bottom**” wall) or the depletion wall (“**top**” wall) does not change this. It only changes the magnitude of the average speed and the position of the maximum through the boundary conditions (Figure 3.4.4). These changes are encapsulated in *slip-lengths* b and t (Equation (3.31a) and Equation (3.31b) respectively), which can be qualitatively thought of as the distance into the impermeable walls at which no-slip would have occurred had the flow extended that far. The variables b and t have been chosen to bring to mind the bottom (accumulation) and top (depletion) walls, respectively. In § 3.4.3, the fluid velocity profile for a Newtonian fluid between two infinite plates separated by a distance h and possessing normalized slip lengths b and t was found (Equation (3.32)). Example flows are shown in Figure 3.4.4 from § 3.4.3. Equation (3.32) for the flow profile

with arbitrary slip lengths at either wall is repeated here for convenience:

$$u(\tilde{y}, \tilde{b}, \tilde{t}) = 6[U] \left[-\tilde{y}^2 + \left(\frac{1+2\tilde{t}}{1+\tilde{b}+\tilde{t}} \right) (\tilde{y} + \tilde{b}) \right].$$

This solution is given in terms of the no-slip average fluid velocity $[U] = \int_0^1 u(\tilde{y}, 0, 0) d\tilde{y}$ for the same pressure gradient (Equation (3.28)) and as always lengths are normalized by channel height $\tilde{\cdot} = \cdot/h$.

To zeroth order, particles carried by the flow move at the same speed as the solvent at the centre of mass of the particle, $u(\tilde{y}, \tilde{b}, \tilde{t})$, but to correct for the curvature of the fluid profile over the surface of a spherical particle of finite radius $\tilde{r}$, Faxén's Law (Equation (5.30)) must be applied as was first done in for FFF in § 5.3. The ideal velocity of the solute particle is then found to be given by

$$\mathcal{V}(\tilde{r}, \tilde{y}, \tilde{b}, \tilde{t}) = \left[1 + \frac{\tilde{r}^2}{6} \nabla^2 \right] u(\tilde{y}, \tilde{b}, \tilde{t}) = u(\tilde{y}, \tilde{b}, \tilde{t}) - 2\tilde{r}^2 [U]. \quad (7.22)$$

By using the slip-velocity Equation (7.22) and the usual concentration distribution (Equation (5.23)) in the definition of the retention ratio from § 5.1 (Equation (5.8)), the ideal retention ratio for finite-sized solute particles in a slit channel with slip at both the depletion and the accumulation walls is expressed as a modification to the no-slip solution:

$$R(\tilde{r}, \Lambda, \tilde{b}, \tilde{t}) = \frac{1 + \tilde{b} + \tilde{t}}{1 + 4\tilde{b} + 4\tilde{t} + 12\tilde{t}\tilde{b}} (R_{\text{no-slip}} + R_{\text{slip}}), \quad (7.23)$$

where $R_{\text{no-slip}}$ is the usual no-slip retention ratio given by Equation (5.31) and

$$R_{\text{slip}} = 6 \frac{\tilde{t} - \tilde{b}}{1 + \tilde{b} + \tilde{t}} \left\{ \tilde{r} \mathcal{L} \left(\frac{[1 - 2\tilde{r}] \tilde{r}^\alpha}{2\Lambda} \right) + \frac{\Lambda}{(1 - 2\tilde{r}) \tilde{r}} + \frac{\tilde{b} + 2\tilde{t}\tilde{b}}{\tilde{t} - \tilde{b}} - \frac{1}{e^{[1-2\tilde{r}]\tilde{r}^\alpha/\Lambda} - 1} \right\}. \quad (7.24)$$

It is easy to verify that $R(\tilde{r}, \Lambda, 0, 0)$ reduces to $R_{\text{no-slip}}(\tilde{r}, \Lambda)$ in the no-slip limit since $R_{\text{slip}}(\tilde{r}, \Lambda, 0, 0) = 0$ and the prefactor in Equation (7.23) is then equal to unity.

7.3.2 Weak Field Results

One of the benefits of writing the retention ratio as the sum of the no-slip solution and a correction term is that the limits of the no-slip term have already been analyzed in detail

in § 5.3. Just as was found in Equation (5.28), here the limits $\tilde{r} \rightarrow 0$, $\tilde{r} \rightarrow 1/2$, and $\Lambda \gg 1$ of $R_{\text{no-slip}}$ are all identical. As before this is because in each of these limits the force is insignificant compared to the effect of steric exclusion from the near wall regions. In this steric-dominated limit, the retention ratio is

$$R(\tilde{r}, \Lambda, \tilde{b}, \tilde{t}) \approx 1 + 2(1 - 3\tilde{b} - 3\tilde{t})(\tilde{r} - 2\tilde{r}^2) + (\tilde{b} - \tilde{t}) \frac{(1 - 2\tilde{r})^2 \tilde{r}^\alpha}{2\Lambda}, \quad (7.25)$$

to first order in $\tilde{b}$, $\tilde{t}$, and Λ^{-1} . At both the smallest and largest particle sizes ($\tilde{r} = 0$ and $\tilde{r} = 1/2$) the retention ratio goes to $R \rightarrow 1$, as it ideally should when hydrodynamic complications are neglected (as discussed in § 7.1). When $\alpha = 1$, the maximum retention ratio is found for a particle of size

$$\tilde{r}_{\text{max}}(\Lambda, \tilde{b}, \tilde{t}) \approx \frac{1}{4} \left(1 + \frac{\tilde{t}}{16\Lambda} - \frac{\tilde{b}}{16\Lambda} \right), \quad (7.26)$$

and the retention ratio for this particle size is:

$$R_{\text{max}}(\Lambda, \tilde{b}, \tilde{t}) \approx \frac{5}{4} \left(1 - \frac{3}{5} [\tilde{t} + \tilde{b}] + \frac{\tilde{b} - \tilde{t}}{40\Lambda} \right). \quad (7.27)$$

True Hydrodynamic Chromatography Limit

In the true HC limit of $\Lambda \rightarrow \infty$ (no external force), the no-slip retention ratio $R_{\text{no-slip}}(\tilde{r})$ is exactly parabolic. Its maximum is predicted by Equation (7.27) to be $R_{\text{max}} = 5/4$ at $\tilde{r}_{\text{max}} = 1/4$ (Figure 7.3.2) and this point marks the transition from HC (for small particles) to Faxén-mode FFF (for large particles). Forgoing a discussion of band broadening, the ability to resolve different sizes is related to the selectivity. In § 6.2, it is shown that the selectivity (both the relative (Equation (6.10)) and device (Equation (6.12)) selectivity) is directly proportional to the slope of the retention ratio as a function of particle size. Therefore, in the interest of an intuitive discussion the slope of the curve, $dR/d\tilde{r}$ is discussed here. The question is thus whether slip can increase the range of the separation and/or the rate of change of retention ratio.

In fact, Equation (7.26) reveals that the range does not change since the maximum must remain centred at $\tilde{r}_{\text{max}} = 1/4$ when there is no external force to break the symmetry. However, when $\Lambda \rightarrow \infty$ in Equation (7.27), R_{max} decreases in the presence of slip at either wall. This reduces the average slope $\langle \partial R(\tilde{r}) / \partial \tilde{r} \rangle = 1 - 3\tilde{b} - 3\tilde{t}$ in the HC regime, leading to reduced

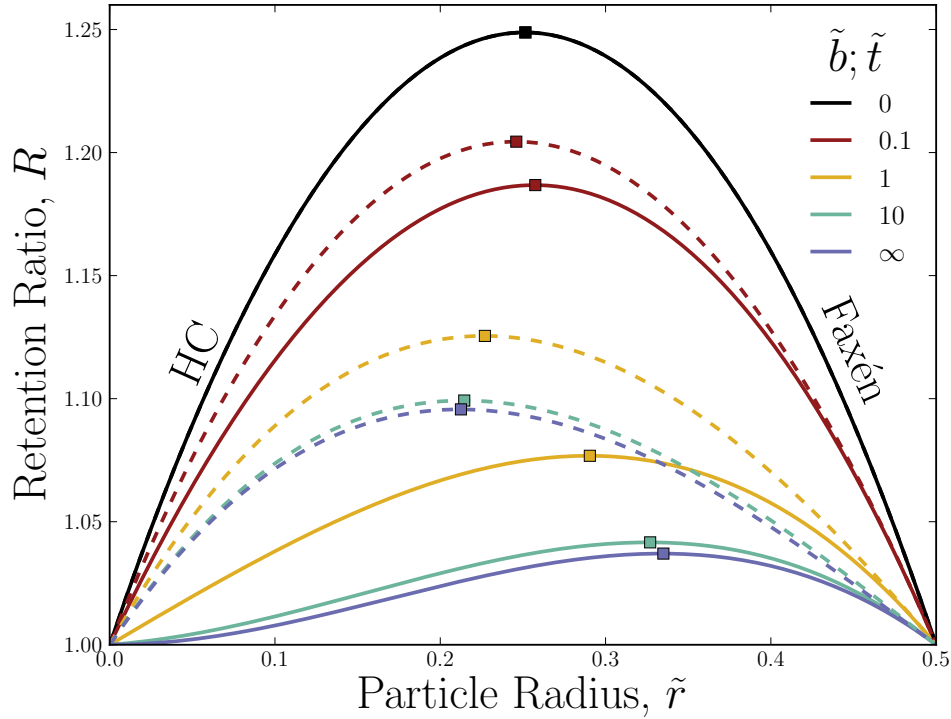


Figure 7.3.2: The retention ratio of FFF with a large but finite device retention parameter $\Lambda = 0.25$. Only hydrodynamic chromatography (HC) and Faxén-mode FFF exist. The transition between the two is marked (■). No-slip FFF is a black (top curve) solid line. The other solid lines show slip at the depletion wall while the dashed lines show slip at the accumulation wall; the colour code for the various slip lengths $\tilde{t}$ and $\tilde{b}$ is given in the legend.

selectivity over the useful range $0 < \tilde{r} < 1/4$. The selectivity near $\tilde{r} = 0$, which is where it is generally the largest (see Figure 7.3.2), is represented by the slope

$$\left. \frac{\partial R}{\partial \tilde{r}} \right|_{\tilde{r}=0} = 2 \left(1 - 3 [\tilde{t} + \tilde{b}] + \frac{\tilde{b} - \tilde{t}}{4\Lambda} \right) \quad (7.28)$$

to first order in both $\tilde{b}$ and $\tilde{t}$. Again, any slip reduces the slope for small particles when $\Lambda \rightarrow \infty$. Thus slip does not improve the performance of the zero field, HC limit.

Weak Field Results

When a weak external field Λ^{-1} is applied, the picture is qualitatively similar but the field breaks the symmetry. In the absence of slip, the retention curve in Figure 7.3.2 is still parabolic with both an HC and a Faxén-mode FFF regime. Equation (7.26) predicts that slip at the accumulation wall should decrease the range of the HC regime by moving the

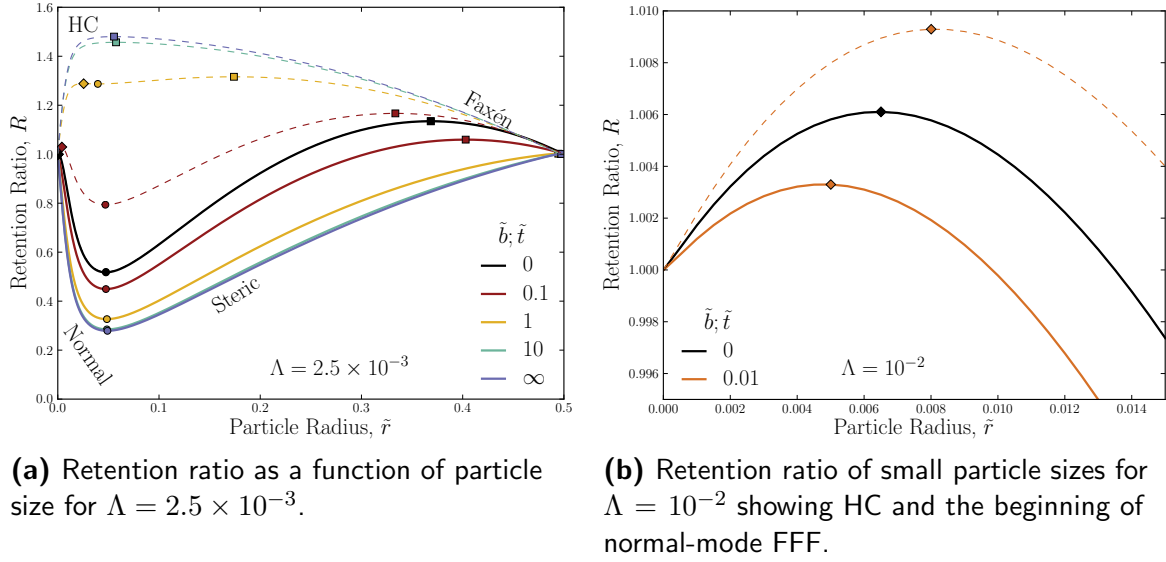


Figure 7.3.3: Retention ratio as a function of particle size for slip at the depletion wall (solid lines) and slip at the accumulation wall (dashed lines). The no-slip solution is given by the solid black line. Diamonds (◆) denote the transition from hydrodynamic chromatography (HC) to normal-mode FFF, circles (●) mark the normal- to steric-mode transition and squares (■) label the transition to Faxén-Mode FFF.

maximum from $\tilde{r}_{\max} = 1/4$ to smaller particle sizes, while slip at the depletion wall should increase the range of HC. When the full form of Equation (7.23) is used in Figure 7.3.2, it is clear that this is exactly what occurs for weak fields. In fact, Figure 7.3.2 also demonstrates the second effect of the weak field: Slip at the depletion wall reduces the maximum retention ratio even more than slip at the accumulation wall does, in qualitative agreement with Equation (7.27). Unfortunately, the small increase in the range $\tilde{r}_{\max}$ found for depletion wall slip is accompanied by a large decrease in the slope $\partial R(\tilde{r})/\partial \tilde{r}$ over the useful range $0 < \tilde{r} < \tilde{r}_{\max}$; this is in agreement with the prediction of Equation (7.28). Clearly, FFF does not benefit from slip at either wall in the HC or low force regimes.

7.3.3 Strong Field Results

In § 5.2.2 it is shown that when the device retention parameter Λ is below some critical value Λ_c , the additional regimes of normal- and steric-mode FFF appear between HC and the Faxén-mode regions. In Figure 7.3.3a and Figure 7.3.3b, the familiar no-slip retention curves are seen. In practice, one must use a single regime since the retention curve is highly non-monotonic. In the no-slip limit, both the narrow, high-selectivity normal-mode regime and the broader, lower-selectivity steric-mode regime can, in practice, be used for

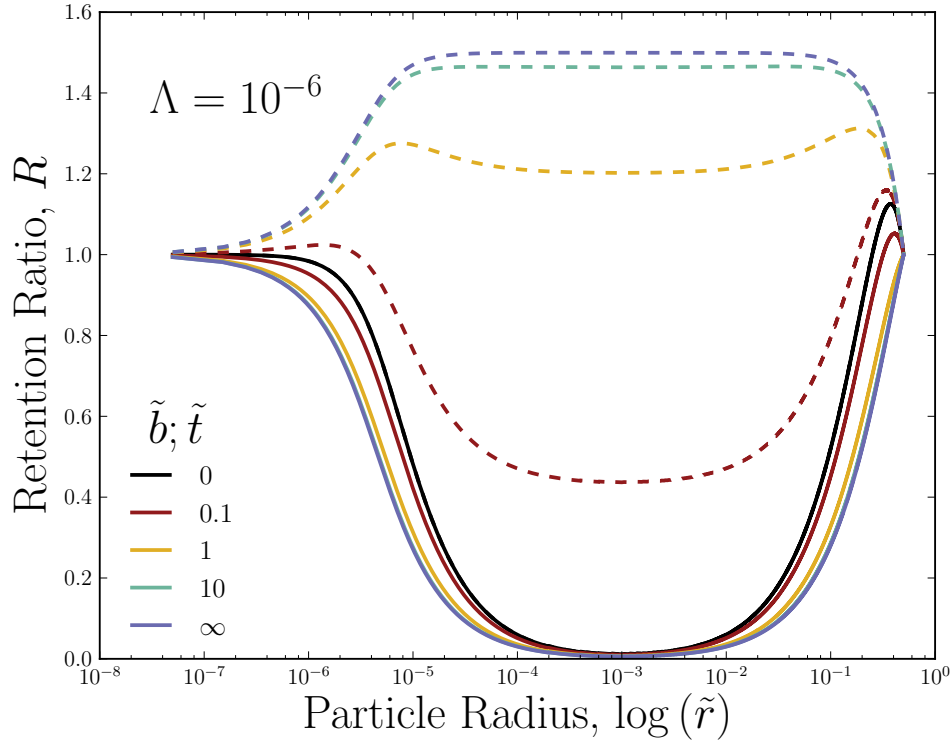


Figure 7.3.4: Retention ratio for a device retention parameter $\Lambda = 10^{-6}$ for slip at the depletion wall (solid lines) and slip at the accumulation wall (dashed lines). The no-slip solution is given by the solid black line.

separating particles, although steric-mode FFF is more susceptible to hydrodynamic effects which increase the particles' friction coefficients. The presence of slip changes the range and mean resolution of all four of the FFF regimes from § 5.3.

Slip at Depletion Wall

Slip at the depletion wall (solid lines in Figure 7.3.3a) improves normal-mode FFF by increasing the retention ratio drop for small particles (the minima (• points) are significantly lowered). Since the range of this regime remains essentially constant, there is a large gain in selectivity here. This improvement can be understood from Figure 3.4.4 (page 109): Slip at the depletion wall causes the slope of the fluid velocity $u(\tilde{y}, 0, \tilde{t})$ to be increased near the accumulation wall. In normal-mode FFF, solutes spend the majority of their time near this wall so the average velocity difference generated by different average heights is increased. However, note that this increased fluid velocity actually becomes smaller as the wall is approached. The $u(\tilde{y}, 0, 0.1)$ and $u(\tilde{y}, 0, 0.75)$ flow profiles in Figure 3.4.4 demonstrate this well. To first order in both $\tilde{y}$ and $\tilde{t}$, the velocity of the fluid near the accumulation wall

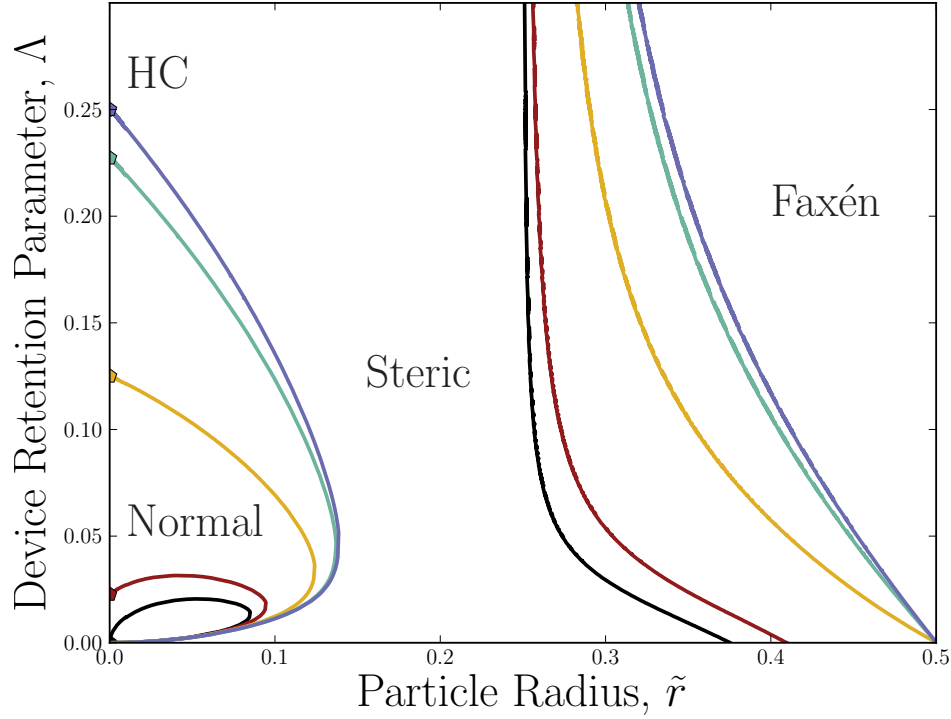


Figure 7.3.5: Operational-mode diagrams for various slip lengths at the depletion wall (and no-slip at the accumulation wall *i.e.* $\tilde{t} > 0$ with $\tilde{b} = 0$). The intercepts (●) are all at the origin.

increases like $\sim \tilde{y} (1 + \tilde{t}) / 2$. As a consequence, when particles are pushed closer to the wall by stronger forces, slip has less of an impact on their velocity in the flow. The increase in slope as a function of $\tilde{t}$ is thus less pronounced in stronger fields. Therefore, the normal-mode increases significantly in slope only if the field is moderate. The significantly increased drop in retention ratio when $\Lambda = 2.5 \times 10^{-3}$ seen in Figure 7.3.3a must be compared to the nearly identical curves over the normal-mode range when $\Lambda = 10^{-6}$ (a more practically realistic value for FFF) seen in Figure 7.3.4. For strong fields, such as shown in Figure 7.3.4, normal-mode FFF occurs over a tiny range of relatively small particle sizes (the same is true for HC) and slip at the depletion wall has less of an impact than it does for more moderate fields.

As is seen in Figure 7.3.3a, slip at the depletion wall also moves the transition point between steric- and Faxén-mode FFF (■ points) to larger particle sizes. This broadens the range over which the steric-mode operation can be used, without significantly affecting its average slope (or selectivity). However, this does not present a benefit in practice since particles nearly the size of the channel ($\tilde{r} = 1/2$) can create problems such as clogging and are affected by non-ideal (increased) drag coefficients.

From Figure 7.3.3a and Figure 7.3.3b it is clear that slip at the depletion wall alters the

range of each regime and that the change is less pronounced for stronger fields in Figure 7.3.4, but how does this change for different device retention parameters and different slip lengths? The $\Lambda - \tilde{r}$ phase diagram Figure 7.3.5 maps out how the ranges of the four FFF-modes change (for $\alpha = 1$). The slip at the depletion wall typically broadens the range of steric-mode FFF. Furthermore, it increases the closed area that defines the normal-mode regime (Figure 7.3.5). The area increases both because its range of particle sizes $\Delta\tilde{r}$ is increased and also because the critical device retention parameter Λ_c above which normal-mode FFF does not exist is raised. When there is slip at the depletion wall, normal- and steric-mode FFF exist in weaker fields (larger Λ) than they would have for no-slip FFF. The critical value Λ_c increases because slip at the depletion wall moves the height at which the velocity of the fluid is maximum away from the accumulation wall.

Moreover, slip at the depletion wall causes the critical device retention parameter Λ_c to occur at smaller particle sizes, reducing both the range and the amplitude of the minuscule HC regime (see Figure 7.3.3b). In fact, when $\tilde{t} \geq (1 + \sqrt{15})/14 = 0.35$, the critical device retention ratio occurs at $\tilde{r} = 0$ and HC does not exist at all below $\Lambda < \Lambda_c$. That is to say that the normal-mode region extends to the smallest particle sizes and Figure 7.3.5 has non-zero intercepts (if and only if $\alpha = 1$). The intercept then grows with slip as $\Lambda_c = \tilde{t}/4(1 + \tilde{t})$, as plotted as filled symbols in Figure 7.3.5. This is fundamentally different from the no-slip case in which a diminutive HC range always exists, even for the strongest fields (or equivalently, the slope of the retention ratio at $\tilde{r} = 0$ is always positive). Figure 7.3.4 shows an example of this phenomenon: HC does not exist for these values of $\tilde{t}$ at $\Lambda = 10^{-6}$.

Slip at Accumulation Wall

If, instead, a no-slip condition $\tilde{t} = 0$ is maintained at the depletion wall and a slip length $\tilde{b}$ is allowed to grow at the accumulation wall, the elution behaviour is quite different. This is shown as the dashed lines in Figure 7.3.3a. The normal-mode operational regime deflates and eventually vanishes, as seen in Figure 7.3.6. Simultaneously, the transition to Faxén-mode operation extends to smaller and smaller particle sizes with increasing slip, and the maximum range and retention ratio of HC also increase. The result is a poor performance in all regimes, until the slip length $\tilde{b}$ is large enough to completely change the nature of the retention curve (top three curves in Figure 7.3.3a).

When the field is strong and the slip length is comparable to or larger than the channel height ($b \gtrsim w$ or equivalently $\tilde{b} \gtrsim 1$), the retention curve once again has only a single

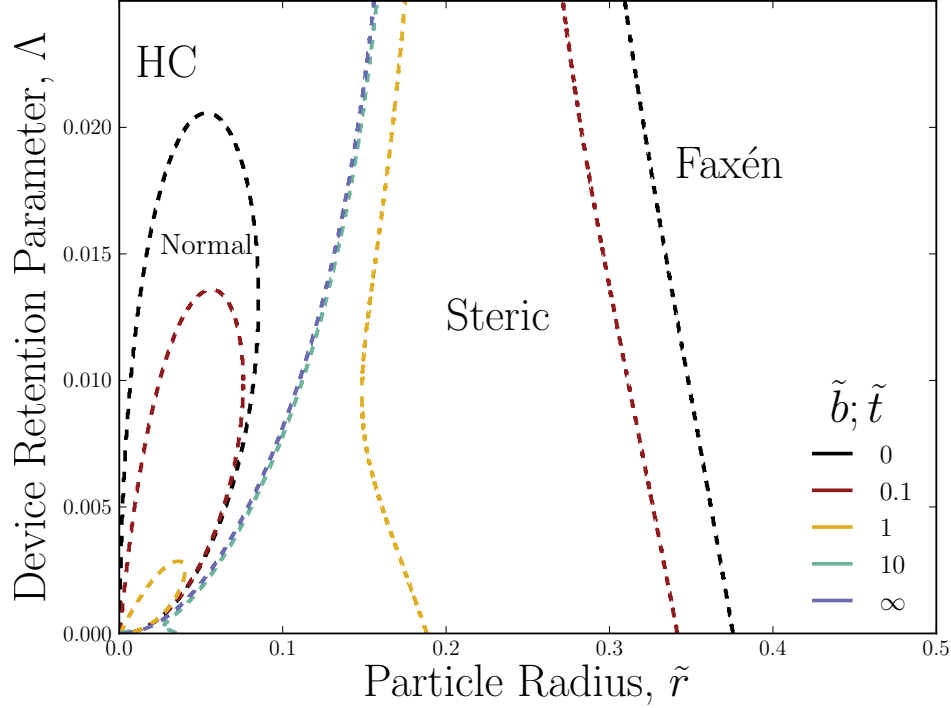


Figure 7.3.6: Operational-mode diagrams for various slip lengths at the accumulation wall (and no-slip at the depletion wall *i.e.* $\tilde{b} > 0$ with $\tilde{t} = 0$).

maximum. This maximum exists at a small value of $\tilde{r}_{\max}$. For this strong field and large slip length limit, the retention ratio goes directly from a steep HC-like regime to a slowly decreasing regime similar to Faxén-mode FFF (squares in Figure 7.3.3a; dashed lines). Remarkably, in Figure 7.3.3a, the slope for $\tilde{r} < \tilde{r}_{\max}$ and $\tilde{b} \rightarrow \infty$ is even greater than it is in normal-mode FFF for no-slip FFF. While increasing the field strength for large $\tilde{b}$ does not significantly increase $\tilde{R}_{\max}$, it does decrease $\tilde{r}_{\max}$ as demonstrated in Figure 7.3.4 suggesting that high selectivity can be achieved in this regime. Although technically this looks like the HC regime because it is the first region with a positive slope, the external field is large while HC implies that it is negligible. Furthermore, the particle size range and slope are more akin to no-slip normal-mode FFF than to no-slip HC. Therefore, this novel limit of small Λ and large $\tilde{b}$ is called *slip-mode FFF*.

In many ways, the mechanism behind slip-mode FFF is more similar to normal-mode FFF than HC. In the slip-mode FFF regime, larger particles feel a stronger force toward the accumulation wall where the fluid velocity is much greater, as shown in Figure 3.4.4. Thus, the retention ratio increases rapidly with particle size just as it dropped rapidly for no-slip normal-mode FFF. However, at some point steric exclusion from the near wall region dominates and the average height increases linearly with particle size. In the no-slip case,

steric exclusion subjects particles to faster flowing solvent and causes the retention ratio to grow with particle size. However, when there is large slip at the accumulation wall, steric exclusion exposes the particles to slower velocities and so the retention ratio slowly decreases with particle size, as if it were in the Faxén regime.

In slip-mode FFF, the external field must be strong ($\Lambda \lesssim 10^{-2}$) for large slip at the accumulation wall to significantly improve the selectivity. When $\tilde{b} \rightarrow \infty$, the maximum in the retention ratio approaches $R_{\max} = 3/2$ and $\tilde{r}_{\max}$ moves to smaller and smaller sizes. The slope is greatest at the smallest particle sizes and increases with field as

$$\lim_{b \rightarrow \infty} \left. \frac{\partial R}{\partial \tilde{r}} \right|_{\tilde{r}=0} = \frac{1}{2} + \frac{1}{8\Lambda}. \quad (7.29)$$

The selectivity can thus be extremely high in this novel FFF method.

Slip at Both Walls

If the slip length at the depletion wall and at the accumulation wall are increased together such that the solvent flow profile maintains its symmetry, the range of each operational mode remains unchanged. Only the difference between the two slip lengths matters for transitions and inversion points. Therefore, there is no reason to have slip at both walls from an optimization point of view.

7.3.4 Other Force Scaling Exponents α

Both the quantitative and qualitative effects of wall slip on field-flow fractionation systems have been considered here. These theoretical predictions are the product of generalizing the ideal retention theory to account for arbitrary slip lengths at both or either wall. Since flow-FFF channels are likely to have some slip, $\alpha = 1$ was chosen and focused upon.

However, other force scaling exponents are of course possible. As is seen in previous sections of this thesis, the qualitative behaviour is often quite similar, though specific differences do exist. Rather than present the data for each scaling exponent, useful metrics for judging the effects of slip on FFF have been condensed into Table 7.3.1 for all possible scaling exponents $\alpha = [1, 2, 3]$. For each mode of operation (or regime) with device retention ratios above or below Λ_c , three metrics are presented to characterize the impact of slip on the resulting retention ratios:

Slip	Regime	Range, $\Delta\tilde{r}$						Retention Imparity, ΔR						Ratio, $\Delta R/\Delta\tilde{r}$					
		$\alpha = 1$		$\alpha = 2$		$\alpha = 3$		$\alpha = 1$		$\alpha = 2$		$\alpha = 3$		$\alpha = 1$		$\alpha = 2$		$\alpha = 3$	
		$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$
$\tilde{t} > 0$	HC	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
	normal	+	NA	+	NA	$\emptyset$	NA	+	NA	+	NA	+	NA	+	NA	+	NA	+	NA
	steric	$\emptyset$	NA	+	NA	+	NA	$\emptyset$	NA	$\emptyset$	NA	+	NA	$\emptyset$	NA	$\emptyset$	NA	$\emptyset$	NA
	Faxén	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
$\tilde{b} > 0$	HC	+	-	+	-	+	+	+	$\emptyset$	+	$\emptyset$	+	$\emptyset$	+	+	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$
	normal	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA
	steric	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA
	Faxén	+	+	+	+	+	-	+	$\emptyset$	+	$\emptyset$	+	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$

Table 7.3.1: Summary of the impact of slip at either wall for the force scaling exponents $\alpha = [1, 2, 3]$. Increases (decreases) in performance with slip are marked by + (-). When there is no clear increase or decrease for the range of Λ the symbol $\emptyset$ is used. Normal- and steric-mode FFF do not exist for $\Lambda > \Lambda_c$ and so are labeled NA (not applicable). These conclusions are based on solutions of the retention ratio as a function of particle radius for slips $0 \leq (\tilde{t}; \tilde{b}) \leq 10000$ and device retention parameters $10^{-4} < \Lambda < 10^{-1}$.

- range $\Delta\tilde{r}$,
- retention imparity ΔR over that range, and
- the ratio $\Delta R/\Delta\tilde{r}$ as a rough measure of slope and selectivity.

This investigation into the impact of slip on FFF ends with the following practical conclusions:

1. Hydrodynamic chromatography does not benefit from slip.
2. The resolution of normal-mode FFF in a moderate external field can be improved by slip at the depletion wall.
3. Slip at the depletion wall typically increases the range of steric-mode FFF with little impact on its resolution.
4. Normal- and steric-mode FFF persist for weaker fields when there is slip at the depletion wall.
5. A new high-resolution mode, slip-mode FFF, is predicted to exist when the field is strong and slip at the accumulation wall is comparable to or larger than the channel height.

These conclusions suggest that experimentalists utilizing flow-FFF should characterize the slip length of their apparatus to determine whether or not slip plays a negligible role. Furthermore, a microfluidic, thermal-FFF device with a large slip length at the accumulation wall could take advantage of the predicted slip-mode FFF of operation. However, such a device

would require quite a substantial slip length at the accumulation wall. Superhydrophobic surfaces are routinely produced with slip lengths on the order of $\sim 10 \mu\text{m}$ [333, 334] and more exotic small-scale systems suggest that slip lengths on the scale of millimeters [343, 344] can be produced; however, using thin Debye layer EOF with a large Smoluchowski slip velocity (Equation (2.105)) likely remains the best candidate system for experimentally verifying the existence and utility of slip-mode FFF at this time.

7.4 Rectangular Channels

The non-ideal complications considered so far in § 7 have involved the long-range nature of hydrodynamic interactions. However, the unified ideal retention theory of § 5.2 makes several simplifying assumptions. Neglecting Faxén’s law is one such simplifying assumption: Including Faxén’s law demanded a complication in the derivation but not in the resulting retention ratio. In the present section, another prevalent simplifying assumption — that elution occurs in a channel of finite height h but infinite width w — is rejected.

Since large aspect ratios cannot be cast in PDMS and other commonly utilized materials for microfluidic device [345], and since wide channels may be inconvenient for device design, an analytical retention theory that accounts for *finite aspect ratios* is desirable. The impact on band broadening [268, 346–348] is investigated previously, but not explicitly the retention curves nor selectivity. As is seen in § 7.1, complications often lead to a reduction in the ability of FFF to resolve between solutes, as is the case with non-parabolic flows due to thermal-gradients [246]; however, this is not always the case, as is seen in § 7.3 and is to be seen here). Another concern is that the presence of two additional walls will increase wall-induced drag that will suppress variations in retention ratio as function of size. This is almost certainly true for relatively large particles, but § 7.1 demonstrated that this retardation is expected to be significant only for solute sizes $\tilde{r} \gtrsim 0.05$.

Instead of using the 2D Poiseuille flow (Equation (3.27)), a **series solution** for the flow profile appropriate for a 3D rectangular elution channel is utilized to derive a series solution for the ideal retention ratio of HC and FFF in finite aspect ratio rectangular channels. This solution is found to be accurate at small solute sizes even if the series is truncated after only a few terms. It is to be seen that finite aspect ratios are not predicted to actually harm the ideal selectivity. Rather than limiting devices’ ability to resolve samples, the series solution suggests that retention curves for normal-mode FFF are well approximated by the simple 2D solution and that steric-mode FFF and HC are improved for small solutes. This work also predicts that large aspect ratios commonly utilized in FFF apparatus are not necessary and that devices with an aspect ratio of unity are viable.

7.4.1 Series Solution

The system is set up in the following way: A solvent of dynamic viscosity η flows in the $\hat{x}$ -direction in response to a pressure gradient dP/dx . The external force f acts perpendicular

to the flow and points in the $\hat{y}$ -direction. The height of the rectangular channel in the $\hat{y}$ -direction is h , which is the characteristic length scale and non-dimensionalizes all other lengths. Finally, the rectangular channel has a width of w in the $\hat{z}$ -direction and so is characterized by an aspect ratio $\tilde{w} = w/h$. In $\hat{z}$, the channel goes from $-w/2$ to $w/2$.

As in the 2D case, the concentration distribution resulting from the competition between potential energy drop fh and thermal energy $k_B T$ is described by the dimensionless retention parameter $\lambda = k_B T / fh$. As before, implicit size dependence in λ is avoided by using the device retention parameter *i.e.* $\lambda = \Lambda \tilde{r}^{-\alpha}$. In HC, $\Lambda = \infty$. The resulting concentration distribution for spherical solutes is

$$n(\tilde{y}, \tilde{z}; \tilde{r}, \Lambda, \tilde{w}) = \begin{cases} n_0 e^{-(\tilde{y}-\tilde{r})\tilde{r}^\alpha/\Lambda} & \text{for } \tilde{r} \leq \tilde{y} \leq 1 - \tilde{r} \text{ and } -\frac{\tilde{w}}{2} + \tilde{r} \leq \tilde{z} \leq \frac{\tilde{w}}{2} - \tilde{r} \\ 0 & \text{otherwise.} \end{cases} \quad (7.30)$$

This is the same as it was for the ideal retention theory (Equation (5.23)) except there is now a steric-limit on the z positions. The cross-sectional average of Equation (7.30) is

$$\langle n \rangle = \frac{n_0 \Lambda}{\tilde{r}^\alpha} \left(1 - 2 \frac{\tilde{r}}{\tilde{w}} \right) \{ 1 - \exp [- (1 - 2\tilde{r}) \tilde{r}^\alpha / \Lambda] \}. \quad (7.31)$$

When the aspect ratio is infinite $\tilde{w} = \infty$, the system becomes 2D; the flow obeys the Poiseuille equation (Equation (3.27)), the concentration obeys Equation (5.23) and so the retention ratio given by Equation (5.31). For this study, the characteristic velocity is chosen to be $[U] \equiv \langle u \rangle = h^2 (dP/dx) / (12\eta) \equiv [U]$, which is the average velocity of the 2D flow is given by Equation (3.28).

The retention ratio for HC and FFF in rectangular channels with finite aspect ratios can be determined by accounting for the non-parabolic flow profile of the carrier solvent. An example flow profiles was simulated using MPCD in § 3.4.3 (specifically Figure 3.4.3). In that section the fluid velocity profile in a rectangular channel was given by the Fourier sum (Equation (3.29)), which is repeated here for convenience:

$$u(\tilde{y}, \tilde{z}; \tilde{w}) = [U] \sum_{n=1}^{\infty} \frac{48}{(n\pi)^3} \left[1 - \frac{\cosh(n\pi\tilde{y})}{\cosh(\frac{n\pi\tilde{w}}{2})} \right] \sin(n\pi\tilde{z}).$$

Example solutions to Equation (3.29) (using only a few terms) are shown in Figure 7.4.1 for aspect ratios 10 and 1.

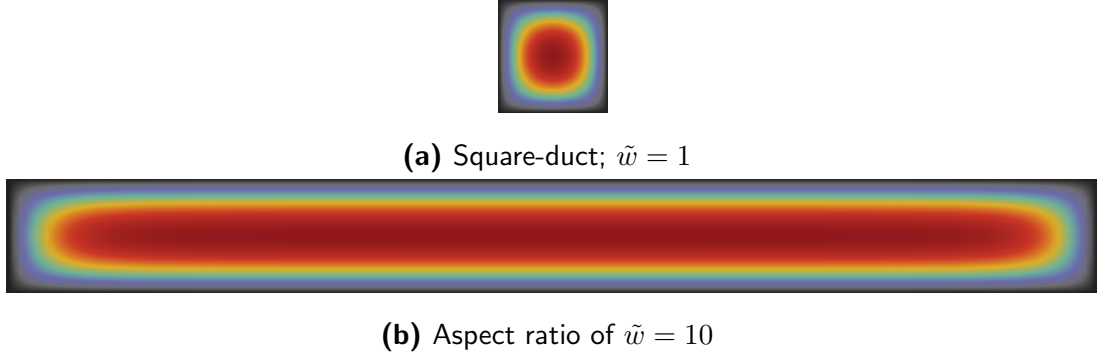


Figure 7.4.1: Example solvent velocity fields for finite aspect ratios. In these figures, the velocity fields are normalized by the mean parabolic Poiseuille velocity $[U] = h^2 (dP/dx) / (12\eta)$ for $\tilde{w} = \infty$. The $\tilde{w} = 10$ geometry has a maximum solvent velocity of $u_{\max} \approx 1.5 [U]$, while the square-duct ($\tilde{w} = 1$) has a maximum of $u_{\max} = 0.88 [U]$.

In order to calculate the retention ratio, Faxén's law (Equation (5.30)) operates on the solvent velocity (Equation (3.29)) to calculate the solute speed $\mathcal{V}$. This produces

$$\mathcal{V}(\tilde{y}, \tilde{z}) = [U] \sum_{n=1}^{\infty} \frac{48}{(n\pi)^3} \left[1 - \frac{(n\pi)^2}{6} \tilde{r}^2 - \frac{\cosh(n\pi\tilde{y})}{\cosh\left(\frac{n\pi\tilde{w}}{2}\right)} \right] \sin(n\pi\tilde{z}). \quad (7.32)$$

The goal is to now calculate the retention ratio by evaluating the averages in Equation (5.8) *i.e.* $R = \langle nu \rangle / (\langle n \rangle \langle u \rangle)$. The denominator of the retention ratio (Equation (5.8)) is the product of Equation (7.31) and Equation (3.30), while the numerator can be determined by integrating the weighted solute speed term by term. Performing the average produces the lengthy, though straight-forward solution for the *rectangular retention ratio*. The solution is so long that it is necessary to define the functional coefficients

$$a_n(\tilde{r}, \tilde{w}) \equiv 1 - \frac{(n\pi)^2}{6} \tilde{r}^2 - \frac{2}{n\pi} \left(\frac{1}{\tilde{w} - 2\tilde{r}} \right) \frac{\sinh(n\pi[\tilde{w} - 2\tilde{r}]/2)}{\cosh(n\pi\tilde{w}/2)} \quad (7.33a)$$

$$b_n(\tilde{r}, \Lambda, \tilde{w}) \equiv \frac{n\pi\Lambda}{\tilde{r}^\alpha} \cos(n\pi\tilde{r}) + \sin(n\pi\tilde{r}) - e^{-(1-2\tilde{r})\tilde{r}^\alpha/\Lambda} \left\{ \frac{n\pi\Lambda}{\tilde{r}^\alpha} \cos(n\pi[1 - \tilde{r}]) + \sin(n\pi[1 - \tilde{r}]) \right\} \quad (7.33b)$$

$$c_n(\tilde{r}, \Lambda) \equiv \left(\frac{n\pi\Lambda}{\tilde{r}^\alpha} \right)^2 + 1 \quad (7.33c)$$

$$d_n(\tilde{w}) \equiv \frac{\tanh(n\pi\tilde{w}/2)}{\tilde{w}} \quad (7.33d)$$

just to fit the equation on the page. This allows the solution for the rectangular retention

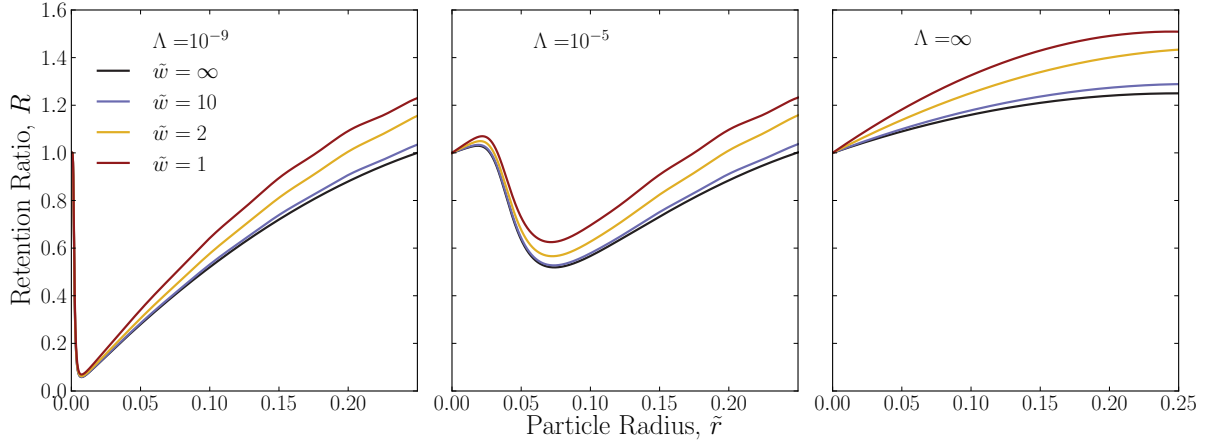


Figure 7.4.2: The retention ratio (Equation (7.34)) as a function of solute size $\tilde{r}$ for $\alpha = 3$ and various external field strengths. Solutions truncated at ten terms.

ratio to be written as

$$R_{3D}(\tilde{r}, \Lambda, \tilde{w}) = \left[1 + \coth \left(\frac{[1 - 2\tilde{r}] \tilde{r}^\alpha}{2\Lambda} \right) \right] \left[\sum_{n=1}^{\infty'} \frac{48}{(n\pi)^3} \frac{a_n b_n}{c_n} \right] \left[1 - \sum_{n=1}^{\infty'} \frac{192}{(n\pi)^5} d_n \right]^{-1}. \quad (7.34)$$

This series solution for the rectangular retention ratio for solute particles in finite aspect ratio channels is the main result of this section. In the absence of an external field, $\Lambda \rightarrow \infty$ and the rectangular retention ratio for HC becomes significantly simpler than the FFF series solution. The *HC rectangular retention ratio* is

$$R_{3D}(\tilde{r}, \tilde{w}) = \left[\frac{1}{1 - 2\tilde{r}} \right] \left[\sum_{n=1}^{\infty'} \frac{48a_n}{(n\pi)^4} \{ \cos(n\pi\tilde{r}) - \cos(n\pi[1 - \tilde{r}]) \} \right] \left[1 - \sum_{n=1}^{\infty'} \frac{192}{(n\pi)^5} d_n \right]^{-1}. \quad (7.35)$$

7.4.2 Consequences of Finite Aspect Ratios

Since the functions of the rectangular retention ratio for FFF (Equation (7.34)) and HC (Equation (7.35)) are series solutions, their behaviour is unclear. Therefore, consider some example parameters. For convenience, the retention curves are plotted for only a single power, $\alpha = 3$. The other physically relevant values of $\alpha = 1, 2$ are qualitatively similar and only minor differences exist. Consider three field strengths:

1. A strong field characterized by a tiny device retention parameter $\Lambda = 10^{-9}$.

2. A moderate field with $\Lambda = 10^{-5}$.
3. No field, which is HC.

For each the ideal parallel-plate retention ratio from Equation (5.31) is shown as well as the finite aspect ratios $\tilde{w} = \{10, 2, 1\}$. The series solutions are truncated after ten terms. Although Equation (7.34) and Equation (7.35) can technically produce solutions for the entire range of solute sizes ($0 < \tilde{r} < 0.5$), only $\tilde{r} \leq 0.25$ is shown because of the conclusions of § 7.1. The finite number of terms used in Equation (7.34) becomes evident at relatively large solute sizes via oscillations in the retention curves.

When the external field is large ($\Lambda = 10^{-9}$), the normal-mode regime of FFF (for which the retention ratio drops rapidly from near-unity to a global minimum) is relatively unaffected with respect to the parallel-plate theory (Figure 7.4.2). In normal-mode FFF, smaller particles elute before larger particles. This is true for all aspect ratios. All this is to say, normal-mode FFF is ideally predicted to be well described by Equation (5.31) for the 2D parallel-plate retention ratio regardless of aspect ratio. In fact, the position of the steric-inversion point does not shift visably from the size predicted by parallel-plate retention theory. This is seen in Figure 7.4.2, or better yet in Figure 7.4.3 (of the selectivity, which will be discussed momentarily) in which the steric-inversion point is the second sharp valley near $\tilde{r} \approx 10^{-2}$.

Past the steric-inversion point is steric-mode FFF, in which larger particles elute before smaller ones. Even for the relatively small aspect ratio of $\tilde{w} = 10$, the rectangular retention ratio is observed to be well approximated by the parallel-plate retention ratio (Figure 7.4.2). This suggests that, even in steric-mode FFF, apparatus need not be designed with astronomical aspect ratios to be well described by the ideal parallel-plate retention theory of Equation (5.31). Commonly, apparatus have large aspect ratios. Smaller aspect ratios could be utilized and parallel-plate retention theory would still be highly accurate.

On the other hand, the slope does increase slightly in the steric-mode regime. Unfortunately, this does not help to separate peaks, which is seen by considering Equation (6.12) for the device selectivity from § 6

$$S_D(\tilde{w}) = \frac{1}{R^2} \frac{dR}{d\tilde{r}}.$$

Here (as in § 6) the channel height is chosen as the characteristic dimension of the elution device because it is the minimum length scale and limits the solute size. Device selectivity

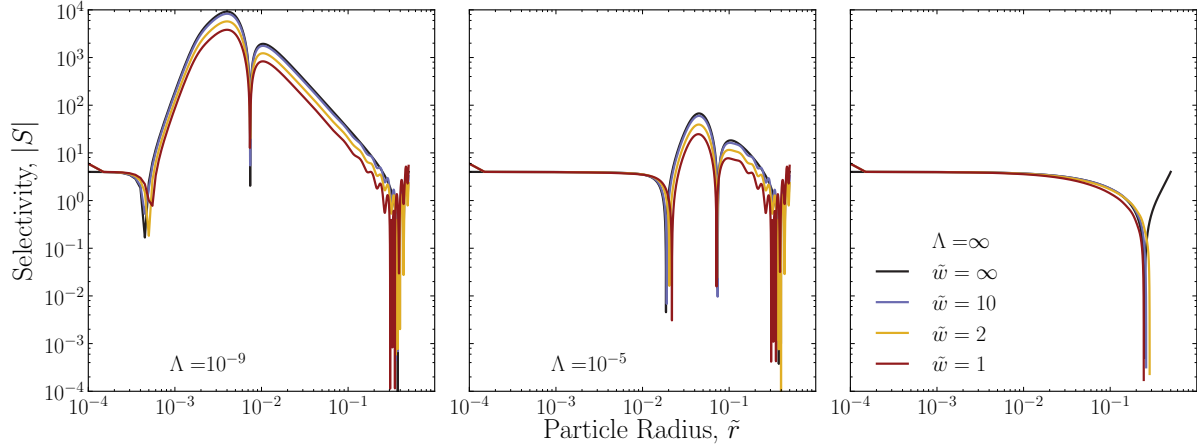


Figure 7.4.3: The device selectivity (Equation (6.12)) corresponding to each retention curve in Figure 7.4.2.

decreases in the normal- and steric-mode regimes as the aspect ratio decreases in Figure 7.4.3 for $\Lambda = 10^{-9}$). The device selectivity is plotted on a log-log graph for the entire range of possible particle sizes since it is not as intrusive. Three dips are visible in S_D (Figure 7.4.3):

1. The first is the transition from the HC-limit at the tiniest $\tilde{r}$ to normal-mode FFF.
2. The second is the steric-inversion point that has already been discussed.
3. The third is the transition to Faxén-mode FFF, which has recently been observed, though in reality is heavily influenced by wall drag (§ 7.1).

The logarithmic view shows that not only does selectivity decrease slightly in steric-mode FFF but also in normal-mode FFF.

By decreasing the field to moderate strengths ($\Lambda = 10^{-5}$ and $\alpha = 3$), the range of the HC portion of the retention curve increases in Figure 7.4.2. Inspecting Figure 7.4.3 shows that the selectivity is rather unaffected by aspect ratio in this regime (though does increase slightly). Likewise, the steric-inversion point remains fixed in terms of the solute size at which it occurs, regardless of $\tilde{w}$.

Other values of the force scaling exponent α are physically relevant for strong field FFF particularly $\alpha = 1$, which corresponds to flow- and thermo-FFF, two common sub-techniques. The retention curves for these are qualitatively similar to Figure 7.4.2 and Figure 7.4.3. The primary difference is that the steric-inversion point is pushed to smaller solute sizes for

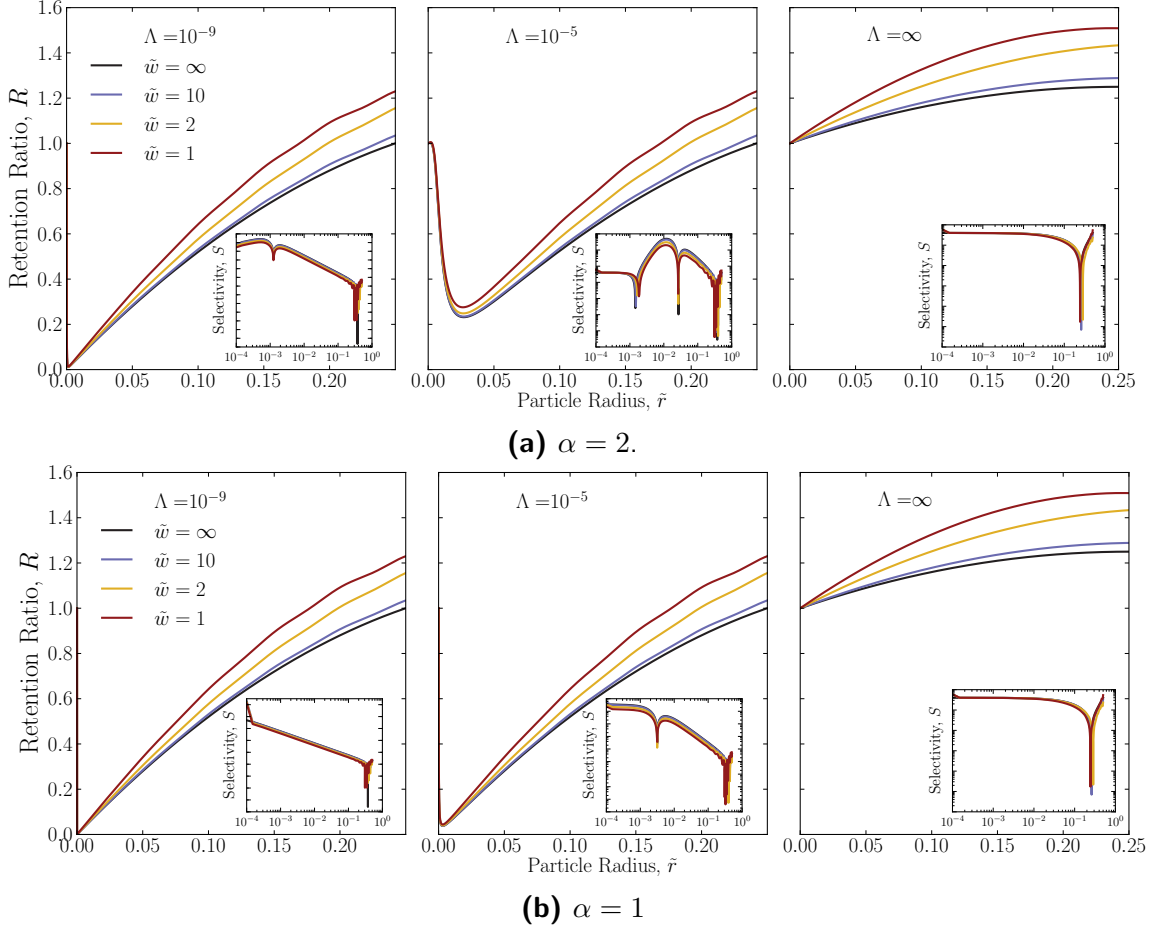


Figure 7.4.4: Same as Figure 7.4.2 and Figure 7.4.3 but with $\alpha = 2$ and 1.

smaller α (Figure 7.4.4). The operational-mode diagrams from § 5.2.2 and § 5.3 demonstrate that this is a property of FFF and is not a consequence of finite aspect ratios (see Figure 5.3.4). As with $\alpha = 3$, Equation (7.34) predicts that the steric-inversion point does not shift to different solute sizes when $\alpha = \{2, 1\}$ (Figure 7.4.4).

Retention curves for aspect ratios smaller than unity were considered as well (not shown). The immediate consequence of $\tilde{w} < 1$ is that width becomes the limiting length scale rather than height. This compresses the retention curve along the $\tilde{r}$ axis. At small particle sizes, the retention ratio in such $\tilde{w} < 1$ channels is larger than in $\tilde{w} > 1$ channels, which follows the trend seen in Figure 7.4.2. However, the ultimate consequence of this is that the total drop in R through normal-mode FFF is reduced. In fact, the retention ratio quite rapidly approaches Equation (5.33) even for strong fields (small Λ).

Next, consider true HC (Figure 7.4.2c). Unlike the finite Λ curves, there is no negative-slope region representing normal-mode FFF. The rectangular retention ratio increases to

greater values than the 2D parallel-plate prediction. This suggests that HC benefits from finite aspect ratios.

It is clear from Figure 7.4.2c that Equation (5.33) with the coefficients $b_{\text{HC}} = 2$ and $c_{\text{HC}} = 4$ is not adequate for HC in rectangular channels with finite aspect ratios. Figure 7.4.2c suggests that it is reasonable to continue assuming a quadratic form for the HC retention ratio. We fit Equation (5.33) to the calculated retention ratio and obtain b_{HC} and c_{HC} as functions of the aspect ratio $\tilde{w}$ by minimizing χ^2 (Figure 7.4.6). For infinite aspect ratios, $b_{\text{HC}} = 2$ and $c_{\text{HC}} = 4$ as expected. As the channel width approaches the height, the constants rise to roughly $b_{\text{HC}} \approx 4$ and $c_{\text{HC}} \approx 8$.

These coefficients are able to suggest whether any aspect ratio is intrinsically more optimized than the others for HC. The device selectivity

$$S_{\text{D}}(\tilde{w}) = \frac{1}{R^2} \frac{dR}{d\tilde{r}} = \frac{b_{\text{HC}} - 2c_{\text{HC}}\tilde{r}}{(1 + b_{\text{HC}}\tilde{r} - c_{\text{HC}}\tilde{r}^2)^2} \quad (7.36)$$

is maximized for small solutes in channels of near-unity aspect ratio (Figure 7.4.5).

By comparing the HC retention ratio to that for an *open-tube cylindrical capillary* of diameter d_{H} , we will now question the validity of assuming that the HC retention is quadratic with respect to particle size (Equation (5.33)). Very briefly, in the absence of an external force the average concentration in a tube is $\langle n \rangle = n_0 [1 - 2r/d_{\text{H}}]^2$, while the flow obeys the Hagen-Poiseuille equation (Equation (3.34)), which is

$$u = 2 \langle u \rangle \left[1 - \left(2 \frac{\rho}{d_{\text{H}}} \right)^2 \right]$$

where $\langle u \rangle = d_{\text{H}}^2 (dP/dx) / (2\eta)$. Applying Faxén's law (Equation (5.30)) estimates the solute velocity field as a function of radial position ρ to be

$$\mathcal{V} = 2 \langle u \rangle \left[1 - \frac{2}{3} \left(2 \frac{r}{d_{\text{H}}} \right)^2 - \left(2 \frac{\rho}{d_{\text{H}}} \right)^2 \right]. \quad (7.37)$$

Therefore, by Equation (5.8) the retention ratio is ideally

$$R = 1 + 4 \frac{r}{d_{\text{H}}} - \frac{28}{3} \left(\frac{r}{d_{\text{H}}} \right)^2, \quad (7.38)$$

which is shown in Figure 7.4.7.

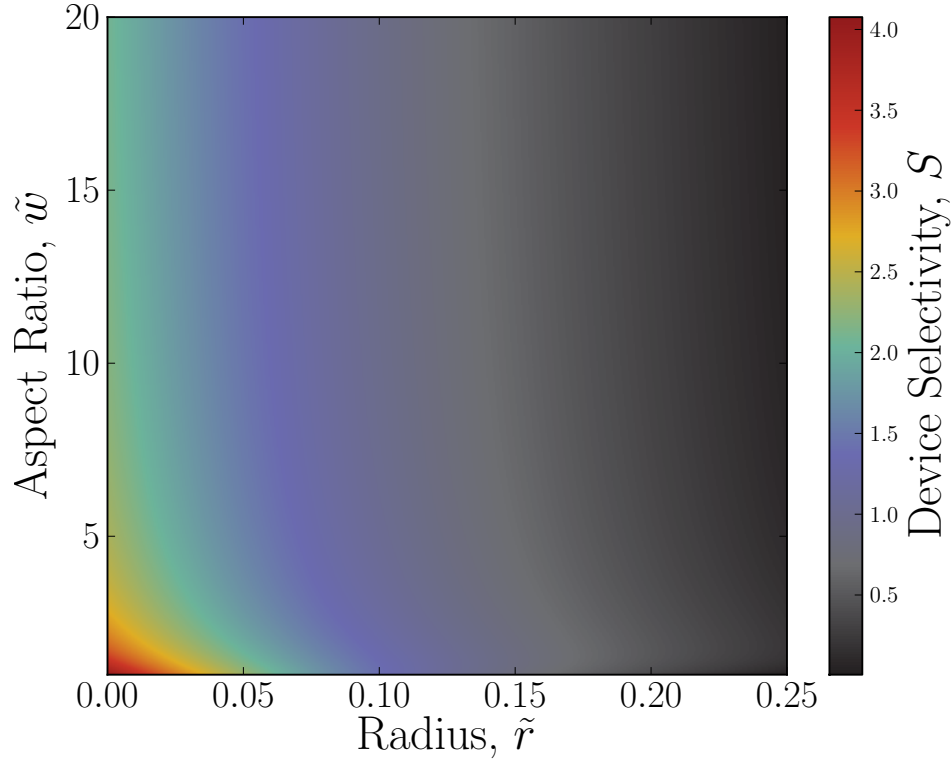


Figure 7.4.5: Device selectivity for HC in a rectangular channel as a function of normalized solute size and normalized width.

To compare a cylindrical capillary and a rectangular channel, an effective *hydraulic diameter* $d_H \equiv 2h / (1 + 1/\tilde{w})$ must be ascribed to the latter [247]. Writing the presumed quadratic form of the HC retention ratio (Equation (5.33)) in terms of hydraulic diameter allows us to compare between different channel geometries:

$$R = 1 + b'_{\text{HC}} \frac{r}{d_H} - c'_{\text{HC}} \left(\frac{r}{d_H} \right)^2, \quad (7.39)$$

where the coefficients are now

$$\frac{b'_{\text{HC}}}{b_{\text{HC}}} \equiv \frac{2}{1 + 1/\tilde{w}} \quad ; \quad \frac{c'_{\text{HC}}}{c_{\text{HC}}} \equiv \left(\frac{2}{1 + 1/\tilde{w}} \right)^2. \quad (7.40)$$

A comparison can now be made between cylindrical and rectangular channels (Figure 7.4.7).

From Equation (7.38) the coefficients for a cylindrical capillary are $b'_{\text{HC}} = 4$ and $c'_{\text{HC}} = 28/3$, while Equation (5.33) gives the coefficients of an infinite aspect ratio channel to be $b'_{\text{HC}} = 4$ and $c'_{\text{HC}} = 16$. Although $b'_{\text{HC}} = 4$ for both geometries, the two retention curves are

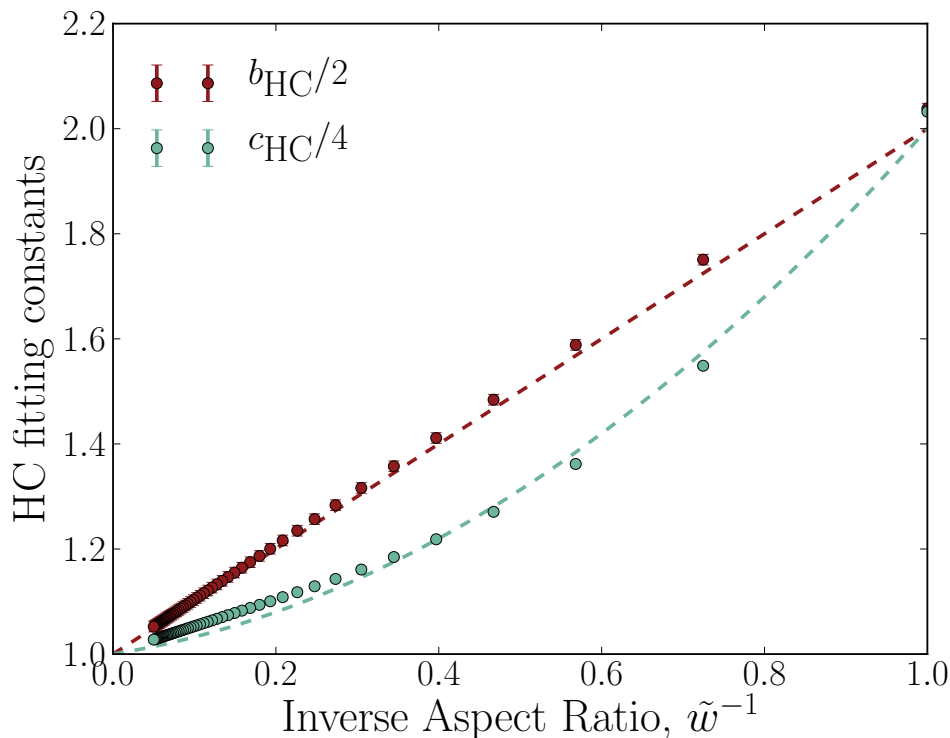


Figure 7.4.6: The parabolic fitting constants for hydrodynamic chromatography from Equation (5.33) as functions of the aspect ratio $\tilde{w}$, normalized by their parallel-plate values. Error bars represent uncertainty on the fit parameters.

seen to be rather different in Figure 7.4.7. Surprisingly, the retention curves for rectangular channels fall between those of the cylindrical and the plate geometries only for $\tilde{w} > 2$; however, for aspect ratios closer to unity, the retention ratios are greater than what was found for cylindrical shapes. The retention ratio of the $\tilde{w} \approx 2$ channel behaves most like that of the cylindrical capillary (Figure 7.4.7). These two retention ratios climb rather rapidly as a function of r/d_H . On the other hand, the retention ratio of larger aspect ratio channels climb more slowly at any given r/d_H (Figure 7.4.7).

Regardless of geometry, the retention curves for the rectangular channels in Figure 7.4.7 appear to follow the presumed quadratic form [253, 254, 256], which is indeed accurate for most particle sizes. However, an investigation of the tiniest particle sizes reveals that the quadratic form breaks down (Figure 7.4.7; inset). Therefore, the commonly presumed form of Equation (5.33) or Equation (7.39) is seen to be an approximation for HC retention in rectangular channels. For particles that are not small, the parabolic form is exceptionally

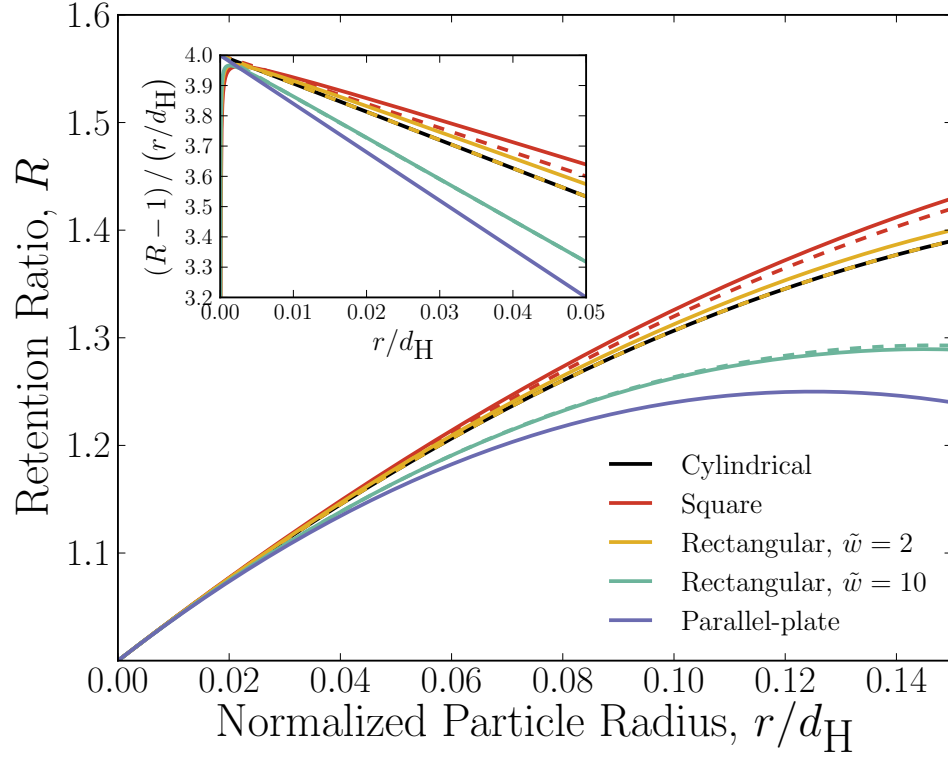


Figure 7.4.7: Hydrodynamic chromatography retention ratio as a function of particle radius normalized by hydraulic diameter for a number of geometries (solid lines). Dashed lines show the parabolic approximation of Equation (7.41) in Equation (7.39). The inset focuses on the small particle size behaviour.

accurate and the coefficients can be approximated by using

$$b'_{\text{HC}} = 4 \quad ; \quad c'_{\text{HC}} = \frac{16\tilde{w}^2 + 4\tilde{w} + 12}{\tilde{w}^2 + 2\tilde{w} + 1}. \quad (7.41)$$

These accurately reproduce the HC retention curves in Figure 7.4.7 (dashed lines) and can be compared to the fitting parameters in Figure 7.4.6.

Summary

Although the ideal retention theory agrees well with literature data for small solutes about the steric-inversion point (Figure 7.1.1), multi-particle collision dynamics simulations show that the ideal retention theory fails at large solute sizes. This is because hydrodynamic interactions with the channel walls become significant and this so-called wall drag leads to lagging, which is described by the slip function (or, equivalently, resistance). Many approximations are explored including Faxén’s classical expansion, the Goldman-Cox-Brenner near- and far-wall approximations and Oseen’s superposition of two solutions for a single wall. It is found that the Goldman-Cox-Brenner far-wall approximation reproduces the simulated retention curve, though superimposing two far-wall approximations (Equation (7.12)) provides a slightly better approximation yet.

Superimposing solutions is the method of choice for numerical theoreticians who use the multipole expansion method to numerically solve for the velocity of a solute eluting through a channel [5]. The resulting slip function provides highly accurate solute velocity profiles that are both continuous at the centre of the channel and in the near-wall region where the solute speed falls to zero. The retention ratio calculated using the results of the multipole expansion find the highest agreement with the simulation data (Figure 7.1.10) and so the form given by Pasol *et. al.* for the slip function is preferred for predicting retention curves.

Moving in the vicinity of microchannel walls causes the colloids to lag behind their ideally predicted speeds, which may have important consequences for particle tracking velocimetry techniques in microfluidic systems. As a consequence, micro-particle image velocimetry that implicitly model the fluid velocity as equivalent to tracer particles velocities run significant risk of systematically underestimating fluid velocities, especially in micro- and nanoscale systems. The MPCD simulations of field-flow fractionation present here suggest that the multipole expansion method produces values that accurately predict the retention ratio, while more traditional approximations fail.

An extremely simple model for hydrodynamic, inertial-lift effects in FFF is presented. It succeeds in reproducing the qualitative behaviour of hyperlayer FFF, but is expected to be accurate only near the steric-inversion point. However, the retention theory produced from this model is interesting in its own right and suggests a novel operational-mode for FFF. Two experimentally demonstrated FFF sub-techniques utilizing forces with different scaling dependence of solute size could be combined. When the two fields oppose one another a sharp peak in the retention curve occurs at a well-defined point beyond the steric-inversion

point. By varying one of the fields the theoretical peak can be shifted until it matches the solute size. Thus this proposed adverse-mode FFF can measure the size of a single solute species to high precision.

Not all complications are hydrodynamic in nature. In the case of slip-FFF, the flow field that carries sample solutes through elution channels is modified by allowing finite slip-lengths at the channel walls. At first, the ideal retention theory developed in this chapter appeared to suggest that slip is nothing more than a minor complication. Slip modifies the FFF and HC retention ratios, but the weak field limit of FFF does not benefit. Slip at the depletion wall increases the range of steric-mode FFF without harming the selectivity. Both normal- and steric-mode FFF persist for weaker fields when there is slip at the depletion wall. Each of these conclusions constitute relatively minor complications that may result in FFF apparatus with finite slip at the channel walls (such as flow-FFF systems that use frits to allow for a cross-flow as the transverse, external field).

However, when there is a large slip at the accumulation wall and a strong external field, an entirely new high-resolution mode, slip-mode FFF, is predicted to exist. Since the separation potential of this techniques could be quite high, researchers could engineer microfluidic systems with a large slip length at one wall. It is proposed that this be done by nanoengineering patterned, ultrahydrophobic surfaces or by generating a thin Debye layer electro-osmotic flow with a large Smoluchowski slip velocity. Another way to achieve a surface with an effective slip length is to coat the wall in a polymeric film. However, polymer brushes are soft matter systems that are partially permeable to solutes. Polymer brushes modify the flow profile and also the concentration distribution of solutes. These effects can be employed by separation devices to generate large differences in elution times for specific ranges of solute sizes. Utilizing polymer brushes within FFF apparatus warrants future attention as a fractionation method distinct from slip-FFF.

Series solutions for the retention ratios applicable to hydrodynamic chromatography (Equation (7.35)) and field-flow fractionation (Equation (7.34)) in elution channels with rectangular cross sections were derived in this chapter. The series solution predicts that normal-mode FFF in the presence of a strong external field is relatively unperturbed from the parallel-plate retention curve by finite aspect ratios. The steric-inversion point at which the retention behaviour transitions from normal-mode FFF to steric-mode FFF remains fixed at the size predicted by parallel-plate retention theory. In the steric-mode regime of FFF, the slope of the retention curve increases as the aspect ratio approaches unity. This suggests that selectivity is improved.

This improvement is most substantial when the external force is zero. In this HC limit of FFF, decreasing the aspect ratio increases the selectivity. The retention ratio is parabolic for HC methods and both the quadratic coefficients increase as the aspect ratio approaches unity. Selectivity is maximized in rectangular HC channels when the aspect ratio is unity. In terms of the hydraulic diameter of the channel, the quadratic coefficients are quite similar to those ideally expected for capillary HC.

*As a theorist, you do have a few tools but you
also have to think.*

Sam Safran [349]

8

Hydrodynamic Chromatography with Coil-Globule Collapse

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In the previous chapter, complications to field-flow fractionation were considered. Wall-drag (§ 7.1), inertial forces (§ 7.2) and finite aspect ratio channels (§ 7.4) are intrinsic to any hydrodynamic chromatography device. However, § 7 demonstrated that considering such complications could lead to optimization (as was the case when channel geometry was considered for rectangular HC in § 7.4.2) or even to novel proposals that harness complexity as a separation mechanism (as in adverse-mode FFF from § 7.2.4 or slip-mode FFF from § 7.3.3).

In this section, a modification to HC is proposed that fractionates via induced changes **in the solute** rather than the separation apparatus. In each of the fractionation techniques considered so far the internal structure of the solutes has not mattered. In electrophoresis, the conformation of the polyelectrolyte chains was explicitly demonstrated not to matter except when the chain is very strongly confined (§ 4.4), while in both the theoretical and numerical investigations into field-flow fractionation the particles were assumed to be hard, spherical particles. However, this need not be the case. This final proposal considers the entropic effect of an ensemble of small particles on a large biomolecule.

Biomolecules have many internal degrees of freedom and can often transition from one stable or meta-stable conformational state to another. Protein folding is an obvious example of this. Living systems perpetually take advantage of such transitions. A good example is the segregation and compaction/de-compaction of chromosomal material during cell division. Physical organization of the chromosome plays an essential role during cell division and in determining gene activity. It is no trivial matter for a cell to tailor DNA structure without spending copious amounts of energy. While eukaryote cells have extensive cellular machinery dedicated to the task [350], bacteria are significantly simpler organisms. Histones, microtubule spindles and the other contraptions of mitosis are absent. What mechanisms for organizing chromosomal material remain available to such simple creatures?

Entropic repulsion between two daughter strands within prokaryote cells can be sufficient for segregation: Excluded volume interactions between the chain segments determine whether they will remain mixed or spontaneously segregate [351–356]. Thus, under high confinement and crowding conditions, entropy can drive two daughter strands to recede to opposite poles of the cell in preparation for cytokinesis. There appears to be no need for the enthalpic interventions employed by higher cellular creatures.

It has been suggested that entropic considerations are essential, not only for segregating daughter strands but also for the compaction and de-compaction of bacterial chromosomes [357, 358]. Even in simple, single celled organisms like *Escherichia coli* the genome

is huge and must fit within the cellular envelope. Molecular crowding by surrounding cytoplasmic proteins enacts an entropic attraction between components of the chromosome and it is proposed that these may be strong enough to cause a phase transition from a swollen conformation to a collapsed state. The volume fraction of cytoplasmic proteins is approximately ~ 0.2 in *E. coli* cells, which is high enough for depletion force to at least not be negligible [359, 360]. Experimental observations of macromolecules within eukaryotic nuclei suggest that these *crowding effects* influence interactions within cells [361–363]. However, questions still remain about the nature of the transition from swollen to collapsed state in simple, prokaryotic chromosomes. Indeed, it has yet to be confirmed whether or not entropic forces are sufficient to account for chromosomal collapse.

Due to complications in experimental systems, computational simulations are needed to verify whether or not non-specific depletion forces are sufficient to cause the collapse of bacterial chromosomal DNA. However, the entropic forces that arise using simulations of the type described in § 3.5.2 have not been fully characterized despite the fact that such depletion forces are ubiquitous in biological systems and mesoscopic simulations could be a powerful tool for studying such systems.

In this section, it is proposed that such *depletion-induced transitions* from a swollen coil to a globular state can be utilized in microfluidic channels to measure internal properties of chromosomes. It is proposed that the sudden change in elution time that accompanies any such collapse could be exploited to gain information about the basic units that make up the biomolecule.

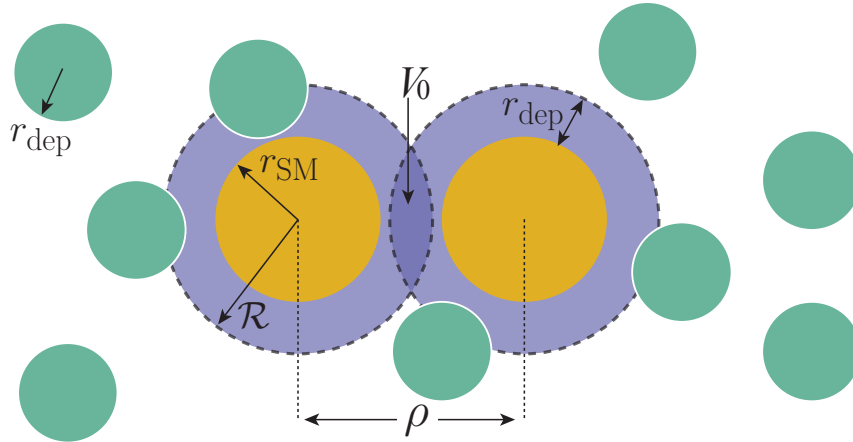


Figure 8.1.1: Two relatively large, spherical and disconnected DNA structural monomers (of radius r_{SM}) in a solution of depletants ($r_{\text{dep}} < r_{\text{SM}}$) each possess a depletant-related excluded volume (of radius $\mathcal{R} = r_{\text{SM}} + r_{\text{dep}}$). When the structural monomers are near ($2r_{\text{SM}} < \rho < 2\mathcal{R}$) an overlap volume V_o exists, causing the number of states available to the population of depletants to be greater than when the monomers are separate ($\rho \geq 2\mathcal{R}$). This leads to an effective force that favours bringing the two structural monomers closer together.

8.1 Depletion Forces

Depletion-induced forces of entropic origin are universal in soft biological systems [364]. They can be found in a myriad of biologically relevant systems and have been used to engineer novel microscopic systems such as lock-and-key complexes [365, 366], and ordered tobacco mosaic virus structures [367, 368].

In § 8.2, a coarse-grained model of bacterial chromosomes is considered. Each chromosome is viewed as a linear chain of DNA *structural monomers* (SMs) that have been formed from a segment of supercoiled plectonemes [353]. Each structural monomer is modelled as a hard, spherical monomer of radius r_{SM} (volume V_{SM}). The structural monomers are surrounded by an ensemble of smaller protein molecules. The proteins are also assumed to be hard, spherical beads (of radius $r_{\text{dep}} < r_{\text{SM}}$ and volume V_{dep}) and to be inert: They can only act entropically on the structural monomers. These model proteins are the *depletants*.

8.1.1 Depletion-Induced Pair Potentials

Because they can produce “**order from disorder**” [369], at first glance entropic forces can feel counter-intuitive. In fact, depletion interactions are universal in biology because they are

purely entropic in nature. Imagine two large, hard, disconnected monomer spheres suspended in a bath of small, hard depletant spheres of radius r_{dep} and volume V_{dep} (Figure 8.1.1). The large monomers each have a thin sheath of radius $\mathcal{R} = r_{\text{SM}} + r_{\text{dep}}$ from which the centres of the depletants are **excluded**. The volume available to the depletants is the volume of the system V_{sys} , less the volume excluded by the structural monomers $2 \times (4\pi\mathcal{R}^3/3)$.

From a kinetics perspective, when the two large spheres are brought together such that their centre-to-centre separation ρ is less than $2\mathcal{R}$, their *excluded volumes* overlap and the depletants can only collide with the monomers from certain angles, causing thermal force to be non-isotropic, pushing the structural monomers together. From a thermodynamics perspective, the free space and, therefore, the number of states available to the depletants increases. This results in a free energy change as a function of available volume for the depletants giving rise to an osmotic pressure. The two descriptions are indeed equivalent.

Asakura-Oosawa Pair Potential

In thermodynamic, **dilute limit** of depletants ($N_{\text{dep}} \rightarrow \infty$ while $\phi_{\text{dep}} \ll 1$), the *osmotic pressure* can be approximated by a van't Hoff relation

$$\Pi_S \approx - \left(\frac{\phi_{\text{dep}}}{V_{\text{dep}}} \right) k_B T \quad (8.1)$$

and the attraction can be discussed in terms of an entropic, attractive, depletion-induced (and therefore statistical in nature) short-ranged pair potential $\mathcal{U}(\phi_{\text{dep}}, \rho)$. In this dilute-limit the pair potential takes the form

$$\mathcal{U} \approx \mathcal{U}_{\text{AO}} \equiv - \int \Pi_S \Sigma \, d\rho = \Pi_S(T, \phi_{\text{dep}}) \cdot V_o(\rho), \quad (8.2)$$

where V_o is the lens-like overlap volume (Figure 8.1.1) that varies as a function of the centre-to-centre separation ρ . For hard spheres, the overlap volume is [44, 370–372]

$$V_o(\rho) = V_{\text{dep}} \left(\frac{\mathcal{R}}{r_{\text{dep}}} \right)^3 \left[1 - \frac{3}{4} \frac{\rho}{\mathcal{R}} + \frac{1}{16} \left(\frac{\rho}{\mathcal{R}} \right)^3 \right]. \quad (8.3)$$

Asakura and Oosawa were the first to use Equations (8.1)-(8.3) to calculate the *depletion-induced pair potential* [373]. This model has been referred to as the penetrating-hard spheres (PHS) model [374] because the structural monomers are assumed to be hard colloids and the

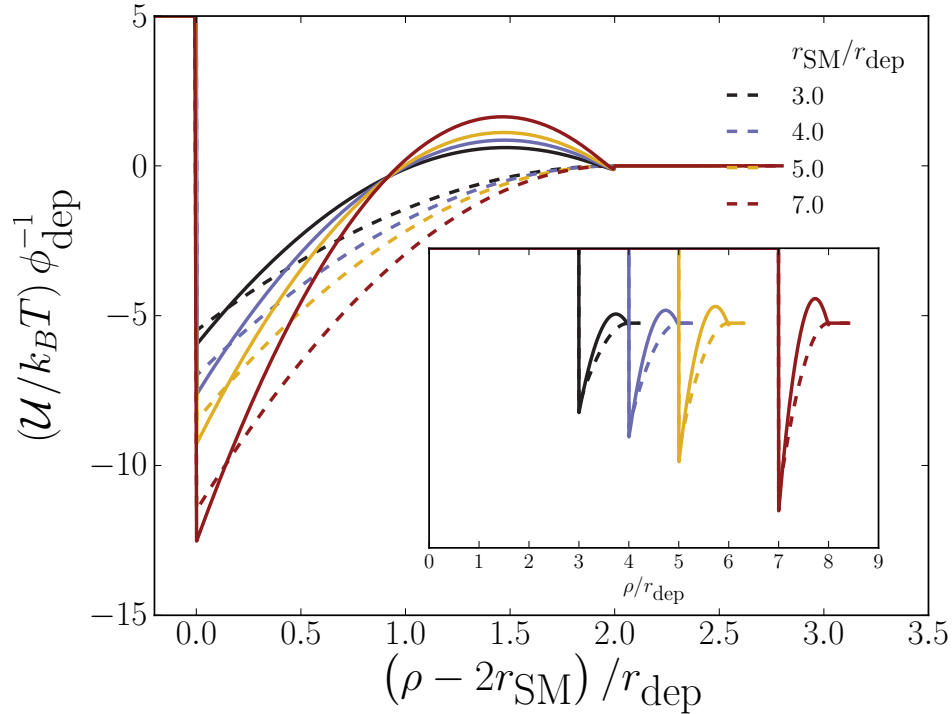


Figure 8.1.2: Depletion-induced attractive pair potential between two structural monomers for a variety of monomer-depletant ratios. The abscissa is a shifted and normalized centre-to-centre separation that emphasizes differences in well depths. The Asakura and Oosawa model (Equation (8.4)) is shown as dashed lines, while the MT-RF predictions are shown as solid lines. Scaling the pair potential removes ϕ_{dep} in the AO model but not in the MT model. The MT-RF pair potentials are for the example volume fraction of $\phi_{\text{dep}} = 0.2$.

depletants are also assumed to be hard with respect to the large structural monomers but the depletants are assumed to be an ideal gas amongst themselves. More often, it is simply called the *AO model*. Explicitly, the AO pair potential is

$$\frac{\mathcal{U}_{\text{AO}}}{k_{\text{B}}T} = \begin{cases} \infty & \rho < 2r_{\text{SM}} \\ \left(\frac{-\mathcal{R}}{r_{\text{dep}}}\right)^3 \left[1 - \frac{3}{4}\frac{\rho}{\mathcal{R}} + \frac{1}{16}\left(\frac{\rho}{\mathcal{R}}\right)^3\right] \phi_{\text{dep}} & 2r_{\text{SM}} \leq \rho \leq 2\mathcal{R} \\ 0 & \rho > 2\mathcal{R} \end{cases}. \quad (8.4)$$

Four points define the nature of the AO model (Figure 8.1.2; dashed lines):

- The range depends only on the size of the depletants.
- Both the volume fraction of depletants and the temperature scale the depth of the well via the osmotic pressure.

- The well deepens as the size ratio $r_{\text{SM}}/r_{\text{dep}}$ is increased.
- The minimum occurs when the two large structural monomers are in contact:

$$\frac{\mathcal{U}_{\text{AO}}^{\min}}{k_{\text{B}}T} = \frac{\mathcal{U}_{\text{AO}}(\rho = 2r_{\text{SM}})}{k_{\text{B}}T} = - \left[1 + \frac{3}{2} \frac{r_{\text{SM}}}{r_{\text{dep}}} \right] \phi_{\text{dep}}. \quad (8.5)$$

The AO model is beautifully simple; however, it has two major limitations, which are discussed next.

Large Monomers - Small Depletants: Division of Time Scales As the depletant size approaches the monomer size, Equation (8.5) predicts there is a non-zero well depth. In the literature, this unphysical result is always avoided by stating that the AO model considers colloids in a bath of much smaller depletants ($r_{\text{SM}} \gg r_{\text{dep}}$), but it is rarely, if ever, explained why the AO theory demands this condition.

In order to understand why the AO theory demands $r_{\text{SM}} \gg r_{\text{dep}}$, consider the opposite case of two **small** monomers in a bath of **larger** depletants. If the smaller spheres are held fixed in place (say using optical tweezers or in a simulation), an excluded volume exists and an osmotic pressure biases them towards each other — just like for larger spheres in a bath of smaller depletants. However, if these smaller monomers are not held in place then the excluded volume is fleetingly short lived compared to the characteristic time scale of the depletants. The depletants explore space with some diffusive time scale $[\tau]_{\text{explore}}$ and the excluded volume has some lifetime $[\tau]_{\text{exist}}$. There is an implicit division of time scales. In order to have the osmotic pressure be described by Equation (8.1), $[\tau]_{\text{exist}} \gg [\tau]_{\text{explore}}$ is needed such that the depletants can act thermodynamically on the structural monomers.

In order to estimate these times, let it be assumed that both the structural monomers and depletants diffuse without any concentration dependence ($\mathcal{D}_i = k_{\text{B}}T/6\pi\eta r_i$). This is reasonable since the AO model is for dilute suspensions. The length scale associated with the excluded volume is $\sim r_{\text{dep}}$ and the length scale associated with the depletants exploring space is the mean distance between depletants (the cubic root of the mean volume per depletant) *i.e.* $\sim (V_{\text{sys}}/N_{\text{dep}})^{1/3} = (V_{\text{dep}}/\phi_{\text{dep}})^{1/3} \sim r_{\text{dep}}/\phi_{\text{dep}}^{1/3}$. Therefore,

$$[\tau]_{\text{explore}} \sim \frac{(V_{\text{sys}}/N_{\text{dep}})^{2/3}}{\mathcal{D}_{\text{dep}}} = \frac{r_{\text{dep}}^2}{\phi_{\text{dep}}^{2/3}} \frac{6\pi\eta r_{\text{dep}}}{k_{\text{B}}T} \quad (8.6a)$$

$$[\tau]_{\text{exist}} \sim \frac{r_{\text{dep}}^2}{\mathcal{D}_{\text{SM}}} = r_{\text{dep}}^2 \frac{6\pi\eta r_{\text{SM}}}{k_{\text{B}}T}. \quad (8.6b)$$

Via the argument above, the lifetime of any temporary excluded volume must be long compared to the time scale of the solution of depletants, which sets the condition:

$$\begin{aligned} [\tau]_{exist} &\gg [\tau]_{explore} \\ r_{SM} &\gg \frac{r_{dep}}{\phi_{dep}^{2/3}}. \end{aligned} \quad (8.7)$$

Since ϕ_{dep} is always less than unity (and more strictly **small**, as is discussed next), the radius of the structural monomers must be much greater than the radius of the depletants in order for Equation (8.4) to be applicable.

Penetrating-Hard Spheres: Zero Volume Fraction Limit The form used for osmotic pressure (Equation (8.1)) in the simplest AO model for depletion interactions is equivalent to an ideal gas approximation, but by construction the depletants must have a finite size. Therefore, this demands that the gas of depletants approaches the zero density limit

$$\phi_{dep} \ll 1. \quad (8.8)$$

Higher densities must be allowed and this requires that the radial density distribution be less step-like and account for correlations beyond the attractive well of the AO model. The Morphometric Thermodynamics Model accounts for first order anti-correlations.

Morphometric Pair Potential

To move beyond the $\phi_{dep} \ll 1$ limit, the free energy cost of **inserting** the large structural monomers must increase above the penetrating-hard sphere assumption of the AO model. The radial density distribution function should be expected to have the standard form for a hard sphere gas. Therefore, one might naively expect the local osmotic pressure is directly proportional to the radial distribution function (since the local density is directly proportional to the radial distribution function and density determines osmotic pressure). Following this line of reasoning, one might be tempted to replace Equation (8.1) with a form for the osmotic pressure that includes the higher virial coefficients of the depletant gas. However, the shape of the resulting pair potential is not altered. Instead, the well depth simply increases to more negative values.

The missing ingredient is the realization that structural monomers getting close to one another are bound to mutually perturb the statistical arrangement of their surrounding shells

of depletants. This results in a repulsive interlude before the depletion attraction starts dominating. The well effectively becomes deeper as it is now the difference between the maximum due to repulsion and the minimum due to attraction, but the minimum itself remains mostly unaffected. One conceptually simple theory (called *morphometric thermodynamics* (MT) [375–377]) reproduces the first repulsive/anti-correlation component of the pair potential.

The morphometric thermodynamics model discusses free energy in terms of the geometric shapes involved and their thermodynamic conjugates. Until this point, only the overlap volume V_o and osmotic pressure Π_S have been discussed. However, there is also a surface area Σ restriction to the depletants with a resulting **entropic surface tension** γ_S and a free energy cost associated with Gaussian curvatures C_1 and C_2 with corresponding **entropic bending rigidities** $\kappa_{S,1}$ and $\kappa_{S,2}$. These are surface tensions and rigidities in the same nature as osmotic pressure is a pressure: They are entropic, thermodynamic quantities. Just as the osmotic pressure is a thermodynamic force that arises because of restrictions on the “placement” of depletants in the overlap volume, the entropic surface tension is a thermodynamic force that arises from restrictions on the “placement” of depletants on shells around one structural monomer due to the presence of the other. Changes in the geometric quantities lead to the total interaction pair potential

$$\mathcal{U} \approx \mathcal{U}_{\text{MT}} = \Pi_S V_o + \gamma_S \Sigma_o + \kappa_{S,1} C_{1o} + \kappa_{S,2} C_{2o}. \quad (8.9)$$

The geometric variables are simple functions of monomer-monomer separation ρ , which are known to be [375]

$$V_o = \frac{4\pi\mathcal{R}^3}{3} \left[1 - \frac{3}{2} \left(\frac{\rho}{2\mathcal{R}} \right) + \frac{1}{2} \left(\frac{\rho}{2\mathcal{R}} \right)^3 \right] \quad (8.10a)$$

$$\Sigma_o = 4\pi\mathcal{R}^2 \left(1 - \frac{\rho}{2\mathcal{R}} \right) \quad (8.10b)$$

$$C_{1o} = \frac{\Sigma_o}{\mathcal{R}} + \pi\mathcal{R} \sqrt{1 - \left(\frac{\rho}{2\mathcal{R}} \right)^2} \sin^{-1} \left(\frac{\rho}{2\mathcal{R}} \right) \quad (8.10c)$$

$$C_{2o} = 4\pi. \quad (8.10d)$$

However, thermodynamic quantities (Π_S , γ_S , $\kappa_{S,1}$ and $\kappa_{S,2}$) are less straightforward. Here,

the *Rosenfeld functionals* [378] are utilized. These are

$$\frac{\Pi_S V_{\text{dep}}}{k_B T} = -\phi_{\text{dep}} \frac{1 + \phi_{\text{dep}} + \phi_{\text{dep}}^2}{(1 - \phi_{\text{dep}})^3} \quad (8.11a)$$

$$\frac{\gamma_S V_{\text{dep}}}{k_B T} = +\phi_{\text{dep}} r_{\text{dep}} \frac{3\phi_{\text{dep}}(1 + \phi_{\text{dep}})}{2(1 - \phi_{\text{dep}})^3} \quad (8.11b)$$

$$\frac{\kappa_{S,1} V_{\text{dep}}}{k_B T} = -\phi_{\text{dep}} r_{\text{dep}}^2 \frac{3\phi_{\text{dep}}^2}{(1 - \phi_{\text{dep}})^3} \quad (8.11c)$$

$$\frac{\kappa_{S,2} V_{\text{dep}}}{k_B T} = -\phi_{\text{dep}} r_{\text{dep}}^3 \left(\frac{-2 + 7\phi_{\text{dep}} - 11\phi_{\text{dep}}^2}{6(1 - \phi_{\text{dep}})^3} - \frac{\ln(1 - \phi_{\text{dep}})}{3\phi_{\text{dep}}} \right). \quad (8.11d)$$

They are not the only accurate approximations that are available in the literature. Notably the second White Bear functionals (WBII) are also commonly employed [379–381]; however, the differences are not great and in a general sense, all such functionals can be written as expansions:

$$\frac{\Pi_S}{k_B T} = - \left(\frac{\phi_{\text{dep}}}{V_{\text{dep}}} \right) f_{\Pi_S}(\phi_{\text{dep}}) \quad (8.12a)$$

$$\frac{\gamma_S}{k_B T} = \left(\frac{\phi_{\text{dep}}}{V_{\text{dep}}} r_{\text{dep}} \right) f_{\gamma_S}(\phi_{\text{dep}}) \quad (8.12b)$$

$$\frac{\kappa_{S,1}}{k_B T} = - \left(\frac{\phi_{\text{dep}}}{V_{\text{dep}}} r_{\text{dep}}^2 \right) f_{\kappa_{S,1}}(\phi_{\text{dep}}) \quad (8.12c)$$

$$\frac{\kappa_{S,2}}{k_B T} = - \left(\frac{\phi_{\text{dep}}}{V_{\text{dep}}} r_{\text{dep}}^3 \right) f_{\kappa_{S,2}}(\phi_{\text{dep}}), \quad (8.12d)$$

where each f_X represents an expansion in terms of volume fraction and virial coefficients. This notation keeps things general but also neat, since obnoxious specific functions of ϕ_{dep} are hidden from view. The AO model corresponds to an $\mathcal{O}(1)$ expansion of each. An expansion to $\mathcal{O}(\phi_{\text{dep}}^2)$ is required to include all four geometric variables. For the Rosenfeld functionals these are

$$f_{\Pi_S} \approx 1 + 4\phi_{\text{dep}} + 10\phi_{\text{dep}}^2 \quad (8.13a)$$

$$f_{\gamma_S} \approx \frac{3}{2}\phi_{\text{dep}} + 6\phi_{\text{dep}}^2 \quad (8.13b)$$

$$f_{\kappa_{S,1}} \approx 3\phi_{\text{dep}}^2 \quad (8.13c)$$

$$f_{\kappa_{S,2}} \approx \frac{\phi_{\text{dep}}}{3} - \frac{2}{9}\phi_{\text{dep}}^2. \quad (8.13d)$$

It is now explicitly clear that adding a correction to the entropic pressure requires the ad-

dition of both entropic surface tension and bending rigidity terms. The surface tension components in particular raise the well depth. The full Rosenfeld functionals (Figure 8.1.2; dashed lines) within the MT model are able to predict the attractive well and also the primary repulsive barrier. Correlations and anti-correlations beyond the primary contributions cannot be reproduced within the MT model.

8.1.2 Simulations of Depletant-Induced Pair Potential

Simplified analytical solutions such as the AO or MT models are appealing for straightforward, low depletant volume fraction systems, but coarse-grained simulations offer a path to studying higher densities and more complicated systems. In order to computationally study this model via Molecular Dynamics simulations, a steep repulsive potential to produce hard steric effects between structural monomers and depletants is needed. To coarse-grain the system, all hydrodynamic interactions that may propagate through the fluid medium are neglected — only thermal motion and the repulsive potentials are included. That is to say, Langevin Dynamics are used. Both the structural monomers and the depletants will be modelled as quasi-“hard spheres” in the framework of Molecular Dynamics. Because depletion forces arise due to excluded volumes between particles that become inaccessible to the smaller depletant particles, any repulsive potential can technically be used to represent the steric repulsion. This work uses the truncated repulsive Lennard-Jones potential, which is also referred to as the Weeks-Chandler-Andersen (WCA) potential (that was introduced in Equation (3.45) of § 3.5.2). This is a simple and commonly employed potential; however, using a potential that is not infinitely steep alters the attractive entropic interactions and must be carefully characterized. In this section, it is seen that an appropriate choice of parameters can significantly increase the depth of the well, which in turn decreases the second virial coefficient and effective volume of the structural monomers.

Since the WCA potential and its two different implementations play an essential role in what follows, readers are encouraged to briefly re-read § 3.5.2, particularly Equation (3.45) for the WCA potential. Please pay close attention to the two implementations:

Shifted-WCA, which is implemented by substituting Equation (3.46) into Equation (3.45) and is denoted sWCA.

Combinatorial-WCA, which uses Equation (3.47) and is denoted cWCA.

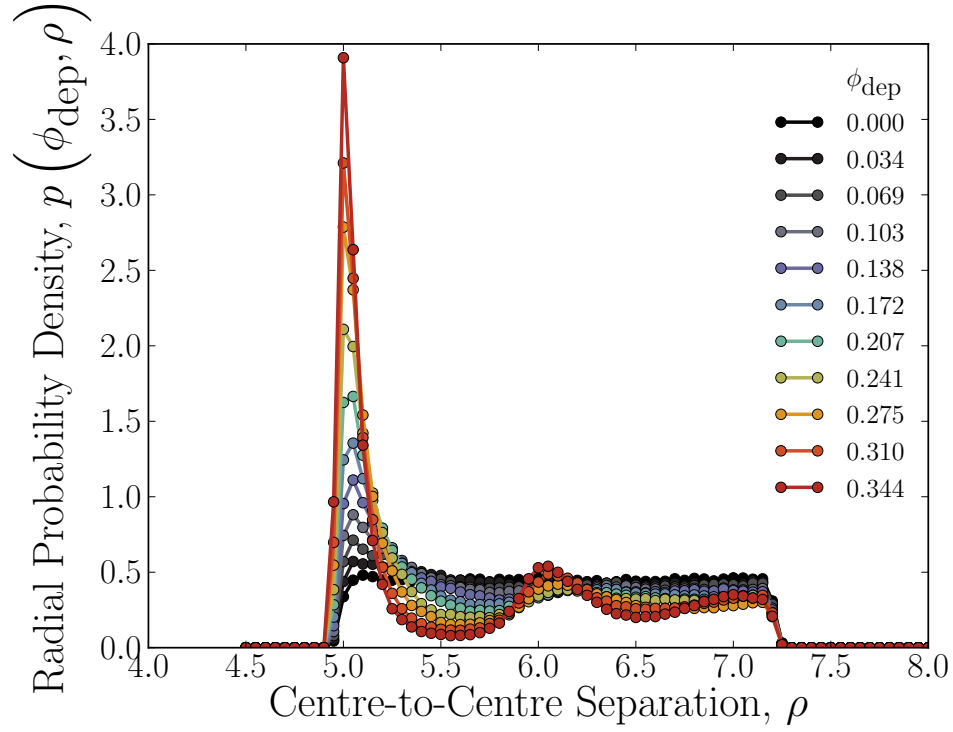


Figure 8.1.3: Radial probability density p of finding two free shifted-WCA structural monomers at a given separation. The structural monomers are of size $r_{\text{SM}} = 2.5\sigma$ in baths of various volume fractions of shifted-WCA depletants of size $r_{\text{dep}} = 0.5\sigma$.

Essentially, the combinatorial-WCA repulsion between two structural monomers U_{cWCA} is slightly softer than its shifted-WCA counterpart U_{sWCA} . The repulsive potential extends further and does not rise as sharply. The combinatorial-WCA potential is longer ranged and less steep, though still rises rapidly as $\sim r^{-12}$.

The radial probability densities of the structural monomers and the depletion-induced pair potential $\mathcal{U}$ for these two WCA models are now to be determined. The combinatorial-WCA model allows more overlap and so the net well depth is significantly deeper than the depth of the shifted-WCA version. This has an impact on the effective second virial coefficient of the structural monomers in a bath of depletants. It is important to keep in mind that both of these WCA potentials are “hard” in that they are both short-ranged, rapidly rising repulsive potentials.

In the following simulations, two structural monomers each with a radius of $r_{\text{SM}} = 2.5\sigma$ are considered. The depletants each have a radius of $r_{\text{dep}} = 0.5\sigma$ and the simulation volume is $(25\sigma)^3$, with periodic boundary conditions. The depletant volume fractions go from $\phi_{\text{dep}} = 0$ to 0.35. This requires that the number of depletants in the simulations be increased from

$N_{\text{dep}} = 0$ to 5500.

In order to measure the radial probability densities $p(\phi_{\text{dep}}, \rho)$, the two structural monomers are bound together with a deep, flat bond that is zero unless the separation exceeds $\rho > 7.25$, after which Equation (3.46) provides the bonding potential. Without such an artificial bond, the probability that two monomers happen to diffusively have a sufficiently small separation within a single simulation can be quite low and statistically significant simulation data cannot be calculated in computationally accessible times.

A different procedure is used to measure the entropic pair potential between two structural monomers. The total could, in principle, have been calculated from the probability distributions via the Boltzmann factor. However, this method was found to be far more computationally costly, especially in regimes of low probability. Therefore, the statistical force on each structural monomer held at a fixed separation is measured. Integrating over the entire range of simulated separations produces the $W(\phi_{\text{dep}}, \rho)$, total pair potential between monomers. The total pair potential W between the two structural monomers is simply the sum of the “molecular” WCA potential U and the entropic component $\mathcal{U}(\phi_{\text{dep}}, \rho)$. When the known WCA potential (Equation (3.45)) is subtracted off only the depletion-induced component remains.

Depletant-Induced Pair Potential: Shifted-WCA

In the absence of depletants, the radial probability density p is homogeneous within the bounds of the artificial bond used for determining the radial probability density (Figure 8.1.3). The probability drops rapidly when the structural monomers are in contact (at $\rho \leq 2r_{\text{SM}} = 5\sigma$) and at the edge of the bond ($\rho \geq 7.25\sigma$). As the volume fraction of depletants increases from zero, it becomes far more likely that the structural monomers are near ($\rho \approx 2r_{\text{SM}}$). By $\phi_{\text{dep}} \gtrsim 0.2$, the likelihood that the two monomers are near is many times greater than the chances that they are separated by a large distance. The short-ranged entropic attractions cause the two large monomers to adhere. In Figure 8.1.3, the monomers are more than $\times 11$ more likely to be in contact when $\phi_{\text{dep}} \approx 0.35$ than when $\phi_{\text{dep}} \approx 0$. The attraction due to the depletants is primarily short-ranged since the radial probability distributions in Figure 8.1.3 decrease rapidly with separation distance. The monomers are most likely to be found within $2r_{\text{SM}} \leq \rho \leq 2\mathcal{R}$ of each other for all ϕ_{dep} . This high probability suggests quite a deep, attractive well develops at large ϕ_{dep} .

Figure 8.1.3 also demonstrates that at high volume fractions of depletants, longer-range correlations other than the primary adhesion exist, which can become significant such that

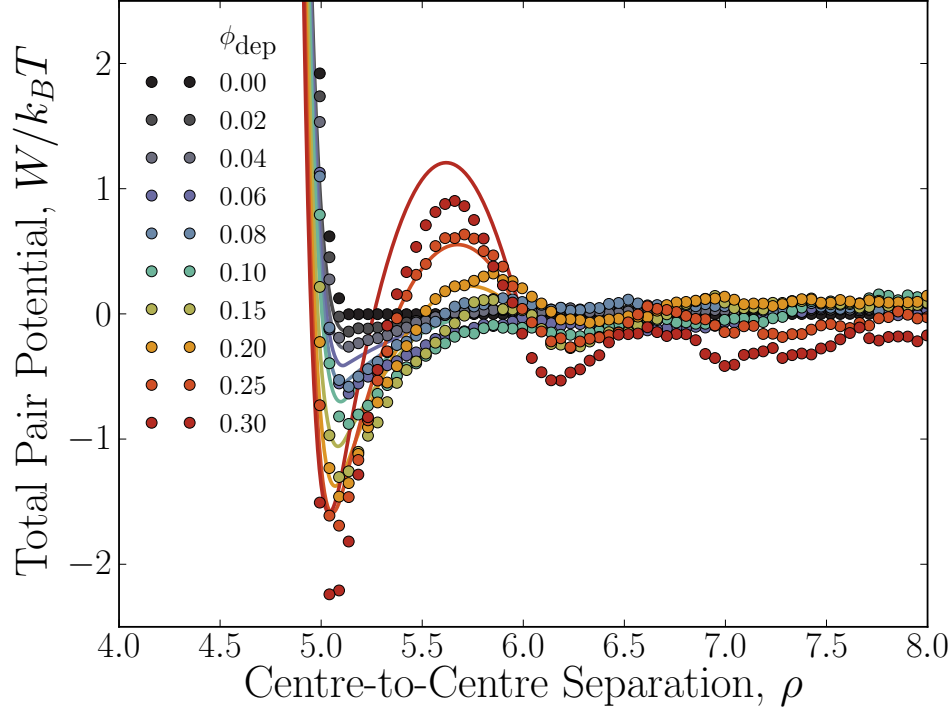


Figure 8.1.4: Simulated total pair potentials W_{sWCA} from force measurements of shifted-WCA monomers of size $r_{\text{SM}} = 2.5\sigma$ in baths of various volume fractions of shifted-WCA depletants of size $r_{\text{dep}} = 0.5\sigma$ (●). Solid lines are the sum of the attraction predicted by the MT model and the shifted-WCA repulsion.

interactions between structural monomers extend further than the $2\mathcal{R}$ range of Equation (8.4) for the AO model.

The total pair potential $W_{\text{sWCA}}(\rho)$, as measured by force integration, is shown in Figure 8.1.4. The strong, short-ranged, shifted-WCA repulsion U_{sWCA} is clearly visible at small separations. Subtracting U_{sWCA} off leaves only the depletion-induced component $\mathcal{U}_{\text{sWCA}}$ (Figure 8.1.5).

At low volume fractions, the simulated pair potentials are well approximated by both the AO and the MT models, but as $\phi_{\text{dep}} \gtrsim 0.1$ the AO model begins to deviate from the measured curves (Figure 8.1.5; inset). On the other hand, the MT model continues to capture the behaviour of the primary attractive well and repulsive barrier (the region within approximately two depletants from the fixed monomer: $2r_{\text{SM}} \leq \rho \lesssim 2r_{\text{SM}} + 4r_{\text{dep}}$) for depletant densities up to $\phi_{\text{dep}} \approx 0.3$. Only at the highest volume fractions does the MT model deviate from the simulation values. At $\phi_{\text{dep}} = 0.3$ the MT model appears to over-predict the shifted-WCA simulation results at both the minimum and the anti-correlation

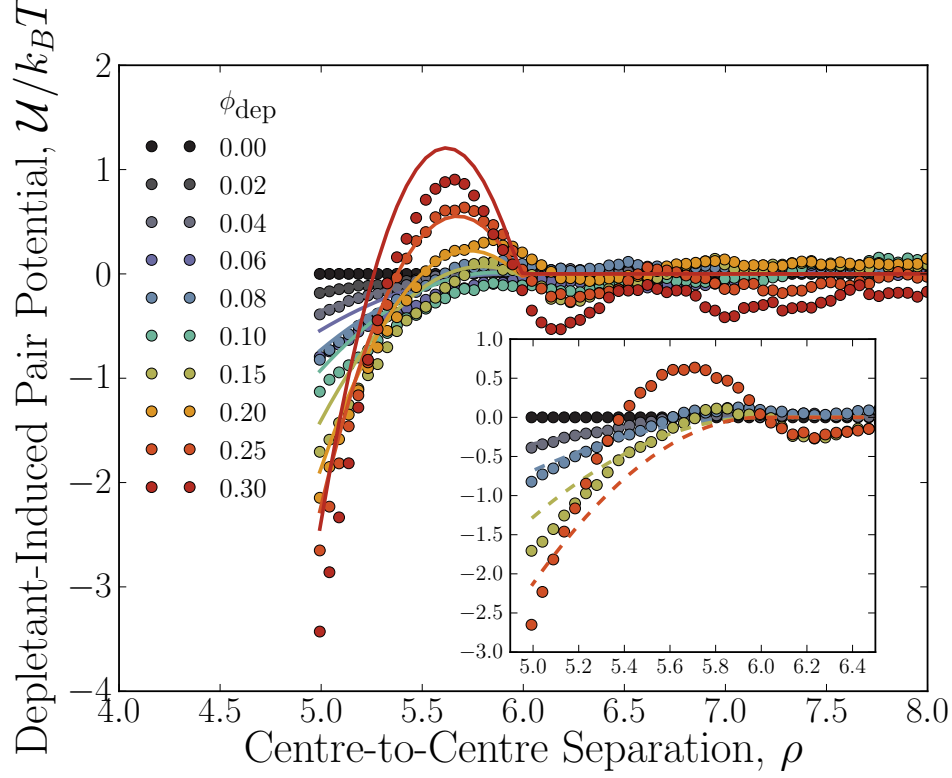


Figure 8.1.5: Simulated depletion-induced pair potentials $\mathcal{U}_{\text{sWCA}}$ as a function of centre-to-centre separation for $r_{\text{SM}}/r_{\text{dep}} = 5$ ($\bullet$). Values obtained from force measurements of shifted-WCA monomers of size $r_{\text{SM}} = 2.5$ and $r_{\text{dep}} = 0.5$. Solid lines show the induced pair potentials predicted by the MT model, while dashed lines in the inset show the AO model.

peak. Of course, the MT model does not capture the secondary features in Figure 8.1.5 because current functionals are not applicable beyond $2\mathcal{R}$ [376].

Although the AO model does not well represent the pair potential as a function of separation, Figure 8.1.5 shows that it does estimate the contact energy well, *i.e.* $W_{\text{sWCA}}^{\min} \approx \mathcal{U}_{\text{AO}}^{\min}$. This is seen quantitatively in Figure 8.1.6. In fact, Figure 8.1.6 shows that the linear AO minimum (solid black line) agrees with the minimum of the MT model (solid blue line) until $\phi_{\text{dep}} \gtrsim 0.3$ and that both are in fair agreement with the simulated $W_{\text{sWCA}}^{\min}$ (green circles), though both predictions are systematically too great. They overpredict the magnitude of the well depth because the simulations include the finitely-ranged sWCA potential as well. In order to better predict the minimum (and indeed the entire total pair potential), the total theoretical pair potential $W_{\text{sWCA}} = U_{\text{sWCA}} + \mathcal{U}_{\text{MT}}$ is calculated (where $\mathcal{U}_{\text{MT}}$ is given by Equation (8.9)). The resulting predictions for the pair potentials are shown in Figure 8.1.4 as solid lines and their minima are plotted as the solid yellow curve in Figure 8.1.6.

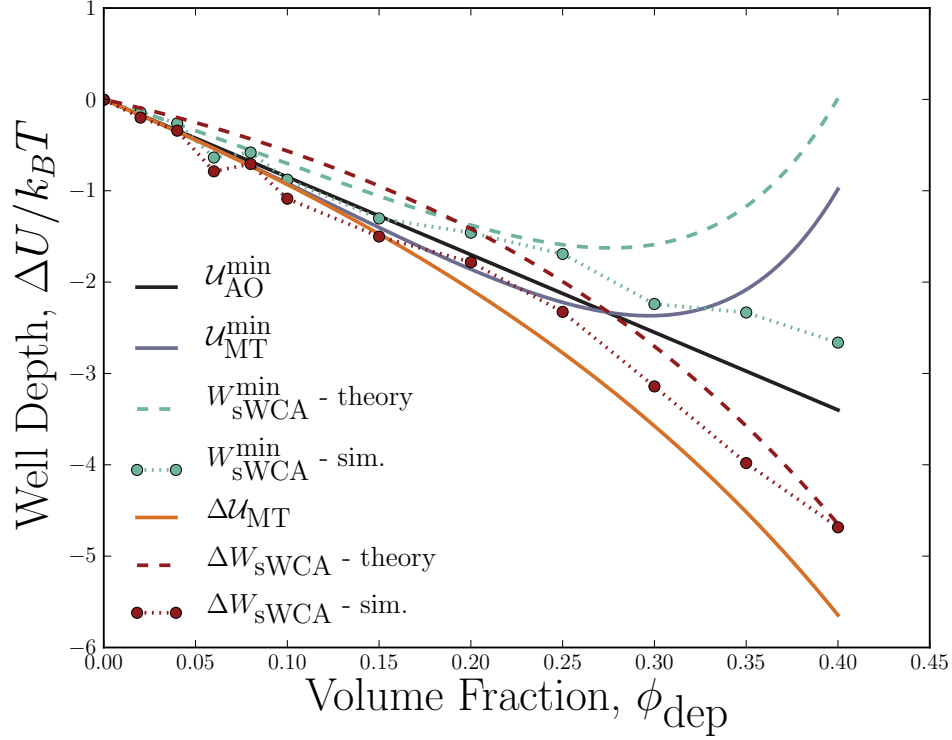


Figure 8.1.6: The minimum value of the pair potential as a function of depletant volume fraction for shifted-WCA structural monomers of size $r_{SM} = 2.5\sigma$ and depletants of size $r_{dep} = 0.5\sigma$. The minimum from simulations (W_{sWCA}^{min} ; green circles) is compared to the AO model (u_{AO}^{min} ; black solid line) and MT model (u_{MT}^{min} ; blue solid line). The green dashed line shows theoretical minima of the MT model plus the shifted-WCA potential (W_{sWCA}^{min}). The corresponding effective well depths (minimum minus maximum) from the simulations and the models are shown as well. The theoretical effective well depth for the MT model (Δu_{MT} ; yellow solid line) is deeper than either the simulations (ΔW_{sWCA} ; red circles) or theoretical sum of the MT model plus the shifted-WCA potential (ΔW_{sWCA} ; red dashed line).

At larger volume fractions, the MT model begins to curve upward, predicting less deep minima, while the AO model continues to decrease linearly. Since the MT model increases, so too does theoretical total potential W_{sWCA} . However, the minima of the simulations do not curve upward but rather continue along their roughly linear decrease. Thus, they qualitatively behave more like the AO model than the MT model despite the fact that the volume fraction is not small.

The AO model, however, does not produce the repulsive peak and so cannot predict the effective well depth that is defined as the difference between the minimum of the contact attraction and the maximum of the anti-correlation barrier. Only the MT model (solid yellow line) and the total potential (dashed red line) can make predictions. The MT model of the effective depth begins similar to the minimum at low volume fractions since the repulsion is

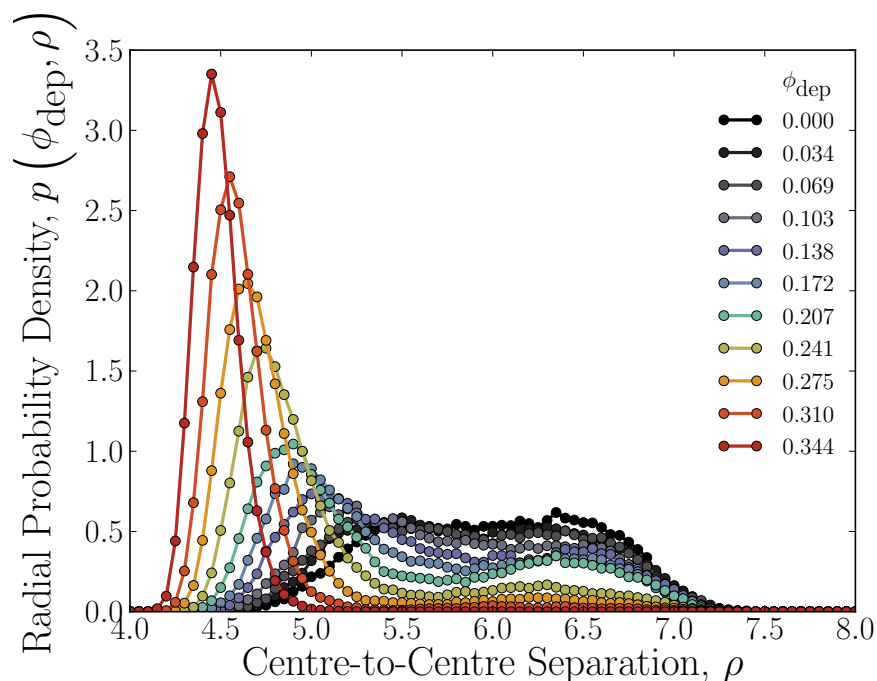


Figure 8.1.7: Radial probability density p of finding two free combinatorial-WCA structural monomers at a given separation. The structural monomers are of size $r_{\text{SM}} = 2.5\sigma$ in baths of various volume fractions of combinatorial-WCA depletants of size $r_{\text{dep}} = 0.5\sigma$. There is a significant probability of overlap, which results in a deep attractive well with a minimum within $r_{\text{min}} < 2r_{\text{SM}}$.

insignificant at small ϕ_{dep} but it rapidly gains curves downward. This qualitatively matches the well depths measured from the shift-WCA simulations. However, the simulations include the repulsive component and so are shifted upward. The total potential (dashed red line) captures this quality.

The well depth competes with thermal energy in determining if structural monomers prefer to agglomerate in clusters or explore the entire available volume. Qualitatively speaking, since the well depth $W_{\text{sWCA}}^{\text{min}}$ in Figure 8.1.6 is only a few $k_{\text{B}}T$ even as $\phi_{\text{dep}} \rightarrow 0.35$, it is expected that extremely high number densities of depletants are required to overcome thermal jostling. This already suggests that if structural monomers are modelled as shifted-WCA beads, depletion forces may not be strong enough to collapse chromosomal DNA from a swollen state except at extremely high densities, as is the subject of § 8.2.

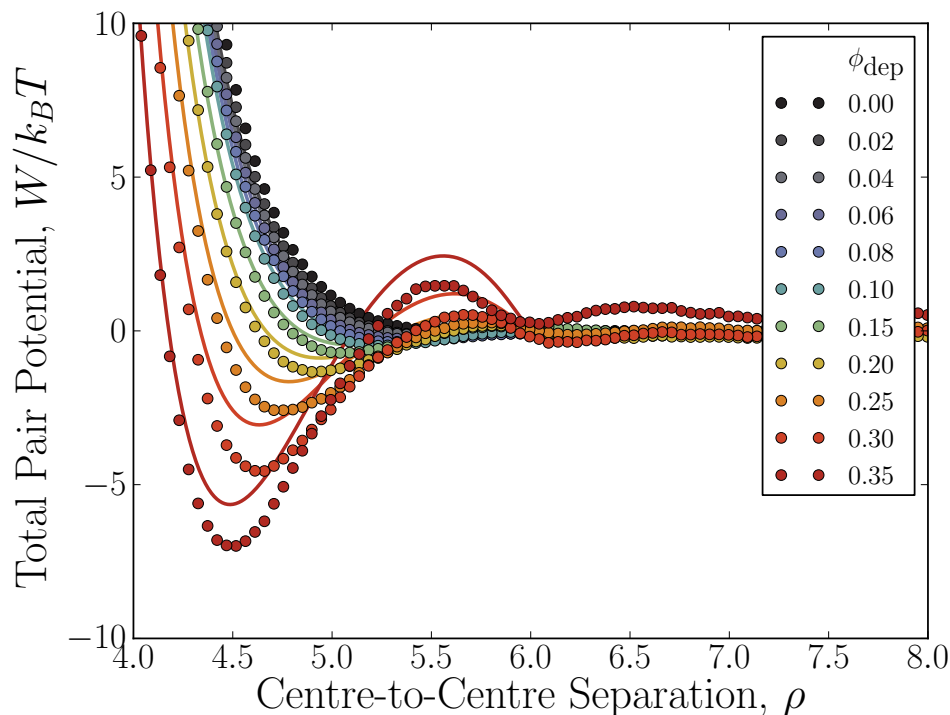


Figure 8.1.8: Simulated total pair potentials W_{cWCA} from force measurements of combinatorial-WCA monomers of size $r_{\text{SM}} = 2.5\sigma$ in baths of various volume fractions of shifted-WCA depletants of size $r_{\text{dep}} = 0.5\sigma$ ($\bullet$). Solid lines are the sum of the attraction predicted by the MT model and the combinatorial-WCA repulsion.

Depletant-Induced Pair Potential: Combinatorial-WCA

Since the attractive portion of the depletion potential is governed by the amount of overlap volume that is achieved, stronger depletion forces are realized by modelling the structural monomers as the slightly softer combinatorial-WCA beads. This is demonstrated in Figure 8.1.7, which shows the radial probability density of finding a given separation distance between the two structural monomers. While the shifted-WCA probability densities rose rapidly as the monomers approached contact at $\rho = 2r_{\text{SM}}$ for all ϕ_{dep} (Figure 8.1.3), the combinatorial-WCA radial probabilities do rise but the maximum is also shifted to smaller separation distances when ϕ_{dep} increases. In fact, at high densities it is most likely that the monomers are separated by a distance significantly less than $\rho = 2r_{\text{SM}}$ (Figure 8.1.7). This overlap amounts to a greater increase in volume for the depletants than was possible for the shifted-WCA model. As will be seen in a moment, the primary well becomes deeper and the secondary correlations/anti-correlations less pronounced than the shifted-WCA equivalents in Figure 8.1.5.

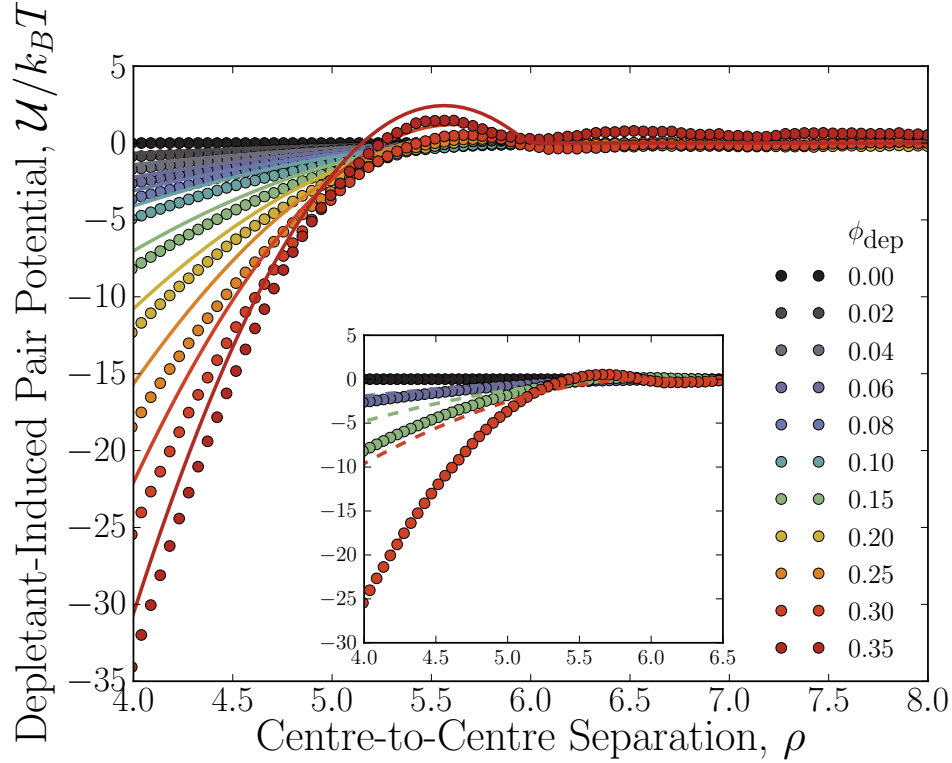


Figure 8.1.9: Simulated depletion-induced pair potentials $\mathcal{U}_{\text{cWCA}}$ as a function of centre-to-centre separation for $r_{\text{SM}}/r_{\text{dep}} = 5$ (●). Values obtained from force measurements of combinatorial-WCA monomers of size $r_{\text{SM}} = 2.5\sigma$ and $r_{\text{dep}} = 0.5\sigma$. Solid lines show the induced pair potentials predicted by the MT model, while dashed lines in the inset show the AO model. The AO model fails to accurately predict the entropic attraction when $\rho < 2r_{\text{SM}}$.

The depletion-induced pair potential is measured in the same manner as in § 8.1.2; however, separations smaller than $2r_{\text{SM}}$ are investigated because they have a non-zero probability and indeed are likely (Figure 8.1.7). The competition between U_{cWCA} and the entropic pair potential $\mathcal{U}_{\text{cWCA}}$ results in a minimum in the total interaction W_{cWCA} for all volume fractions. A comparison between Figure 8.1.4 and Figure 8.1.8 shows that the well is much deeper in the combinatorial-WCA model than the shifted-WCA. This is because of the additional overlap that can occur. Since the U_{cWCA} repulsion rises less dramatically, the combinatorial-WCA wells are broader, extending to smaller separations. For high volume fractions, the combinatorial-WCA repulsion does not dominate W_{cWCA} until much smaller separations than in the shifted-WCA model.

Once again, the WCA potential can be subtracted from W in order to produce the depletion-induced pair potential $\mathcal{U}_{\text{cWCA}}$ (Figure 8.1.9). The depletion-induced pair potential continues into separations $\rho < 2r_{\text{SM}}$, which differs from the strict definitions of the AO

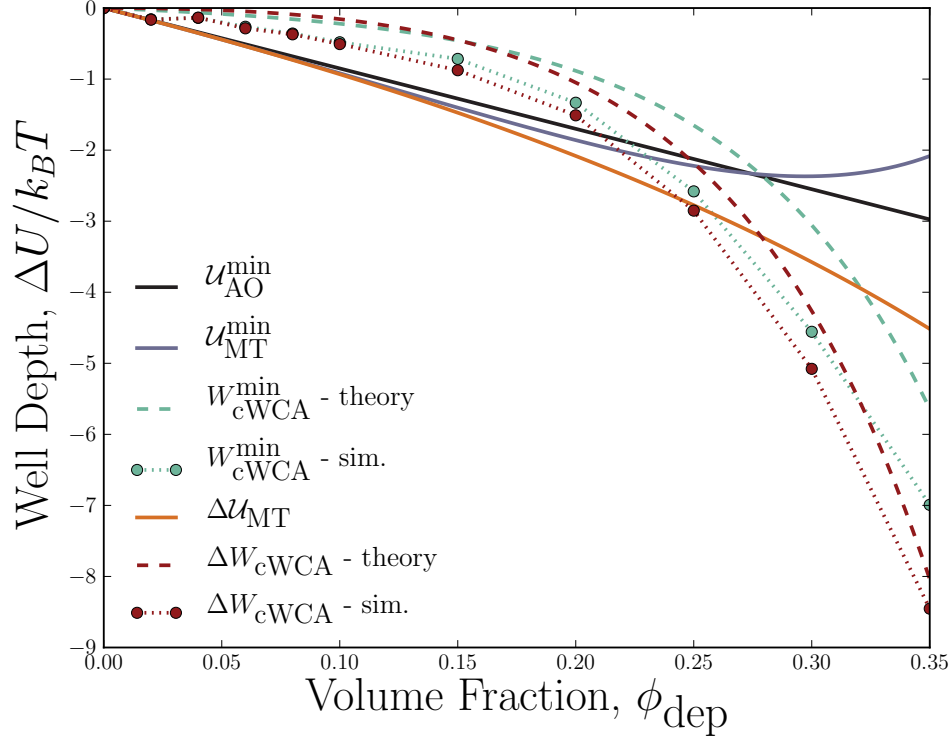


Figure 8.1.10: The same as Figure 8.1.6 but for the combinatorial-WCA simulation model. Unlike the shifted-WCA case (Figure 8.1.6), neither the minimum of the AO nor MT models well approximates the value from simulations. The theoretical effective well depth from the MT model (Δu_{MT} ; yellow solid line) is deeper than the simulations (ΔW_{cWCA} ; red circles) and theoretical sum of the MT model plus the combinatorial-WCA potential (ΔW_{cWCA} ; red dashed line) at small volume fractions of depletants, while it is more shallow at high volume fractions. Notably, the simulations (red circles) and theory (red dashed line) for ΔW_{cWCA} are significantly deeper in the combinatorial-WCA model than in the shifted-WCA model (Figure 8.1.6).

and MT models. By construction, AO and MT models have infinitely hard cores at $2r_{\text{SM}}$. However, this hard core is added artificially in Equation (8.4) and can simply be removed. The AO potential crosses the simulated U_{cWCA} potential quite near $\rho \approx 2r_{\text{SM}}$, but when significant overlap occurs the AO potential U_{AO} cannot reproduce the measured depletion-induced pair potential as Figure 8.1.9 (inset) shows. On the other hand, allowing the MT pair potential U_{MT} to extend to smaller separations does a remarkable job of reproducing the depletant-mediated component of the pair potential. For this reason, summing U_{MT} (but not U_{AO}) and U_{cWCA} once again reproduces the total interaction potentials in Figure 8.1.8.

Because the potentials are much softer, the resulting minima are not expected to follow the ideal models and indeed they do not (Figure 8.1.10). However, since summing the MT model and the cWCA potential reproduces the pair potentials so well, it also predicts the

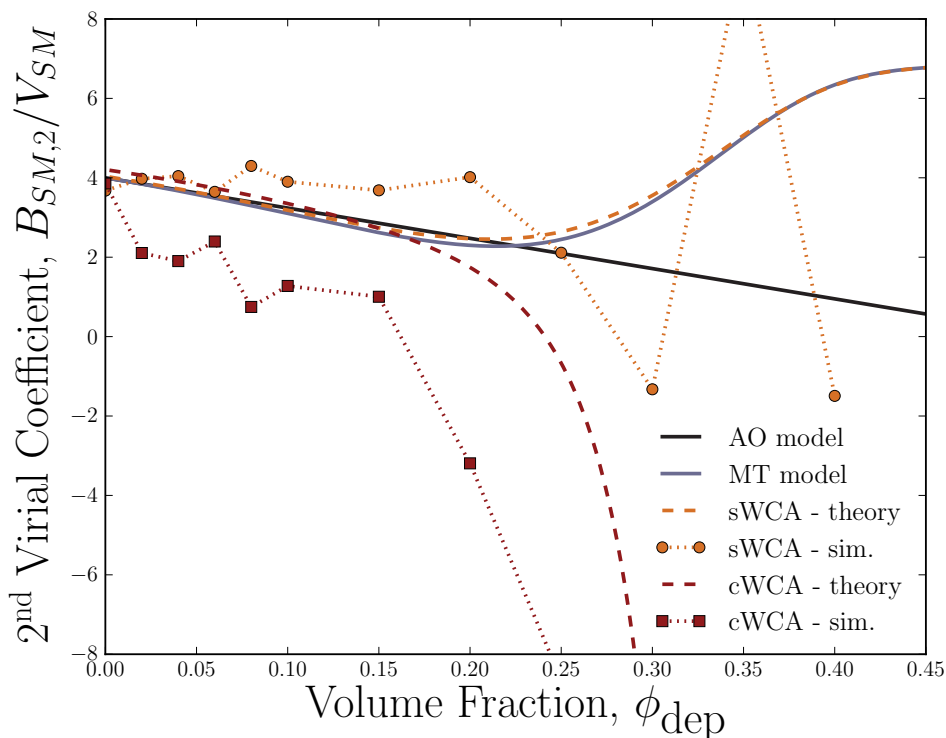


Figure 8.1.11: Second virial coefficient for shifted-WCA monomers with $r_{SM}/r_{dep} = 5$ as a function of ϕ_{dep} .

behaviour of the minima and the well depths as functions of ϕ_{dep} quite well. As was also the case with the shifted-WCA model, the predictions systematically over-predict the simulated values.

8.1.3 Virial Coefficients: Implicit Depletants

Until this stage, only pairs of structural monomers in a bath of depletant particles have been considered. In preparation for discussing long chains of structural monomers in § 8.2, it is important to consider an entire ensemble of particles with attractive potentials due to depletion-induced interactions. The effective excluded volume and three-body interaction term of the monomers is required. Effectively, an equation of state for a dilute solution of hard structural monomers in a bath of depletants must be found. Consider only the structural monomers and include the depletants implicitly as the depletant-mediated pair potentials described in § 8.1.2.

The *second virial coefficient* for a dilute solution of hard structural monomers interacting

pair-wise is

$$B_{\text{SM},2} = -2\pi \int_0^\infty \left(e^{-u(\phi_{\text{dep}},\rho)/k_{\text{B}}T} - 1 \right) \rho^2 d\rho. \quad (8.14)$$

The solution is ideally a hard sphere gas with a correction due to short-ranged, entropic interactions such that the virial coefficient is the hard sphere value less a correction due to a likelihood to “stick”:

$$B_{\text{SM},2} \approx 4V_{\text{SM}} \left[1 - \frac{\Delta B_{\text{SM},2}}{V_{\text{SM}}} \right]. \quad (8.15)$$

It is a perturbation away from the hard-sphere value that is sought here. The second virial coefficient can be numerically calculated using Equation (8.14) for the simulated total pair potentials of both WCA models. Consider first the harder, shifted-WCA simulations.

The computed $B_{\text{SM},2}$ linearly decrease from a value that is extremely close to the hard-sphere value of $B_{\text{SM},2} = 4V_{\text{SM}}$ (Figure 8.1.11; yellow circles). The simulated shifted-WCA second virial coefficient is roughly linear with volume fraction of depletants until approximately $\phi \approx 0.25$, after which point the data become too noisy to make any conclusions. The combinatorial-WCA model has a much deeper well and the second virial coefficient is far less than the shifted-WCA curve (Figure 8.1.11; red squares). The combinatorial-WCA $B_{\text{SM},2}$ falls quite rapidly and at high volume fractions, the second virial coefficient drops to significantly more negative values than shown in Figure 8.1.11. Can the second virial coefficient as a function of volume fraction of depletants be theoretically predicted?

AO Solution: $\phi_{\text{dep}} \ll 1$

Assuming that the structural monomers are hard and $\phi_{\text{dep}} \ll 1$ simplifies matters immensely. This is the AO model and the depletion-induced attractions appear as a correction on the hard-sphere result, specifically taking the form

$$\frac{B_{\text{SM},2}}{4V_{\text{SM}}} \approx 1 - \phi_{\text{dep}} g, \quad (8.16)$$

where $g = g(r_{\text{dep}}/r_{\text{SM}})$ is a third order polynomial given by

$$g\left(\frac{r_{\text{dep}}}{r_{\text{SM}}}\right) \equiv \frac{3}{2} + \frac{15}{8} \left(\frac{r_{\text{dep}}}{r_{\text{SM}}}\right) + \frac{3}{4} \left(\frac{r_{\text{dep}}}{r_{\text{SM}}}\right)^2 + \frac{1}{8} \left(\frac{r_{\text{dep}}}{r_{\text{SM}}}\right)^3. \quad (8.17)$$

The AO approximation of the second virial coefficient (Equation (8.16)) shows that in this limit, the second virial coefficient is reduced as the volume fraction of depletants is increased, reflecting the deepening of the implicit depletion attraction. As is expected for the AO model, the correction from the hard-sphere value is linear in ϕ_{dep} . The linear AO prediction compares well to both the shifted- and the combinatorial-WCA simulations for $r_{\text{SM}}/r_{\text{dep}} = 5$ when the volume fraction of depletants is low (Figure 8.1.11). For $\phi_{\text{dep}} \lesssim 0.15$, the approximation predicts the second virial coefficient of the shifted-WCA simulations rather well.

This AO solution can be described as an *implicit-depletant van der Waals gas*. A solution of structural monomers with a number density n_{SM} in which the depletants are only accounted for implicitly through the second virial coefficient possesses an equation of state that is quite similar to a standard van der Waals gas. Unlike a standard van der Waals gas in which only the first term has $k_{\text{B}}T$ dependence, in an AO solution the pressure scales with $k_{\text{B}}T$, reflecting the entropic basis of the attractive forces. Otherwise, there is a 1 : 1 correspondence:

$$P \approx k_{\text{B}}T n_{\text{SM}} [1 + n_{\text{SM}} B_{\text{SM},2}] = k_{\text{B}}T \left[\frac{1}{(n_{\text{SM}}^{-1} - b)} - a n_{\text{SM}}^2 \right] \quad (8.18a)$$

$$a \equiv 4V_{\text{SM}}\phi_{\text{dep}}g \quad (8.18b)$$

$$b \equiv 4V_{\text{SM}}. \quad (8.18c)$$

Since constructing this equation of state demanded that $|\mathcal{U}_{\text{AO}}^{\text{min}}| \ll k_{\text{B}}T$, this model will not predict a phase transition from dispersed structural monomers to an agglomeration. Indeed, this can be verified by finding the critical point (the inflection point on an $P - \phi_{\text{dep}}$ phase diagram). Doing so requires that the volume fraction at which agglomeration occurs is unphysically high.

MT Solution

Since the MT model presented in § 8.1.1 can describe the depletion-induced pair potentials to higher depletant densities than the AO model, inserting Equation (8.9) into Equation (8.14) and numerically calculating the second virial coefficient is expected to be more accurate to higher depletant volume fractions. However, the resulting function of depletant volume fraction using the MT model for $r_{\text{SM}}/r_{\text{dep}} = 5$ (Figure 8.1.11; solid light blue line) is no better at predicting the simulation curves than the AO model.

At low depletant densities, the numerical value for the MT model of $B_{\text{SM},2}$ agrees with the simulation values but, surprisingly, the second virial coefficient calculated numerically from the MT model is not particularly more accurate than the AO model for $\phi_{\text{dep}} \lesssim 0.2$. At intermediate values ($\phi_{\text{dep}} \approx 0.25$), the simulations begin to decrease more quickly while the numerical MT value erroneously begins to rise to high values of $B_{\text{SM},2}$. In fact, the numerical solution becomes greater than even the hard sphere gas value of $4V_{\text{SM}}$ and never decreases below zero, suggesting that the MT model fails above $\phi_{\text{dep}} \gtrsim 0.25$. This behaviour of the MT model has been discussed in detail for various relative sizes and choices of functionals elsewhere [377]. Although it generates more accurate pair potentials, the MT model does not provide more accurate second virial coefficients than the AO model.

MT plus WCA Solution

While discussing the simulation models, it is shown that $\mathcal{U}_{\text{MT}} + U = W$ (using Equation (8.9) and Equation (3.45)) does an admirable job predicting the total pair potentials measured from both types of simulations *i.e.* W_{sWCA} and W_{cWCA} . These theoretical pair potentials can be integrated to predict the second virial coefficient as a function of volume fraction of depletants. Since the theoretical W_{sWCA} incorporates the MT model and the steep shifted-WCA U_{sWCA} potentials, the predicted $B_{\text{SM},2}$ is nearly identical to the ideal MT model and even rises unphysically at large ϕ_{dep} values (Figure 8.1.11; dashed yellow line).

The theoretical second virial coefficient predicted for the combinatorial-WCA and the MT model decreases nearly parallel to the $B_{\text{SM},2}$ of the AO model at low volume fractions of depletants. However, even at $\phi_{\text{dep}} = 0$ (in which no depletants are present and the only interaction is through the WCA potential U_{cWCA}) the second virial coefficient is larger than the AO and MT models. The slight difference is because the combinatorial-WCA model is not perfectly hard and therefore even in the absence of depletants has slightly different $B_{\text{SM},2}$. Notice that for combinatorial-WCA the second virial coefficient at $\phi_{\text{dep}} = 0$ is $B_{\text{SM},2}(0) = 4.20 V_{\text{SM}}$, which suggests that it has an effective statistical value that is 5% larger than V_{SM} .

At large volume fractions ($\phi_{\text{dep}} \gtrsim 0.15$) the predicted $B_{\text{SM},2}$ of the MT and combinatorial-WCA theory drops below the ideal AO model and begins to plunge to very negative numbers. It becomes negative at $\phi_{\text{dep}} = 0.245$. The behaviour is mirrored in the combinatorial-WCA simulation values for $B_{\text{SM},2}$.

Third Virial Coefficient

Just as with $B_{\text{SM},2}$, a first approximation to the *third virial coefficient* is the value predicted for a hard sphere gas,

$$B_{\text{SM},3} \approx 10V_{\text{SM}}^2. \quad (8.19)$$

However, estimating the third virial coefficients by perturbations to Equation (8.19) based on the pair potentials from § 8.1.2 does not improve $B_{\text{SM},3}$. This is because the interactions are **not additive**. Consider a collision between three structural monomers: Two of the structural monomers create a lens-shaped excluded volume V_{12} (Equation (8.3)). The third structural monomer forms a lens-shaped excluded volume with each of the other two; however, a sizable portion of these volumes overlap with V_{12} . Pair-wise interactions would triple the entropic contribution of this overlap volume. The problem is most exasperated when the depletants are relatively large (here $r_{\text{SM}}/r_{\text{dep}} = 5$). This over estimation becomes more significant for higher virial coefficients. It suffices for the purposes of § 8.2 to simply conclude that the hard-sphere value (Equation (8.19)) adequately estimates the third virial coefficient.

Having developed a coarse-grained model that explicitly includes depletant particles for simulating depletion-induced interactions, it is now possible to study entropically driven collapse of bacterial chromosomes.

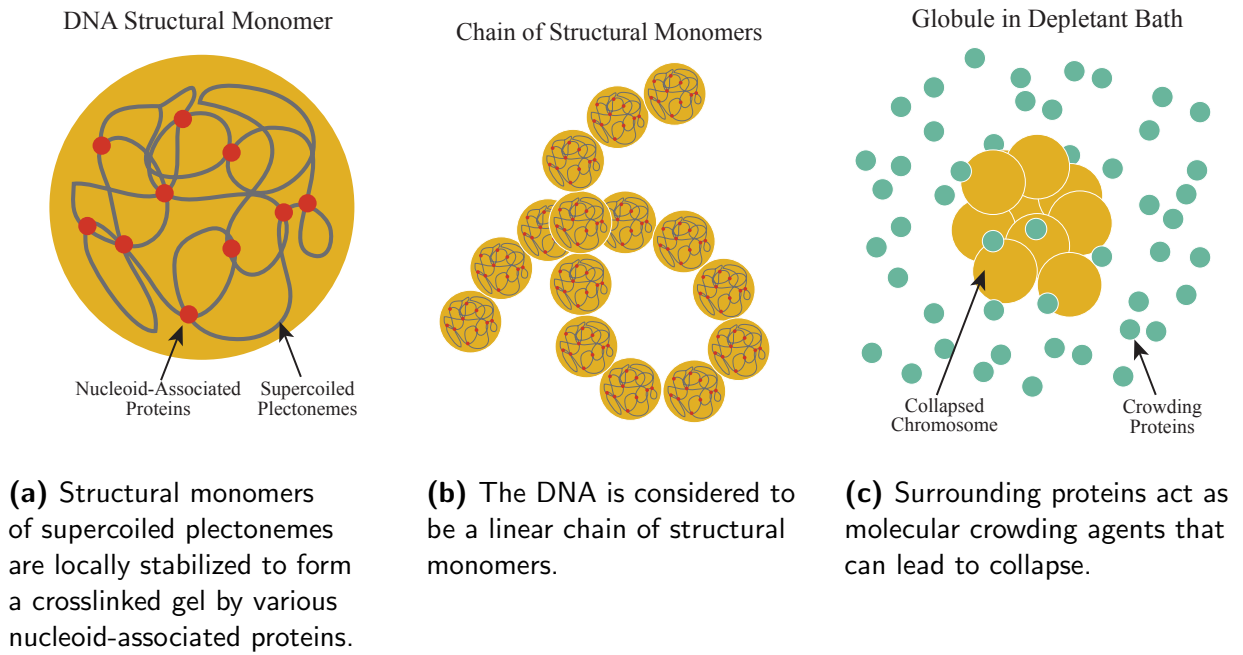


Figure 8.2.1: The coarse-grained model of bacterial DNA after Jun and Wright [353].

8.2 Depletants/Chromosome System

Computational WCA simulations of non-specific depletion forces are carefully characterized in § 8.1. The question now becomes whether or not non-specific depletion forces are sufficient to cause the collapse of models of simplistic bacterial chromosomal DNA. In this section, a simplified computational model treats both the supercoiled DNA structural monomers and the smaller protein crowding agents as combinatorial-WCA spheres. By presenting a simple theoretical model, the action of depletants on supercoiled bacterial DNA will be quantitatively cast as an effective solvent quality. The model predicts that the radius of gyration of the model chromosome falls with increased molecular crowding through a good solvent regime until it passes a Θ -point into poor solvent behaviour and finally collapses into a dense globular state.

8.2.1 Coarse-grained Model of Simple Chromosome Chains

A coarse-grained model of bacterial chromosomes imparts simplicity but also allows the conclusions of this section to be generally applicable to the action of depletants on large biomolecules. Here, each chromosome is viewed as a linear chain of DNA structural monomers

(SMs). Structural monomers are also called “structural units” [353, 382] and “compacted domains” [352] in the literature. Each structural monomer is a distinct topological domain [383] of supercoiled plectonemes which is stabilized by crosslinking by nucleoid-associated proteins (Figure 8.2.1(a)) [353, 384, 385]. It is likely that many different proteins (so called structural-maintenance-of-chromosomes proteins [382, 384, 386, 387]) crosslink the structural monomers. Prokaryotic organisms lack histones and much of the other machinery used by eukaryotes to organize their chromosomes. The chromosome separates into a dense protein-poor nucleoid of structural monomers and an exterior of protein-rich cytoplasm [388, 389]. Each structural monomer is thus modelled as a hard spherical monomer of radius r_{SM} (volume V_{SM}) that sterically excludes all non-nucleoid-associated proteins from entering its interior.

Both the structural monomers and the cytoplasmic protein depletants are modelled as inert, hard spherical particles. As is described in § 8.1, these coarse-grained simulations consist of representing DNA structural monomers and many smaller depletants as hard spheres diffusing within an implicit Langevin solvent.

In particular, in this section, the combinatorial-WCA form is utilized for a system of structural monomers of sizes $r_{\text{SM}} = \{1.5, 2, 2.5\} \sigma$ and depletants of size $r_{\text{dep}} = 0.5\sigma$. When the system of structural monomers and depletants interact via the combinatorial-WCA model, the depletion-induced pair potential between structural monomers is deeper than for infinitely hard spheres or the shifted-WCA model (Figure 8.1.6 and Figure 8.1.10). This is because a slight soft-core overlap occurs, which increases the excluded volume that the depletants cannot occupy, which (in turn) increases the osmotic pressure and deepens the attractive well depth. The results of § 8.1 provide both a coarse-grained simulation method and a corresponding theoretical model to study the behaviour of model chromosomes in a bath of depletant proteins. The following simulations will be used to test the hypothesis that depletant-mediated attraction can be sufficient to cause the collapse of bacterial chromosomes from a swollen state to a globular state. The number of monomers used in this study is $N_{\text{SM}} = 15$, which corresponds to the lower bounds of estimates of N_{SM} from experimental measurements of $N_{\text{SM}} \approx 16$ [382]. Since a typical bacteria chromosome consists of ~ 4.6 million base pairs (*E. coli*), each structural monomer contains ~ 300 kbp. The physical-size of structural monomers has been estimated as low as 80 nm [390] (or 87 nm [353] or 158 nm [361, 391]) and as high as 440 nm [382].

In the simulations, structural monomers are polymerized into a chain via FENE springs as described in § 3.5.2. The size ratios of $r_{\text{SM}}/r_{\text{dep}} = \{3, 4, 5\}$ over-approximate the size of the depletants compared to a more realistic $r_{\text{SM}}/r_{\text{dep}} \approx 20 - 100$ for typical proteins. In order

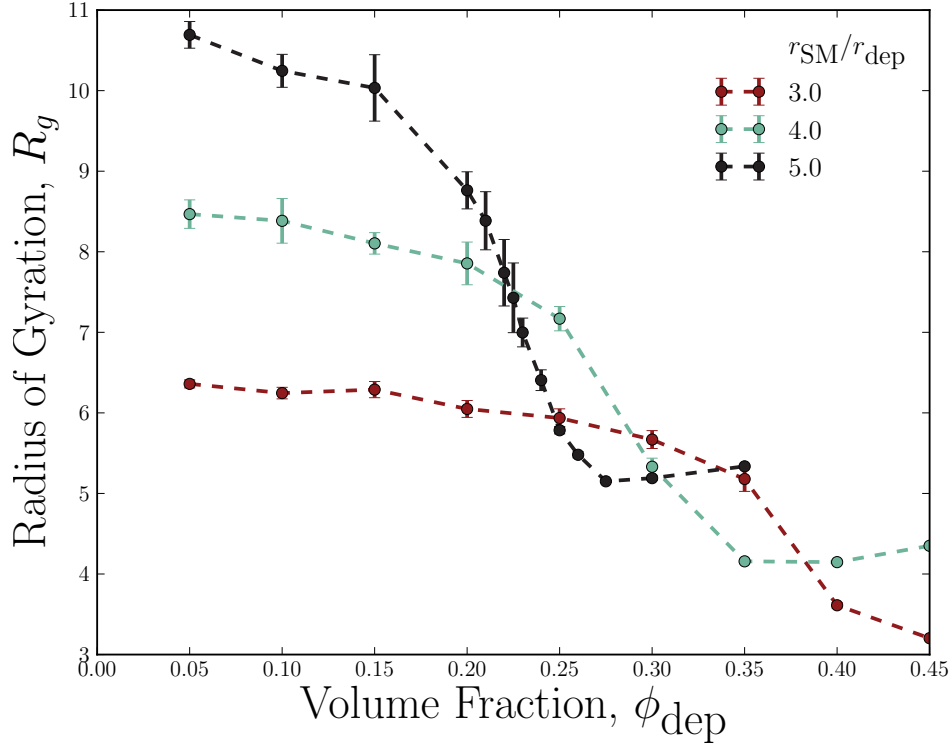


Figure 8.2.2: The radii of gyration of $N_{\text{SM}} = 15$ chains of combinatorial-WCA structural monomers as a function of depletant volume fraction, ϕ_{dep} . The observed transition to a collapsed state varies as a function of the size ratio $r_{\text{SM}}/r_{\text{dep}}$.

to minimize finite size effects, periodic boundary conditions were implemented on control volumes of $V_{\text{sys}} = \{30^3, 40^3, 60^3\} \sigma^3$ for $r_{\text{SM}} = \{1.5, 2, 2.5\} \sigma$, respectively. This required N_{dep} as large as 294801 in order to achieve a maximum volume fraction of $\phi_{\text{dep}} = 0.45$.

8.2.2 Polymer Collapse: Combinatorial-WCA Simulations

For model chromosome chains of $r_{\text{SM}} = \{1.5, 2, 2.5\} \sigma$ and in the absence of depletant proteins, excluded volume interactions between structural monomers swell the DNA to a radius of gyration greater than the ideal value of $R_g \approx r_{\text{SM}} N_{\text{SM}}^{1/2} / \sqrt{6}$. Qualitatively, the radius of gyration R_g is expected to decrease as the volume fraction of depletants ϕ_{dep} is increased because the well is deepened. Indeed, Figure 8.2.2 demonstrates that as the number of depletants (of radius $r_{\text{dep}} = 0.5\sigma$) is increased, the simulated radius of gyration decreases. In fact, the radius of gyration collapses from a swollen state to a globular state when combinatorial-WCA simulations are performed (Figure 8.2.2).

Since $r_{\text{SM}}/r_{\text{dep}} = 5$ is the largest ratio of sizes considered for the combinatorial-WCA model, its radius of gyration falls from a swollen to a collapsed state at the lowest volume fraction, as would be qualitatively predicted from the AO-model pair potential (Equation (8.4) from § 8.1.1). At the smaller ratio of $r_{\text{SM}}/r_{\text{dep}} = 4$ the drop occurs at higher volume fractions ($0.2 \lesssim \phi_{\text{dep}} \lesssim 0.35$) and at the smallest ratio $r_{\text{SM}}/r_{\text{dep}} = 3$ the drop occurs at $0.2 \lesssim \phi_{\text{dep}} \lesssim 0.4$, which are the highest volume fractions investigated.

The $r_{\text{SM}} = 5\sigma$ curve appears to have a global minimum (Figure 8.2.2). This is likely not a true minimum but rather appears because at the highest densities the chain collapses into conformational states with relatively deep local minima that require uncommonly large fluctuations in order to escape and find the global minimum. Packing effects appear to make these states metastable. At high densities, these states are long lived on the time scale of the simulations, while at intermediate depletant densities ($\phi_{\text{dep}} \approx 0.27$) such states are less stable and so the true minimum is more likely to be observed.

Simulations were also performed in which the structural monomers were modelled using the shifted-WCA potential. It was demonstrated that the well depths produced in this model were far less deep in § 8.1 and indeed a phase transition from a swollen state to a globular state like those observed for the combinatorial-WCA model was not observed for shifted-WCA simulations (not shown). A small decrease did occur for $r_{\text{SM}}/r_{\text{dep}} = 5$ but by the highest volume fractions accessible to simulations ($\phi_{\text{dep}} \approx 0.4$) a substantial drop had not. It is likely that a transition cannot be observed in the shifted-WCA model but can be observed in the combinatorial-WCA model because the well depths are deeper and so the phase transition is shifted to lower volume fractions.

The transitions in Figure 8.2.2 are somewhat abrupt but they **do not** appear to be discontinuous. In particular, the uncertainty on the mean of the radii of gyration during the transition are not comparable to the drop itself. The probability distribution of the radius of gyration $p(R_g)$ in the absence of depletants is broad since the fluctuations of a polymer in good solvent are large. Figure 8.2.3 shows the radius of gyration for $r_{\text{SM}}/r_{\text{dep}} = 5$ in non-zero volume fraction baths. As the volume fraction of depletants is increased, the mean decreases and at the highest volume fractions of depletants the probability distribution becomes sharply peaked about the collapsed state (Figure 8.2.3). The chains have collapsed to a globular-state and do not fluctuate significantly. However, throughout the transition, coexistence of the swollen-state and the globular-state is not observed. Each of these probability distributions are *unimodal*, containing only a single maximum and at no point during the transition can co-existence between swollen and globular states be identified. Thus, the

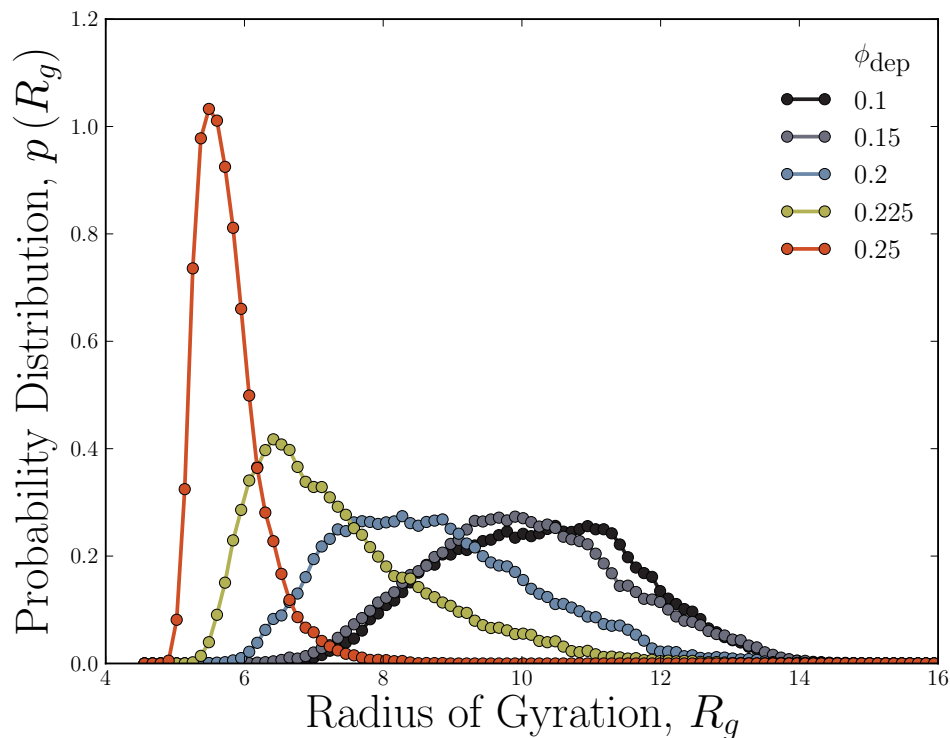


Figure 8.2.3: Probability distributions of R_g for the size ratio of $r_{\text{SM}}/r_{\text{dep}} = 5$ and various volume fractions. The volume fractions shown are in the transition region, near the critical volume fraction of depletants ϕ_{dep}^* but remain unimodal. There appears to be no coexistence of both swollen and collapsed coils.

collapse is a *continuous phase transition* as is expected for a freely-jointed polymer undergoing a phase transition [392], but this finding is in disagreement with the experimental report of Pelletier *et. al.* [382].

8.2.3 Chromosome-Depletant Flory Theory

How can this transition be discussed in a simplified way, while reproducing the phase transition from a swollen to a globular/collapsed state (*i.e.* Figure 8.2.2)? Consider a classic *Flory theory* for chromosomal DNA in an **implicit** bath of protein depletants. More clever theories may build a free energy for the entire system of chromosomal material and cytoplasmic proteins [393]. Here, a far more simplistic view is taken: Rather than explicitly accounting for the protein depletants, they are only implicitly included through the virial coefficients $B_{\text{SM},2}$ and $B_{\text{SM},3}$.

In § 8.1, a theoretical prediction for the second virial coefficient of the structural monomers

that arises due to depletant-mediated attractions is presented. In this simplified free energy, the second virial coefficient will provide the *effective volume* of the structural monomers $\nu_{\text{eff}} \equiv B_{\text{SM},2}/4$ and the third virial coefficient, which was approximated as constant and equal to the hard-sphere value, gives an estimation of the three-body interaction coefficient $\omega_{\text{eff}} \approx B_{\text{SM},3}/10 \approx V_{\text{SM}}^2$. When the depletants are included implicitly, the free energy of the system (implicitly) belongs to the chromosome chain alone. The free energy of the system then has only two terms

$$F = F_{\text{ent}} + F_{\text{int}}, \quad (8.20)$$

which are:

1. An **entropic spring free-energy cost** due to connectivity

$$\frac{F_{\text{ent}}}{k_{\text{B}}T} = \frac{3}{2} \frac{R_{\text{g}}^2}{N_{\text{SM}} r_{\text{SM}}^2} + \frac{N_{\text{SM}} r_{\text{SM}}^2}{R_{\text{g}}^2}. \quad (8.21)$$

This is a common interpolation between the free energy of a swollen state ($F_{\text{ent}} \propto R_{\text{g}}^2$) and a collapsed state ($F_{\text{ent}} \propto R_{\text{g}}^{-2}$) [392, 394].

2. An **effective interaction free energy** that can be written as an expansion

$$\frac{F_{\text{int}}}{k_{\text{B}}T} = \left[\left(\frac{3}{4\pi} \right) \left(\frac{N_{\text{SM}}^2}{R_{\text{g}}^3} \right) \nu_{\text{eff}} + \left(\frac{3}{4\pi} \right)^2 \left(\frac{N_{\text{SM}}^3}{R_{\text{g}}^6} \right) \omega_{\text{eff}} + \dots \right], \quad (8.22)$$

where ν_{eff} is the effective excluded volume, ω_{eff} is the three-body interaction coefficient, *etc.*

This is a simplistic but robust and general way to approach a generic polymer. One need only minimize F with respect to R_{g} to calculate the expectation value of the radius of gyration to be given by

$$\begin{aligned} 0 = & \underbrace{3 \left(\frac{R_{\text{g}}}{r_{\text{SM}} N_{\text{SM}}^{1/2}} \right)^5}_{\text{swelling}} - \underbrace{2 \left(\frac{R_{\text{g}}}{r_{\text{SM}} N_{\text{SM}}^{1/2}} \right)}_{\text{compression}} \\ & - \underbrace{\left(\frac{9}{4\pi} \right) \left(\frac{\nu_{\text{eff}}}{r_{\text{SM}}^3} \right) N_{\text{SM}}^{1/2}}_{\text{2-body interactions}} - \underbrace{\left(\frac{27}{8\pi^2} \right) \left(\frac{\omega_{\text{eff}}}{r_{\text{SM}}^6} \right) \left(\frac{R_{\text{g}}}{r_{\text{SM}} N_{\text{SM}}^{1/2}} \right)^{-3}}_{\text{3-body interactions}} + \dots, \end{aligned} \quad (8.23)$$

where all the numerical coefficients are near unity and should not be given fundamental consideration.

The current strategy is to consider the limiting cases of Equation (8.23) and model the interaction coefficients of the polymer chain as the virial coefficients of an ensemble of free structural monomers interacting in a bath of small particles that are included only implicitly via depletion forces *i.e.* through ν_{eff} and ω_{eff} . Qualitatively speaking, ν_{eff} controls the *solvent quality* through the usual definition

$$\chi = \frac{1}{2} - \frac{\nu_{\text{eff}}}{4V_{\text{SM}}}. \quad (8.24)$$

Therefore, the chromosome-depletants system is discussed in terms of an effective solvent quality due to the depletion forces [363]. Each solvent regime is an idealization in which all but two of the terms in Equation (8.23) are considered to be insignificant and neglected.

Good Solvent

If the coil is in a swollen state then three-body interactions are rare and the compression term is dropped in Equation (8.23) such that

$$\frac{R_g}{r_{\text{SM}}} = 6^{1/5} \left(\frac{\nu_{\text{eff}}}{V_{\text{SM}}} \right)^{1/5} N_{\text{SM}}^{3/5}. \quad (8.25a)$$

This is the *good solvent* regime in which the monomers form a self-avoiding random walk. In the limit that $\nu_{\text{eff}} \rightarrow V_{\text{SM}}$ (corresponding to an absence of depletants in this situation), the good solvent regime concludes with the extreme *athermal solvent*

$$\frac{R_{g,\text{athermal}}}{r_{\text{SM}}} = 6^{1/5} N_{\text{SM}}^{3/5}. \quad (8.25b)$$

Poor Solvent

If the chain is in the collapsed state then $R_g \ll r_{\text{SM}} N^{1/2}$ so $F_{\text{ent}} \approx 0$ and only the two interaction free energy terms remain such that

$$\frac{R_g}{r_{\text{SM}}} = 2^{1/3} \left(-\frac{\omega_{\text{eff}}}{V_{\text{SM}}\nu_{\text{eff}}} \right)^{1/3} N_{\text{SM}}^{1/3}, \quad (8.25c)$$

where the negative sign within the brackets is appropriate since ν_{eff} is expected to be negative. This looks as we would expect for a polymer in *poor solvent*.

The poor solvent collapse can only endure for so long. Eventually the polymer is in its fully globular state and

$$\frac{R_{g,\text{glob}}}{r_{\text{SM}}} \simeq N_{\text{SM}}^{1/3}, \quad (8.25d)$$

which, of course, scales the same as the poor-solvent case but no longer varies with increased ϕ_{dep} through ν_{eff} . This extremum is referred to as a *non-solvent*.

Theta-Solvent

The analogy of solvent quality as a framework for discussing entropic effects of depletants suggests there will exist some depletant volume fraction that corresponds to a *Θ -solvent* condition. This will be denoted $\phi_{\text{dep}}^{\Theta}$ and in this situation it is controlled by the volume fraction of depletants, instead of temperature as in a traditional solvent. It is the point between good solvent and poor solvent conditions, which occurs when the radius of gyration scales as an ideal random walk

$$\frac{R_g}{r_{\text{SM}}} \simeq N_{\text{SM}}^{1/2}. \quad (8.25e)$$

This suggests that $\nu_{\text{eff}} \simeq 0$. On the other hand, if $R_g = r_{\text{SM}} N_{\text{SM}}^{1/2}$ is substituted into Equation (8.23) then

$$\nu_{\text{eff}} = \left[\left(\frac{3}{2\pi} \right) \left(\frac{\omega_{\text{eff}}}{r_{\text{SM}}^3} \right) + \text{const.} \right] N_{\text{SM}}^{-1/2} \propto N_{\text{SM}}^{-1/2},$$

which only goes to zero in the limit of long chromosome chains. For finitely long chains of $N_{\text{SM}} = 15$, the Θ -regime exists over a **narrow range** of depletant volume fraction.

8.2.4 Phase Transition is Second Order

Since the effective volume $\nu_{\text{eff}} = B_{\text{SM},2}/4$ and the three-body term $\omega_{\text{eff}} = B_{\text{SM},3}/10$ for the structural monomers are approximated in § 8.1, the radius of gyration of the chromosome chain can be calculated as a function of ϕ_{dep} from Equation (8.23).

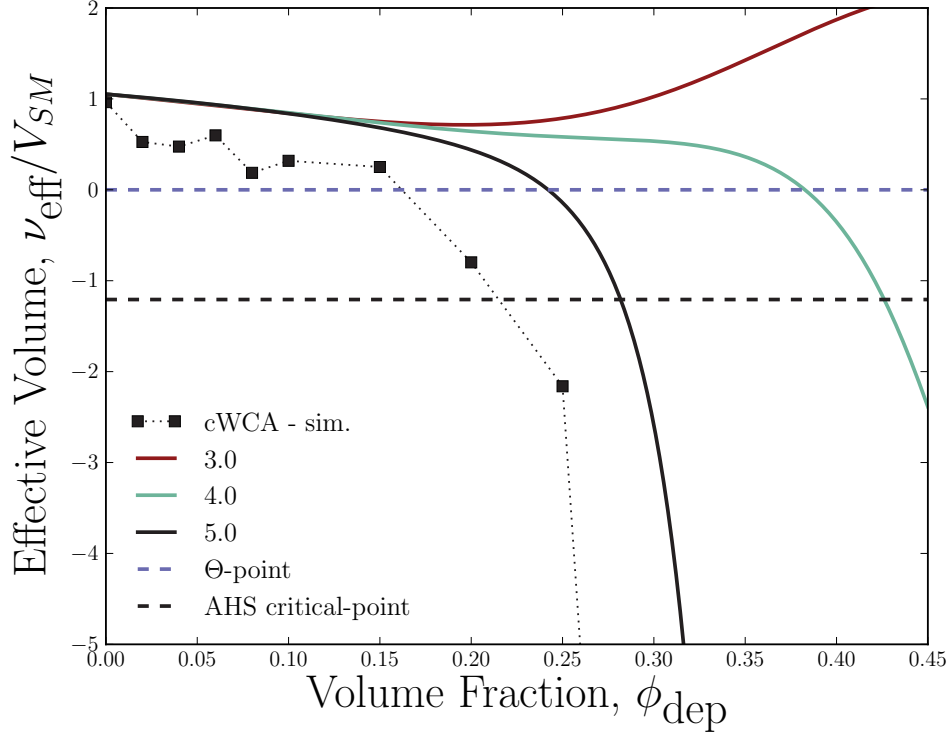


Figure 8.2.4: The effective volume of structural monomers interacting via depletion-induced pair potentials. When no depletants are present the effective volume is the physical volume V_{SM} . The effective volume remains near V_{SM} at low volume fractions but drops rapidly above the Θ -point. For $r_{SM}/r_{dep} = 5$, the Θ -point is $\phi_{dep}^{\Theta} = 0.16$ and the AHS prediction of the critical point is $\phi_{dep}^{*AHS} = 0.21$.

In § 8.1, the second virial coefficient is calculated by inputting the total interaction energy $W_{cWCA} = \mathcal{U}_{MT} + U_{cWCA}$, where $\mathcal{U}_{MT}$ is the attractive depletion-induced pair potential, as modelled by the MT model and U_{cWCA} is the repulsive combinatorial-WCA potential. In terms of the effective volume, the total interaction energy W_{cWCA} is substituted into

$$\nu_{eff} = -\frac{1}{2}\pi \int_0^{\infty} \left(e^{-W_{cWCA}(\phi_{dep}, \rho)/k_B T} - 1 \right) \rho^2 d\rho. \quad (8.26)$$

Doing so produces an effective excluded volume that is well approximated as V_{SM} at low volume fractions of depletants, but drops rapidly to large negative numbers at higher ϕ_{dep} values just as happens with the $r_{SM}/r_{dep} = 5$ simulations (Figure 8.2.4).

While the predicted $r_{SM}/r_{dep} = 5$ and 4 effective volume curves behave qualitatively similarly, the $r_{SM}/r_{dep} = 3$ curve is quite different. Rather than dropping at large volume fractions, ν_{eff} begins to climb back up. This is the characteristic behaviour of the MT model for small size ratios and high volume fractions of depletants. In this case, these artificial and nonphysical effects appear to dominate ν_{eff} . The rise of effective volume curve seen in

Figure 8.2.4 suggests that this occurs at rather small volume fractions for $r_{\text{SM}}/r_{\text{dep}} = 3$.

The effective volume of the structural monomers when $r_{\text{SM}}/r_{\text{dep}} = 5$ crosses zero at $\phi_{\text{dep}}^{\Theta} = 0.25$ (Figure 8.2.4; red dashed line). Not coincidentally, this is approximately where the R_g collapse occurs in Figure 8.2.2. The same qualitative statements can be said of the $r_{\text{SM}}/r_{\text{dep}} = 4$ prediction for the effective volume, except that the predicted Θ -point is shifted up to $\phi_{\text{dep}}^{\Theta} = 0.33$. Since the volume fraction is already dropping rapidly, the Θ -point $\phi_{\text{dep}}^{\Theta}$ acts as a lower boundary on the critical point for the phase transition ϕ_{dep}^* to be slightly different.

Qualitatively speaking, the volume fraction at which the effective volume is zero is a critical point ϕ_{dep}^* : The steric-repulsion between monomers no longer dominates over the depletion-induced attractions at $\phi_{\text{dep}}^{\Theta}$. At volume fractions higher than this, attractions are more significant and collapse eventually occurs. Technically, the Θ -point $\phi_{\text{dep}}^{\Theta}$ must occur at lower volume fractions than the critical point of collapse ϕ_{dep}^* , but Figure 8.2.4 shows that the effective volume is plummeting to large, negative numbers, suggesting that the critical point follows soon after.

When attractive potentials are extremely short ranged (as with depletion forces) there is a quasi-universality to the critical value [377]. The critical second virial coefficient at which a phase transition to agglomeration occurs is $B_{\text{SM},2}^* = -1.207 (4V_{\text{SM}})$ in ensembles of Adhesive Hard Spheres (AHS) [395]. It has been argued that when $r_{\text{SM}}/r_{\text{dep}} \gg 1$ (as is discussed for the AO model in § 8.1.1) that binary hard sphere mixtures of structural monomers and depletants are accurately equivalent to AHS colloidal gases [377]. This immediately suggests that the critical effective volume for which a phase transition is expected is $\nu_{\text{eff}}^{*\text{AHS}} = -1.207V_{\text{SM}}$, rather than $\nu_{\text{eff}}^{\Theta} = 0$. The AHS value for the critical volume fraction of depletants when $r_{\text{SM}}/r_{\text{dep}} = 5$ is $\phi_{\text{dep}}^{*\text{AHS}} = 0.28$ (Figure 8.2.4; intersection of solid blue line and dashed black line) and when $r_{\text{SM}}/r_{\text{dep}} = 4$ is $\phi_{\text{dep}}^{*\text{AHS}} = 0.37$.

Consider the simulated curve for the radius of gyration with $r_{\text{SM}}/r_{\text{dep}} = 5$ in Figure 8.2.2: the chain reaches its globular state by $\phi_{\text{dep}}^{*\text{cWCA}} = 0.27$. This point acts as the proxy for the critical point in the simulations. In order to encompass all of these possible critical points, we write

$$\nu_{\text{eff}}^X \equiv c^X V_{\text{SM}}, \quad (8.27a)$$

where $X = \{*\text{cWCA}, *\text{AHS}, \Theta\}$, $c^{*\text{AHS}} \approx -1.207$ and $c^{\Theta} \approx 0 - 0.05$.

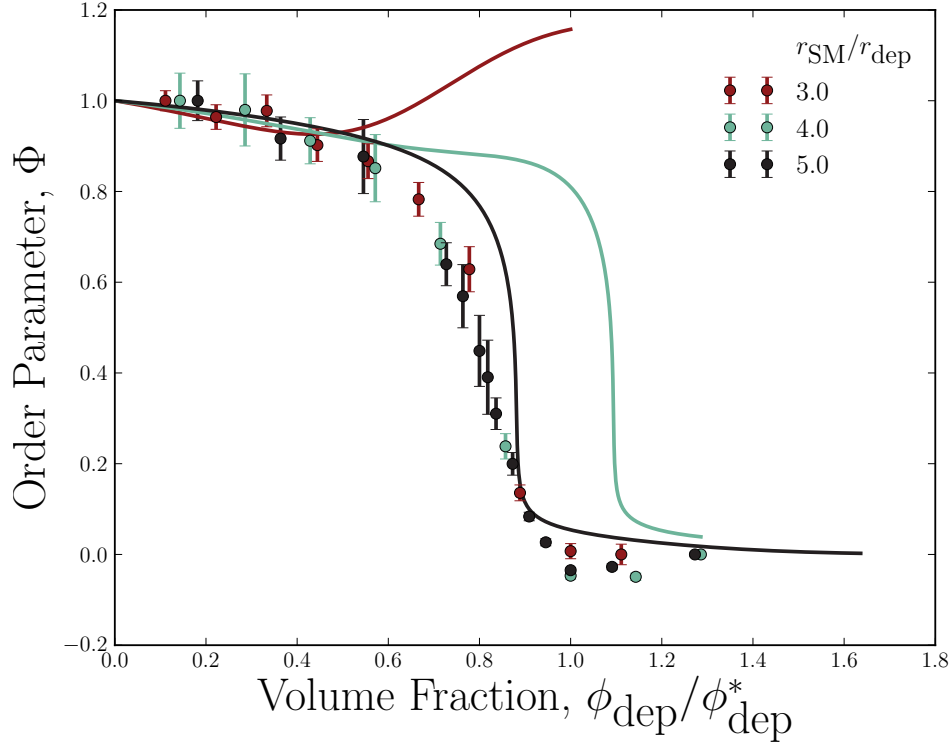


Figure 8.2.5: The order parameter $\Phi \equiv (R_g - R_{g,\text{glob}})(R_{g,\text{athermal}} - R_{g,\text{glob}})$ reduces the radius of gyration axis of Figure 8.2.2. When plotted against the rescaled volume fraction $\phi_{\text{dep}}/\phi_{\text{dep}}^{\text{cWCA}}$ the simulation curves for $r_{\text{SM}}/r_{\text{dep}} = \{3, 4, 5\}$ collapse to a single curve. The theory results from substituting the effective volume from Equation (8.26) into the Flory theory (Equation (8.23)).

The critical point of the combinatorial-WCA simulations $\phi_{\text{dep}}^{\text{cWCA}}$ can be used to rescale the volume fraction of depletants and an order parameter for the phase transition can be defined. This *coil-globule order parameter* is

$$\Phi \equiv \frac{R_g - R_{g,\text{glob}}}{R_{g,\text{athermal}} - R_{g,\text{glob}}}, \quad (8.28)$$

where $R_{g,\text{athermal}}$ is the $\phi_{\text{dep}} \rightarrow 0$ limit (Equation (8.25b)) and $R_{g,\text{glob}}$ is the collapsed, non-solvent value (Equation (8.25d)). When the order parameter Φ is plotted against the rescaled volume fraction, the three simulated curves from Figure 8.2.2 collapse onto a single curve (Figure 8.2.5). The measured order parameter starts at unity when the number of depletants is zero, decreases slowly over low depletant volume fractions and transitions to zero at the critical point $\phi_{\text{dep}}/\phi_{\text{dep}}^{\text{cWCA}} = 1$. For higher volume fractions the order parameter remains $\Phi = 0$. Plotting the simulations and predictions in this manner should collapse the curves.

The order parameter curve is predicted by substituting the predicted effective volume for

the pair potential simulations (Equation (8.26)) into the Flory theory (Equation (8.23)) to determine the radius of gyration as a function of depletant volume fraction (Figure 8.2.5). The simulated radii of gyration are well represented by the Flory theory curve when $r_{\text{SM}}/r_{\text{dep}} = 5$ and 4. Since the theory fails to predict the effective volume when $r_{\text{SM}}/r_{\text{dep}} = 3$, the Flory theory is also inadequate for that size ratio. The low volume fraction region of negative curvature is the well-predicted region, while simulations appear to begin to collapse sooner. This causes the radius of gyration to be over-predicted during the transition. Furthermore, the simulated transition occurs more slowly than predicted, but overall the agreement is satisfactory for $r_{\text{SM}}/r_{\text{dep}} = 5$ and 4.

The simulated data are relatively well collapsed in Figure 8.2.5, but the theoretical curves are not precisely collapsed by normalizing by $\phi_{\text{dep}}^{*\text{WCA}}$. The two predicted curves are shifted along the abscissa, as is well demonstrated by considering the inflection point in Figure 8.2.5 (inset). The inflection point occurs at $\phi_{\text{dep}}/\phi_{\text{dep}}^* = 0.90$ for $r_{\text{SM}}/r_{\text{dep}} = 5$ but at $\phi_{\text{dep}}/\phi_{\text{dep}}^* = 0.95$ for $r_{\text{SM}}/r_{\text{dep}} = 4$.

This section presents WCA simulations of coarse-grained DNA chains in baths of smaller depletant particles. These simulations demonstrate that depletion-induced attraction between DNA's structural monomers can indeed be sufficient to cause the collapse from a swollen state to a globular state. In these simulations, the transition is second-order similar to what one would expect for a freely-jointed polymer chain going from good-solvent to poor solvent conditions.

The effective solvent quality is quantified by predicting the effective excluded volume of each structural monomer. This effective volume can be well approximated for combinatorial-WCA simulations by modelling the total interaction as the WCA potential plus the Morphometric Thermodynamics model for the depletant-mediated pair potential. It must be noted, however, that this model breaks down for small ratios of structural monomer size to depletant size. For large size ratios the sum of the WCA potential plus the Morphometric Thermodynamics model produces reasonable expectation values for the critical value of volume fraction at which the transition is expected to occur. Through this effective volume, the critical volume fraction can be predicted and the theoretical prediction of the radius of gyration as a function of volume fraction of depletants agrees with the simulation collapse. Both simulations and theory for this simplified model of supercoiled DNA in a bath of protein depletants predict that depletion-induced attractions are sufficient to cause a second order collapse to a globular state.

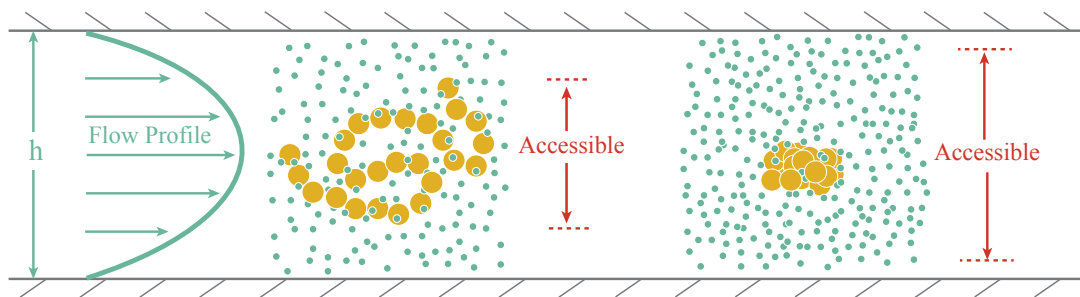


Figure 8.3.1: When performing hydrodynamic chromatography, a swollen chain has a hydrodynamic radius of approximately R_g and so R_g defines the near-wall excluded region. Adding a volume fraction of depletants ($\phi_{\text{dep}} > \phi_{\text{dep}}^*$) causes the coil to phase transition to a globular state, which has a much smaller effective radius. A large change in the accessible region of the channel is predicted to correspond to a large change in retention ratio.

8.3 HC of Depletants/Chromosomes

A substantial amount of work has been done here to demonstrate that depletant forces are sufficient to cause a second order phase transition of a model chromosome from a swollen state to a collapsed state. This is a worthwhile conclusion in its own right but separation science is the cynosure of this thesis. Thus, it is now asked, “Can depletion-induced collapse be exploited within fractionation technologies?”

Consider performing planar hydrodynamic chromatography on the simplified chromosomes. Although they are not strictly hard spherical colloids, the polymer chains have a radius of gyration R_g , which roughly defines the spherical volume occupied by the coil. The HC retention theory discussed in § 5 can thus be utilized by approximating $r \approx R_g$. The retention behaviour of solutes eluting via HC is discussed in detail in § 5.1.5 and throughout this thesis: In short, the retention ratio is a parabolic function of solute size (Equation (5.20), Equation (5.33), Equation (7.35), Equation (7.38), Equation (7.39), *etc.*) and so traditionally offers an indirect measure of a polymer’s contour length.

Consider now a coil co-eluting with small, tracer-like depletants dispersed within the carrier fluid (*polymer/depletant HC*). In this thesis, it is assumed that the flow is slow such that the interactions between solutes (depletants and structural monomers) are not perturbed from the situation explored in § 8.1 and § 8.2. The coil will have a swollen R_g when the volume fraction is below the critical value of ϕ_{dep}^* , but if the volume fraction of depletants

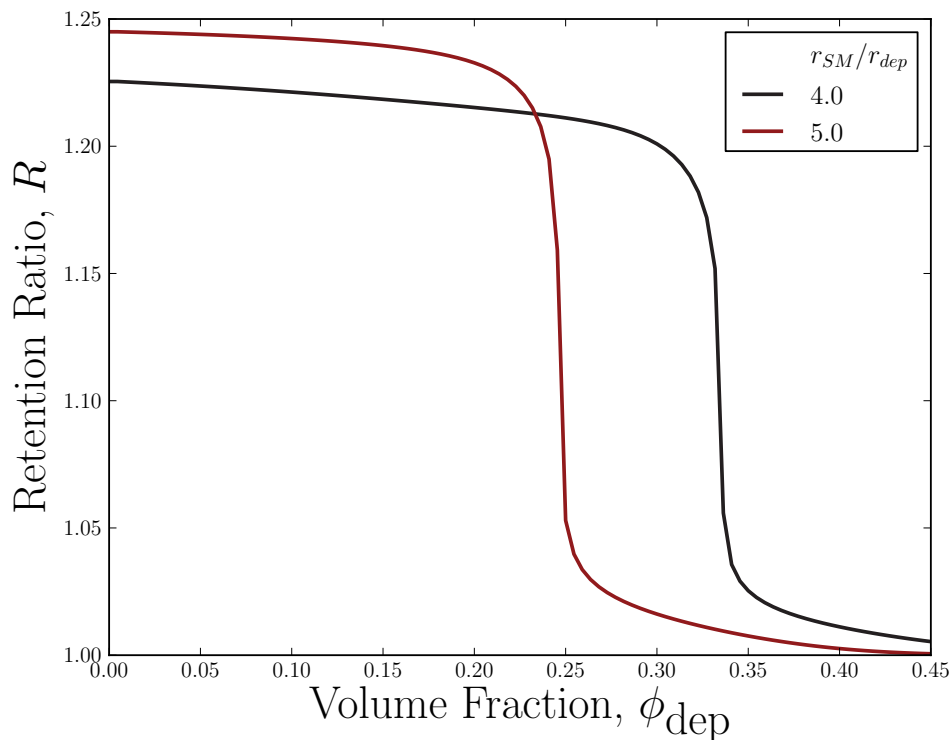


Figure 8.3.2: Retention ratio of a chain of structural monomers in a bath of depletants as a function of volume fraction of depletants. The critical volume fraction is clearly visible for the two sizes of structural monomers.

increases above ϕ_{dep}^* , the radius of gyration of the model chromosome collapses (as seen in § 8.2). This situation is schematically drawn in Figure 8.3.1. Since this collapse leads to a drop in the effective radius r , the retention time of the coil suddenly changes at ϕ_{dep}^* . Furthermore, the critical depletant volume fraction is controlled by the effective volume of the structural monomers that arises due to the depletion-induced attraction (through Equation (8.14)) and is ultimately controlled by the ratio of structural monomer size to depletant size $r_{\text{SM}}/r_{\text{dep}}$ (Equation (8.4)), independent of contour length. Thus, identifying the critical volume fraction potentially provides structural information about the structural monomers that compose the chromosome chain.

The polymer chains considered previously for which the Flory theory predicted a collapse had structural monomer sizes of $r_{\text{SM}}/r_{\text{dep}} = \{4, 5\}$ and $N = 15$. Respectively, the radius of gyration of each is $R_g = \{25.7, 32.2\} \sigma$, where σ is the size scale of the WCA simulations. The polymer/depletant HC retention ratio for these two model chromosomes is shown in Figure 8.3.2 for a channel height of $h = 150\sigma$. This channel height is relatively arbitrary but was chosen to give the largest drop in retention ratio. The retention curves clearly display the

critical volume fraction of depletants for each of the two chains. Since this point is controlled by the ratio of sizes between the structural monomers and the depletants, polymer/depletant HC could be used to identify internal changes within bacterial chromosomes as reflected by changes in their effective structural monomer size.

Summary

The proposal of this chapter is to utilize the second order phase transition of bacterial chromosome material from a swollen to collapsed state that occurs in the presence of depletants to explore variations or changes in the internal structure of chromosomes. However, the nature of this transition is still debated within the literature and computational simulations are needed to verify whether or not non-specific depletion forces are sufficient to cause the collapse of simplistic bacterial chromosomal DNA.

In order to coarse-grain large structural monomer/depletant systems, two types of truncated Lennard-Jones (WCA) simulations are presented. In both models, a steep repulsive potential models steric interactions:

1. The shifted-WCA model is the “harder” of the two models. At low volume fractions of depletants, the pair potential is well approximated by the simplest model of depletion-induced interactions (the AO model), while at intermediate volume fractions the more accurate MT model is required to predict the simulation values. At high volume fractions, higher order correlations and anti-correlations become non-negligible and neither model accurately reproduces the measured pair potentials.
2. The combinatorial-WCA is the softer of the two models since it is longer ranged and less steep (though still “hard”). Because it is softer, the radial probability cannot be used to determine the depletant-induced pair potential as a function of separation, although the force integration method can. The simulated pair potentials are well predicted by summing the MT model for the entropic interactions and the WCA potential. At low depletant volume fractions, the simulated minimum as a function of volume fraction is larger than predicted by the AO model but is much deeper at large volume fractions.

The second virial coefficient for an ensemble of structural monomers is calculated from the simulated pair potentials. At low volume fractions, the simulated second virial coefficient agrees with the AO model’s prediction of a linear decrease from the hard-sphere value. At high volume fractions, neither the AO nor MT models can reproduce the measured values. The simulated second virial coefficients are not better represented by the MT model than the simplest AO model. By calculating the second virial coefficient from the combination of the MT model (extended to separations less than the assumed radius of the structural monomers) and the WCA potential, the simulated values are reproduced. In particular, the second virial coefficient of the combinatorial-WCA model is negative at finite volume fractions of

depletants and decreases rapidly. Although the second virial coefficient is approximated as a function of volume fraction, the third virial coefficient is approximated as the hard-sphere value because depletant-induced interactions are non-additive and reduced with each interacting body.

The second virial coefficient is pivotal in building a Flory theory for the radius of gyration of model chromosomes as a function of depletant volume fraction. Doing so includes the depletants implicitly and so their effects on the chain are equivalent to a solvent quality. The predicted radius of gyration as a function of depletant volume agrees well with Langevin dynamics simulations that explicitly include depletants for size ratios of $r_{\text{SM}}/r_{\text{dep}} = 5$ and 4. The simulations demonstrate that depletant-mediated entropic forces are sufficient to cause the collapse of a chain of structural monomers from a swollen state to a globular state. The radius of gyration collapses in a manner that is consistent with a second order phase transition.

The rapid collapse of the chromosome at the predicted volume fraction of depletants suggests that chromatographic methods that are sensitive to global size could gain information about difference and/or changes in the structural monomers of simple bacterial chromosomes. By performing hydrodynamic chromatography of supercoiled DNA in the presence of depletants, the critical volume fraction of depletants can be identified. This is polymer/depletant HC, which is proposed here as a method for investigating the internal structure of simple chromosomes.

I haven't done anything. I have done absolutely nothing.

Robijn Bruinsma [396]

9

Conclusions

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Separation science is a broad field and, therefore, although this thesis has focused on the translation of a few specific techniques to microfluidic systems, it explored many topics. It began by presenting the known theoretical foundations for separation science. These topics were wide ranging and so an attempt was made to phrase this information into a framework fitting separation science. Most important for chromatographic techniques, it presented the convection-diffusion equation. Since convection and diffusion occur within a fluid medium, the same theoretical framework was used to discuss electro-hydrodynamic interactions.

In micro- and nano-fluidic systems the situation took a particular form. Small length scales ensure that low Reynolds numbers and near-unity Péclet numbers characterize such fractionation devices. Ionized charges within the fluid medium lead to charge distributions in the vicinity of confining walls and solutes. These distributions further define micro- and nano-fluidic systems as they tend to screen electrostatic and hydrodynamic interactions in a manner that is not characteristically observed in macroscopic separation systems.

These systems are ideally suited to mesoscopic simulations. In particular, multi-particle collision dynamics (MPCD), a coarse-grained, particle-based Navier-Stokes solver for modelling hydrodynamic interactions, can efficiently reproduce low Reynolds numbers flows and near-unity Péclet numbers. Implementing the MPCD algorithm is simple in concept. Point-like fluid particles stream for a discrete time. Particles are then binned and particles in a given cell randomly exchange momentum in a random way that conserves the net momentum of the cell. The collision operation is a non-physical scheme that produces thermal noise but also hydrodynamic fields on long enough length and time scales.

Although simple many details about the MPCD algorithm were recorded here. Multiple options for collision operators and many possible boundary conditions were discussed, as were thermostats and schemes for generating flows. Most importantly for this thesis, the incorporation of solutes that are coupled to the fluid were introduced. Various ways of coupling both hard, colloid-like particles and polymer chains of Molecular Dynamics (MD) beads were given. These topics formed the background information needed to perform the research reported here.

9.1 Electrophoresis

The MPCD algorithm was first applied to simulating the electrophoresis of polyelectrolyte chains. In order to do so in a mesoscopic manner a new algorithm was developed. This

mean-field MPCD-MD Debye-Hückel algorithm is able to model the electro-hydrodynamics of charged macromolecules. Principally, the simulated polymers possess a finite Debye length and a heuristic model for counterion condensation was implemented. Finite Debye lengths were achieved by projecting the approximate counterion charge distribution onto the MPCD fluid particles so that the ions (and their long-range, computationally-expensive electrostatic interactions) need not be explicitly included. Because of this, the MPCD-MD Debye-Hückel algorithm was able to reproduce the electrophoretic mobility of charged oligomers as a function of chain length. It also allowed studies of electrophoretic mobility as a function of Debye length and as a function of charge distribution along the back-bone of the chain (polyampholytes).

Furthermore, the MPCD-MD Debye-Hückel algorithm was used to test and explore fundamental assumptions of electrophoresis of polyelectrolytes. The electrophoretic mobility of polyelectrolytes confined by a radial potential was explored. It was confirmed that the chains remain free-draining, regardless of conformation as was expected because of electrohydrodynamic screening by the Debye layers. However, the mobility increases sharply at strong confinements. These results were explained as arising due to changes in the effective friction coefficient of local segments of chains due to orientation effects on length scales smaller than the Debye length.

These confinement simulations lay the groundwork for further studies. One should ask what the effect of different potentials might be, wonder what changing the Debye length of the monomers would do to the mobility as a function of confinement, imagine the impact of persistence length and question how confinement may alter charge condensation along the chain, for instance. Looking even further, the simulations should be extended to simulate more realistic models of nanofluidic confinements. To do so, smooth, no-slip boundary equations should first replace the confining potential. Bounce-back boundary conditions will introduce direct friction to the polyelectrolyte and also the surrounding fluid. The walls will further screen hydrodynamic interactions, but they could also be charged and electroosmotic flow could be included. Simulations in this direction are currently relevant because many research groups are obtaining conflicting experimental results on the electrophoresis of DNA in nanochannels [145, 146].

9.2 Hydrodynamic Chromatography and Field-Flow Fractionation

Another family of separation techniques is that of field-flow fractionation (FFF). Traditionally, the sub-techniques of normal-mode FFF and of steric-mode FFF are used to separate samples, with hydrodynamic chromatography (HC) as the theoretical zero-force limit of the technique. In this thesis the disparate retention theories describing each of the three were united into a single unified retention theory by expressing the average elution time as an explicit function of solute size. This allowed maps of operational-mode space to be constructed and the transitions between different modes of operation to be traced.

Metrics were needed in order to compare FFF to other separation techniques and between operational modes of FFF, but existing metrics utilized by the FFF community were relative in nature and so novel metrics better suited to the questions asked in these studies were proposed. The new device selectivity metric maintains the advantages but bypasses some of the non-intuitive aspects of the traditional definition. The device selectivity was applied to each of the ideal operational modes of FFF alongside the traditional definition in order to highlight the similarities and differences between the two.

The unified retention theory also revealed that the ideal assumptions break down at the largest particle sizes. Since large particles are subject to a velocity gradient across their surfaces, the retention theory was extended to account for Faxén's law. This led to the prediction of an undiscovered operational mode of FFF, which was denoted Faxén-mode FFF.

MPCD simulations of HC and FFF were performed (in conjunction with collaborators' experimental studies) in order to verify the existence of the Faxén-mode operational regime. Results for large particle HC qualitatively agree with predictions but large particles elute more slowly than was predicted. This is the result of increased drag due to hydrodynamic interaction with the no-slip channel walls. In order to theoretically account for this increase in the effective friction coefficient of the solutes, many simple models were investigated. Only an effective friction coefficient produced by multi-pole expansion calculations [5] adequately reproduced the retention curves. By numerically incorporating these wall-lag effects, quantitative agreement between theory and MPCD simulations (and experiments) was achieved.

The microfluidic walls do not only induce resistive forces. If particle Reynolds numbers are large enough, they can also lead to lateral migration due to inertial (lift) forces. A theory

for multiple constant transverse fields was built as a simplistic model of these inertial forces. Although it is likely too naive to adequately handle lift, this model led to the proposal of adverse-FFF, a novel separation technique in which a second (opposing) external field is applied.

Channel walls clearly play an essential role in determining the rich behaviour of solutes eluting in FFF. Therefore, ideas to improve separation by actively modifying channel surfaces were explored. An idea developed here is the use of large slips at the channel walls. It was found that large slip lengths at the accumulation wall in the presence of a strong external field produce a novel fractionation mode, which was called slip-mode FFF. The large slip lengths necessary for slip-mode FFF could be created by nano-engineering superhydrophobic surfaces or using electro-osmotic flow (with a Debye length smaller than other length scales relevant to the separation process).

The last complication to FFF presented was the finite width of elution channels. A series solution was derived for the retention theory in 3D rectangular channels. Interestingly, the FFF retention curves did not change substantially, which is an asset for microfluidic engineers who wish to design channels using common materials. Separation using HC was predicted to benefit slightly from near-unity aspect ratios.

9.3 Hydrodynamic Chromatography with Coil-Globule Collapse

Not all the proposed separation techniques depend on altering the separation apparatus. The use of depletants to cause conformational changes in polymers was explored. Using depletants in conjunction with HC (polymer/depletant HC) could be utilized to deduce information about monomer size. This was discussed with respect to depletant-chromosome systems because the structural monomers of simple bacterial DNA are worth studying via chromatographic techniques, and also because questions remained about the nature of depletion-induced collapse. In order to study this system, coarse-grained simulations of entropic forces were carefully characterized.

The work done to characterize the coarse-grained simulations provides opportunities beyond proposing separation techniques. Simulating such systems is computationally costly and the deeper wells of the entropic pair potentials in combinatorial-WCA, compared to

shifted-WCA simulations, may allow future scientists to simulate soft matter systems in which depletion attraction plays an important role but would otherwise be too computationally expensive to include.

9.4 Idealized Models of Miniaturization

Throughout this work, the strategy has been to apply the simplest, most idealized models to the complications that arise out of miniaturization. In this way, not only are non-intuitive results predicted but also the phenomena leading to them are clear. This approach meant that proposals for novel separation methods were nearly always suggested following the study of some complication. Miniaturization does lead to complications, but these complications are opportunities to be taken advantage of, just as much as they are difficulties to be overcome.

Cet étudiant est (sans dessin/sans-dessein).
Gary Slater [397]



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Imagining things and doing things (as it turns out) are entirely different things.

David Pine [399]

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I learned this from [Paul] Chaikin so it must be right.

Dave Weitz [400]



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*People think osmotic pressure is pressure but it's
not pressure.*

Paul Chaikin [401]

D

Acronyms and Mathematical Nomenclature

D.1 Acronyms

AHS Adhesive Hard Spheres

AO Asakura-Oosawa

BC Boundary Condition

BD Brownian Dynamics

COI Characteristic-Of-Interest

DH Debye-Hückel

DPD Dissipative Particle Dynamics

EOF Electro-Osmotic Flow

FENE Finitely Extensible Nonlinear Elastic

FFF Field-Flow Fractionation

FT Fourier Transform

HC Hydrodynamic Chromatography

LA Long-Ajdari

LB Lattice Boltzmann

LJ Lennard Jones

MD Molecular Dynamics

MPCD Multi-Particle Collision Dynamics

MT Morphometric Thermodynamics

OB Oseen-Burgers

PHS Penetrating-Hard Sphere

SM Structural Monomer

SRD Stochastic Rotation Dynamics

WCA Weeks-Chandler-Andersen

cWCA combinatorial-WCA

sWCA shifted-WCA

D.2 Mathematical Nomenclature

Operators

$\mathbf{x}$ Set of thermodynamic variables

$\underline{\mathbf{x}}$ Tensor set of thermodynamic variables

$\vec{x}$ Vector

$\hat{x}$ Tensor

$\frac{\partial y}{\partial x}$ Partial derivative of y with respect to x

$\frac{dy}{dx}$ Derivative of y with respect to x

$\frac{Dy}{Dx}$ Material derivative of y with respect to x

$\langle x \rangle$ Average

$\bar{x}$ First moment of a zone

σ_x Standard deviation of a zone

$|x|$ Absolute value

j_x Flux of x

$\vec{\nabla}$ Del operator

∇^2 Laplacian operator

$\tilde{x}$ Nondimensionalization (often with respect to height h , though not always)

$x^\top$ Transpose $\delta(x)$ Dirac-delta function $\Theta(x)$ Heaviside functionFT $[x]$ Fourier transform $\mathcal{L}(x)$ Langevin function, $\coth(x) - 1/x$

Numerical Constants

 $\hat{\mathbb{I}}$ Identity matrix $\hat{x}$ Unit vector

Dimensionless Numbers

Pé Péclet number

Re Reynolds number

Re_p Particle Reynolds number

Sc Schmidt number

Variables (Latin alphabet)

Lower case

 a Size of a cell (often an MPCD cell) b “Bottom” slip length b_{HC} Linear coefficients in parabolic HC theory, *i.e.* $R = 1 + b_{\text{HC}}\tilde{r} - c_{\text{HC}}\tilde{r}^2$ c_{HC} Quadratic coefficients in parabolic HC theory, *i.e.* $R = 1 + b_{\text{HC}}\tilde{r} - c_{\text{HC}}\tilde{r}^2$ c_f Coefficient relating force to particle size, *i.e.* $f = c_f r^\alpha$ c_{H} Henrys function d Distance from contact point to centre of mass $\mathbf{d}$ Dimensionality d_{H} Tube diameter (or effective hydraulic diameter) e Unit charge f Force g Acceleration h Channel height k Spring constant k_{B} Boltzmann constant m Mass n Number density, *i.e.* concentration $\hat{n}$ Normal unit vector p Probability (or radial probability density) q Frequency r Radius $\tilde{r}_{\text{HN}}$ Transition from HC to normal-mode FFF $\tilde{r}_{\text{NS}}$ Steric-inversion point *i.e.* transition from normal-mode FFF to steric-mode FFF $\tilde{r}_{\text{SF}}$ Transition to Faxén-mode FFF $\tilde{r}_{\text{L}}$ Size of buoyant particles in adverse-mode FFF r_{SM} Radius of structural monomers r_{dep} Radius of depletants

t Time	L Length (often channel or contour length of a linear polymer)
$\hat{t}$ Tangential unit vector	L Angular momentum
t “Top” slip length	$[L]$ Characteristic length scale
t_{test} Welch’s generalized two sample t-statistic	M Velocity scaling applied to MPCD particle position by BCs
t_o Void time	N Number of constituents (often degree of polymerization)
t_R Retention time	N_c Number of particles in a cell (often an MPCD cell)
v Linear velocity of a body	N_{cut} Number of monomers from junction of a ring polymer at which a cut is made
w Channel width	N_x Number of theoretical plates (N_R relative; N_D device)
$\vec{x}$ Position, (x, y, z) in Cartesian coordinates and (ρ, z, θ) in cylindrical coordinates	P Pressure
Upper case	Q Charge (often of a monomer)
A Amplitude	R Retention ratio
$B_{\text{SM},n}$ n^{th} virial coefficient of a solution of structural monomers	R_{eff} Effective radius of a radial harmonic potential
C_1 Gaussian curvature	R_g Radius of gyration
C_L Lift coefficient	R_H Hydrodynamic radius
D Shift applied to MPCD particle position by BC	R_s Resolution
E Electric field	S Entropy
F Generalized thermodynamic force	S_x Selectivity (S_R relative; S_D device)
F_x Fractionating power (F_R relative; F_D device)	S_θ Orientation order parameter
G Gravitational acceleration	T Temperature
K Resistance (or translation) tensor (inverse is slip function)	

U	Potential energy (often the truncated	ζ	Friction coefficient
		ζ	Zeta potential
γ_S	Entropic surface tension (associated with restrictions to surface area) Lennard-Jones [WCA] repulsive potential)	η	Dynamic viscosity
		η'	Dilation viscosity
$[U]$	Characteristic velocity scale	κ	Thermal conductivity
V	Volume	$\kappa_{S,1}$	Entropic bending rigidities (associated with restrictions to Gaussian curvature)
V_{sys}	System volume	λ	Retention parameter
W	Total pair potential	λ	Mean-free path
X	Generalized thermodynamic coordinate	λ_D	Debye length

Variables (Greek alphabet)

Lower case

α	Force scaling exponent, <i>i.e.</i> $f \sim r^\alpha$	μ	Chemical potential
ϑ	Angle of rotation	μ	Hydrodynamic mobility
γ	General kinetic coefficients for a linear transport process	μ_{el}	Electrophoretic mobility
γ_{rel}	Thermostat rescaling coefficient	ν	Kinematic viscosity, <i>i.e.</i> η/ϱ
γ_S	Entropic surface tension (associated with restrictions to surface area)	ν_{kin}	Kinematic component of viscosity
δ	Stern layer thickness	ν_{col}	Collision component of viscosity
ε	Coefficient of restitution	ν_{eff}	Effective volume
ϵ	Strain ($\dot{\epsilon}$ is the strain rate)	ξ	Random number or thermal noise
$\varepsilon_0 \varepsilon_r$	Effective dielectric constant (including screening effects)	ϱ	Mass density
		ρ	Radial distance
		ρ_0	Bond length
		σ	Monomer size (often MD size scale)
		σ_{cond}	Electric conductivity

σ_{el} Surface charge
 τ Thermostat relaxation time scale
 $[\tau]$ Characteristic time scale
 φ Asymmetry ratio
 ϕ Volume fraction
 χ Solvent quality
 χ Contact point
 ψ Source/Sink
 ω Angular velocity
 ω_{eff} Three-body interaction coefficient

Upper case

Γ The sum of the inverses of retention parameters for multiple forces, *i.e.* $\Gamma = \sum \Lambda_i^{-1} \tilde{r}^{\alpha_i} = \sum \lambda_i^{-1}$
 Δ WCA shift term
 Λ Device retention parameter
 Ξ Common term in functions for MPCD viscosity, *i.e.* $\Xi = \langle N_c \rangle / (\langle N_c \rangle - 1 + e^{-\langle N_c \rangle})$
 Π Viscous stress tensor
 Π_S Osmotic/entropic pressure (associated with restrictions to volume)
 Σ Area
 Φ Coil-globule order parameter
 Ψ Electrostatic potential

Ω Number of states

Ω_{ϑ} Rotation

Variables (Script alphabet)

$\mathcal{D}$ Diffusion coefficient

$\mathcal{F}$ Affinity

$\mathcal{F}$ Term encompassing steric effects beyond diminished channel height

$\mathcal{H}$ Hydrodynamic interaction tensor

$\mathbb{I}$ Moment of inertia

$\mathcal{J}$ Impulse

ℓ Separation distance

ℓ_p Persistence length

$\mathcal{Q}$ Volumetric discharge rate

$\mathcal{N}$ Torque

$\mathfrak{z}$ Characteristic-of-interest

$\mathcal{R}$ Structural monomer radius plus depletant radius, *i.e.* $\mathcal{R} = r_{\text{SM}} + r_{\text{dep}}$

$\mathbb{R}$ MPCD collision operator

$\mathcal{S}$ Surface (typically of a boundary condition)

$\mathbb{T}$ Stress tensor

$\mathcal{U}$ Depletion-induced pair potential

$\mathcal{V}$ Particle velocity

Since you said what you should say, you said everything.

Alexander Grosberg [402]

E

Published Manuscripts

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Review

Modeling the separation of macromolecules: A review of current computer simulation methods

Theory and numerical simulations play a major role in the development of improved and novel separation methods. In some cases, computer simulations predict counterintuitive effects that must be taken into account in order to properly optimize a device. In other cases, simulations allow the scientist to focus on a subset of important system parameters. Occasionally, simulations even generate entirely new separation ideas! In this article, we review the main simulation methods that are currently being used to model separation techniques of interest to the readers of *Electrophoresis*. In the first part of the article, we provide a brief description of the numerical models themselves, starting with molecular methods and then moving towards more efficient coarse-grained approaches. In the second part, we briefly examine nine separation problems and some of the methods used to model them. We conclude with a short discussion of some notoriously hard-to-model separation problems and a description of some of the available simulation software packages.

Keywords:

Computer simulations / Electrophoresis / Microfluidics / Modeling / Separation methods
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1 Introduction

Computers are getting cheaper and more powerful every year. At the same time, separation systems are getting smaller and faster. The convergence of these two trends has led to a situation where it is now possible to simulate the key parts of some separation systems at the molecular level. An example of this is the atomistic simulation of the translocation of an ssDNA molecule through a nanopore [1], a process that may lead to the \$1000 genome sought by the NIH in the USA.

Transport-based separation systems generally represent a compromise between the physical separation of several molecular species (*e.g.* due to their different velocities in the

device) and their spatial spreading due to various diffusion-related processes. Since most separation devices employ sieving, liquids and electric forces, modeling efforts must generally include long-range electrostatic forces, long-range hydrodynamic forces, frictional and diffusion contributions, conformational effects (for macromolecules), entropic factors, gradients of various types, and interactions with surfaces and obstacles. It is the role of the theoretician to reduce the number of factors to a bare minimum in order to design models that can be solved either analytically or numerically. For example, long-ranged hydrodynamic interactions (HI) are often neglected in the case of gel-based separations.

Simulating complex processes using numerical models that include various levels of detail is now widely seen as the third approach to scientific discovery, complementing the well-established experimental and theoretical methods. Computer simulations are more than mere attempts at reproducing experimental results. Because we have full control over the simulation parameters, and because we can measure every conceivable property (including correlations between properties) of a system during a numerical experiment, simulations allow detailed autopsies and diagnostics not normally achievable in a laboratory. They also allow us to explore numerous systems and geometries at relatively low cost.

Historically, several discoveries were first made on a computer, *i.e.* *in silico*. In the field of electrophoresis, one of us (G.W.S.) and his co-workers [2] first discovered the phenomenon of DNA band inversion using a computer simulation of the biased reptation model [3, 4]. Experiments

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Abbreviations: BD, Brownian dynamics; BFA, bond-fluctuation algorithm; DPD, dissipative particle dynamics; FENE, finitely extensible nonlinear elastic; HI, hydrodynamic interactions; LB, lattice Boltzmann; LD, Langevin dynamics; LJ, Lennard-Jones; LMC, lattice Monte Carlo; Mbp, megabase pairs; MC, Monte Carlo; MD, molecular dynamics; OMRC, Ogston–Morris–Rodbard–Chrambach; SRD, stochastic rotation dynamics; WCA, Weeks–Chandler–Andersen

later confirmed the existence of this counterintuitive phenomenon.

In view of the growing importance of computer simulations in the field of separation methods, we believe that this review is timely. Our hope is that it will be of equal interest to the experimentalist who wants to understand the relevant literature and use simulations to guide his/her laboratory work, to the theoretician who wants to explore novel systems using numerical methods, and finally to the computational scientist who wants to use new tools.

This review article has three main parts. In Section 2, we describe several approaches that are currently being used to model separation processes. We first examine methods that include molecular details, then describe algorithms that simplify the description of the liquid phase, and finish with several coarse-graining methods that generate even simpler numerical models. In Section 3, we review nine well-known problems and the simulation methods that are being used to study them. In Section 4, we look at the future and discuss a few problems that have proven hard to simulate and understand with the current methods. An Appendix then lists some of the most well-known software packages in the field; however, it is important to note that many (perhaps most) simulation studies have actually been based on simulation programs written by the researchers themselves. The choice of material is obviously that of the authors; we recognize that several important algorithms and problems are missing from this review. Among those, approaches based on the continuum description of the flow and the distribution of analytes [5, 6] and reptation models of gel electrophoresis [4, 7–12] are perhaps the most obvious.

2 Simulation methods

In this section we describe some of the most popular simulation techniques used in computational studies of electrophoretic separation methods. It is obviously impossible to review them all – as a matter of fact, new methods are proposed every year. In that light, we present some of the main methods in a way that we believe is both logical (from the more microscopic to the most simplified) and compact. For a more rigorous discussion on levels of coarse-graining see [13]. In our descriptions, we do not strive for the level of detail that would allow readers having no prior knowledge to do their own simulations. Rather, we present a bird's-eye view of the hierarchy of different computational methods. Correspondingly, the number of references is large as the interested reader will need to consult specialized texts in order to fully appreciate the intricacies of these simulation methods.

2.1 Molecular dynamics

The molecular dynamics (MD) technique is used to follow the evolution of a large number of interacting particles by

numerically integrating the classical equations of motion. Although MD is often used to simulate systems at the molecular level, it is also suitable for modeling larger-scale systems by implementing coarse-grained methodologies. Hence, it is a common approach to simulating polymer dynamics.

When performing MD simulations, the first step is to calculate the net force on each particle. Newton's second law $\mathbf{F} = m\mathbf{a} = m\ddot{\mathbf{r}}$ then provides the particle's acceleration. Numerically integrating by $\mathbf{v} = \mathbf{v}_0 + \mathbf{a}\Delta t$ yields the particle velocity at a short time Δt later; a second integration $\mathbf{x} = \mathbf{x}_0 + \mathbf{v}\Delta t$ gives its position (in practice, more accurate numerical integration techniques such as the velocity-Verlet algorithm [14, 15] are employed). After performing these calculations for each particle, the new configuration of the system at time $t + \Delta t$ is obtained. This process is repeated to generate trajectories for each particle that, together, comprise a series of snapshots describing the evolution of the system. For conceptual simplicity, we begin by describing a fully atomistic MD simulation (each atom is represented by one particle). However, as this is generally too computationally expensive, we subsequently introduce various techniques to “coarse-grain” the models and make MD a viable tool for studying separation methods. Although we will discuss only a few key topics, there are a great number of excellent books [14–17] and review articles [18–20] that can be used to explore the rich field of MD simulations in greater depth.

2.1.1 The force field

The interactions between the particles in the system obviously play a major role. The mathematical forms and parameters dictating these interactions are known as the “force field”; the key concepts used in this review are discussed below.

2.1.1.1 Lennard-Jones

The interaction between two free, uncharged atoms implies two primary effects. First, there is a short-ranged repulsion preventing overlap. Second, there is a long-range attraction arising from weak but favourable interactions due to induced dipole effects (dispersion forces). The Lennard-Jones (LJ) potential [21] is commonly used to model these effects:

$$U_{\text{LJ}}(r_{ij}) = 4\varepsilon_{ij} \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right] \quad (1)$$

where r_{ij} is the separation between the particles i and j , ε_{ij} is the depth of the potential well and σ is the effective size of the particle (see Fig. 1 for a plot of this potential). The numerical values used for ε and σ dictate the details of the interaction.

2.1.1.2 Coulombic forces

The other primary non-bonded interaction arises from the electrostatic interaction between pairs of particles given by

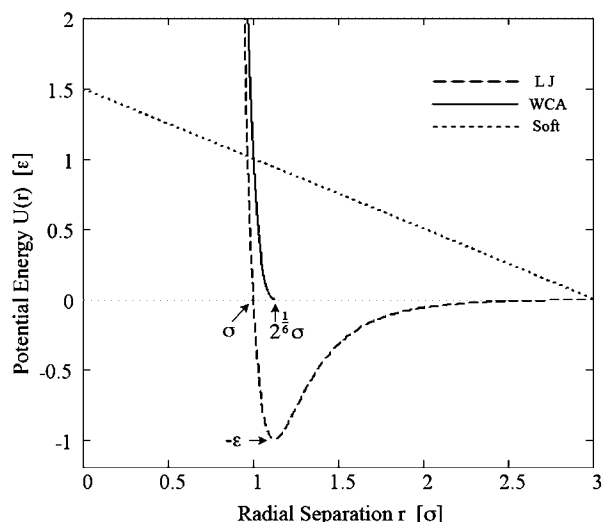


Figure 1. Plot of the full LJ potential, the purely repulsive WCA potential, and the soft potential common in DPD. The positive soft potential of DPD extends far further and avoids the singularity of the LJ and WCA potentials.

the Coulombic potential

$$U_{\text{Coul}}(r_{ij}) = \frac{1}{4\pi\epsilon_0} \frac{q_i q_j}{\epsilon r_{ij}}$$

Here, q_i and q_j are the effective charges on each particle, ϵ_0 is the permittivity of free space, and ϵ is the dielectric constant of the medium. In atomistic simulations with an explicit, polar solvent model, including ϵ is not necessary. However, for simulations with either implicit or non-polar solvent models (including the mesoscopic models common in coarse-grained simulations), ϵ is an effective dielectric constant that includes the screening effects due to the medium (*e.g.* $\epsilon = 80$ for water). Although the magnitude of the net charge is obvious for free ions, in molecules where charges are shared *via* bonds the value of the effective partial charges is a vital component of the force field. Unlike the LJ interaction that decays relatively quickly, the Coulombic interaction is long ranged. While a cut-off distance (beyond which contributions are not considered) is appropriate in one dimension, the long-ranged contributions are important in two or three dimensions. For this reason, inclusion of electrostatic effects can be computationally expensive and many sophisticated techniques have been developed to address this particular problem. For a review of many of these methods see [22, 23]; further methods are discussed in [24, 25].

2.1.1.3 Bonded interactions

In MD simulations, elements of chemical bonds are captured by implementing potentials to maintain bond lengths and bond angles. Although other forms are used, a common choice for both is a harmonic potential such that the bond stretching $U_{\text{BS}}(r_{ij})$ and bond bending $U_{\text{BB}}(\theta_{ijk})$

potentials are given by

$$U_{\text{BS}}(r_{ij}) = \frac{1}{2} k_{ij} (r_{ij} - r_0)^2 \quad (2)$$

$$U_{\text{BB}}(\theta_{ijk}) = \frac{1}{2} k_{ijk} (\theta_{ijk} - \theta_0)^2 \quad (3)$$

Here, k_{ij} and k_{ijk} are force constants, θ_{ijk} is the angle formed by the bonds joining atoms i, j, k , and r_0 and θ_0 are the equilibrium separation and bond angle respectively. Hence, in this model, atoms are bonded together *via* Hookean springs while the bond angles oscillate around the equilibrium value. In atomistic simulations, the various parameters are an essential part of the force field as they dictate the details of these interactions. Note that while we discuss only bond stretching and bending here, terms can be added to model effects such as restrictions to torsional angles and *cis versus trans* configurations [26].

2.1.2 Coarse graining

A fully atomistic MD simulation is quite detailed in the features it replicates and is appropriate to study dynamics on the nanosecond and nanometer scales. To probe larger systems on longer time scales, the use of coarse-graining techniques is essential.

2.1.2.1 United atoms

The first step in coarse-graining a system is to lump groups of atoms together and simulate them as a single “particle”. For example, in atomistic MD simulations of proteins, it is common to group the hydrogens of amino acid side chain carbons in with the carbon to form a united atom. Reducing the number of particles results in reduced computing time while preserving the dynamics of interest. One can extend this idea and model entire side chains (or even entire monomers) as single particles. From this, we can then simulate any polymer as a string of bonded generic beads – each of which represents a monomer. Of course, the interaction between adjacent beads is vital; such local effects can be incorporated by using, *e.g.* a potential between adjacent bonds in the chain to give a finite stiffness to the backbone (for example, the bond angle potential discussed above [27]).

We can extend the level of coarse-graining even further by lumping polymer beads together and simulating n monomers as a single particle that now represents a subchain of the polymer. If n is large enough such that the length of the subchain is equal to or exceeds the Kuhn length (a measure of the stiffness of the polymer chain), correlations between subchains are negligible and we can simulate the coarse-grained polymer as a freely jointed chain [28]. This is a standard model for studying a polymer *via* MD simulations as it allows for computational simplicity and efficiency while preserving the dynamics of interest. What is lost is the direct correspondence to the system in study and hence an extra layer of abstraction between simulation and reality is introduced.

2.1.2.2 The WCA potential

In coarse-grained simulations, it is common to use the purely repulsive Weeks–Chandler–Andersen (WCA) potential (also known as the truncated LJ potential) to model particle interactions [29]. This is an LJ potential that is shifted and truncated at its minimum value (see Fig. 1) so that it ends smoothly at a distance $r_c = 2^{1/6}\sigma$:

$$U_{\text{WCA}}(r_{ij}) = \begin{cases} 4\varepsilon_{ij} \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right] + \varepsilon_{ij} & \text{for } r_{ij} < r_c \\ 0 & \text{for } r_{ij} \geq r_c \end{cases} \quad (4)$$

However, the standard LJ interaction is often used to study systems with varying affinities between different types of particles (this is implemented by varying the ε_{ij} 's).

2.1.2.3 Finitely extensible bonds

In a coarse-grained model, the use of harmonic potentials to connect adjacent beads can lead to unphysical bond stretching effects. For this reason, a finitely extensible force is needed such that there is a hard limit on the bond length. The most common choice is the finitely extensible nonlinear elastic (FENE) potential:

$$U_{\text{FENE}} = -\frac{1}{2}kr_0^2 \ln \left(1 - \frac{r^2}{r_0^2} \right) \quad (5)$$

Here, k indicates the stiffness of the bond and r_0 gives the maximum bond length (the potential shoots to infinity as r approaches r_0). For appropriate values of k and r_0 , the occurrence of “bond crossing” is extremely rare and hence behaviour appropriate for a self-avoiding polymer is produced [30]. It is interesting to note that this form is an approximate solution to the inverse Langevin function; in some cases, it may thus account for the entropic restoring force, which arises from stretching a subchain [28].

2.1.3 Simulating a polymer

Putting the above together, the simplest model of a polymer in a coarse-grained simulation is a freely jointed chain of beads (which can either carry a net charge or be neutral) interacting by the WCA potential (or the LJ potential when short range attractions are desired) and with immediately neighbouring monomers bonded *via* the FENE potential. Extension to branched polymers is trivial. Although we have sacrificed many details, we have preserved effects such as excluded volume, the entropic elasticity, and the non-crossability of bonds. Although this model may seem very crude, it does allow for a realistic scaling behaviour of polymeric properties such as the radius of gyration and the diffusion coefficient with respect to the degree of polymerization. This approach also represents the most realistic model of polymers for which experimentally relevant simulations are feasible. Note that although this “bead-spring” model is a common approach, other models can be employed. For example, algorithms can be implemented to

maintain the bond distance at a fixed length [31, 32] resulting in a “bead-rod” model (or a “pearl-necklace” model for the case of the rod length being comparable to the diameter of the bead) [33].

Now that we have a model for the polymer, we can add other components. For example, a common scenario involves simulation of polyelectrolytes with free ions. To accomplish this, ions can be defined as WCA particles carrying a net charge. Additionally, one can include features such as obstacles or boundaries (*e.g.* walls) by building them out of particles or defining them using mathematical constraints. The final, and critical, component of the simulation system is the solvent. As the model used for the fluid impacts both the dynamics that are observed and the computational time, Sections 2.2–2.4 will focus on various ways of treating the fluid.

At this point, it is important to mention that choosing a method of controlling the temperature is often a crucial consideration in MD simulations; in fact, it is also one that is often intimately connected to the choice of solvent model. Implementing a thermostat is particularly critical when an external field is adding energy to the system – as in electrophoresis. Although there are schemes based on rescaling the velocities [34] or periodically assigning random velocities [35], such algorithms can lead to artefacts as local momentum is not conserved (a particular problem when hydrodynamics are of interest [36]). In coarse-grained simulations, the method chosen for treating the fluid often involves a particular temperature control scheme *via* the coupling of the analyte (*e.g.* the polyelectrolyte) to the fluid. An important case is the thermostat developed for dissipative particle dynamics (DPD) (Section 2.3.1), which can be used independently of the fluid model and, for several reasons, has been a significant advancement in the field [37]. Finally, in the case of performing Langevin dynamics (LD) or Brownian dynamics (BD), the temperature is explicitly in the equation of motion (Section 2.4). Although discussing the details of these algorithms (and others [38–41]) is beyond the scope of this review, the interested reader is encouraged to investigate these various schemes starting with the citations given and the discussion of solvent models in the following sections.

2.2. Explicit fluid

At the atomistic level, many models exist that explicitly describe a water molecule [42–45]. In fact, developing appropriate models for simulating water alone is the subject of active research. In coarse-grained simulations of solvents, we group atoms, and even molecules, together and simulate them as a single bead. The use of the WCA potential for this solution of beads is found to provide a good solvent, regardless of temperature [46]. The advantage of using an explicit fluid is that it is conceptually obvious and preserves the long-range HI that correlate the movement of objects in a fluid. However, in practice, this results in much of the simulation time being dedicated to calculating the details of

the fluid bead dynamics, which are often not of interest. For these reasons, several techniques for modeling the fluid to maintain the HI while neglecting the computationally costly details of the fluid motion have been developed. These models are discussed in the following section.

2.3 Mesoscopic fluid

It is possible to describe hydrodynamic effects without resorting to an explicit description of fluid molecules or the discretization of the continuous Navier–Stokes equations. In particular, a clever use of conservation laws allows mesoscopic methods to employ local algorithms that recover the solution to the hydrodynamics equations in the large-scale/long-time limit while at the same time bypassing the molecular details of the fluid and thus dramatically reducing the computational cost of the simulation. In the following sections we will review three mesoscopic approaches, namely DPD, stochastic rotation dynamics (SRD) and lattice Boltzmann (LB).

Following the description of these mesoscopic methods, Section 2.4 will introduce LD and BD. A comparison of the different methods discussed ensues in Section 2.5 where a schematic is presented to summarize each of the methods.

2.3.1 Dissipative particle dynamics

In DPD, the fluid is modelled by large particles interacting *via* a *soft* potential [47–49]. This allows for a reduction in computing time in two ways. First, as each DPD fluid bead represents a cluster of fluid molecules moving together in a coherent manner, the simulation tracks a much lower number of interacting objects. Second, since with a soft potential the forces cannot be arbitrarily large, we can reduce computing times by increasing the integration time step. All particles interact by three forces: a conservative force $\mathbf{F}^C$, a dissipative force $\mathbf{F}^D$, and a random force $\mathbf{F}^R$:

$$\mathbf{F}_i = \sum_{i \neq j} \left(\mathbf{F}_{ij}^C + \mathbf{F}_{ij}^D + \mathbf{F}_{ij}^R \right) \quad (6)$$

where it is assumed that the interactions are negligible beyond a cut-off radius r_c . Pairwise potentials ensure that momentum and angular momentum are conserved. The force $\mathbf{F}_{ij}^C$ represents and can include any conservative forces that act on the particle. A common choice is a soft repulsion (see Fig. 1) acting along the line of centres such as

$$\mathbf{F}_{ij}^C = \begin{cases} a_{ij}(r_c - r_{ij})\hat{\mathbf{r}}_{ij} & \text{for } r_{ij} < r_c \\ 0 & \text{for } r_{ij} \geq r_c \end{cases} \quad (7)$$

where a_{ij} parametrizes the maximum repulsion. The dissipative force is an inter-drag force between a pair of soft fluid particles moving through each other opposing their relative motion $\mathbf{u}_{ij}$ and dissipating heat:

$$\mathbf{F}_{ij}^D = -\omega^D(r_{ij})\zeta(\hat{\mathbf{r}}_{ij} \cdot \mathbf{u}_{ij})\hat{\mathbf{r}}_{ij} \quad (8)$$

where ζ is the friction constant between the two clusters. The random noise force is given by

$$\mathbf{F}_{ij}^R = \omega^R(r_{ij})\sqrt{2\zeta k_B T} \frac{\gamma_{ij}}{\sqrt{\Delta t}} \hat{\mathbf{r}}_{ij} \quad (9)$$

where γ_{ij} is a random number of zero mean and unit standard deviation. To satisfy the fluctuation–dissipation theorem, the dissipative and random forces are interrelated through the weight functions, as $\omega^D(r) = [\omega^R(r)]^2$.

The random and dissipative forces act as a source and a sink for heat, respectively. Therefore, DPD, unlike BD or LD is an implicit thermostat [37, 50] that conserves linear and angular momentum and thus recovers hydrodynamics in the macroscopic limit. It should be noted that DPD does not conserve total energy, but only mass and momentum. Solutes can be included as DPD beads and by the inclusion of a bead–spring type force in Eq. (6), DPD can simulate polymers as well [50, 51]. In fact, DPD can be used as an effective thermostat that conserves HI, independently of the fluid model [37].

2.3.2 Stochastic rotation dynamics

The DPD beads represent clusters of many particles but all bead–bead interactions must still be evaluated. In SRD [52–54], also called multiparticle collision dynamics [55, 56] or real-coded lattice gas [57, 58], collisions between fluid particles are replaced by multiparticle collision events that omit the molecular details and eliminate the need to calculate the forces between the fluid particles. These events are defined to conserve mass, momentum, and energy such that the hydrodynamic equations of motion are obeyed on sufficiently long length and time scales [59]. SRD simulations occur in two steps. During the first, or *streaming*, step, the particles move ballistically, and their positions $\mathbf{r}_i(t)$ are updated in discrete time intervals δt :

$$\mathbf{r}_i(t + \delta t) = \mathbf{r}_i(t) + \mathbf{v}_i(t)\delta t \quad (10)$$

The second, or collision, step transfers momentum between particles. The simulation domain is partitioned into cells. The number of particles in each cell may vary from one cell to another but the total number is conserved. Each cell has a centre of mass velocity $\mathbf{v}_{CM}$, which corresponds to the local macroscopic velocity. The collision step is a simple non-physical scheme that is constructed to conserve momentum. Multiparticle collisions within each cell are represented by the operation

$$\mathbf{v}_i(t + \delta t) = \mathbf{v}_{CM}(t) + \mathbf{R}(\mathbf{v}_i(t) - \mathbf{v}_{CM}(t)) \quad (11)$$

By making the collision operator, $\mathbf{R}$, a rotation through an angle α about a randomly chosen axis, conservation of energy, isotropy and a Maxwell–Boltzmann velocity distribution are met in the continuum limit. Other choices allow SRD to operate as a thermostat as well [60]. Unfortunately, Galilean invariance is broken by the discretization of space into cells. However, this can be completely remedied by performing the collision operation in a cell grid, which is shifted each time step by a random vector [61, 62].

When integrating SRD into a standard MD simulation the solute particles can be coupled to the momentum of the fluid by including them in the SRD collision step (Eq. 11) [55, 56, 63–65]. In typical simulations of a polymer, on the order of 10^2 MD time steps are performed between SRD collision events [54, 66–68]. This separation of time scales ensures that the momentum transferred to the polymer during the collision step is well distributed throughout the chain [69]. Another common scheme uses a hybrid SRD/MD approach in which not only the solute–solute but also the solute–solvent interactions are handled by an MD algorithm, while solvent–solvent interaction is simulated by SRD [53, 70, 71].

2.3.3 Lattice Boltzmann method

LB coarse-grained models [54, 72, 73] are based on a solution of the discretized Boltzmann transport equation. The main quantity in the LB approach is the velocity field, rather than fluid particles, and it is an inherently statistical approach, where discrete momentum distributions are represented on a spatial grid.

The discretization of positions and momenta using finite sets of directions greatly simplifies the problem. The most frequent mesh types for the LB simulations are the D2Q9, D3Q15, and D3Q19 lattices, where $DkQn$ refers to the number k of dimensions and to the discrete number n of velocity vectors, $\mathbf{e}_i$.

A set of distribution functions $\Gamma_i(\mathbf{r}, t)$ is defined on each lattice site $\mathbf{r}$. Each of these can be interpreted as the fraction of fluid that will move with the i th discretized velocity at time t . The discretized Boltzmann equation provides a generic description for the time evolution of the probability density, but there is freedom in the choice for the actual form of the collision integral. A common formulation is the Bhatnagar–Gross–Krook approximation, where

$$\Gamma_i(\mathbf{r} + \mathbf{e}_i \Delta t, t + \Delta t) = \Gamma_i(\mathbf{r}, t) - \frac{\Delta t}{\tau} (\Gamma_i(\mathbf{r}, t) - \Gamma_i^{\text{EQ}}(\mathbf{r}, t)) \quad (12)$$

where τ is the phenomenological relaxation time, which prescribes the timescale for the relaxation of the actual population Γ_i to the equilibrium particle distribution function Γ_i^{EQ} . In the low velocity approximation, Γ_i^{EQ} can be expressed as

$$\Gamma_i^{\text{EQ}} = w_i \rho \left[1 + \frac{\mathbf{e}_i \cdot \mathbf{u}}{c_s^2} + \frac{(\mathbf{e}_i \cdot \mathbf{u})^2}{2c_s^4} - \frac{u^2}{2c_s^2} \right] \quad (13)$$

where ρ is the hydrodynamic density and c_s is the speed of sound, which is determined by mesh properties. The weights, w_i , must be suitably chosen to recover the macroscopic Navier–Stokes equations and are dependent on the mesh configuration [54]. The viscosity of the LB fluid is determined by the choice of the relaxation rate.

LB can be coupled to small suspended spheres by treating them as point particles that interact with the fluid

through a friction force proportional to the relative velocity obtained *via* linear interpolation from the surrounding lattice sites [74, 75]. By adding fluctuation terms to both the fluid and the embedded particles, LB can operate as an adequate thermostat [74]. The correct treatment of a fluctuating LB algorithm has been recently addressed in several papers [76, 77].

2.4 Langevin and Brownian dynamics

Going beyond mesoscopic models, further coarse graining can be achieved by avoiding direct simulation of the fluid altogether. The timescale of individual collisions with solvent molecules (causing frictional drag and Brownian motion) is much smaller than the time scales relevant to electrophoresis. It is computationally advantageous to coarse grain out the fine details of the collisions and simply include their statistical effect on the solute. One may consider implicitly the two main effects of the fluid acting on the particle: (i) a frictional force opposing its motion and (ii) random kicks arising from collisions with the solvent. The frictional (or dissipative) force $\mathbf{F}^D(t)$ removes energy from the particle while the fluctuating Brownian force $\mathbf{F}^B(t)$ adds energy to the particle. Hence, at this coarse-grained level, the fluid is included solely in a statistical manner governed by the fluctuation-dissipation theorem. By replacing the explicit fluid with a drag and a Brownian force, we lose the long-range particle–particle interactions mediated by the fluid. This makes such an approach particularly tempting when the HI are negligible or not of primary concern. Nevertheless, we will see how they can still be included.

2.4.1 Pure Langevin and Brownian dynamics

In LDs, starting with Newton's second law as we did for pure MD, we now add a dissipative drag force $\mathbf{F}^D(t)$ and a Brownian force $\mathbf{F}^B(t)$ (in addition to the conservative forces we had before, $\mathbf{F}^C(t)$) to end up with Langevin's equation

$$m\mathbf{a}(t) = m\ddot{\mathbf{r}}(t) = \mathbf{F}^C(t) + \mathbf{F}^D(t) + \mathbf{F}^B(t) \quad (14)$$

where m is the mass of the particle. For the dissipative term, one usually assumes Stokesian drag on a spherical particle, $\mathbf{F}^D(t) = -\zeta \mathbf{v}(t)$, ζ being the friction coefficient of the particle in the fluid. The velocity $\mathbf{v}(t)$ is then the velocity of the particle with respect to the local solvent velocity. This is an important detail if one wants to consider flow or long-range HI. Although $\mathbf{F}^B(t)$ is due to the solvent molecules colliding with the particle, it can only model the net effect of a large number of collisions. The Brownian force is taken as a centred Gaussian random variable [78] with zero mean and variance $2\zeta k_B T / \Delta t$, where Δt is the integration time step. The fact that the variance is related to the friction coefficient ζ is again a consequence of the fluctuation–dissipation theorem. On the time scale of

interest the values of the Brownian force are uncorrelated at different time steps.

It can be shown that the energy transferred to the particle from a single collision with a solvent molecule decays on the viscous time scale m/ζ [79]. If, as is typically the case, this is much smaller than the timescale over which $\mathbf{F}^C(t)$ changes (overdamped limit), we may set $m\mathbf{a}(t) = 0$ in the Langevin equation and obtain the following discretized equation of motion:

$$\mathbf{r}(t + \Delta t) = \mathbf{r}(t) + \frac{\Delta t}{\zeta} [\mathbf{F}^B(t) + \mathbf{F}^C(t)] \quad (15)$$

which defines BD. For a more rigorous derivation see [80].

2.4.2 Incorporating long-range hydrodynamic interactions

Traditionally, LD does not include HI between particles. This approximation is valid for certain systems (*e.g.* when HI are screened out), but in many cases HI have a significant impact on the dynamics [81].

To see how we can incorporate them, let us consider a particle i . As it is moving with a certain velocity, it slows down due to the drag force. Since the drag force exerted by the fluid on a particle must be equal and opposite in direction to the force exerted by the particle on the fluid, this causes the fluid to move. Hence, the effect particle i has on particle j is that the latter experiences a modified drag as it is no longer surrounded by a stationary fluid. The magnitude of this coupling depends on their relative separations. On the time scale of interest this perturbation can generally be considered to be felt instantaneously. In a many-particle system, a common approximation is to ignore screening issues and to consider a superposition of all pairwise HI [82].

Consideration of the fluctuation–dissipation theorem implies that any modulation of the drag term must be accompanied by correctly modifying the magnitude of the Brownian term. An interesting consequence is the correlation of the Brownian forces on different particles at the same time. In practice, the relationship between the drag and the Brownian term is determined *via* interaction tensors (mainly the Oseen or Rotne–Prager–Yamakawa tensor). Application details can be found in [79, 80, 82–87].

2.5 Comparison of fluid models

We have presented a hierarchy of fluid models from the most detailed explicit solvent models to the mesoscopic and, finally, implicit (LD and BD) approaches. Using explicit models (especially the atomistic variant) is essential when high accuracy and the chemical details (such as different hydrophobicities of different parts of the analyte) are required. In most other cases, simpler approaches should be used. All three mesoscopic methods (DPD, SRD, and LB) use a simple (but often sufficient)

model to describe fluid dynamics and can also act as thermostats that define the local temperature when coupled to MD particles.

Owing to their conceptual differences, these methods use different types of parameters to describe the fluid, which results in a different suitability for specific problems. For example, while the fluid viscosity is directly accessible in LB methods, it becomes a combination of different parameters and can be controlled only indirectly in DPD [88, 89]. Similarly, while DPD and LB can only approximate the continuous-time dynamics of the fluid when the discrete time step is small, SRD is proven to yield correct long-time hydrodynamics for any step size. However, SRD's transport properties depend explicitly on the chosen time step [59]. More differences between the methods arise if confined fluids or interactions with large obstacles or particles are studied. Here, the ability to treat different boundary conditions becomes important, which is covered in detail in the literature [53, 63, 90–94].

All three methods share a similar computational efficiency, and computation times depend mainly on the implementation, the computer system, and also the investigated system. However, the speedup over explicit fluid simulations can be a factor 20 or higher [74, 95].

These mesoscopic models describe compressible fluids in which hydrodynamic forces need time to propagate through the medium. For this reason, as well as considerations of computational speed, performing LD with proper inclusion of HI can be preferable in some cases [96]. Furthermore, if the full treatment of HI is not necessary, the use of pure LD or BD is advisable.

We summarize the different ways of treating the solvent in MD simulations with a schematic that depicts the most important features of different fluid models (Fig. 2).

2.6 Monte Carlo simulations

In addition to the coarse-graining introduced previously, it is reasonable to ask whether it is possible to also coarse-grain the dynamics itself in order to achieve further computational speedup. This is the motivation behind the development of the various Monte Carlo (MC) methods.

In the broadest sense of the term, any computational method involving randomness can be called an MC method (the name says it all!). This broad definition would, however, include, *e.g.* BD, already considered under the banner of MD. In the spirit of the preceding discussion, we would therefore call an MC method any approach that sacrifices at least some of the dynamical details of the MD methods. How much is sacrificed can vary. At one extreme are methods designed to study equilibrium properties by quickly exploring large parts of the phase space. In this case, unphysical moves, such as rotations of large parts of chains in the pivot algorithm [98], can be introduced on purpose to speed up the simulation. Since in simulations of electrophoresis and related separation methods we are primarily interested in the dynamics of the

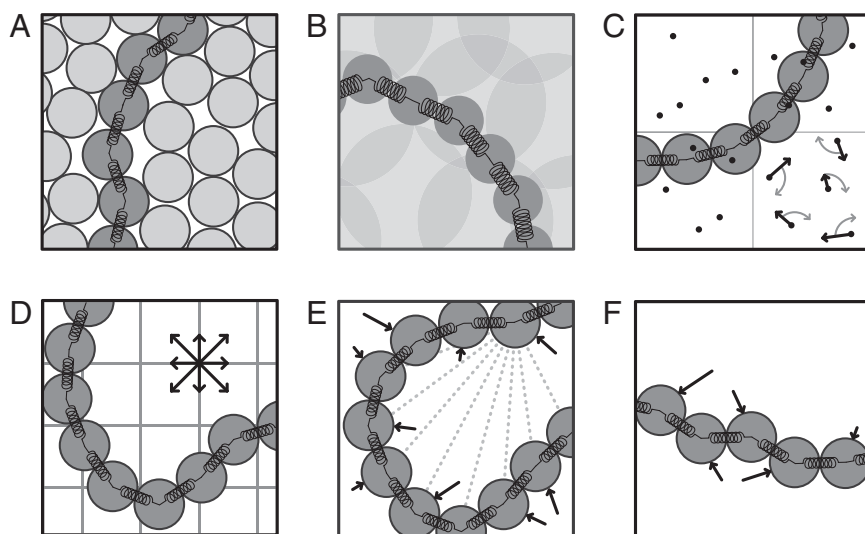


Figure 2. Schematic depicting different methods of modeling the solvent in MD simulations. In each case, a representative polymer is shown as a chain of beads connected by springs. (A) The explicit fluid approach models the solvent as beads interacting through a short-range potential and simulated to the same level of detail as the polymer. Fluid particles are often chosen to be the same size as the monomers. (B) The DPD method typically models the solvent as large, overlapping, softly repulsive spheres. (C) The SRD method replaces collisions between particles by a multiparticle collision operator, which models the local collisions as a rotation of particle velocity through an angle about a randomly chosen axis. (D) In LB the fluid is discretized to a grid. Arrows symbolize the discrete set of allowed velocities. The mesh shown is a D2Q9 (eight velocity vectors plus the zero velocity in two dimensions). (E) LD/BD represents the fluid implicitly by including a friction and a random term in the equation of motion of each monomer. HI can be included by introducing an additional long-range force between monomers (illustrated for one bead as a series of dashed lines). (F) Pure LD/BD neglects HI — friction and Brownian forces are the only effects of the solvent that are included. After Padding and Louis [97].

analytes, such MC approaches are less useful for our purposes, although they can be applied to some auxiliary problems, such as generating an equilibrated entangled sieving polymer solution in which the analytes would then migrate, studying the properties of capillary coatings, or finding the free energy of a DNA molecule as a function of its coordinate along a nanopore. We do not discuss these methods here; they are reviewed, *e.g.* in [99–101]. Instead, we concentrate on dynamical MC algorithms.

MC methods can be divided into two groups. In off-lattice MC methods, particles and monomers of polymer chains can occupy any positions in space. But one can also discretize the space allowing the particles to reside only at the nodes of a lattice. Such methods are known as Lattice MC (LMC). Since the sets of possible configurations and moves are then discrete, they can be described using integer arithmetic and often special computational techniques, such as multispin coding ([102], Section 15), which allow significant savings in computer time and memory compared with off-lattice MC. Of course, the cost of increased efficiency in LMC is even less realism!

Our ideal goal when simulating electrophoresis problems is to make quantitative predictions for the mobility and diffusivity of the analyte. Although this is possible in simple cases, MC simulations are generally used to predict trends (including scaling laws) and qualitative features, which in many cases is quite acceptable.

We start our discussion of MC methods with approaches to simulating hard particles. We then discuss some

chain simulation methods. As discussed in Section 1, we will not describe the MC methods used to simulate DNA reptation models [103].

2.6.1 Methods for particles

Consider a particle undergoing Brownian motion in the presence of an external force (*e.g.* an electric field) and some obstacles (*e.g.* gel fibres). In MC, this becomes a simple biased random walk. At each time step, a move is randomly selected from a predefined set, and a test is used to accept or reject it. A simulation is simply a series of such moves. Different MC algorithms are defined by: (i) the set of moves and the probability of selecting a particular move from that set; (ii) the acceptance test for the selected move; and (iii) the time step *per* move. Although the physics behind these three elements can be subtle (the main issue is generally the definition of the time scale), the simulation itself is often quite simple.

The most popular MC approach is Metropolis MC [104], a method designed to study equilibrium configurations. In the simplest lattice variant, at each step the particle simply tries to move to any of the neighbouring sites on the lattice, and all possible moves can be selected with equal probability. Moves can be accepted or rejected. If the change in energy from the initial to the final configuration is negative ($\Delta U < 0$), the move is accepted; otherwise, it is accepted with probability $\exp(-\Delta U/k_B T)$ (the Boltzmann factor). If the move is rejected, the particle remains in its previous

position, but the clock is still advanced by one unit of time. This is known as the Metropolis test. The latter guarantees that the correct equilibrium properties are obtained. However, reproducing the correct dynamics is problematic. First of all, how the MC unit of time (an attempted move) is related to actual time is usually left undefined, which makes quantitative predictions difficult. Moreover, in a high field the method is even qualitatively wrong: once $|\Delta U|/k_B T \gg 1$, all steps along the field are accepted and all those against the field are rejected; as a result, the velocity saturates and the diffusion coefficient vanishes. A different approach is then required.

In the 2-D lattice variant of such an approach [105], the system is modelled as a square lattice, where each node is either free or is an impenetrable obstacle. At each time step, the particle can move in one of four directions $(\pm\hat{x}, \pm\hat{y})$. Unlike in the Metropolis algorithm, the probabilities of selecting each of these moves are no longer equal; instead, if an external force $\vec{F} = F\hat{x}$ is applied to the particle, they are given by [105]

$$p_{\pm x}(\varepsilon) = \frac{1}{(1 + e^{\mp 2\varepsilon})(1 + \tanh(\varepsilon)/\varepsilon)} \quad (16)$$

$$p_{\perp}(\varepsilon) = \frac{1}{2(1 + \varepsilon \coth(\varepsilon))}$$

where the scaled force ε is given by

$$\varepsilon = \frac{Fa}{2k_B T} \quad (17)$$

with a the mesh size of the lattice. Note that the algorithm is rejection-free in the absence of obstacles; however, moves leading to an overlap between the particle and an obstacle are rejected. The time step is no longer arbitrary as it is given by the expression

$$\tau(\varepsilon) = \frac{\tau_B}{1 + \varepsilon \coth(\varepsilon)} \quad (18)$$

where $\tau_B \equiv \tau(\varepsilon = 0)$ is the mean duration of a (Brownian) jump in the absence of an external field. The latter is directly related to the free-space diffusion coefficient D_0 :

$$\tau_B = \frac{a^2}{2D_0} \quad (19)$$

It is this relation that connects the MC and experimental times. The above choice of transition probabilities and the time step can be shown [105] to give the correct average velocities for fields of arbitrary strength. However, the dispersion coefficient is correct only in the limit of a vanishingly small field [106]. In this limit, the dispersion coefficient D can actually be obtained more efficiently by using the Nernst–Einstein relation:

$$D = \lim_{F \rightarrow 0} k_B T \mu(F) \quad (20)$$

where $\mu(F) = v(F)/F$ is the mobility. In a non-vanishing field, the correct dispersion coefficient can be obtained by varying the time step (making it a random number) [106]. If a constant time step is desired (as is the case for the

numerically exact algorithm that we describe next), the MC moves themselves must be modified [106, 107].

2.6.1.1 Exact calculation method

Standard MC methods require a large amount of simulation data in order to have a low statistical error. In recent years, our group has developed a numerical method that allows one to compute the exact mean velocity and diffusion coefficient of a particle moving on a lattice with impenetrable obstacles. This method, which basically gives the exact solution to the MC simulation, is both faster and more precise. We now show the basics of this approach. For further details, the reader can refer to [108, 109].

The first step is to obtain the transition matrix T whose elements T_{ij} are probabilities that a particle on site j jumps to site i in a single time step. If at time t , the probability of presence of the particle on site i is $n_i(t)$, then after a single time step,

$$n_i(t + \tau) = \sum_j T_{ij} n_j(t) \quad (21)$$

Implicitly, this assumes that the time step is unique; since the latter depends on the field intensity, the method works only in a uniform field (unless the field is so weak that the field dependence of $\tau(\varepsilon)$ can be neglected). The steady state, defined by the equality $n_i(t + \tau) = n_i(t) \equiv n_i$, is thus the normalized eigenvector of T with the eigenvalue of unity (the normalization condition is $\sum_i n_i = 1$). This eigenvector, which can be obtained with an arbitrary precision by a simple numerical calculation, is the exact solution of the LMC algorithm.

Once the steady-state occupation probabilities $n_i(\varepsilon)$ are computed, the mean velocity of the particle, $v(\varepsilon)$, can be obtained by averaging over all sites, $v(\varepsilon) = \sum_i n_i(\varepsilon) v_i(\varepsilon)$, where the average local velocity on site i is

$$v_i(\varepsilon) = \frac{p_{+x}(\varepsilon)L_+(i) - p_{-x}(\varepsilon)L_-(i)}{\tau(\varepsilon)} \quad (22)$$

with the displacements $L_{\pm} = a$ if there is no obstacle in the given direction and zero otherwise.

The dispersion coefficient D in the zero-field limit can be calculated using Eq. (20). In a non-vanishing field, D can be obtained with a numerically exact method based on the generalized Taylor–Aris dispersion theory [110].

2.6.2 Methods for chains

Simulating polymer chains presents additional challenges since connectivity and (often) non-crossability of chains have to be maintained during the simulation. In MC simulations of polymer chain dynamics, only one monomer (or, at most, a small local group of monomers) is moved at each step. This choice is necessary in order to keep the frequency of moves leading to overlaps sufficiently low. However, a unit of time should now correspond to one attempted move per monomer.

2.6.2.1 Off-lattice methods

In the most straightforward and commonly used off-lattice MC method for bead–spring chains [111], a single step consists in displacing a monomer chosen at random along each axis, by an amount chosen from the uniform distribution on $[-\Delta/2, +\Delta/2]$, where Δ is a predefined constant. The event is accepted or rejected according to the Metropolis criterion, but all attempts make the clock advance. If the arbitrary parameter Δ is too small, the evolution of the system may be too slow; if Δ is too large, the rejection rate is too high. The optimal Δ normally corresponds to an acceptance rate of about 50%, although a lower Δ can make the dynamics more realistic. The same potentials as for MD (e.g. the FENE and WCA potentials) can be used. Besides this, one can use much simpler potentials, such as a square-well bond potential that is zero within a specified range and infinity outside, with the range chosen (just as in FENE) to avoid chain crossings. This improves the efficiency as the computation of complicated potential functions is avoided. However, in this case the chain will have no tension and so square-well potentials should be avoided in strong fields (see below).

In the bead–rod model [112], the bond lengths have to be preserved explicitly. The simplest moves are then rotations of a monomer around the axis connecting its two neighbours (for an end monomer, the rotation is on the sphere with the centre at its neighbour). Note, though, that in a strong electric field, when the chain is stretched, such moves are rather inefficient and this may lead to unphysical artefacts.

2.6.2.2 Bond-length-preserving lattice methods

In lattice models of polymers, monomers still hop between lattice sites, like in single-particle models. To avoid introducing a bond potential and still make sure that neighbouring monomers remain close in space, only those motions that keep all bond lengths within a certain range are allowed. The first models were particularly restrictive in this respect, keeping all bond lengths strictly fixed. Verdier and Stockmayer [113] have used a simple cubic lattice requiring the polymer bonds to coincide with the lattice bonds and thus making them all of unit length. In the first version of the model, only single-monomer motions were allowed. This was later found too restrictive and various two-monomer motions were added, the most popular of which is the so-called crankshaft motion [114]. Unfortunately, the dynamics in such models become very slow if we introduce excluded volume interactions by forbidding two monomers to reside on the same site. Moreover, Madras and Sokal [115] showed that for *any* model with *any* finite set of moves where neighbours along the chain remain neighbours on the lattice some configurations cannot be reached (*i.e.* the algorithm is non-ergodic). Nevertheless, in those cases where excluded volume interactions can be neglected, the Verdier–Stockmayer and other similar approaches can still be useful.

2.6.2.3 Bond-fluctuation algorithm

The deficiencies of the fixed bond length MC models led to the development of lattice methods with fluctuating bond lengths. The most popular one is the bond-fluctuation algorithm (BFA) first proposed by Carmesin and Kremer for 2-D problems [116] involving both linear and branched polymers. Although BFA is not strictly ergodic, its non-ergodicity problems can probably be neglected for all practical purposes. The algorithm was extended to 3-D by Deutsch and Binder [117].

In the BFA (Fig. 3), the monomer is represented by a 2×2 square (four lattice sites) in 2-D and a $2 \times 2 \times 2$ cube (eight lattice sites) in 3-D. A lattice site can be occupied by only one particle at a time. In 2-D, the bond lengths between connected monomers must be less than 4, while in 3-D bond lengths must be $\leq \sqrt{10}$, excluding $\sqrt{8}$. These simple conditions allow for a self-avoiding walk in which there is no crossing of bonds. Each attempted move consists of first picking a monomer at random and moving it by one lattice site along one of the lattice axes. As long as the new conformation does not create monomer overlaps or create a forbidden bond length the move is accepted. As before, a unit time corresponds to one attempted move *per* monomer.

For simulations of electrophoresis, obstacles can be placed at some lattice sites and the field is treated as in other models, by using the Metropolis test. In principle, other interactions, such as intra- and interchain interactions between monomers, can be included as well, but in most cases this is unnecessary (an exception is for the proper modeling of polymer stiffness, an important factor for DNA simulations).

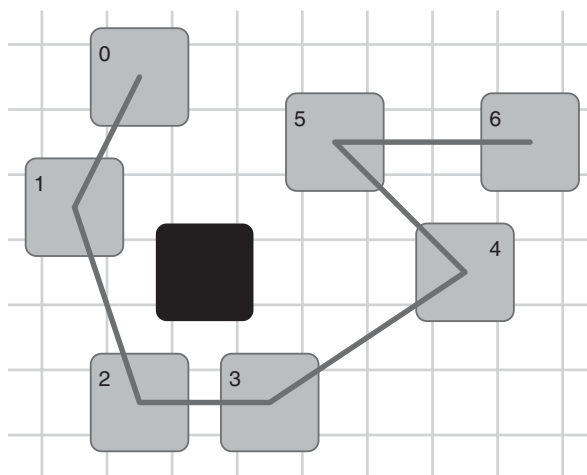


Figure 3. A polymer chain in the 2-D BFA. Each gray square represents a monomer occupying 2×2 sites. All allowed bond lengths from 2 (between monomers 2 and 3) to $\sqrt{13}$ (between monomers 3 and 4) are present in this figure. Allowing only these bond lengths and forbidding the monomers to overlap with the obstacle (black) is sufficient to ensure that the chain does not pass through the obstacle and does not cross itself. After Ref. [118]

2.6.2.4 Problems in strong fields

LMC models considered here have severe problems in strong electric fields. For instance, in gel electrophoresis simulations, the speed of the polymer decays exponentially as a function of the field, while experimentally, the electrophoretic mobility is essentially field-independent in the strong-field limit. To understand the reasons for this failure, consider a chain hooked upon a post with the two unequal arms pointing along the field and sliding off the post [119]. The sliding time is inversely proportional to the field intensity. But in LMC, since only local moves (*e.g.* single-monomer moves) are allowed, the only way for the chain to move is *via* chain slacks originating at the end of the short arm and propagating against the field. The probability for the slack to go all the way to the post decays exponentially with the potential energy difference between the tip of the arm and the post. If the short arm of the hooked conformation is of length L_s , the mean time between successful events will thus increase roughly like $\exp(+FL_s/kT)$, where F is the force on the monomer. This increase being an artefact of the model (the tension does not propagate along the backbone), the model can work only if the argument of this Boltzmann factor is much smaller than unity. Since L_s is proportional to the number of monomers in the chain, N , the maximum allowed external force scales like $F \propto N^{-1}$. This is too restrictive to be useful in practice. In order to solve this problem, one must modify the lattice models by adding *non-local moves*, an idea first implemented by Deutsch and Reger [119] and Duke and Viovy [11] for reptation models. Azuma and Takayama [120] added such moves to the BFA. Although their approach is not very carefully justified, it produces qualitatively reasonable results.

2.6.2.5 Numerically exact methods for chains

LMC algorithms for chains can sometimes be solved exactly, similar to how this is done for single particles. This was done by Boileau and Slater [121] for the BFA. The major complication is that each possible chain conformation and location should be considered a separate state and the number of such conformations grows exponentially with the chain length. For this reason, the approach is only practical for very short polymers (linear or branched). Of course, the solutions are only exact for the dynamics of specific algorithms, which are themselves approximate. We should also mention a numerically exact solution of the MC approach to studying polymer translocation through nanopores [122] that reduced this problem to a 1-D biased random walk by using a calculated dependence of the entropy of the chain on the number of monomers that have passed through the nanopore.

3 Simulation examples

In this section, we discuss nine current problems in the fields of separation science and electrophoresis. We examine

the systems by focusing on the type of simulation methods that are being used to study them. The choice of a numerical model is directly related to the question being asked. For example, coarse-grained methods are ideal for generic investigations of the basic mechanisms leading to separation while microscopic methods may be required when more specific problems are to be solved. To give the reader a broad view of the importance of simulations in our field, we have selected a wide range of problems.

3.1 EOF

EOF is ubiquitous in electrophoresis. EOF occurs when there is an electric field with a tangential component at a charged surface in contact with a liquid. The mobile counterions that make up the Debye layer next to the surface viscously drag the rest of the fluid. Although it is sometimes possible to solve for the fluid velocity profile analytically [123], computational modeling is often used to garner a better understanding of EOF. Let us examine the simple example shown in Fig. 4 where we can see the results of some MD simulations, which are described in detail by Tessier and Slater [124]. The solid line shows the net charge distribution, which has a maximum next to the wall followed by an

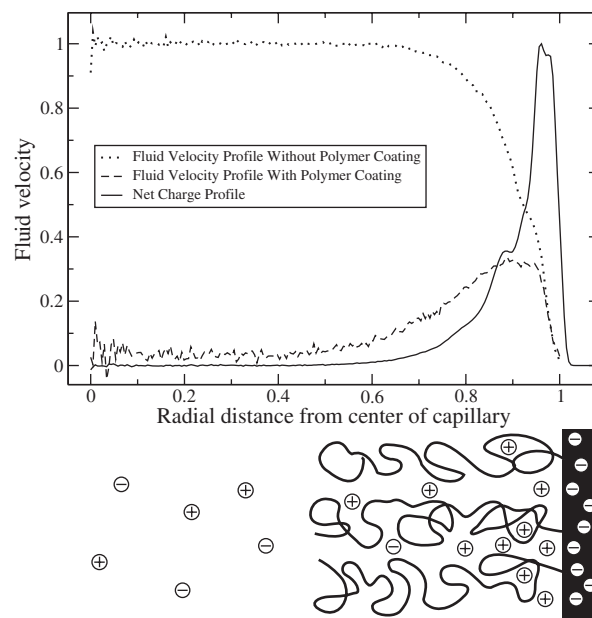


Figure 4. The radial profiles of the fluid velocity from MD simulations with and without a polymer coating, as well as the net charge density profile. The fluid velocities have been normalized by the bulk fluid speed in the coating-free case while the charge density has been normalized by its maximum value. The radial distance is in units of the inner radius of the tube. The fluid density is $0.8\sigma^{-3}$ while the bulk and surface charge densities are $0.02\sigma^{-3}$ and $0.1\sigma^{-2}$, respectively. The coating has a grafting density of $0.05\sigma^{-2}$ and degree of polymerization $N=20$. Further details can be found in [128, 124]. The schematic representation of the system is roughly aligned with the graph.

exponential decay as predicted by Debye–Hückel theory. The slight bump in this curve is the result of packing of the water beads near a fixed corrugated wall. This effect is even more evident if one looks at the individual ion density profiles. The fluid velocity (dotted curve) changes in the region where there is a net charge but takes on a bulk value outside of the thin charged region near the wall.

For microfluidic devices with complex geometries the EOF profile is often non-trivial to find. For the entropic trapping device developed by Han and Craighead [125], simulations were carried out using DPD with a slip boundary condition to reproduce a realistic EOF profile in order to include its effects in more complex simulations [126]. Similarly, it has been shown that the LB method can also be used to effectively model EOF [127]. The problem of EOF in complex geometries has also been investigated by solving the Navier–Stokes equation numerically [6]. In fact, the only fluid model presented in this review that has not been used for modeling EOF to our knowledge is the recently developed SRD algorithm.

In the context of electrophoretic separation, the presence of EOF often has a deleterious effect on the resolution. For example, the EOF increases dispersion in capillary electrophoresis because the EOF is non-uniform due to the non-uniform charge distribution on the wall [5, 124, 129]. For this reason, polymer coatings are often used to quench the EOF. They have the additional benefit of preventing wall–analyte interactions that cause additional dispersion in the system [130]. Simulations of polymer coatings tend to use a system size that is thicker than both the Debye layer and the polymer coating. Beyond the counterions and polymer coating the fluid velocity profile reaches a plateau. The fluid speed in this plateau region (referred to as the bulk speed) is the same regardless of the system size and thus these miniature (often nano-scale) simulation systems provide realistic models for much larger experimentally relevant systems.

Simulations of EOF in the presence of a polymer coating are fairly recent due to the high computational overhead involved in simulating them. Recent numerical investigations [124, 128] have been able to reproduce the scaling predictions of Harden *et al.* [131] for grafted polymer coatings using coarse-grained MD simulations. These studies looked in particular at the case when the polymer coating is thicker than the Debye length and looked at two regimes: the mushroom (isolated chains) and brush (high grafting density). In both regimes the MD simulations were able to confirm some of the predicted scaling behaviours of the bulk EOF with respect to the properties of the polymer layer such as the scaling with respect to the degree of polymerization N and grafting density. The dashed line in Fig. 4 shows a simulation where a polymer coating of length $N = 20$ beads and grafting density of $0.05\sigma^{-2}$ is used (here σ is the bead size in the WCA potential, Eq. (4)). Even these relatively short polymers clearly quench the majority of the EOF in the bulk of the fluid (note that in experiments the thin region near the wall where flow is generated makes up

only a very small fraction of the total system size). Simulations by Qiao and He [132] using the DPD algorithm investigated the same situation showing interesting nonlinearities in the EOF due to dynamic coupling between the polymer's conformation and the fluid velocity profile. The simulations also confirmed the fluid velocity profile as a function of the distance from the wall for a quenched polymer brush.

More detailed atomistic simulations by Qiao [133] have shown that for cases where the Debye layer is on the same scale as the polymer layer more complex behaviour can result. They found that at low grafting densities hydrophilic polymer coatings can actually increase the thickness of the Debye layer and thus the potential difference between the wall and the bulk fluid (termed the zeta potential) which increases the bulk EOF. This effect was attributed to a reduction in the amount of water in the region of the polymer layer, which caused the counterions to move further from the surface. At higher grafting densities, a larger suppression of EOF as the friction between the polymers and the fluid becomes larger was shown.

3.2 Free-flow electrophoresis

Free-flow electrophoresis is widely used to separate and characterize biomolecules. When a polyelectrolyte in gel-free solution is subject to a constant electric field, its average drift velocity depends not only on the applied field but also on interactions between the polyelectrolyte, the counterions and the solvent. Because the interrelation between the different forces is quite complex, free-solution electrophoresis is not easily accessible to complete analytical treatments. Results do exist for long-chain limits where certain simplifications are applicable [134–136] but experimental evidence indicates that these models are not sufficient to explain the behaviour of short chains [137–139].

In the schematic of the free-solution electrophoresis of flexible polyelectrolytes (Fig. 5), we see that the mobility is a function of length that approaches a constant with increased chain length so that separation of longer macromolecules by electrophoresis is not possible. This limit, called the free draining regime, is well described by analytical methods [134–136].

The behaviour of short chains, exhibiting not only length dependence but a non-monotonic behaviour in the transition from oligomers to long flexible chains, is not adequately described by current theoretical approaches. Here, modern simulation methods introduced in Section 2 provide much insight. The ability to coarse-grain certain interactions more than others facilitates probing different aspects of the behaviour one at a time.

In particular, fully atomistic MD (Section 2.1) can look at small oligomers, thereby focusing on chemical details, and has been employed to accurately describe the dynamic behaviour of short (3 and 6 units) fragments of ssRNA [140]. The diffusion coefficient (corrected for finite-size effects

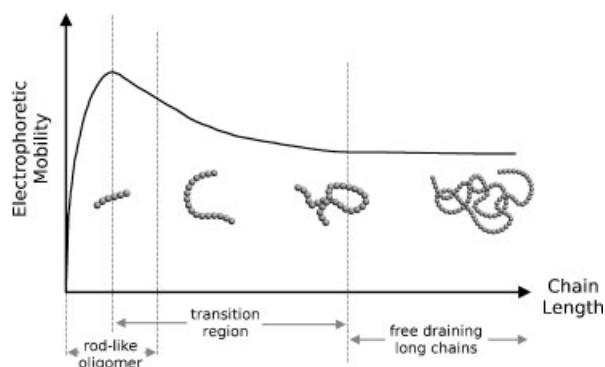


Figure 5. Schematic of the dependence of the electrophoretic mobility on the length of a flexible polyelectrolyte in free solution. Short rod-like oligomers show a sublinear increase in electrophoretic mobility with chain length. The mobility approaches a constant for long globular chains in the free draining regime. Between these two behaviours lies a transition region in which both counterion and hydrodynamic effects must be accounted for.

[141] and solvent viscosity) and the increase in electrophoretic mobility from 3 to 6 nucleotides are consistent with experimental results. The simulations show the importance of the counterions in reducing the effective charge by transiently binding to the polyelectrolyte resulting in the sublinear increase in the mobility with chain length that we see in Fig. 5. The fact that the mobility follows the increase in effective charge demonstrates how the hydrodynamic friction of short, rod-like polyelectrolytes depends only weakly on the length of the chain.

Longer-chain behaviour can be understood only by investigating the hydrodynamics of polyelectrolytes undergoing conformational changes from a rod-like to a globular state. As the number of counterions bound to the polyelectrolyte increases with length, in this midlength regime their contribution remains essential. Two recent studies using mesoscopic techniques (SRD (Section 2.3.2) and LB (Section 2.3.3)) have investigated the transition region between short fragments and long chains in detail [142, 143]. They show that without HI, mobility would actually decrease with length and approach a constant value for large molecular weights. However, by including HI, simulations accurately reproduce the experimentally observed non-monotonic behaviour of the mobility. By determining the effective charge, estimates of the effective friction were determined, and a transition from logarithmic to linear scaling with length was observed [144]. The microscopic interpretation of this phenomenon is still being discussed, but all of these studies [142–144] emphasized the importance of the interplay between HI and counterion condensation. This change is attributed to the correlated movement of the counterions in the vicinity of the polyelectrolyte, effectively cancelling long-range HI this signals the transition to a free draining regime.

When the chain length is increased further, screening eliminates the need to explicitly account for hydrodynamic

effects. In this case, implicit fluid techniques without hydrodynamics (Section 2.4) but with explicitly included counterions have been applied to study polyelectrolytes in electric fields, showing that below a critical field strength the static and dynamic properties of the polyelectrolyte remain unaffected and continue to agree with experimental data. However, when using high electric field strengths that exceed the fields in experiments by several orders of magnitude, alignment of the polyelectrolyte with the field and an increased electrophoretic mobility due to dissociation of counterions is observed [145, 146]. Whether these effects are of any practical relevance is not clear.

The above works emphasize the importance of correlations between counterions, the polyelectrolyte and the resulting screening of HI. Unlike the electrophoretic motion, the diffusive motion of the polyelectrolyte is not correlated with counterion motion and HI remain unscreened [143, 147]. It has been shown that the diffusion of long polyelectrolytes can be correctly modelled when the counterions are neglected as long as hydrodynamic effects are included [148]. In this simulation, the Rotne–Prager–Yamakawa formulation (Section 2.4.2) was used to quantitatively predict equilibrium and non-equilibrium diffusivity of DNA molecules up to 126 μm in length.

3.3 Polymer-obstacle collisions

In many electrophoretic methods, size selectivity is due to the interaction between the analyte and obstacles of some sort. To better understand this interaction, Deutsch and Madden [149, 150] have done pioneering 2-D BD simulations of a polymer migrating through an ordered matrix of obstacles. These authors found that in a very strong field, the polymer migrated through the gel in an unexpected fashion, very different from the conventional reptation picture [103]. The chain goes periodically through a sequence of states: a coil collides with a post, extends its arms around it, slides off the post leaving it in a fully extended state, and then collapses into a coil again. This process, termed *geometration*, was later observed in videomicroscopy experiments [151, 152]. Collision with a post is an important part of geometration; obviously, this process can be studied computationally in more detail if a system with just a single obstacle is considered.

Nixon and Slater [153] did the first such computational study using the BD approach in 2-D. They considered a chain of beads (without excluded volume) connected by FENE-like springs inside a narrow channel with an obstacle. The field was assumed to be uniform, *i.e.* the field lines penetrate the obstacle. The chain started in the random coil state. As the collision begins, the coil gets deformed and becomes pancake-shaped. The subsequent behaviour of the polymer is similar to that observed by Deutsch and Madden, but more clearly seen, as other neighbouring obstacles do not interfere. Based on these simulations, the authors developed an analytical theory predicting both the average

retardation of the polymer due to the collision and the variance of this retardation.

More detailed simulation studies of collisions using a very similar 2-D BD approach were carried out by Sevick and Williams [154] and Saville and Sevick [155]. The latter studied the properties of collisions as a function of the impact parameter, the initial distance in the direction perpendicular to the field between the centre of mass of the chain and the centre of the obstacle. Different obstacle sizes were also considered. These authors found that besides the “hooking” collisions studied by Nixon and Slater, another possibility is the “rolling off” collisions where the coil “rolls over” the surface of the obstacle and does not deform much during the collision (Fig. 6). For “rolling off” collisions that become dominant for large obstacles, the duration of the collision depends mostly on the obstacle size, rather than the chain length. Among “hooking” collisions, besides the conventional type (called U/J collisions, because the hooked chain conformation resembles these letters), collisions with multiple hooking (later termed W collisions [156], as the conformation can resemble a “double-U”) were also found.

More recently, Randall and Doyle [157] found in their fluorescence microscopy experiments that besides U/J and W collisions, yet another type of collision (called X for “extending”) is possible. These collisions resemble the U/J type, but the longer arm of the chain is not fully unwound at the beginning of the unhooking process, containing a coil at the end that unwinds gradually as the unhooking proceeds. To study this collision type in detail, Kim and Doyle [158] carried out 3-D BD simulations with excluded volume interactions. Unlike previous work, they did not make the assumption of a uniform electric field, instead calculating the field assuming that the obstacle (cylindrical in shape) is a perfect insulator. They indeed found X collisions, along

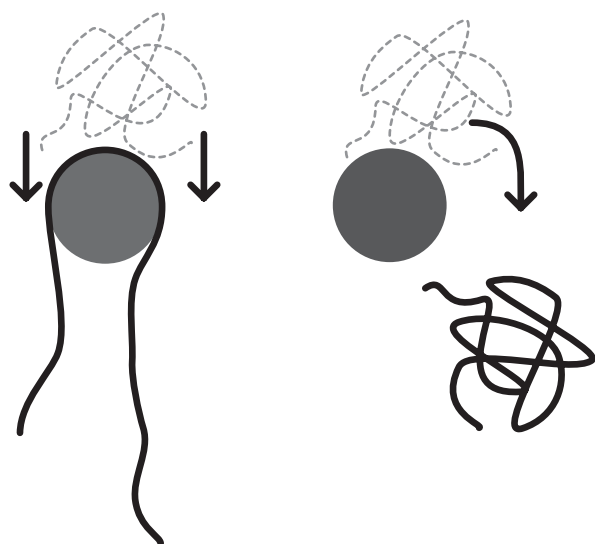


Figure 6. Different types of collisions of a chain with an obstacle: a collision involving hooking of the chain upon the obstacle (left) and a “rolling off” collision during which the chain remains a coil (right).

with previously known U/J and W types. It turned out that, surprisingly, the X type is the most dominant one in a broad range of chain lengths and field strengths. Given that this remains the case even in simpler models, perhaps previous authors simply did not distinguish between completely and incompletely unwound chains (for instance, [153] did not distinguish between these states).

The work discussed so far deals with the case of strong fields, when the thermal effects are negligible or at least secondary. A very recent paper by Holleran and Larson [159] also considers the case when, on the contrary, the field is weak and thermal diffusion dominates. In this case the arms are never extended and the polymer remains a coil that drifts slowly past the obstacle. In fact, in this regime the polymer, at least semi-quantitatively, can be considered as a rigid particle and be studied using, e.g. the exact MC methods described in Section 2.6.1. The paper is also interesting because of its use of a novel computational model of the chain (developed by the authors and described in a separate publication [160]), where the springs connecting the beads and not the beads themselves are repelled by the obstacles, which ensures that the chain cannot penetrate the obstacle even when the distance between the beads is much larger than the obstacle size. This allows the authors to treat very long chains (longer than 1000 Kuhn lengths).

All simulations discussed so far used BD neglecting Coulomb interactions between monomers and HI. Coulomb interactions are screened by counterions. The justification for neglecting HI offered by Kim and Doyle [158] is that both their simulation and the experiment it models [157] were carried out in a slit, in which case HI should be screened. However, even in the bulk the results are at least qualitatively correct, as the comparison with recent simulations by Kenward and Slater [161] using *explicit solvent* shows. Introducing a solvent can certainly produce some quantitative changes. Neglecting HI assumes that the polymer is free-draining, which is true in free solvent, but not when the chain is slowed down by an obstacle. The result is the modification of the friction force on the chain, which also becomes conformation-dependent. Kenward and Slater also studied collisions between two polymer chains, of which only one is driven by an external force, but both are mobile. Such collisions are important in the case of electrophoresis in polymer solutions [162, 163]. In this case, hydrodynamic effects influence the conformations of the chains. Similarly, studying situations where the colliding chain is driven by a flow, rather than an external field, is, of course, possible only when the solvent is included in some way; Kenward and Slater considered this case as well.

Finally, we mention other computational approaches applied to this problem. Starkweather *et al.* did off-lattice bead-rod MC simulations of a chain colliding with an immobile random coil [164] and a mobile chain [165]. As mentioned MC methods often have problems in strong fields, and their use is especially dangerous when applied to this problem, as it involves U-shaped chain configurations. The authors had to restrict themselves to moderate field

strengths, when the chain is far from being fully stretched. André *et al.* [156] used a special algorithm tracking the evolution of different loops and arms of the chain after the impact.

3.4 Ogston: Modeling sieving in hydrogels

A specific electrophoretic regime exists when the size of the analyte is smaller than or comparable to the mean pore size of the gel. This regime is often called the Ogston regime for electrophoretic sieving. Although the concept is technically restricted to rigid analytes, it is possible to extend its use to flexible polyelectrolytes such as DNA if one assumes that the chain takes on a spherical conformation with an effective radius R . By coarse-graining out the fine details of the individual monomers and considering the analyte as a solid sphere, we can discretize our system on a lattice (Fig. 7) and use simulations or exact calculation methods to study the electrophoretic mobility or diffusion coefficient of the analyte in this regime. A straightforward lattice approach to modeling the gel system would be to consider a gel fibre as an impenetrable obstacle. In Fig. 7, the analyte and the obstacles are of the same size (the lattice step size); although this is the case that we will consider below, it is equally easy to study larger obstacles and/or larger analytes that occupy more than one lattice unit (more on this later).

The first such numerical model was used by Slater and Guo [166] to test the key hypothesis of the Ogston–Morris–Rodbard–Chrambach (OMRC) model. According to the OMRC model, the mobility of the analyte in this regime is linearly proportional to the fractional gel volume that it can occupy, a purely geometric parameter that can be computed quite easily for the model shown in Fig. 7. All the other assumptions of the OMRC model (*e.g.* a low field intensity) being compatible with the numerical model, the results of the study represented a direct test of the fractional volume hypothesis. The Slater and Guo exact numerical calculations showed that the mobility of the analyte is higher in an ordered gel, compared with a random one, even if the fractional volume is the same. This was the first demonstration that the OMRC model is incomplete; in fact, these

authors also showed that the OMRC model corresponds to a mean-field model valid for an annealed gel (a gel with rapidly moving obstacles) [166].

As mentioned earlier, it is possible to extend the exact method to treat larger particles [167]. In this case, molecules are also viewed as rigid spherical particles, but can be larger than the obstacles. These results are valid in the zero-field limit since the interactions with the obstacles are assumed to be hard-core, *i.e.* the particles do not deform when colliding with an obstacle. It is also possible to extend this calculation method to treat attractive interactions between the analyte and the gel structure [168].

DNA molecules can also be modelled using the MC exact calculation method without making the hard sphere approximation [121]. Indeed, one can use multiple particles linked by bonds to represent a flexible chain. For example, such polymers can be described by self-avoiding walks and modelled by the BFA. This extension opens the door to a fundamental study of the electrophoretic sieving of oligomers, rod-like molecules, vesicles, star-shaped macromolecules, *etc.* One could argue that the MC exact calculation model is not a sufficiently good representation of a gel matrix since the field is assumed to be uniform throughout the gel. In reality, the field lines are affected by the gel structure (the obstacles). It has been shown [169] that the LMC exact method can also be extended to treat spatial variations of the electric field, and that this has little impact on the results at low field intensity.

It is possible to apply the exact method to treat high fields instead of vanishingly small external fields [105]. For non-deformable analytes, this can reproduce the trapping that sometimes occurs in real electrophoresis experiments. Multiple obstacle geometries have been studied and it is possible to properly model experimental observations of trapping and pulsed field de-trapping. A modified version of the initial algorithm [170] also allows the simultaneous calculation of the mobility and of the dispersion coefficient; this is obviously needed in order to predict the resolution of a specific device.

3.5 Microfluidic ratchets

The Brownian motion of particles is what gives rise to diffusion. It is possible to exploit these natural thermodynamic fluctuations for the separation of particles by adding an external force that biases the dynamics. Brownian ratchets are devices that use an asymmetry, either temporal or spatial, to drive the motion of a Brownian particle even when the net external force is zero. For example, a temporal asymmetry could be a zero-mean field alternating between a short high-intensity forward pulse and a longer low-intensity backward pulse. A spatial asymmetry could take the form of asymmetrically shaped obstacles or walls. Using any or all of these types of asymmetry, a non-zero net velocity can be observed in the presence of a field even if the net force is zero (hence the name *ratchet*). The random motion of the

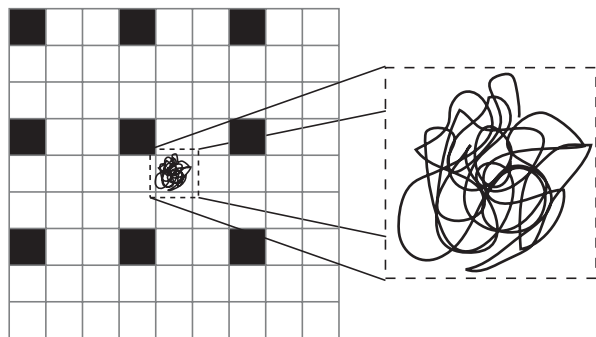


Figure 7. Modeling a polyelectrolyte in the Ogston regime with an LMC model.

particle then plays a major role (this is why it is called a *Brownian ratchet*).

As an example, consider the case of particles in a properly designed microfabricated array of obstacles (see Fig. 8). LMC methods can be used to simulate Brownian ratchets of this kind (see Section 2.6.1.1). First, the system is represented as an array of impenetrable obstacles. A starting point is chosen and the particle is moved randomly with the probabilities defined in Eq. (16). With this method, long computational times would be required in order to obtain statistically precise results. A faster and more precise computational method was described in Section 2.6.1.1. The increased accuracy is vital at very low fields, where the statistical uncertainty associated with the normal approach is often larger than the studied behaviour.

Gauthier and Slater [171] examined several ratchet systems using this simulation approach. The system shown on the right-hand side of Fig. 8 is interesting since it uses a symmetric array of obstacles together with an asymmetric pulsed field. These authors observed several current reversals (changes in direction) for different values of the field. For certain choices of parameters, particles of different sizes but having the same charge were predicted to move in opposite directions, a remarkable result (in some cases, a particle can even move against the direction of the net field, a phenomenon called absolute negative mobility). A Brownian ratchet of this type was later built experimentally by another group [172]. An aqueous solution of charged spheres of radius $2\ \mu\text{m}$ was placed in a periodic array of misaligned posts with alternating small and large gaps. The applied asymmetric external field was a superposition of a constant (E_{DC}) and an alternating signal ($\pm E_{\text{AC}}$). The system behaved as expected and absolute negative mobility was observed – a nice example of the kind of new ideas that simulations can suggest.

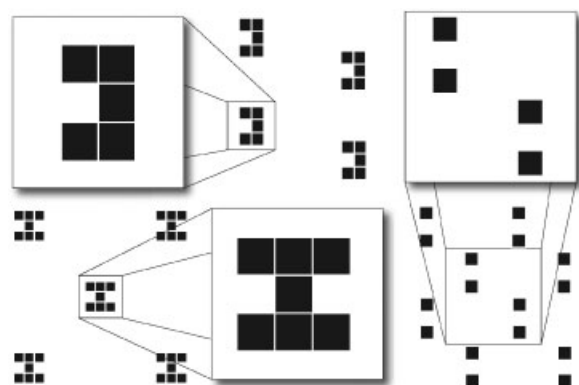


Figure 8. Three types of obstacles that can be used to design a Brownian ratchet separation system. Left, top: trap-shaped obstacles with a left-right spatial asymmetry. An unbiased AC electric field would lead to a net velocity pointing in the negative x direction. Left, bottom: symmetric obstacles with traps in both directions. Here, one would need an asymmetric pulsed field to drive the Brownian ratchet. Right: the distribution of obstacles proposed by Gauthier and Slater [171]: misaligned rows of obstacles. The lattices are not explicitly shown for clarity purpose.

Tessier *et al.* [173, 174] studied the system proposed by Han *et al.* [175] in the ratchet regime, both spatial and temporal. For the spatial asymmetry, the system was modified to introduce a geometrical asymmetry. The BFA (see Section 2.6) was used to model the polymer. Tessier *et al.* also simulated the system using a zero integrated pulsed field [176]. In both cases, separation was predicted. These predictions have yet to be tested.

3.6 Nanopore translocation

By threading ssDNA through a narrow pore (Fig. 9) and identifying the bases as they pass through, nanopores offer a promising avenue for the development of sequencing technologies [177]. In contrast to gel electrophoresis, nanopore sequencing could offer rapid (thousands of bases *per second*) sequencing of a single DNA molecule; a revolution which would have a great impact in fields associated with the life sciences. The same approach can also be used for other purposes, such as sizing molecular contour lengths. A significant advancement occurred in 1996 when Kasianowicz *et al.* demonstrated that RNA and DNA could be detected passing through a biological nanopore (α -haemolysin) by monitoring the disruption of ionic currents [178]. Subsequently, there have been a great number of theoretical [179–182] and experimental studies [183–188] focused on nanopore translocation (note that the number of nanopore related publications is staggering and in this very brief review, we are limited to providing only a few selected publications for each topic). Providing a bridge between theory and experiment, many computer simulations have also been performed and, in fact, most of the techniques discussed in Section 2 have been used to study the translocation process.

At the coarsest level, LMC simulations (see Section 2.6.2) are often performed in conjunction with theoretical studies to test the resulting predictions [181, 182, 189–193]. Other studies have used off-lattice MC to study translocation driven by an external or adsorption force [194–196]. Finally, one approach has mapped the translocation onto a 1-D diffusion process and then employed an exact numerical technique to obtain results [122, 197]. Although such studies are able to investigate very long polymers at low fields, the details of the dynamics are not produced and, additionally, the model is coarse to the extent that effects such as HI can be included only in an approximate manner. At the opposite end of the spectrum, fully atomistic MD simulations of DNA inside a channel have also been performed. These studies have revealed interesting details about the viability of distinguishing between bases of DNA translocating through a synthetic nanopore [1] and the dependence of various quantities on the DNA orientation inside the biological α -haemolysin channel [198]. They are, however, limited in terms of time scale and are unable to simulate the full translocation process. In between these extremes, many coarse-grained MD simulations using an implicit fluid [199–201], an explicit fluid

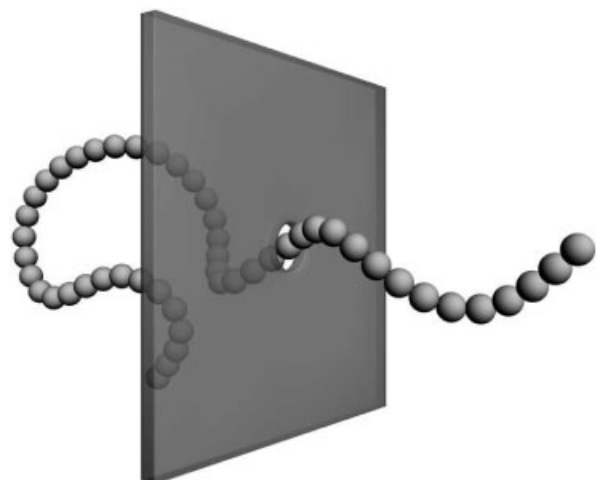


Figure 9. Schematic of a polymer translocating through a solid-state nanopore.

[202–204], or a mesoscopic fluid model [205–208] have been performed in an attempt to include the critical factors while requiring a reasonable level of computational power.

Using one or more these approaches, these studies have investigated the role of underlying physical mechanisms such as conformational entropy [197], HI [205], the solvent effect [209], counterions [204], pore-polymer interactions [210], and the pore geometry [211, 203]. Additionally, the dependence on the nature of the polymer has been studied by simulating charged polymers [212] and heteropolymers [213, 214]. Furthermore, considering application to sequencing, it is not surprising that a great number of studies have also examined driven translocation by implementing a pulling force [215], an adsorption force [196], or an external field (discussed below). As is obvious from this list, which is in itself incomplete, there is not enough space in this brief review to cover all of these results. Rather, as a single example, we will discuss some of the results for a key aspect of the general translocation problem: the scaling of the translocation time τ with the degree of polymerization N of the polymer for driven translocation.

In experiments on the biological α -haemolysin pore, both Kasianowicz *et al.* and Meller *et al.* found a linear dependence of the translocation time on the polymer length ($\tau \sim N$) for short ssDNA fragments [178, 188]. In contrast, the experiments of Storm *et al.* found a scaling of $\tau \sim N^{1.27}$ when driving long DNA strands (6500–97000 base pairs) through a solid-state nanopore [183]. This result is in agreement with their prediction that $\tau \sim N^{2\nu}$ for long polymers when HI are taken into account ($\nu \cong 3/5$ is the 3-D Flory exponent). From the analytical side, considering translocation driven by a chemical potential gradient, Muthukumar predicted linear scaling [189] while Kantor and Kardar predicted $\tau \sim N^{1+\nu}$ [191]. The latter group also performed bond-fluctuation MC simulations but were unable to verify the prediction due to limited polymer lengths. However, additional simulation studies for long

polymers have found a scaling of $\tau \sim N^{1+\nu}$ using 2-D fluctuating bond MC [216] and using the exact numerical method [197]. Finally, Dubbeldam *et al.* predicted a scaling of $\tau \sim N^{2/(2\nu+2-\gamma_1)}$ (where γ_1 is the surface exponent) [217] and found results consistent with this from off-lattice MC simulations. Moving towards a more detailed simulation of the dynamics, driven translocation has also been studied using LD and BD simulations. Performing 2-D LD simulations, one study found a scaling of $\tau \sim N^{1+\nu}$ [201] – a result consistent with the Kantor and Kardar prediction and the MC simulations cited above. Meanwhile, others have found a linear dependence [199, 200]. The discrepancies between these results may be attributed to differences in the particular system setups such as the pore length, polymer length, magnitude of the external field, and polymer model.

A limitation of all the simulation results cited thus far (MC and LD/BD) is that they neglect HI. As long ranged correlations through the fluid are conceivably important in the translocation process, much of the current work focuses on MD simulations using mesoscopic fluid models. Using the DPD approach, He *et al.* have found a linear dependence of τ on N [207]. Meanwhile, Izmitli *et al.* [205] and Fyta *et al.* [206], each using an LB fluid model but with different polymer models, have both found an exponent of 1.28, a result in good agreement with the experimental data of Storm *et al.* corresponding to $\tau \sim N^{2\nu}$. Both of these studies also directly tested the impact of HI by performing the same simulations without the LB solvent. Izmitli *et al.* found a negligible change as the exponent rose to 1.31 while Fyta *et al.* observed a slightly larger effect with the exponent rising to 1.36. The key parameter here appears to be the molecular size: is there a critical polymer size below which the HI are negligible because the translocation is then dominated by the polymer-pore interaction and not by the polymer-fluid interaction? In our opinion, a combination of careful simulations and experimental studies will be needed to answer this question.

As demonstrated by this one example, a wide range of simulation techniques have been employed to elucidate various details of the translocation process. In fact, given this wide array, one must keep in mind the scope of a particular simulation approach and the limitations of a chosen system setup when considering the results of each study. Ultimately, however, this diverse amount of information is an advantage in fully characterizing the system. While there are still many hurdles remaining before a nanopore sequencing device is realized (the development of a base identifying detector, slowing down the overall process, having the DNA find the pore), current simulations are giving insight on the fundamentals of polymer translocation.

3.7 Entropic trapping

In Ogston-type models of electrophoresis, it is assumed that analytes cannot pass through constrictions that are smaller

than their diameter. This is true for hard particles, but polymer coils can deform and still pass through holes that are much smaller than their radius of gyration R_g . However, as deformed coils are no longer completely random, this has an entropy cost and a polymer entering a narrow space has to overcome an *entropic barrier*. In a porous medium, such as a gel, such barriers are especially important when the average pore size is comparable to R_g so that there are pores both smaller and larger than R_g . In this situation, *entropic trapping* is possible, in which case the polymer can stay trapped for a long time in a large pore, since all ways out of it involve overcoming entropic barriers. The consequences of this were first studied in off-lattice MC simulations (Section 2.6.2) by Baumgärtner and Muthukumar [218–220]; one interesting result is a much stronger size dependence of the diffusion coefficient (and thus, *via* the Nernst–Einstein relation (Section 2.6.1), of the electrophoretic mobility) than predicted for larger polymers spanning many pores (the reptation regime). This was later confirmed experimentally [221].

Entropic trapping in gels, while an important issue, is still just one of the factors influencing the separation. On the other hand, Han *et al.* [124, 175, 222, 223] have fabricated and studied a device where, by design, entropic trapping is the dominant contribution to separation. The device is an array of cavities separated by long and narrow *slits*. The size of the cavities is much larger than R_g for the typical DNA sizes whose separation is desired, whereas the width of the slit is much smaller than R_g in one direction, but, importantly, is still $\gg R_g$ in the other direction. Han *et al.* found that the mobility *increases* as the size of the polymer increases. This is rather counterintuitive, given that larger polymers should deform more passing through the slit and this should be more entropically costly. Han *et al.* explained this by suggesting that rather than entering the slit as a whole, the polymer stays around the slit and loops (or *hernias*) get inserted in the slit (Fig. 10). Such insertion has an entropy cost proportional to the insertion length, but it also causes the decrease of the electrostatic energy proportional to the square of the length. As a result, the free energy increases until the top of the free energy barrier is reached, but then starts decreasing. The escape rate, as always in transition-state theory, depends exponentially on the barrier height, but also depends on the prefactor (the attempt frequency). It was argued that the barrier height is inversely

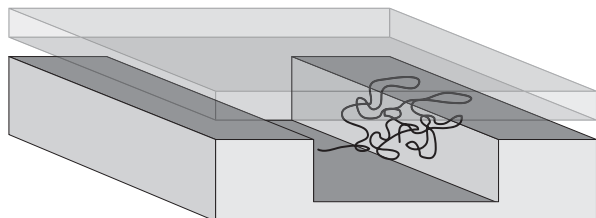


Figure 10. Schematic drawing of a part of the entropic trap device of Han *et al.* with a DNA chain near the entrance to a narrow slit. Several hernias are inserted in the slit.

proportional to the field strength, but is independent of the polymer size, and so the separation is entirely determined by the prefactor. This prefactor should be proportional to the size of the part of the polymer exposed to the slit, as this determines the number of hernias that can form simultaneously; this size is proportional to R_g which increases with the polymer size, and therefore the escape rate indeed increases with the size, as observed experimentally.

This simple theory, while appealing, was in need of verification by simulations, especially given that it relied on the independence of the free energy barrier height of the polymer size; since this barrier height enters in the exponent, even a slight dependence may completely overwhelm the dependence contained in the prefactor. With this in mind, several simulations have been carried out. Tessier *et al.* [173] used the MC BFA (Section 2.6.2.3). The field used in the simulation was computed by numerically solving the Laplace equation. The results for the mobility obtained in the simulations are in qualitative agreement with the experiments. Overall, the simulations confirmed the theory by Han *et al.*, but also further refined it. In particular, it was found that for weaker fields the mean trapping time indeed depends exponentially on the inverse field with the slope on the semi-logarithmic plot independent of the molecular size, confirming that the activation energy is indeed size-independent. On the other hand, for longer chains there was a deviation from the perfect exponential at higher fields attributed to the change in the shape of the coil near the entrance to the slit. At moderate fields the coil around the slit acquires a pancake shape and the radius of gyration behaves as that of a 2-D random walk; but as the field gets stronger, the escape into the slit is so fast that the coil has no time to deform. This has obvious consequences for the escape rate, as the prefactor depends on the extent of the coil along the slit entrance. The field dependence of the critical hernia length, as estimated by Tessier *et al.*, is also largely in agreement with the theory.

Chen and Escobedo [224] looked more directly at the free energy barrier associated with the entrance into the slit. Since free energy calculations are done in equilibrium, one of the equilibrium MC methods (in their case, configuration-bias MC [225]) was used (see Section 2.6). The advantage of this approach is that reliable results can be obtained even for very small fields, when the escape is so slow that good statistics cannot be obtained in dynamical simulations. The result of these free energy calculations is that over a large range of parameters, the barrier height is indeed polymer-size-independent, however, deviations are observed at very low fields for very short chains (the regime that Tessier *et al.* could not study very carefully). These deviations are not unexpected: at very low fields, the critical hernia length is very large, and if the chain is short, may actually become comparable to or even (in theory) exceed the chain length.

Streek *et al.* [226] used BD simulations (Section 2.4) to study essentially the same system. Based on their results, these authors suggest that in addition to entropic trapping, there exists another separation mechanism, due to size-dependent trapping of chains in the corners of the

cavity. The authors claim that it is this new mechanism, rather than entropic trapping, that is dominant. This may well be true at the rather high fields that they consider and especially in the case of constrictions that are wider than the radius of gyration of the chain (considered in a separate publication [227]), when the entropic barriers are essentially negligible. On the other hand, it is clear that at very low fields for narrow constrictions the entropic trapping mechanism should dominate, as no mechanism based on diffusion alone, without any activation barriers involved, would be able to compete. The intermediate case, when the entropic barrier height is $\sim k_B T$, is the most interesting practically and deserves further study; the answer may depend on many details, such as the size of the cavities.

We should also mention newer work likewise using BD. Panwar and Kumar [228] recognized that when the trapping barrier is not very high, two other time scales besides the trapping time contribute: the time it takes the DNA to approach the constriction and the crossing time. They studied the field and chain length dependencies of all three times. These authors used a bead-rod model of the DNA; Lee and Joo [229] did a similar study for a bead-spring chain.

All work described here uses methods that do not take hydrodynamic effects into account. For this reason, the DNA behaves as a free-draining chain when in reality a trapped chain is not free-draining. As Tessier *et al.* [173] point out, this implies that the field intensity needed to overcome the entropic barrier is underestimated by a factor of $\sim N^{2/5}$, where N is the chain length. As always at this level of modeling, quantitative comparisons with experiment are difficult, because the effective charge of the DNA is different from the bare charge due to counterion condensation. These effects need to be taken into account in future work.

3.8 Surface electrophoresis

A novel electrophoretic separation technique based on the DNA's interaction with a surface was first reported by Pernodet *et al.* [230] in 2000. By adsorbing DNA to a surface, length dependent separation on a flat surface without any restrictions or any sieving matrices was achieved (see Fig. 11A). It was found that the interactions between the molecule and the substrate essentially act as a length dependent source of friction, enabling electrophoretic separation. The initial experimental observations were accompanied by MD simulations [231, 232] and have been followed up by further studies [233, 234] under different conditions. The results showed that the DNA-surface interaction is a key parameter for the process: a strong attraction leaves the molecules fully adsorbed and no separation is possible, too weak an attraction lets molecules desorb and resume bulk behaviour, where likewise no separation is possible. Additionally, the interaction can be noticeably influenced by choosing a specially patterned surface [235, 236], an exciting and unique approach to designing optimized and custom-made separation systems.

Since the exact nature of the interaction and the resulting separation mechanism remain elusive at this point, there is a pressing need for more elaborate theoretical and computational studies that include electrostatic and hydrodynamic effects alike as they are crucial when the molecules approach the surface. This has been neglected so far.

3.9 Confinement-driven separation

Recent progress in design and fabrication of microfluidic devices on a sub-micrometer length-scale [237–239] demands a good understanding of the statics and dynamics of the polyelectrolytes under steric confinement. We can distinguish several regimes of confinement. In a device that is much larger than the size of the polyelectrolytes, given by their radius of gyration R_g , the conformations are unperturbed and isotropic (weak confinement). Reducing the dimensions of the devices to the order of R_g , the conformations of the polyelectrolytes start to become restricted by the walls and show deviations from the equilibrium (strong confinement, see Fig. 11B). With further reduction of the device size, the polyelectrolyte becomes extremely restricted and the static and dynamic properties undergo significant changes [240, 241] (Fig. 11C).

The decrease in size of microfluidic devices used in actual experiments and the growth in size of the systems that are addressable by means of computer simulations – due to advancement of simulation methods together with the increase of computer power – led to a cross-over creating systems that can be worked on from both sides. Recent experiments in slit-like nanochannels studied the static and dynamic properties of single molecules [242–248] and showed how confinement can be used as a tool to change polymer conformations as well as the dynamics through

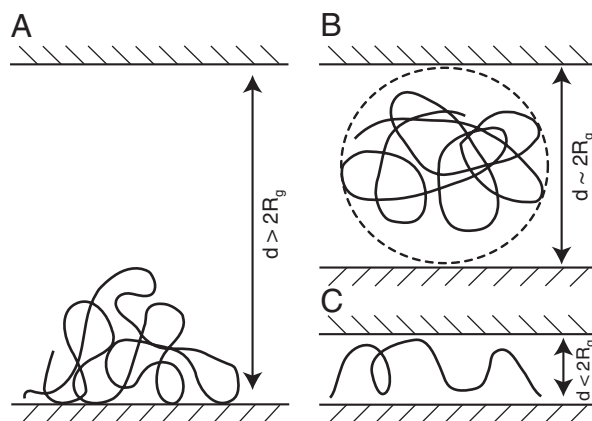


Figure 11. Different regimes of confinement used in some gel-free separation techniques: (A) weak confinement ($d > 2R_g$) in the presence of an attractive surface, (B) stronger confinement ($d \sim 2R_g$) that starts to influence the chain conformations, and (C) strong confinement ($d < 2R_g$) in which the chain conformations are determined by the walls of the channels.

modulation of the HI. Consequently, the role of hydrodynamics in confinement has been the focus of several recent computer studies [75, 96, 248–254]. The results indicate that, under weak confinement, the HI between polymer and wall drive the polymer to the centre of the channel if an external flow is applied. However, in high confinement, a migration towards the channel walls was observed. Since this interesting physical phenomenon depends on the ratio of the size of the polymer to the width of the channel as well as on the strength of the driving force, it seems to be an ideal candidate for free-solution separation of polyelectrolytes. Recently reported measurements on the diffusion and the electrophoretic mobility of DNA in strongly confined systems [255–257] indicate a possible electrophoretic separation mechanism based on the modified dynamics in strong confinement. A systematic simulation study of this subject has yet to be done in order to verify these results. In particular, electrostatic interactions and the influence of counterions on the HI have been neglected so far, but they should be assumed to be of great importance if the length scales of the system become comparable to the Debye length, below which electrostatic interactions are not fully shielded by the solvent [258, 254].

4 Outlook

This review has hopefully convinced the reader that the computational approach has been useful in understanding a large variety of electrophoretic separation systems and relevant electrokinetic phenomena. Compared with theory, many situations that cannot be studied theoretically without gross simplifications can be treated computationally; in fact, simulations provide valuable clues to theorists as to *exactly what* simplifications and assumptions they are allowed to make. Compared with experiments, simulations have an unmatched ability to look at the microscopic level; but perhaps the most important is the possibility to “switch on and off” different effects (such as HI) at will, something that is not available to experimentalists and yet is extremely helpful in developing a better understanding of the systems and phenomena of interest.

Considering the future of both the simulation methods and their applications to separation phenomena, the most straightforward approach is the “brute-force” one: harnessing the inevitable increases in computational power to conduct more detailed simulations of larger systems for longer times. However, in striving for more realistic simulations in this manner, it is important to recognize just how wide the gulf between experiments and detailed simulations is. To see this, consider atomistic MD simulations that are already being used to study DNA inside a nanopore (Section 3.6). While these simulations are yielding interesting results, significant increases in computational power would greatly enhance what they are able to study. For example, detailed atomistic simulations may be able to aid in the design of a probe to read the bases as they pass through the

channel. However, current atomistic MD simulations are typically limited to trajectories of hundreds of nanoseconds for limited system sizes. On the other hand, in the lab, the dynamics of interest can evolve on a time scale up to seconds or even longer. Consequently, while any increase in computational power is enthusiastically welcomed, the gap between detailed simulation and experiment remains large and the development of efficient techniques to appropriately coarse-grain the simulations is equally vital to broadening the scope and relevancy of computational studies.

Hence, as a final thought to this review, we examine a number of emerging or long-standing-but-difficult areas where new ideas and computer simulations will be needed over the next few years.

4.1 Multiscale simulations

In simulations, often different regions of the system require different levels of detail. For instance, a molecular-level fluid model may be required in the vicinity of the analyte, but at larger distances a faster mesoscopic approach may be sufficient. Likewise, the accuracy of a translocation simulation may be improved if the polymer is simulated atomistically in the vicinity of the nanopore. To avoid simulating the whole system with the highest level of detail needed only in a small region, multiscale simulations can be used. The biggest challenge is the seamless coupling between regions with different level of detail, which is especially difficult if the region boundaries are required to move. Several multiscale methods for fluids have appeared recently (see [259–264]). Such methods should see increased use in electrophoresis simulations. Besides, more “trivial” *serial* multiscale studies, where the outcome of a more detailed simulation is used to construct a coarse-grained model and *vice versa* [265], will be useful.

4.2 Mesoscopic solvents

SRD is a relatively new method of mesoscopically handling solvents and as such its full potential has yet to be captured. Despite being a model that is conceptually and relatively simple to implement, few of the electrophoretic simulation examples discussed above have taken advantage of SRD's ability to treat relatively large and multicomponent systems with complex and dynamic boundary conditions. Furthermore, careful choice of the multiparticle collision operator allows for hydrodynamics to be “turned off” and simply replaced with a Brownian heat bath in order to explore HI effects in the system.

4.3 Hydrodynamic effects in gel electrophoresis

The separation of small analytes by gel electrophoresis is often called “sieving”. “Small” typically means that the size

of the object is smaller than the mean pore size of the gel matrix. The word sieving suggests that separation is related to steric interactions restricting the motion of the analyte. Although detailed obstruction models have been proposed, their validity is not clear because they ignore HI and do not properly treat electrostatic interactions. Since we have three length scales here (the Debye length, the particle's radius and the mean pore size), many different regimes must exist. Understanding the simultaneous screening of the electrostatic and HI in a sieving matrix, especially if the latter is an entangled polymer solution, will require new ideas and probably extensive coarse-grained MD simulations.

4.4 Electrophoresis in polymer solutions

Electrophoresis in capillaries and microchips using polymer solutions as sieving matrices is increasingly important. Conventional wisdom is that well-entangled polymer solutions behave essentially like gels. However, the absence of crosslinking may be important, especially in strong fields typically used for capillary electrophoresis. Recent videomicroscopy experiments [266, 267] seem to suggest that the DNA is able to drag matrix polymers even well above the entanglement concentration. Simulations are needed to help understand this process. The consequences for the basic geometration mechanism of DNA motion can perhaps be understood even in rather simple models similar to that by Deutsch [149], but with “draggable” obstacles. Electrophoretic motion in more dilute solutions, around the critical entanglement concentration, is of interest as well.

4.5 Drag-tags for free-solution separation

Attaching suitable molecules to DNA fragments can restore size-dependent mobility regardless of the free-draining property [268–270]. The read length is optimized by choosing large but perfectly monodisperse drag-tags. As it is an experimental challenge to produce such polymer labels, two recently proposed alternatives seem promising: Haynes *et al.* [271] proposed to use branched polymers with well-defined architecture, whereas Grosser *et al.* [272, 273] introduced non-ionic surfactant micelles as drag-tags with very large hydrodynamic friction. Computer simulations can be used to characterize these new labels and provide ideas on how to extend these approaches.

4.6 EOF

As mentioned in Section 3.1, it has been shown that the mesoscopic fluid models such as LB and DPD provide realistic EOF profiles. It has also been shown that they provide realistic HI. One would suspect that given that they do these two things accurately, they will probably also be able to model EOF in the presence of polymer coatings.

If this is the case, mesoscopic models could allow for the simulation of more complex coatings and a wider range of parameters due to the increase in speed of computation.

4.7 DNA denaturation

As a means of sequence dependent separation, denaturing gradient gel electrophoresis exploits a rapid decrease in the mobility of a dsDNA fragment when sections of it denature. It is currently unclear whether the experimentally observed blocking is an actual trapping or a steep reduction in mobility. The bubble dynamics could play an important role in the blocking, thus a static helical-coil configuration may or may not be enough to give the correct picture. Unfortunately, simulating accurate bubble dynamics sets a coarse graining length scale to the length of a single base pair. A MD model that could incorporate the correct “breathing” dynamics of a dsDNA [274] in the presence of a gel while being able to treat dsDNA lengths of interest could be an important tool for investigating the blocking phenomenon.

4.8 Separation of large DNA

The idea of a Human Genome Project became realistic when PFGE made it possible to separate DNA molecules as large as a few megabase pairs (Mbp), a necessary step for chromosome mapping and sample preparation. After a few years of rapid progress, PFGE saturated at about 5 Mbp. Of course, the physics of PFGE is expected to be complicated for molecules that are millimeters in contour length! Agarose gels, with their sub- μm pores, are probably not the best material to extend the usefulness of PFGE. Recent ideas, based on new separation concepts, have yet to become commercial products. Computer simulations played a key role in the development of PFGE, and will again be needed in order to optimize the separation of such huge molecules and to design new and faster devices.

4.9 Protein separations

While the electrophoretic separation of nucleic acids, small ions and spherical particles has been modelled extensively, the same cannot be said of protein electrophoresis. Proteins are complex molecules, with non-trivial charge distributions on their surface. They can be separated in their native state or denatured, in free-flow electrophoresis or in gels, under uniform conditions or in the presence of gradients of various kinds. Although some models developed for particles or DNA can possibly be used for proteins as well, this has never been carefully tested. Computer simulations will be required to optimize the separation of proteins, which remains a difficult issue in the laboratory. However, generic simulation methods are not likely to work well since the precise shape and charge distribution that characterize a specific protein must be taken into account.

In conclusion, as computers become more powerful and new algorithms are developed, the future of computational studies of separation phenomena looks even brighter!

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6 Appendix

MD simulation packages

While the theory behind an MD simulation is conceptually straightforward, in practice it can be a large undertaking to code from scratch. This is particularly true when one wishes to implement more involved algorithms in order to efficiently calculate long-ranged electrostatic interactions or incorporate mesoscopic fluid models. For this reason, there exists a multitude of simulation “packages” in which the routines necessary for performing the simulation have already been implemented. Hence, the user can simply supply input information pertaining to their system, select simulation features and parameters, and then use the packages to execute the simulation, produce trajectory files, and, frequently, perform analysis. Thus, to aid the interested reader, we present in this appendix a table of the more prominent MD simulation packages (note that the list given here is by no means exhaustive; see, for example, the Wikipedia article on MD at http://en.wikipedia.org/w/index.php?title=Molecular_Dynamics&oldid=231786544).

In addition to providing the simulation package name (and related references), we also include some details for each package. The information given here is meant to convey what the package is most often used for and also to indicate any special features unique to this package. These entries should not be interpreted as restrictive, but rather as highlighting interesting or distinct features among a group of software which all accomplish similar goals.

For example, consider delineating the packages between “atomistic” or “coarse-grained” simulations. In principle, all the cited packages are capable of performing fully atomistic simulations. But as such simulations require a

fully atomistic force field it is easiest to start with a package that either comes with some force fields implemented (the first four packages in the list) or, at least, is designed to be compatible with the force fields from other packages (the next two entries). Similarly, all of the packages could be used for coarse-grained simulations. In fact, due to its efficiency in calculating non-bonded interactions, GROMACS, a biomolecule-oriented package, has been used for coarse-grained polymer simulation work. However, in choosing a more coarse-grained oriented package, one is more likely to find other useful elements such as the implementation of mesoscopic fluid models as found in the last two entries.

Finally, we include a “Free” column. A check mark here indicates that the program is free for academic use (at a minimum). This column is included to encourage the interested reader to download and begin experimenting without any monetary investment.

Name ^{a)}	Details	Free
GROMACS [275] www.gromacs.org	Includes force fields for fully atomistic Efficient calculation of non-bonded interaction	✓
CHARMM [276] www.charmm.org	Includes force fields for fully atomistic Pioneer for MD simulations	
NAMD [277] www.ks.uiuc.edu/Research/namd/	Includes force fields for fully atomistic Capable of steered and interactive MD	✓
AMBER [278] amber.scripps.edu	Includes force fields for fully atomistic AMBER force field is compatible with and used in many of these MD packages	
DL_Poly [279] www.ccp5.ac.uk/DL_POLY/	Compatible with GROMACS or AMBER force fields Includes potentials for non-biological materials	✓
LAMMPS [280] lammps.sandia.gov	Compatible with CHARMM, AMBER, and GROMACS force fields. Includes DPD	✓
ESPResSo [281] www.espresso.mpg.de	Designed for coarse-grained Includes many algorithms for electrostatics Includes LB, DPD	✓

a) Parallelized versions of all these packages exist to take advantage of high performance computing clusters.



Electrophoresis: When hydrodynamics matter

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ABSTRACT

The combination of hydrodynamic and electrostatic interactions leads to non-trivial effects that can be observed in various electrophoretic and electro-osmosis systems. In this article, we focus our attention on problems involving polyelectrolytes. First, we examine the free-draining behavior of polyelectrolytes such as DNA, a remarkable phenomenon that makes it impossible to use free-solution electrophoresis to fractionate nucleic acids. We show that the common assumption that hydrodynamic interactions are screened and therefore irrelevant in this system is wrong, and that one must be very careful when dealing with electro-hydrodynamics, especially when mechanical forces are also present. In the limit of small forces, one can superimpose the mechanical and hydrodynamic flow fields and make predictions that are often in excellent agreement with experiments. For DNA, the full electro- and hydrodynamics can then be reduced to the conformationally dependent superposition of a polymer sedimenting through a fluid and a polyelectrolyte being electrophoresed. This superposition or Electro-hydrodynamic Equivalence Principle has been used to explain a variety of problems and to propose methods that can allow the electrophoretic separation of DNA.

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1. Introduction

The electrophoresis of polyelectrolytes, particularly nucleic acids, has long been a topic of interest due to its practical importance. Essential to many electrophoretic assays of biomacromolecules is separation based on length, a key step in DNA sequencing. Unfortunately, due to the screening of hydrodynamic interactions, long polyelectrolytes cannot be separated in free solution and instead a polymer network is generally used as a sieving matrix [1]. Since hydrodynamic interactions are screened by the polymer network, traditional theoretical and computational studies have ignored them [2,3]. However, there are a wide variety of systems where polyelectrolytes are subject to an electric field in the absence of a polymer network, which have become of increasing interest in recent years. In these systems, hydrodynamic interactions are no longer screened and can in fact play a primary role in polyelectrolyte dynamics. This review introduces the basic theoretical framework used to understand electro-hydrodynamics of charged polymers and discusses several illustrative applications related to analytical biochemistry.

2. Electrophoresis of charged particles and polymers

The charged monomers of polyelectrolytes interact in non-trivial ways. We will first consider the electrophoretic motion of a single,

charged bead of radius R in an electrolyte solution of valency z , permittivity ϵ and viscosity η . Since this topic has been reviewed before [4], only material of direct interest to this article is presented.

2.1. Charged particles

A charged particle attracts counter-ions from the surrounding solvent and together with thermal fluctuations establishes a local concentration gradient called the *double layer*. The thin inner region around the particle, in which ions are effectively adsorbed to the surface, is called the *Stern layer*. The Stern layer creates an effective surface charge and establishes a potential (the ζ -potential) between its outer surface and the bulk solution. Beyond this layer is the *diffuse layer* in which ions are free to move. The potential ψ in the diffuse layer is related to the charge distribution through the Poisson–Boltzmann equation in situations where ion position correlations are not important. There is no known analytical solution for the Poisson–Boltzmann equation around a sphere. However, the *Debye–Hückel approximation* for small potentials ($ze\psi/k_B T \ll 1$) gives the approximate result $\frac{\psi(r)}{\zeta} \approx \frac{R}{r} \exp\left[-\frac{(r-R)}{\lambda_D}\right]$. In this linear limit, the counter-ion concentration decays exponentially over a characteristic length scale called the *Debye length*, λ_D , to the bulk solution's neutral background [4]. At length scales greater than λ_D , the counter-ions screen the particle's electric field.

The length scales R and λ_D determine the electrophoretic behavior. There are two revealing limits: the *Hückel* and *Smoluchowski* limits. In the Hückel limit $R/\lambda_D \ll 1$, a particle of charge Q may be

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treated as a point charge and balancing the driving and drag forces leads to a velocity

$$\vec{v} = \frac{Q\vec{E}}{6\pi\eta R} = \frac{2\zeta\epsilon}{3\eta} \vec{E}. \quad (1)$$

Note that the *electrophoretic mobility*, defined as the ratio $\mu \equiv v/E = 2\zeta\epsilon/3\eta$, is often used instead of the velocity. In the opposite (Smoluchowski) case of thin Debye layers, $R/\lambda_D \gg 1$, the interface appears planar to the counter-ions. The *electro-osmotic flow* (EOF) of the fluid approaches the *Smoluchowski slip velocity* in the rest frame of the surface

$$\vec{v}_s = \frac{\zeta\epsilon}{\eta} \left(1 + e^{-R/\lambda_D}\right) \approx \frac{\zeta\epsilon}{\eta} \vec{E}. \quad (2)$$

The particle is thus driven through a stagnant fluid at the Smoluchowski mobility $\mu = \zeta\epsilon/\eta$ and since λ_D is the only relevant length scale, the particle's size or shape does not enter the problem explicitly.

Notably, the mobilities in these two limits differ only by a numerical constant for a sphere. The mobility of spheres with finite Debye layers naturally fall somewhere between the Hückel and Smoluchowski limits, which suggests

$$\mu = \frac{\zeta\epsilon}{\eta} \times f_H(R/\lambda_D) \quad (3)$$

where Henry's function f_H is a coefficient of order 1 that satisfies both limits.

2.2. Oligomers

If we now consider a chain of charged beads (or monomers) of size b , we can see the transition from Hückel to Smoluchowski behavior as the number of monomers N increases. Since $\lambda_D > b$, a polyelectrolyte monomer ($N=1$) is clearly in the Hückel limit. Thus, if a second monomer is bonded to it, their diffuse layers overlap. Because the shearing of the fluid happens over the length scale λ_D (the viscous drag depends on the size of the ion sheath), the drag of the dimer does not increase significantly. Since the charge increases and the drag does not, the mobility of the connected beads is much greater than that of the isolated monomer. As we continue to add monomers, the dimer becomes an *oligomer*. In a good solvent, sufficiently long oligomers adopt a self-avoiding coil configuration with a characteristic size $R_g(N)$, the *radius of gyration*. If $R_g \ll \lambda_D$ the entire oligomer is in the Hückel limit. However, as R_g becomes greater than λ_D , the viscous drag on the oligomer grows linearly with further increase of the contour length. With both the drag and the charge growing linearly with N , the mobility eventually saturates. This rise and saturation of μ is shown in the upper curve of Fig. 1.

Note that this is not what happens if hydrodynamic interactions (HI) are ignored. In the absence of HI, the drag on the monomers is generated locally and not over the extent of the ion sheath. In this case, overlapping diffuse layers are of no consequence, and the drag increases with N right from the start. Simulations that neglect HI completely fail to capture cooperative shearing and so the mobility decreases monotonically, as seen in the lower curve of Fig. 1 [5,6]. The reason for this somewhat surprising decrease in mobility is due to charge condensation effects. As the contour length $L=Nb$ increases the electrostatic potential energy within the Debye layer increases, causing counter-ions to condense out of the diffuse counter-ion cloud into the Stern layer. This so-called *Manning condensation* results in a reduced effective charge of each monomer, thereby reducing the electrophoretic mobility of the polyelectrolyte chain if HI are neglected. Recent

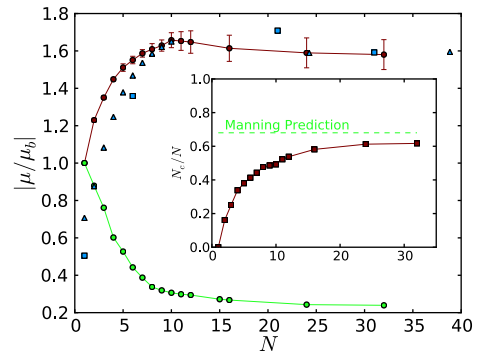


Fig. 1. Electrophoretic mobility of oligomers normalized by the mobility of a single monomer μ_0 . Computer simulations that neglect HI are shown in green. Simulations that do include hydrodynamics (shown in red) agree well with experimental capillary electrophoresis (blue triangles) and electrophoretic NMR (blue squares) mobility. Inset: Fraction of monomers onto which a counter-ion has condensed, N_c/N , from simulations with hydrodynamic effects compared to the Manning prediction [5]. Produced from data provided by C. Holm.

simulations (reproduced in the inset of Fig. 1) confirm that counter-ion condensation increases with chain length [5,6].

Interestingly, the competition between the Manning condensation effect and the increase in mobility due to cooperative shearing effects in overlapping Debye layers is consistent with the observed non-monotonic dependence of μ on N shown in Fig. 1 [5,6]. The mobility reaches a maximum for intermediate sizes before decreasing towards the asymptotic mobility $\mu_0 \equiv \mu(N \rightarrow \infty)$. Presently, no theoretical argument correctly models the maximum in mobility of charged oligomers.

2.3. Free-draining behavior

Sufficiently long DNA molecules migrate with the same electrophoretic mobility μ_0 regardless of their contour length L . This plateau in mobility occurs because the effective charge and the effective friction both scale linearly with L [7,8]. This well-known effect precludes the use of free solution electrophoresis as a separation method for most polydisperse DNA samples. The key feature responsible for this effect is the screening of the HI between the DNA monomers. As a result, the DNA chain friction coefficient is simply N times a characteristic local monomer friction coefficient: $\xi = N\xi_b$. This so-called *free-draining* behavior is due to the complimentary response of charged monomers and their associated counter-ions to an electric field. When a moderate field is applied, the force on the monomer is equal and opposite to the force on its double layer. To first order, the momentum flux on the double layer is viscously transferred to the monomer and vice versa; the field pulls the monomer one way, and the counter-ions in the opposite way, and the viscous forces on the fluid from each cancel as schematically shown in Fig. 2.

For a thick Debye layer, the hydrodynamic flow field decays exponentially [9], whereas for thin Debye layers the residual hydrodynamic field goes as r^{-3} [3]. In either limit, the effective HI decay rapidly with distance. We contrast this with the fluid response to non-electric forces, such as applied mechanical forces acting on specific monomers or body forces such as gravity that act on all monomers, for which the HI set up by fluid flows decay as r^{-1} [10]. The resulting motion of all chain segments is coupled together, leading to collective and cooperative action, and the chain appears as a consolidated object opaque to fluid flow [11]. Thus, the friction on the polymer depends explicitly on its conformation and scales as its hydrodynamic radius $R_H \sim R_g$. Interestingly, Brownian thermal forces on the monomers also generate long-ranged hydrodynamic interactions between the monomers of a polyelectrolyte, even in the presence of an electric field. Thus, the motion of a polyelectrolyte during electrophoresis is a superposition of the

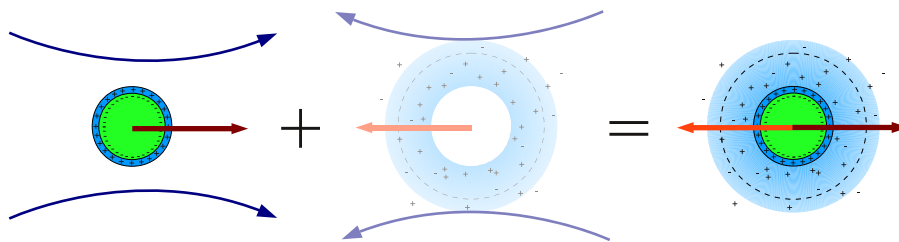


Fig. 2. The electric field pulls on the monomer and its ion sheath with equal and opposite force. To first order, the perturbation of the fluid due to the shear from the monomer is canceled by the shear due to the counter-ions. HI can only exist on length scales shorter than λ_D .

convective flow of a free-draining polymer superimposed with diffusive motion governed by a Stokes-like diffusion coefficient that scales like $1/R_H$ [7]. As a consequence, one cannot use a Nernst–Einstein equation to link the electrophoretic mobility μ to the diffusion coefficient D of a polyelectrolyte subjected to an electric field. The qualitatively different effects of electric and non-electric forces on the transport of polyelectrolytes in solution provide opportunities for novel methods for their separation. This will be the focus of much of this review.

3. Electro-hydrodynamic equivalence principle

The previous section presented a bleak picture for free-flow electrophoretic fractionation of long DNA fragments. However, if electric and non-electric forces act in concert, the picture is different. An electric force acts on both the polyelectrolyte and the surrounding ions, but a non-electric force $\vec{F}$ can be chosen to act only on the monomers. Under simultaneous action of the two forces, the resulting motion is, in the linear regime, a superposition of the two responses. The steady state drift velocity $\vec{v}$ is achieved when the drag force F_ξ balances the electric force F_E and the non-electric force $\vec{F}$ [2•]

$$0 = \vec{F} + \vec{F}_\xi + \vec{F}_E \quad (4)$$

$$= \vec{F} - \hat{\xi} \cdot (\vec{v} - \hat{\mu} \cdot \vec{E})$$

where $\hat{\xi}$ and $\hat{\mu}$ are the effective hydrodynamic friction and electrophoretic mobility tensors. Since the counter-ions are unaffected by $\vec{F}$, the total force on the ion sheath and on the chain no longer cancel and a long ranged hydrodynamic flow field ($\sim 1/r$) appears, which generates collective responses in the chain. The transport coefficients $\hat{\xi}$ and $\hat{\mu}$ are then explicitly dependent on the polyelectrolyte conformation and in principle have a tensorial character. In particular, the drift velocity $\vec{v}$ is, in general, a function of the polyelectrolyte's size N , in contrast to the universal plateau mobility μ_0 observed in free-solution. In other words, the polyelectrolyte is no longer free-draining [10] and size fractionation is possible.

The result of Eq. (4), first proposed for polymers in the seminal work of Long et al. [2•], suggests a *Principle of Electro-hydrodynamic Equivalence* [2•]. The term $\hat{\mu} \cdot \vec{E}$ can be thought of as an effective local velocity $\vec{v}_s$. In full, the equivalence principle states that when polyelectrolytes with small Debye lengths are acted on by a weak electric field $E \ll \zeta/\lambda_D$ and simultaneously by a mechanical force F , one can replace a consideration of the full electrostatics and hydrodynamics with an effective local flow which is simply the Smoluchowski slip velocity (Eq. (2)). The remainder of the review will be spent discussing examples of such non-electric forces in the context of electrophoresis problems.

4. Tethered polyelectrolytes and stall forces

To illustrate the power of the equivalence principle, we consider a tethered polyelectrolyte in an electric field [12]. The tension induced

by anchoring the polymer in place acts as the non-electric force and the net chain velocity is $\vec{v} = 0$. Thus, according to Eq. (4), we can identify a *stall force* [2•]

$$\vec{F}_{stall} = -\hat{\xi} \cdot (\hat{\mu} \cdot \vec{E}). \quad (5)$$

We note that as a direct consequence of this principle, $\vec{F}_{stall}$ is significantly less than the sum of the electrical forces on each monomer. In the frame of the tether, the electric field generates a flow of solvent due to its action on the free counter-ions in the Debye layer of the polyelectrolyte. The magnitude of this velocity is given by Eq. (2). In the frame of the solvent, however, it is the polymer that translates through the solvent at a rate given by Eq. (2). In either view, the polyelectrolyte experiences an effective conformation-dependent hydrodynamic drag $\hat{\xi}$ [2•] and it will deform under sufficiently strong electric forces due to the associated viscous forces. This creates an interesting self-consistent drag-conformation-velocity problem.

The conformations of tethered polymers in flows are well understood [13–15]. When the drag force $F_{flow} = \xi v$ is less than thermal forces, $F_{therm} \approx k_B T/R_H$, the polymer is an undeformed coil with hydrodynamic radius $R_H \sim N^{3/5}$; while if $F_{flow} > F_{therm}$, deformation occurs. The transition between these regimes takes place at the critical field

$$E^*(N) \cong \frac{k_B T}{\mu_0 \xi R_H} \sim N^{-6/5}. \quad (6)$$

For $E > E^*(N)$ the chain is deformed more rapidly by the flow than can be relaxed by internal diffusion, resulting in deformed steady-state conformations. The polymer then unfurls into the so-called *trumpet* regime in which the conformation is a series of increasingly large blobs. The blob size increases because chain tension decreases from a maximum value at the tethering point to zero at the free end of the polymer (see Fig. 3) [13,14]. When $E > N^{1/5} E^*$, a small fully-extended chain section appears near the tether point (the so-called *stem-flower* regime [13,14]), eventually leading to a fully extended chain for $E > N^{6/5} E^*$ [16].

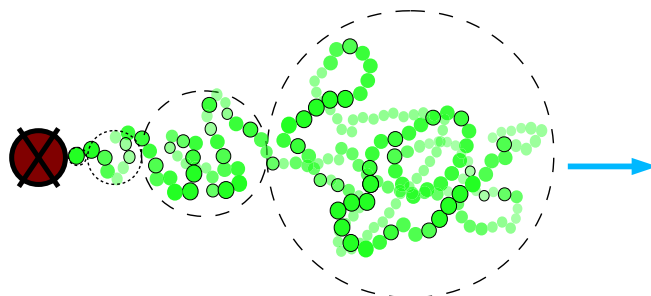


Fig. 3. The trumpet regime of a polyelectrolyte tethered to a stationary post in the presence of an electric field ($1 < E/E^* < N^{1/5}$) or a moderate flow field.

The equivalence principle has been verified for highly extended conformations both by explicit molecular dynamics simulations [20,21] and experimentally by fluorescence for dsDNA stained with ethidium bromide [12,19] and epifluorescence microscopy of YOYO-1 labeled λ -DNA [17•]. By tethering DNA to insulating elliptical posts (which do slightly perturb the otherwise uniform electric field), it has been demonstrated that tethered DNA is indeed not free-draining. In fact, comparison between extension by an electric field and extension by a flow field reproduced in Fig. 4 shows very nearly identical results and demonstrates that the equivalence principle is a very useful approximation. The slight systematic errors in Fig. 4 suggest that at high fields full electro-hydrodynamics are not completely linear and Eq. (4) is no longer strictly appropriate. Stigter and Bustamante [22] describe the mobility in this regime through a numerical theory that incorporates the alignment of DNA with the applied field. Polyelectrolytes are modeled as worm-like chains of ellipsoidal segments with HI. According to this *Stigter model*, the resulting extension produced by an electric field compared to a flow field given by Eq. (2) differs except at the smallest deformations. For a given stall force, flow fields stretch polyelectrolytes to a greater extent than electric fields demonstrating that strictly speaking the equivalence principle is only valid for small deformations [22]. However, as a working approximation it is quite sufficient.

5. End-labeled free-solution electrophoresis (ELFSE)

Tethering a polyelectrolyte to a post may be a good test of the equivalence principle but the practical potential of Eq. (4) lies in the idea that by frustrating the free-draining nature of polyelectrolytes, DNA can be separated based on size. In many ways tethering a polyelectrolyte to a neutral object (a *label* or *drag-tag*) that is free to convect is equivalent to polymers tethered to a post when viewed in the rest frame of the polymer. In fact, this theoretical framework can be used to study polyelectrolytes dragging an uncharged label (ELFSE) as well as charged labels pulling a neutral polymer (called *Free-Solution Conjugate Electrophoresis* [23–25]). The drag-tag can be a colloid [26], a micelle [27], a neutral polymer [28•] or any microscopic object that provides a drag force. Let us consider two types of drag-tag: (i) a hard sphere as shown in Fig. 5, and (ii) a second polymer (or polyelectrolyte) as in Fig. 6.

5.1. Spherical drag-tags

If we apply the equivalence principle to both objects independently (i.e., we assume that there is no hydrodynamic or electrostatic interactions between the drag-tag, which may be charged too, and the polyelectrolyte) then from Eq. (4) the forces on the polyelectrolyte

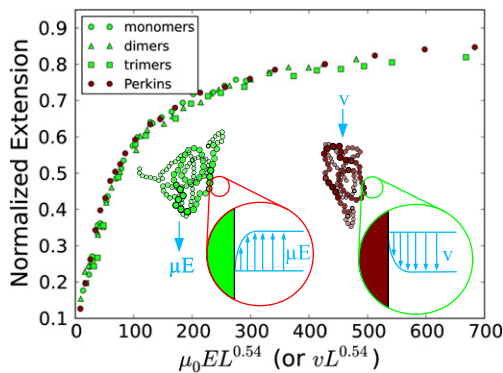


Fig. 4. The fractional extension of a λ -DNA fragment plotted against the combination $\mu_0 EL^{0.54}$ as measured by Ferree and Blanch [17•] compared to the extension by flow measured by Perkins et al. [18] (x-axis is now $vL^{0.54}$). A similar analysis was done by Long et al. [12,19]. Produced from data provided by S. Ferree and T.T. Perkins.

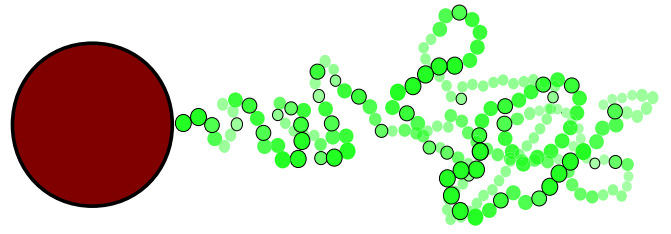


Fig. 5. The electrophoretic motion of a polyelectrolyte pulling a neutral colloidal particle.

(subscript p) and drag-tag label (L) are $F_p = \xi_p(v - \mu_p E)$ and $F_L = \xi_L(v - \mu_L E)$. For a spherical label of radius R and mobility μ_L , $\xi_L = 6\pi\eta R_L$. By balancing the forces, the mobility of the composite object is found to be

$$\mu_{ELFSE} = \frac{v}{E} = \frac{\xi_p \mu_p + \xi_L \mu_L}{\xi_p + \xi_L}. \quad (7)$$

This expression is deceptively simple since ξ_p is conformationally dependent and so is a function of the effective velocity of the fluid ($v - \mu_p E$) stretching the polymer, i.e. $\xi_p \rightarrow \xi_p(v - \mu_p E)$. The mobility must be solved self-consistently [1].

5.2. Diblock copolymer drag-tags

When the drag-tag is another polyelectrolyte, the difference isn't directly in Eq. (7) but rather in the form of the friction coefficient of the drag-tag [29]. In this case, the conformations (and so the friction coefficients) of both blocks are functions of their respective effective flows: $\xi_p \rightarrow \xi_p(v - \mu_p E)$ and $\xi_L \rightarrow \xi_L(v - \mu_L E)$. A key ingredient of the dynamics is that as the field induces elongation of the two polymers, ξ_p and ξ_L increase, which results in a reduction of the net velocity v . Again, we have a complicated self-consistent problem. Simulations using *dumb-bell models* of ELFSE complexes show that at high enough fields, this coupling can result in a nonmonotonic mobility as a function of electric field [30]. For the important case of uncharged drag-tags [31], the composite mobility reduces to

$$\frac{\mu_{ELFSE}}{\mu_p} = \frac{\xi_p}{\xi_p + \xi_L} = \frac{1}{1 + \xi_L/\xi_p}. \quad (8)$$

It has been implicitly assumed that the drag-tag and the DNA are hydrodynamically separated objects throughout this discussion but this is not necessarily true. This assumption is most suspect when the complex is not deformed by electrophoretic motion. In this case, there is no reason to assume that the drag-tag and the DNA occupy different space (most likely the label is immersed within the DNA conformation or vice-versa). Experiments [28•] and simulations [32] indicate that the field strengths normally applied are too small to induce segregation.

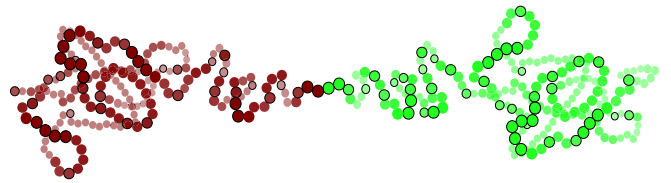


Fig. 6. A diblock copolymer as an ELFSE complex. As the electric forces move the charged block through the fluid, it must drag the other. Both chains deform in opposite directions and the mobility of the complex must be self-consistent with the conformation of the complex.

There is a subtle point here [33]: One may be tempted to argue that when the charged DNA engulfs the uncharged polymeric label the DNA double-layer ensures that the entire complex is free-draining (i.e., perhaps the drag-tag is free-draining too). This is not the case. The friction coefficients of the drag-tag and the DNA still depend explicitly on the conformation even when unsegregated and randomly mixed. The net electrophoretic mobility of random coil polyampholyte chains is highly dependent of the charge distribution but, when the Debye layer is small, it is essentially an average of the mobility of each monomer [33]. The ELFSE complex can be viewed as a di-polyampholyte. In this case, Eq. (8) is still applicable, but since the friction terms have different meanings, it is more practical to rewrite it as

$$\frac{\mu_{\text{ELFSE}}}{\mu_p} = \frac{1}{1 + \alpha/N} \quad (9)$$

where N is the number of DNA monomers and α is the number of bases contained in a DNA blob that has the same hydrodynamic radius as the drag-tag [34•]. Since α is a relative friction coefficient, it depends on the persistence length of DNA. This expression has been tested experimentally and was found to be in superb agreement with data. The value of α can actually be predicted with good accuracy from the basic physical properties of the DNA and its drag-tag [24,35].

Early experimental work on ELFSE showed that DNA with streptavidin drag-tags tethered to both ends were significantly slower than one would expect on the basis of the drag generated by a single streptavidin [36]. It wasn't until end-effects were looked at in detail that this enhancement was understood and identified as potentially useful [37]. In brief, charged end monomers contribute slightly more to the overall mobility of the complex because they are more likely to reside outside the core of the coil [34•]. Replacing an end-monomer by an uncharged label thus has an effect slightly larger than predicted by Eq. (9). Therefore, it was predicted that an ELFSE label of size $2\xi_L$ placed at one end of a DNA molecule would slow it down less than two labels of size ξ_L placed at the two ends. Experimental comparison of DNA with synthetic polypeptoid drag-tags at one or both ends confirmed the predictions of enhanced drag [38].

The improved understanding of ELFSE allows for the design of efficient drag-tags. Indeed, some engineered labels have been considered. For example, branched drag-tags [39] and soft micelles [40,41,27] both hold great potential for ELFSE because of their large hydrodynamic size [35].

6. Collisions with posts

Arrays of isolated posts have been investigated in recent years as a means of electrophoretic separation of DNA by length [42] (dense arrays are essentially similar to sieving gels). In this scenario, a polyelectrolyte chain that collides and catches on a post will have a residence time on the post before escaping that depends of the chain length. Intuitively, this process should be able to provide molecular separation if the collision frequency and system parameters are chosen properly.

In general, we may divide polymer-obstacle collisions into four stages: (i) free flow approach, (ii) deformation, (iii) unhooking escape, and (iv) partial or complete conformational relaxation before the next collision. Here we are most interested in regimes (ii) and (iii), when the post interacts with the chain. Since the post exerts a transient reaction force on the chain during collision and the subsequent escape, the equivalence principle holds during this process. So, the effective drag force on the chain while it is associated with the post depends on both chain length and conformation, just as was described above for end-tethered polyelectrolytes.

As shown in Fig. 7, there are several possible ways a polyelectrolyte can collide with a post. We will focus our attention on finite sized posts of diameter $D < R_g$. If the impact parameter between the

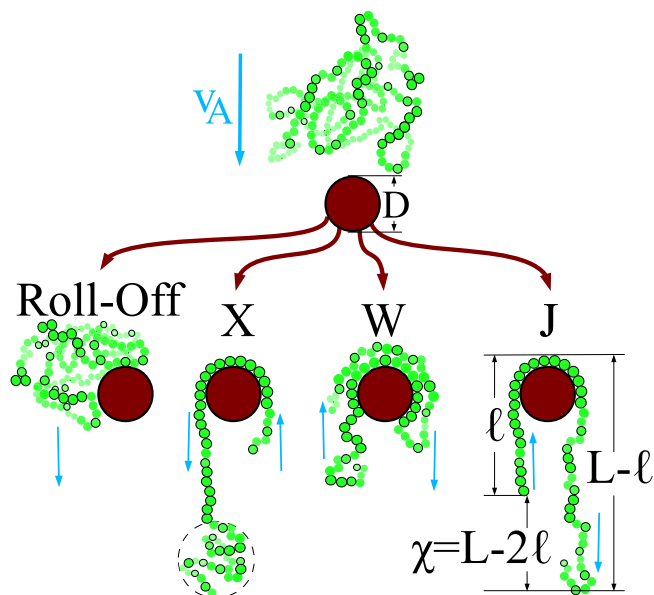


Fig. 7. A schematic description of the polymer-post collision process. The chain approaches the post relatively undeformed before it collides and either rolls off or deforms. The different arms compete to drag the polymer over the post until one side is chosen and the chain un-hooks. The hooked chains can be categorized as (i) J-collisions, (ii) W-collisions, and (iii) X-collisions.

post and the center of mass of the chain is less than R_g , then the probability of a collision event occurring is certain. However, as the impact factor becomes larger than R_g the probability of a collision quickly decays [43]. When a collision does occur, the polyelectrolyte can simply roll off the post (a possibility that is especially important if the driving field is too small to unravel the chain), or it can deform around the post and temporarily hook [44]. The propensity of the chain to either roll off or hook to the post is determined by the Deborah number $De = \tau_{rx}/\tau_A$, the ratio of the natural relaxation time of the polymer to the characteristic time scale of the shearing $\tau_A \approx D/\mu_0 E$. When $De < 10^0$, minimal stretching occurs and the polyelectrolyte rolls off the post. For $10^0 < De < 10^2$ hooking occurs with a probability that is highly dependent on De [44]. Finally, in the regime $De > 10^2$, the hooking probability as a function of impact parameter becomes independent of De , and the chain deforms significantly [44].

If the chain does hook to the post, it will adopt a transient pulley-like conformation in which each arm competes to escape. Such conformations are often categorized as being in one of the three states shown in Fig. 7: J-collisions, X-collisions, and W-collisions. Simple collisions that result in a single strand of polymer hooked around the post with stretched arms downstream are referred to as J-collisions. An X-collision is a very asymmetric variant of the J-collisions with a short, extended arm and a long, partially relaxed arm that forms a stem-flower conformation. In an X-collision, the chain unhooks before the long arm can fully extend. A W-collision is a relatively rare event in which a polymer is partially trapped on the post in a folded conformation with numerous strands in contact with the post. This is a metastable state that often becomes a J-collision during the escape process [45,46]. We refer readers interested in W- and X-collisions to the excellent review by Dorfman [42] who covers collisions in some detail. In the following, we focus our attention on the case of J-collisions.

J-collisions are conceptually simple because the arms can be treated as two extended threads. In the large force limit of $E \gg E^*$, both arms are fully stretched at all times t [47]. The pertinent lengths are defined in Fig. 7. The shorter arm has a length $\ell(t) < L/2$, where $L = Nb$ is the contour length of the polymer, and the longer one has a length $L - \ell(t)$. The difference between the two arms is $\chi(t) = L - 2\ell(t)$.

The two arms are competing so unhooking dynamics result from the balance of the forces on the two arms. The net force on the chain due to the electric field that results from the excess length χ is $\xi_\chi v_A$, where $v_s = \mu_0 E$ is the equivalent electrophoretic flow rate. As the chain unhooks at a rate $d\ell/dt$, it resists with an overall drag force $-\xi_j d\ell/dt$. Notice that ξ_χ is the friction coefficient of the excess length while ξ_j is the friction coefficient of the entire chain in the J-collision conformation. Thus, the unhooking process obeys the equation of motion

$$\frac{d\ell}{dt} = v_s \frac{\xi_\chi}{\xi_j}. \quad (10)$$

Assuming the equivalence principle applies, the friction coefficients for an extended, rod-like chain section of n monomers of size b and friction coefficient ξ_b , scales as $\xi_{\text{rod}} \sim n\xi_b/\ln n$. This provides estimates for both ξ_j and ξ_χ . Integrating Eq. (10) gives the length of the shorter arm $\ell(t)$ throughout the unhooking process. From this, the escape time τ_e can be found as the time at which $\ell(t = \tau_e) = 0$. Averaging the initial length $\ell(0)$ over all possible values ($0 - L/2$) gives the mean unhooking time as [15]

$$\langle \tau_e \rangle = \left(\frac{Nb}{2\mu_0 E} \right) \times g(N) \quad (11)$$

where $g(N)$ is a weak (logarithmic) function of N that differs depending on the nature of the force applied and the role of the HI (in practice, $g \approx 1$). The collision process has been studied in detail for various situations by Kenward and Slater [48] using a theoretical framework that includes HI but excludes electrophoretic forces.

However, for weak electric field strengths the two arms of the J-conformation are not fully stretched, and this simple expression is no longer valid. Field strength actually plays a cardinal role in determining unhooking dynamics [47]. For moderate fields in the range $N^{1/5} < E/E^* < 10N^{1/5}$, there is a broad transition regime between extended arms (the case presented above) and undeformed coils. At these fields, the arms adopt the trumpet conformations discussed in Section 4. Early work on incorporating hydrodynamic interactions into the weak field regime applied the equivalence principle to each arm and predicted different scalings for the escape time of freely draining and hydrodynamically coupled chains [16]. To the best of our knowledge, these scaling laws have not been verified by experiments or simulations. A more recent model proposes an escape time similar in form to Eq. (11) but with E -dependence incorporated [45,46]. The unhooking time in moderate fields is

$$\tau_e = \frac{\mathcal{L}}{2\mu_0 E} \ln \left(\frac{\mathcal{L}}{\mathcal{L} - \ell} \right) \quad (12)$$

where $\mathcal{L}$ is the effective contour length of the stretched chain. The effective length goes as $\mathcal{L} \sim N^\gamma$ where the scaling γ is set by the field strength E and lies within the range $0.54 < \gamma < 0.75$ [45,18] for weak fields and $\gamma \rightarrow 1$ for the strong field limit discussed previously. The escape time has been verified many times using Brownian Dynamics simulations that neglect HI completely [47,49] and compared to experiment [46] but results that test the role of HI are lacking.

7. Confinement effects

7.1. Confinement between plates

Confining a polyelectrolyte in a narrow channel, capillary, or between closely spaced parallel walls sterically limits its possible conformations, inducing chain extension in the unconstrained dimensions. The confinement increases the probability of interaction between the monomers of the polyelectrolyte and the molecules of the interface,

thereby inducing a non-electric force during electrophoresis due to added friction of the monomers in transient contact with the wall. This non-electric frictional force is presumably an extensive quantity that increases linearly with the number of monomers in contact with the wall:

$$\vec{F} = \vec{F}_w \approx \omega N \xi_w \vec{v}. \quad (13)$$

where ξ_w is the monomer-wall friction factor, N is the degree of polymerization of the polyelectrolyte, and ω is the fraction of monomers in transient contact with the wall. The confining walls have a second effect in this scenario. The proximity of the walls screens the hydrodynamic interactions between monomers (by symmetry of the angular pre-averaging of the hydrodynamic interaction tensor [50,51]). Therefore, the friction coefficient of the chain is typical of a free-draining chain [52], $\vec{F}_\xi = N \xi_b \vec{v}$. Putting $\vec{F}_\xi$, $\vec{F}_w$ and $\vec{F}_E = N \xi_b \mu_0 \vec{E}$ into Eq. (4) gives

$$\mu = \frac{\mu_0}{1 + (\xi_w/\xi_b)\omega} \quad (14)$$

In the weak field limit ($E \leq 300$ V/cm), the electrophoretic mobility of λ -DNA confined between narrowly separated plates ($D \approx 19$ nm) is consistent with Eq. (14) for $\omega \sim N^{1/2}$ [53]. This result may be understood qualitatively by appealing to established scaling notions for confined polymers. For a polymer chain with contour length $L = Nb$ confined in one or more dimensions to a gap of size D , one might expect the fraction of monomers ω in contact with the wall to be an increasing function of the ratio L/D . Consider the case of a polymer confined in solution within a narrow gap, $D \ll R_g$, between parallel planar surfaces with a uniform distribution of adhesive sites. For gaps smaller than the persistence length of the polymer, $D < p$, the polymer adopts a quasi-2D self-avoiding conformation in good solvent conditions with lateral radius of gyration $R_{||} \sim N^{3/4}$. As each monomer is in the vicinity of the walls, the fraction of monomers in contact at any instant with adhesive sites on the wall scales as $\omega \sim \Theta R_{||}^2/N \sim N^{1/2}$, where Θ is the surface number density of adhesive sites. A version of this argument has been previously proposed for larger gap sizes, $p \ll D \ll R_g$ [53], with the same conclusion. At even larger fields, λ -DNA continues to move through nanoslits in a stick-slip manner [54]. This suggests that the mean friction ξ_w may be field-dependent and a result of entropic or dielectrophoretic trapping.

7.2. Single adsorbing wall

In fact, it is possible to restrict the conformational freedom of a polyelectrolyte with only a single wall. Consider DNA near an adsorbing flat surface. The surface enacts a non-electric force on the polymer and the deformed DNA conformation is the net result of the competition between adsorption and the entropy loss due to confinement. The equivalence principle can thus be utilized and applying an electric field parallel to the surface can produce a molecular size dependent mobility.

This phenomenon was first reported by Pernodet et al. [55]. The additional friction between the surface and the (adsorbed) DNA appears to be responsible for the fact that DNA fractionation is now possible. Exactly how the mobility scales with contour length remains an unsettled issue. The earliest work found $\mu \sim N^{-\beta}$ where $\beta = 0.87$ for double-stranded DNA on Si wafers [55] and yet later investigations observed $\beta \approx 0.25$ [56,57]. A deterministic model has been proposed that indicates β may in fact be field dependent [58]. At the smallest fields this model predicts that $\beta = 1$ while at the largest fields it suggests β goes to zero and the mobility again becomes size independent.

Experiments of DNA electrophoresing along self-assembled, corrugated surfaces of nonporous silica beads indicated that surface friction rather than topology controls fractionation [56]. Engineering

complicated chemically nanopatterned surfaces can augment small changes in conformation (and presumably friction) improving performance [57,59–61].

8. Electro-hydrodynamics of Soft Interfaces

In this section we look at recent progress in the understanding of the use of polymer coatings for modulating the shear of a fluid at a solid–liquid interface. This field can be divided into two sub-topics: polymer coatings for modulating the electro-osmotic flow generated by an immobile charged surface (EOF) [62], and the electrophoresis of soft colloids [63]. In EOF researchers are interested in the fluid velocity far from the wall while in soft colloid electrophoresis researchers are interested in the mobility of the polymer grafted colloid. Understanding one gives insight into the other since the physics which governs the two is very similar. Below we connect recent advances in understanding of the modulation of EOF with parallel advances in the field of soft colloid electrophoresis.

Much of the theory of soft interfaces comes from the soft colloid electrophoresis community [63,64] and is based on continuum hydrodynamic and electrokinetic transport equations for particles with porous, compliant polymeric coatings. Alternative approaches have utilized scaling models from polymer physics [65•] or coarse-grained simulation methods to study the response of polymer layers to EOF [66–70].

One prediction in both the soft colloid electrophoresis and soft interface EOF communities is that for a thick enough coating both the mobility of the colloid and the EOF mobility generated by the coating are simply the free-draining mobility of individual polymers, μ_0 . The reason for this is simply the fact that the polymers shear the fluid such that the difference in the velocity of the backbone and the nearby fluid is the Smoluchowski slip velocity. Thus for soft colloids being electrophoresed the polymers move $\mu_0 E$ faster than the fluid, while for grafted polymers the surrounding fluid is pumped with a velocity $-\mu_0 E$ as schematically shown in Fig. 8. The equivalence between EOF and the electrophoretic mobility was investigated in experimental studies utilizing heteropolymers of variable charge densities. Heteropolymers made of neutral and charged monomers were synthesized and the electrophoretic mobility of the polyelectrolytes was compared to the EOF produced when the same heteropolymer was used as a polymer coating [71]. The two values showed very close agreement, although the EOF mobility was slightly lower.

Efforts to model the effects of polymer coatings on EOF have focused on end-grafted polymers in the limit of thin Debye layers (the Smoluchowski limit) [65•]. This scaling theory makes predictions for

EOF profiles within and outside of the polymer layers as a function of the grafting density, the degree of polymerization, electrophoretic mobility of the polymers, the electric field, and the electro-osmotic flow in an uncoated channel. Partial inhibition of EOF by neutral polymer layers on charged surfaces was predicted, and the implications of the electro-hydrodynamic equivalence principle were considered. In the limit of thicker Debye layers, there have been numerous theoretical studies of the electrophoresis of polymer-coated colloids [63,64]. As an example, for polyelectrolyte grafted colloids at low-to-moderate ζ -potentials, detailed predictions based on continuum electrokinetic transport analysis have been made for the deviation of the electrophoretic mobility from free-draining behavior [63]. In both cases the difference in the fluid velocity between the interface and the bulk approaches the Smoluchowski slip velocity as the coating becomes thicker, although the difference for thinner coatings differs. In addition, the fluid flow profile, while qualitatively similar, is quantitatively different in the two cases.

Recently, there has been increasing effort to develop accurate and efficient computer simulations of EOF near soft interfaces. For instance, predictions of the scaling approach of Harden et al. [65•] for the dependence of the EOF mobility on grafting density and degree of polymerization has been confirmed by MD simulations for neutral chains grafted to a charged surface [66]. Other simulations of rod-like polymers fixed rigidly to a surface (to ensure uniform density as a function of height from the surface) focused on the fluid velocity profile near the wall [67], which agreed with theory [65•]. When the polymers were allowed to stretch, a non-linear relationship between the EOF mobility and the applied electric field was observed, but with a different form from that predicted for flexible grafted chains [68]. While these studies all focused on neutral coatings new computational methods based on the equivalence principle [21,72] should allow for simulations of charged coatings.

As an example of the similarity between EOF near soft interfaces and soft colloid electrophoresis, one can consider the effect of solvent quality on the ability of neutral polymer coatings to quench EOF [68,69], and compare this to soft colloid electrophoresis experiments, which look at the temperature dependence of PNIPAM coated charged colloids [73]. Both approaches address how neutral polymers affect the shear of fluid at a charged surface as the solvent quality varies. In each case, there is significant slip at the interface when the solvent quality is poor and the polymer coating is collapsed on the surface. When the solvent quality is good and the polymers swell to form a thick layer, both approaches demonstrate that the slip is virtually eliminated. Moreover, both approaches show that the change in the height of the polymer layer is strongly correlated with a change in the slip at the

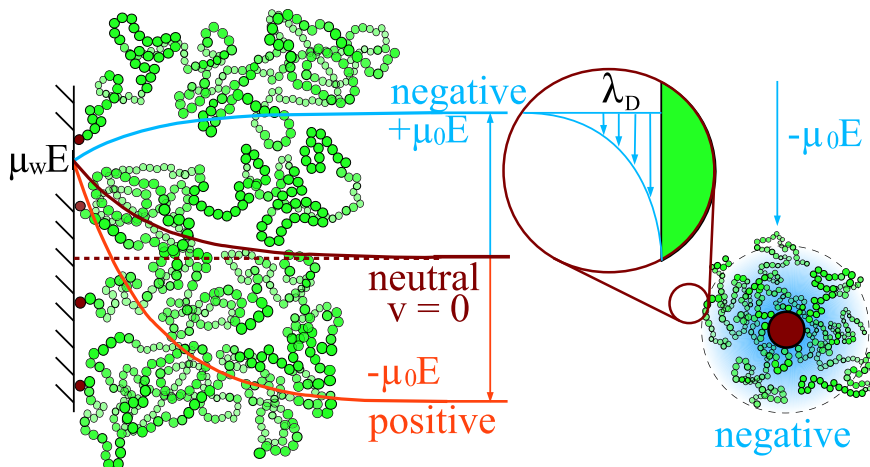


Fig. 8. A thick enough neutral coating will reduce the EOF from the bare wall value of $v_f = \mu_w E$ to zero outside of the polymer layer. A positively charged coating will cause the velocity to be reduced even further to $-\mu_0 E$, where μ_0 is the free-solution mobility of the polymer itself. On the other hand, a negatively charged layer will contribute to the EOF increasing the fluid velocity up to $\mu_0 E$. A soft colloid electrophoreses at the same velocity as the EOF produced by its brush.

interface. Similar results have been seen in experiments on the modulation of EOF with polymer coatings with varying degrees of hydrophilicity [70,74].

9. Future perspectives

Electro-hydrodynamics of polyelectrolytes is an important topic with numerous practical applications, most notably DNA fractionation. While long chains subject only to an electric field are free-draining, polyelectrolytes subject to both an electric field and a mechanical force are not—a fact not widely appreciated in the literature. In many such situations, the Principle of Electro-hydrodynamic Equivalence can be used to predict the mobility. This review covered several prototypical systems currently being studied but many more exist and inevitably new systems will emerge.

While not covered in this review, nanopores have received extensive attention; however, only recently have researchers recognized the importance of hydrodynamics. Grosberg and Rabin have discussed the role of the equivalence principle as molecules transition from the bulk solution into the nanopore [75]. During the translocation process itself, the stall force required to hold a polyelectrolyte fixed in the pore is indeed given by Eq. (5) [76–78]. On the other hand, hydrodynamics on the scale of the polymer coil do not appear to play an important role and in certain cases can be ignored.

Separation by confinement was discussed in this review but only between two parallel plates. Confining DNA to dimensions smaller than its persistence length is possible and such strongly confined systems exhibit markedly different behavior than that discussed here [79]. Current experiments focus on micro- or even nano-scale systems but still rely on relatively simple geometries due to the difficulty in manufacturing non-trivial patterns. Fabrication approaches continue to improve the ability to tailor device geometries, although it is unclear what complex structures will be best for the fractionation of DNA or other biologically relevant macromolecules (or even cells and organelles). Entropy-exploiting devices [80], nanopits [81], and future engineered surfaces are among the systems that will likely lead to superior separation potential.

While current devices have been designed based on purely physical intuition, in the near future device geometries and dimensions will be pre-optimized based on computer simulation results made possible by increases in computing power and new simulation techniques [82]. In particular, simulation methods based on the equivalence principle have emerged in recent years for several mesoscopic fluid models such as dissipative particle dynamics [72], Brownian dynamics [83], and lattice-Boltzmann [21] fluids, which have shown realistic behavior with drastically less computation time. These and other future developments should lead to crucial advancements in this important field.

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Simulations of Free-Solution Electrophoresis of Polyelectrolytes with a Finite Debye Length Using the Debye-Hückel Approximation

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We introduce a mesoscale simulation method based on multiparticle collision dynamics (MPCD) for the electrohydrodynamics of polyelectrolytes with finite Debye lengths. By applying the Debye-Hückel approximation to assign an effective charge to MPCD particles near charged monomers, our simulations are able to reproduce the rapid rise in the electrophoretic mobility with respect to the degree of polymerization for the shortest polymer lengths followed by a small decrease for longer polymers due to charge condensation. Moreover, these simulations demonstrate the importance of a finite Debye length in accurately determining the mobility of uniformly charged polyelectrolytes and net neutral polyampholytes.

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The complex interplay between electrostatics and hydrodynamics can give rise to surprisingly counterintuitive phenomena. Notably, several papers by Long *et al.* [1–4] predicted a variety of interesting physical behavior for polyelectrolytes in which hydrodynamics play a crucial role. Despite this, early simulations of polyelectrolyte electrophoresis generally neglected long ranged hydrodynamic interactions because they focused on understanding traditional gel electrophoresis through a cross-linked polymer matrix, where the matrix screens hydrodynamic interactions [3]. New methods have emerged over the past twenty years which use microfabricated devices to conduct electrophoresis [5]. In many of these systems, hydrodynamic interactions are only partially screened, and thus, to simulate electrophoresis algorithms which take them into account are necessary.

There are a wide variety of methods in the literature to simulate electrohydrodynamics [6]. Recent work has combined traditional molecular dynamics (MD) simulations for electrostatic interactions with either a lattice-Boltzmann fluid [7,8] or one based on multiparticle collision dynamics (MPCD) [9,10]. These methods reproduced reasonable electrophoretic behavior but the computational cost of calculating interactions explicitly is quite large. Several methods to eliminate costly electrostatic calculations use an infinitely thin Debye layer approximation, where there is a Smoluchowski slip between the charged object and the surrounding fluid. Our previous work was able to produce correct behavior for a variety of systems using a modified coupling of monomers to a lattice-Boltzmann fluid [11]. Others have reproduced free draining behavior of long polyelectrolytes using a dissipative particle dynamics fluid [12]. Simulations have also been done on electro-osmotic flow (EOF) in the thin Debye layer limit using both Navier-Stokes equations [13] and dissipative particle dynamics [14]. None of these methods reproduce the fluid velocity profile within the Debye layer and they are, thus, only valid when the Debye length is significantly smaller than the other relevant length scales.

There have also been several interesting approaches based on Brownian dynamics methods which use a tensor for the long range interactions [15,16]. Rex and Löwen [16] looked at the role of electrohydrodynamics in lane formation of colloidal suspensions undergoing electrophoresis. The study of Kekre, Butler, and Ladd [15] examined polyelectrolytes confined between parallel plates subject to both an electric field and a pressure driven flow. Their simulations were able to convincingly reproduce the intriguing results of Zheng and Yeung [17]. Neither of these approaches require thin Debye layers nor use costly electrostatic calculations. However, both suffer the inherent difficulties of Brownian dynamics simulations, namely nontrivial boundary conditions [15] and poor scaling with system size [18].

It has long been known that the electrophoretic mobility of long polyelectrolytes is independent of the degree of polymerization N . However, for short polymers, it is known that there is an initial increase in mobility followed by a slight drop to the free draining limit [19]. The rise for small values of N is due to the overlap of the counterion clouds of neighboring monomers, something also observed for colloidal suspensions [20]. In cases when the polymer's radius of gyration is smaller than the counterion cloud thickness, the overlapping Debye layers cause cooperative shearing of the surrounding fluid, which leads to an increased mobility. In general, the mobility $\mu(N)$ is seen as the ratio of an effective charge Q_{eff} to an effective friction coefficient Γ_{eff} : $\mu(N) = Q_{\text{eff}}/\Gamma_{\text{eff}}$. This cooperative shearing of the nearby fluid causes Q_{eff} to increase more rapidly than Γ_{eff} for small values of N [7–10,21], resulting in an increase in the mobility with respect to N .

For highly charged chains at sufficiently large N , the electric forces pull counterions out of the solution so that they condense onto the backbone of the chain. This phenomenon, known as Manning condensation [22], causes the mobility to be a nonmonotonic function of N . A slight maximum exists at intermediate lengths because

the effective charge per monomer decreases for longer polymers lengths even after the effective friction per monomer becomes relatively constant. These results have been reproduced using hybrid simulations with explicit charges [7–10]. As mentioned earlier, this suffers the drawback of requiring computationally expensive electrostatic calculations (due to the many salt ions), limiting the length and time scales which can be explored. The cost becomes extremely large for short Debye lengths, where a large amount of additional positive and negative salt ions are present.

In this Letter, we develop a simulation technique using MPCD that is valid for finite Debye lengths. By applying the Debye-Hückel approximation for the distribution of counterions surrounding a charge, we assign effective charges to MPCD fluid particles within a few Debye lengths of a charged monomer. This effectively approximates the counterion sheath without resorting to simulating the explicit behavior of individual counterions and salt ions. The computation time is significantly reduced and since the algorithm remains strictly local, simulation times scale linearly with system size and are independent of the implicit salt content. The use of MPCD also allows for the inclusion of complex boundary conditions with ease, a notable drawback of Brownian dynamics approaches. In order to validate our algorithm, we examine the electrophoretic mobility of linear polyelectrolytes as a function of length and compare to experimental results as well as simulation results which use explicit charges [7–10].

We treat the polymers as being composed of monomers which have a purely repulsive Weeks-Chandler-Andersen potential [23] between all monomer pairs:

$$\frac{U_{\text{WCA}}(r)}{\epsilon} = \begin{cases} 4 \left[\left(\frac{\sigma_{\text{LJ}}}{r} \right)^{12} - \left(\frac{\sigma_{\text{LJ}}}{r} \right)^6 \right] + 1, & r \leq 2^{1/6} \sigma_{\text{LJ}}, \\ 0, & 2^{1/6} \sigma_{\text{LJ}} < r, \end{cases} \quad (1)$$

where σ_{LJ} is the length scale of the interaction and ϵ is the energy scale [23]. We set $\sigma_{\text{LJ}} = 0.5\sigma$, where σ is the MD length scale. The mass of the MD beads is set to $3m$, where m is the MPCD mass scale. The N monomers in the polymer are linked together by finitely extensible nonlinear elastic bonds of the form

$$U_{\text{FENE}}(r) = -0.5kR_0^2 \ln[1 - (r/R_0)^2], \quad (2)$$

where $R_0 = 0.75\sigma$ is the maximum extension and $k = 120\epsilon/\sigma^2$ controls the strength of the bond. In addition, there is a Debye-Hückel potential between charged MD beads (but not MPCD beads):

$$\frac{U_C(r)}{k_B T} = \begin{cases} \frac{\lambda_B q_i q_j}{r} \exp(-r/\lambda_D), & r < r_{\text{cut}}, \\ 0, & r > r_{\text{cut}}. \end{cases} \quad (3)$$

Our simulations set the Debye length $\lambda_D = 1\sigma$ and every monomer is given a valence of $q = 1$. The Bjerrum length is set to $\lambda_B = 1.5\sigma$, an appropriate value for reproducing experimental conditions [7], while $r_{\text{cut}} = 5\lambda_D = 5\sigma$. The integration time step was set to 0.01τ (where τ is the simulation time unit), a common value in MD simulations.

Our polymer is embedded in a coarse-grained MPCD fluid model, which was developed based on stochastic rotation dynamics proposed by Malevanets and Kapral [24]. In MPCD, detailed interactions between fluid molecules are omitted and replaced with a multiparticle collision that is not physical but rather is constructed to locally conserve mass, momentum, and energy.

MPCD simulations occur in two steps. During the streaming step, particles move ballistically and without interaction such that their positions $\vec{r}(t)$ are updated in discrete time intervals δt as $\vec{r}(t + \delta t) = \vec{r}(t) + \vec{v}(t)\delta t$. The collision step transfers momentum between particles by partitioning the simulation domain into cells. Each cell has a center of mass velocity $\vec{v}_{\text{CM}}$, which corresponds to the local macroscopic fluid velocity. Collisions within each cell reassign random velocities to each cell's constituents while preserving $\vec{v}_{\text{CM}}$. The collision step ensures that the hydrodynamic equations of motion are obeyed on sufficiently long length and time scales [25].

The implementation of MPCD used in this study integrates an Andersen thermostat directly into the collision operator $R = \vec{v}_{\text{ran}} - \langle \vec{v}_{\text{ran}} \rangle_{\text{cell}}$, where $\langle \cdot \rangle_{\text{cell}}$ is the average within a given cell and $\vec{v}_{\text{ran}}$ are randomly generated velocities for each of the particles in that cell [26,27]. The distribution of $\vec{v}_{\text{ran}}$ is chosen such that the temperature $k_B T = \epsilon$. Polymers are coupled to the fluid by including monomers in the MPCD collision step [28]. The density of the fluid is set to $5/a^3$, where a is the MPCD cell size. The MPCD streaming time is set to 0.05τ . Similar to previous MPCD-MD hybrid simulations, we set $a = 2\sigma_{\text{LJ}}$ [9,10].

To include the effect of electrohydrodynamics, MPCD particles neighboring monomers are given an effective charge. We assume that the counterion density, $\rho_e(r)$, is proportional to the electrostatic potential, which obeys the Debye-Hückel approximation

$$\rho_e(r) \propto \begin{cases} \exp(-r/\lambda_D)/r, & r < r_{\text{cut}}, \\ 0, & r > r_{\text{cut}}, \end{cases} \quad (4)$$

where r_{cut} is a cutoff radius kept at $5\lambda_D$ (beyond which the results are independent of the cutoff). All MPCD particles within r_{cut} of an MD bead receive a small partial charge proportional to Eq. (4) and normalized such that the sum of the partial charges is $1e$. When an MPCD bead is within r_{cut} of several MD particles the partial charges are summed. A force of magnitude $Q_i E$ (where Q_i is the charge on particle i and $E = 1$ in this work) is then applied to both the charged MPCD particles and the MD beads. This method of assigning the counter force to the fluid

ensures both local momentum conservation and a reasonable distribution of the force on the surrounding fluid. This mean field approach is similar in spirit to the algorithm employed by Duong-Hong *et al.* [12], who artificially applied a force to dissipative particle dynamics particles surrounding a polymer chain, and for electrokinetics, provides a modest increase in performance for small systems (which would be dramatic for larger systems with large amounts of neutral solvent containing both negative and positive ions). In fact, the electrostatic portion of the MPCD algorithm is independent of box size (since salt ions are not explicitly treated), unlike explicit ion simulations, which increase at best as the box length cubed. Thus, the algorithm is ideal for the migration of individual (or dilute solutions of) polyelectrolytes in intricate micro- and nanofluidic structures, where experimental systems are increasingly large and complex.

Additionally, the effect of charge condensation can be included in this model by considering an MPCD counterion to be condensed if the charge on the particle exceeds some threshold value, Q_T that is chosen to reproduce a reasonable effective charge for long polymers. This is estimated to be $Q_{\text{eff}} \approx b/\lambda_B \approx 0.3$, where b is the bond length [7]. When a counterion is condensed, the charge on the MPCD particle is set to zero and the charge is subtracted from the corresponding MD beads from which it gained its charge via Eq. (4). After the charge of both the MD and MPCD particles is set, a force qE is added to all charged particles to account for the effects of the external electric field. This heuristic approach to including counterion condensation caused by the nonlinear portion of the Poisson-Boltzmann equation is shown to provide good results while being computationally inexpensive. Importantly, the profile far from the charged particle should always be linear; thus, the use of the Debye-Hückel approximation introduces only a small sacrifice in accuracy very close to the charged particle.

Consider a charged polymer subject to an electric field in free solution (free-solution electrophoresis) as discussed in the introduction. Figure 1 shows the mobility as a function of the degree of polymerization for a variety of simulation approaches (open symbols) as well as experimental results (filled symbols) [7].

The open triangles show simulation results when monomers have their velocity reduced by $-\mu_0 E$ prior to the collision step. This is essentially the infinitely thin Debye layer limit [11]. Reducing the velocity by $\mu_0 E$ prior to coupling the beads to the fluid causes their steady-state velocities to be $\mu_0 E$ greater than the local fluid velocity instead of equal to it. In such methods, the mobility is independent of N . Since each monomer collides independently with the fluid, both the effective charge and the effective friction grow linearly with N resulting in a mobility independent of length. This is the drawback of all simulation techniques based on the thin Debye layer assumption: while

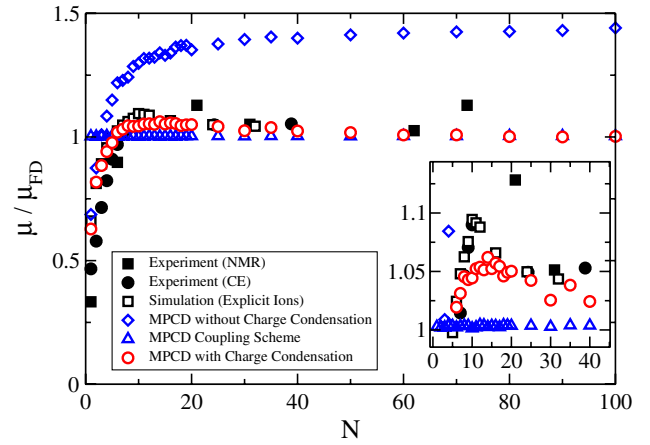


FIG. 1 (color online). The mobility of short polyelectrolytes as a function of the degree of polymerization N normalized by the free draining value μ_{FD} . The results of our hybrid simulations with and without counterion condensation (open circles and open diamonds, respectively), experimental results (filled symbols) and simulations using explicit ions (open squares). The open triangles are simulations using a thin Debye layer approximation. The experimental data and explicit ion simulation results are reproduced with permission from Ref. [7].

they may capture the correct behavior for large objects, when the length scale of the object is comparable to the Debye length, complex behavior cannot be reproduced.

The open diamonds show the result when the Debye-Hückel approximation is used to apply a counter force without attempting to take into account charge condensation. In this case, there is a sharp rise of the mobility for small values of N followed by a plateau. While the initial rise is qualitatively correct, these simulations fail to reproduce the experimental data in two respects. First, the rise in mobility continues for larger values of N than in experiment. Second, these simulations cannot reproduce the nonmonotonicity around $N = 10$ because condensation is neglected.

The open circles in Fig. 1 show our simulation results when charge condensation is included. The results agree well with experiments (filled symbols) and explicit ion simulations (open squares) [7]. The sharp initial rise at small N , the plateau to the free draining mobility at large N , and the slight maximum at intermediate N are all present. Figure 2 shows the effective friction Γ_{eff} and effective charge Q_{eff} values for both simulations using explicit ions [7], as well as the mean field MPCD-MD Debye-Hückel approach proposed here. The average effective charge Q_{eff} as a function of N for our approach agrees well with the simulations which used explicit ions. The effective friction Γ_{eff} also shows very similar behavior between our approach and explicit ion simulations. Notice that the effective friction coefficient for the MPCD algorithm with charge condensation is considerably lower than when there is no charge condensation. This is because the charges which adsorb to the polymer are those which are closest to the backbone and are, thus, the most

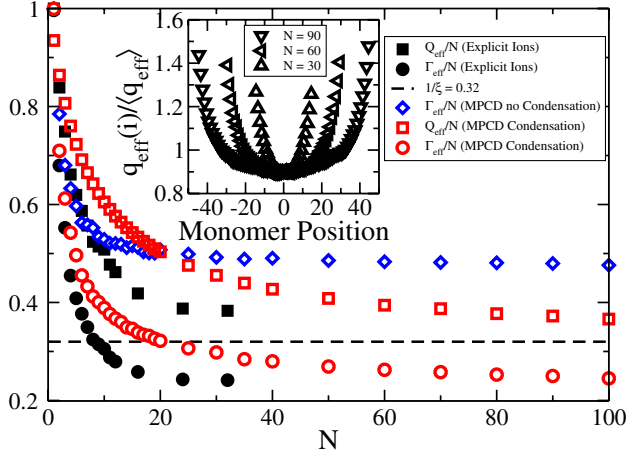


FIG. 2 (color online). Effective friction Γ_{eff} (circles) and effective charge Q_{eff} (squares) per monomer as a function of the degree of polymerization N . Filled symbols are our hybrid simulation results while open symbols are simulations using explicit ions reproduced with permission from Ref. [7]. The diamonds show the effective friction when there is no charge condensation. Inset: Effective charge of a monomer as a function of monomer position normalized by the average effective charge on all monomers.

strongly hydrodynamically coupled to it. This is why the decrease in the mobility due to charge condensation in the large N limit is only about 40%, despite the effective charge being reduced by about 66%.

Despite using a simple approach based on the Debye-Hückel approximation, our method displays not only the right effective charge as a function of N but also a remarkably accurate distribution of effective charge as a function of monomer number as seen in the inset of Fig. 2. The results should be compared to those of Limbach and Holm [29], who looked at charge condensation with explicit ions in detail. The increased charge on the ends produces slightly higher mobility [4,11]. The fact that both the average effective charge as well as its distribution along the chain agree with results using explicit ions indicates that the simplistic approach taken to charge condensation is reasonable.

Figure 3 shows the mobility μ_0 of polyelectrolytes with length $N = 20$ for a range of λ_D . As is typical in electrokinetics, mobility increases with λ_D . One fascinating topic with very few simulation results is polyelectrolytes with complex charge distributions. For instance, consider a net neutral diblock ring polymer cut to create a net neutral linear triblock polyampholyte with a negatively charged centre block and unequal, positively charged ends. It is known that if the ring polymer is cut at the positive-negative junction ($x = 0$) creating a diblock polyampholyte, the mobility is zero. However, if the cut position is at any other point ($x \neq 0$), then the net neutral complex has a nonzero mobility [4]. The inset to Fig. 3 shows the importance of finite Debye length on this phenomenon: the smaller $\lambda_D = 0.5$ produces a non-negligible mobility while the larger

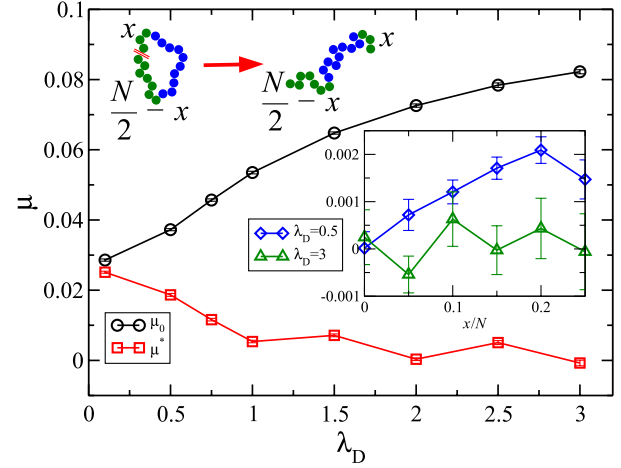


FIG. 3 (color online). Mobility of a uniformly charged polymer, μ_0 , as a function of the λ_D , along with mobility of a net neutral block copolymer, μ^* , rescaled by a constant factor. The inset shows the mobility of a net neutral block copolymer ($N = 20$) as a function of cutting position x for two values of λ_D .

$\lambda_D = 3$ results in a significantly reduced mobility (the large Debye length limit is zero, regardless of cut position). Nonzero mobility requires that λ_D be smaller than the scale of the charge heterogeneity of the polyelectrolyte complex, which, regardless of cut position, is never true for the $\lambda_D = 3$ case. The maximum mobility occurs for a symmetric chain with equally long ends ($x = 0.25N$) [11].

Figure 3 also shows the maximum mobility of this polymer, μ^* as a function of λ_D . The data have been rescaled such that the limit $\lambda_D = 0$ yields $\mu^* \approx 2\mu_0$ [4]. The fact that the uniformly charged polymer and block polyampholyte copolymer show opposing trends in mobility with respect to λ_D highlights the different physical mechanisms involved in the two cases: μ_0 increases with λ_D because of increased cooperative shearing, while μ^* decreases because the nonzero mobility of the polyampholyte is the result of locally generated EOF which becomes less local as the Debye length increases.

In this Letter, we introduced a mean field approach based on the Debye-Hückel approximation. The method consists of assigning an effective charge to MPCD particles near charged monomers using a weight given by the Debye-Hückel approximation. If the charge on a given MPCD particle is over a certain threshold, it is then given no charge and the charge is subtracted from the monomers, thereby mimicking the effects of charge condensation. The model was verified by reproducing the free-solution electrophoretic behavior of short polyelectrolytes as a function of the degree of polymerization. Furthermore, the effective friction, charge, and charge distribution along the chain backbone showed good agreement with more computationally expensive simulations which use explicit ions. Our method successfully recovered the expected increase in mobility with Debye length (a notoriously difficult

problem for nonexplicit techniques). The ability of the local Debye-Hückel approximation to produce reasonable electrophoretic behavior for various polymer lengths means that it could be used to efficiently simulate electrohydrodynamics in more complex situations. To demonstrate this, simulations of a net neutral polyampholyte were performed which showed how mobility depends on charge conformation and Debye length.

While we illustrated the utility of our approach by examining the free-solution electrophoresis of charged polymers, the idea of using the Debye-Hückel approximation to simulate realistic behavior should also be applicable to other challenging electrohydrodynamic problems, including the dynamics of confined charged polymers, EOF in complex microstructures, polyelectrolyte mediated EOF, colloidal electrophoresis, and induced-charge electrophoresis and electro-osmosis.

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Operational-modes of field-flow fractionation in microfluidic channels

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ABSTRACT

Through a careful consideration of the retention ratio for field-flow fractionation (FFF), we show that a single unified ideal retention theory can predict a wide range of separation behaviours including hydrodynamic chromatography, normal-mode FFF and steric-mode FFF by introducing the concept of a *device retention parameter*. We determine the critical device retention parameter above which normal-mode does not exist and there is no clear distinction between hydrodynamic chromatography and steric-mode FFF. Numerical analysis of the elution order as a function of particle size quantitatively predicts the transitions between these regimes. The resulting map of the operational-modes shows each of the regions and their connectivity, and so may guide future device design. By extending this analysis to account for the variation of stress over particle surfaces, a hitherto unreported regime called *Faxén-mode* FFF is predicted, which has the same elution order as normal-mode FFF. This mode arises when particle sizes approach the channel height, as can occur when microfluidic devices are utilized for FFF. The transition from steric-mode to Faxén-mode FFF is numerically mapped and approximations for each transition are presented.

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1. Introduction

Field-flow fractionation (FFF) is an elegant separation technique based on a simple concept: separation of a mixture of different species can occur when solute particles, carried through a channel by a nonuniform, laminar flow profile, are acted on by an external field applied perpendicular to the flow. A schematic demonstrating the principles of FFF is given in Fig. 1 for the case of a symmetric parabolic flow profile. The perpendicular field pushes the ensemble of solutes against the accumulation wall but diffusion disperses the solute and resists the inhomogeneity. An exponential concentration distribution results in equilibrium. In particular, each species of solute has a different concentration profile. Since solute particles near the channel wall are subject to slower flowing solvent than particles near the centre, samples with a mean height close to the accumulation wall are carried along with a slower average velocity than samples with a larger mean height.

The simplicity of FFF disguises the flexibility of the technique. FFF has been used to separate particles as small as 1 nm [1–4] and as large as 100 μm [5,6]. It has been used to size separate everything from colloids [7–10] to macromolecules [11–15] to cells [16–19]. It can do this because of the wide variety of fields that can be used. Sedimentation, cross flows (both symmetrical and asymmetrical), and thermal fields are most commonly used. However,

electrical, pressure, magnetic, dielectrophoretic, acoustic, photophoretic have all been demonstrated as acceptable physical fields [20,21]. The miniaturization of FFF techniques for wide-spread use in microfluidic devices appears tenable. Electrical- [22,23], dielectrophoretic- [24,25], and thermal- [26,27] FFF appear particularly amenable to micro- and nanofluidic devices [28,29]. However, miniaturization must be approached with caution. Effects due to miniaturization can be subtle and easily overlooked. The consequences of using microfluidic channels as FFF devices have been discussed in the literature [30–32]. Initial discussions centred on the role of channel dimensions in FFF [30]. In ideal situations, efficiency and speed are independent of channel height while selectivity is reduced with decreased channel height. At that time, there seemed to be a consensus that there was no immediate advantage to decreasing channel size.

More recently, it was pointed out that a subset of FFF techniques does in fact benefit from miniaturization. In particular, the resolution of electrical-FFF is increased as channel height is decreased, allowing for shorter analysis times in microchannels [31]. Improvements to both the resolution and the separation efficiency have been quantitatively demonstrated [32]. It is now recognized that in practice miniaturization entails both a decrease in channel height and also length which results in less peak spreading [32].

Experimentalists seeking to reap the benefits of using microfluidic channels in FFF apparatuses must recognize that, depending on sample and chosen channel height, the size of solute particles may vary from relatively small to large and confined. Although this does not necessarily preclude analytical interpretation of resulting chromatograms, it does require a unified retention theory that can

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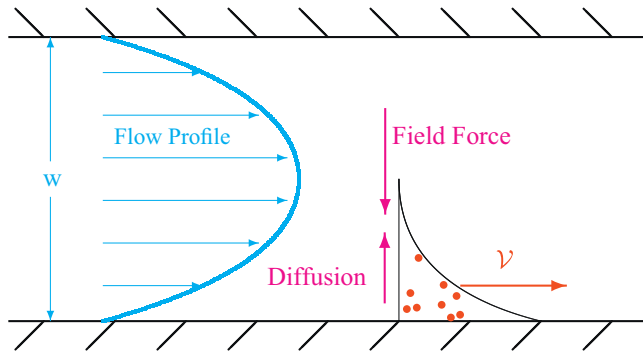


Fig. 1. Schematic of normal-mode FFF. An external force, perpendicular to the flow profile pushes the solute against the accumulation plate. The competition between the force and the diffusivity establishes a concentration gradient. The mean velocity of the solute ($\langle v \rangle$) is a function of the solute concentration $c(\tilde{y})$ and solvent velocity profiles $v(\tilde{y})$.

predict the fractionation for samples of everything from the tiniest tracer particles eluting in the hydrodynamic chromatography limit of FFF to the largest beads that fit snugly in the channel. In practice, a microscopic system size is needed for these two limits to be achieved in a single device.

In this paper, we take a simplistic view of the FFF process. We do not explicitly account for the fact that we are concerned with micro- and nanoscopic channel heights. Rather, we extend the traditional theory of Giddings [5,33,34] into operation regimes that at one time were quixotic but in today's age of microfluidics and nanotechnology are within reach. The theory is ideal in the sense that the flow field is assumed to be slow and unperturbed, and particles' interactions with the wall are assumed to be solely steric, without frictional or hydrodynamic interactions. Similarly, complicating effects such as particle slip, nonparabolic solvent flow and attraction to the accumulation wall are intently ignored [35].

2. Background

2.1. Normal-mode FFF

Consider a cross-sectional area with a solvent flow velocity field v parallel to the plates and solute concentration profile c . The cross-sectional average velocity of solute through a plane is

$$\langle v \rangle = \frac{\langle cv \rangle}{\langle c \rangle} \quad (1)$$

where $\langle \cdot \rangle$ denotes cross-sectional average over the channel height. One should compare $\langle v \rangle$ to the average velocity of the solute carrier fluid, $\langle v \rangle$. The retention ratio R is defined as the ratio of these two mean velocities. Since both elute through the same length of channel, the ratio of the time it takes the solute to elute (called the retention time) and the time it takes the solvent to elute (called the void time) is equivalent to the ratio of mean velocities as

$$R = \frac{t_0}{t_R} = \frac{\langle v \rangle}{\langle v \rangle} = \frac{\langle cv \rangle}{\langle c \rangle \langle v \rangle}. \quad (2)$$

If no-slip at each wall is assumed and any distortion due to the perpendicular field is neglected, (e.g. thermal-FFF tends to skew the velocity profile [36,37,20]), the velocity of the fluid as a function of the distance from the accumulation wall normalized by the channel height $\tilde{y} = y/w$ (hereafter $\tilde{\cdot}$ denotes scaling by channel height, w) is given by the Poiseuille equation

$$v(\tilde{y}) = 6\langle v \rangle (\tilde{y} - \tilde{y}^2). \quad (3)$$

Any perpendicular external force f can be used to create a nonuniform concentration distribution but we assume the concentration profile is independent of the mean flow velocity $\langle v \rangle$ [38].

Table 1

External forces f and corresponding scaling exponents α and device retention parameters Λ for examples of common FFF techniques. Variable names are the same as used by Cölfen and Antonietti in Table 1 of Ref. [20] with the notable exceptions that here the particle size is r , the centrifugal acceleration is G and the Stokes–Einstein equation $D = k_B T / 6\pi\eta r$ has been used, where η is the solvent viscosity.

FFF-method	f	α	Λ
Sedimentation	$\frac{4\pi}{3} G \Delta \rho r^3$	3	$\frac{3}{4\pi} \frac{k_B T}{G \Delta \rho w^4}$
Flow	$6\pi\eta \frac{w \tilde{y}_c}{V_0} r$	1	$\frac{k_B T}{6\pi\eta w^3} \frac{V_0}{V_c}$
Thermal	$6\pi\eta \frac{D_T \Delta T}{w} r$	1	$\frac{k_B T}{6\pi\eta D_T \Delta T}$
Magnetic	$\frac{4\pi}{3} \rho \chi_m H_m \Delta H_m r^3$	3	$\frac{3}{4\pi} \frac{k_B T}{w^4 \rho \chi_m H_m \Delta H_m}$
Electrical	$6\pi\eta \mu_e E r$	1	$\frac{k_B T}{6\pi\eta \mu_e E w}$

Point-particles in a solution with thermal energy of $k_B T$ have a concentration profile with the form

$$c(\tilde{y}) = c_0 \exp\left(-\frac{f\tilde{y}}{k_B T}\right) = c_0 \exp\left(-\frac{\tilde{y}}{\lambda}\right) \quad (4)$$

where c_0 is the concentration of solute at the accumulation wall, $\tilde{y} = 0$. The retention parameter λ is strictly defined as the ratio between the thermal and potential energy scales

$$\lambda \equiv \frac{k_B T}{f w}. \quad (5)$$

For point-particles, the mean concentration height thus has the form

$$\langle \tilde{y} \rangle = \frac{\langle \tilde{y} c \rangle}{\langle c \rangle} = \lambda + \frac{1}{1 - e^{-1/\lambda}}. \quad (6a)$$

In the strong force limit, λ becomes small and

$$\langle \tilde{y} \rangle \simeq \lambda - e^{-1/\lambda} \simeq \lambda. \quad (6b)$$

In this limit, we can interpret λ as the dimensionless length scale of the concentration distribution. In the negligible force limit, λ is large and expanding the exponential in Eq. (6a) to third order leads to

$$\langle \tilde{y} \rangle \simeq \frac{1}{2} \left[1 - \frac{1}{6\lambda} + \frac{1}{360\lambda^3} + \dots \right] \simeq \frac{1}{2} \quad (6c)$$

as we would expect for a uniform concentration.

In much the same way as was done for $\langle \tilde{y} \rangle$, an analytical expression for the retention ratio can be found by evaluating the averages in Eq. (2) for Eqs. (3) and (4). The resulting retention ratio is independent of flow rate:

$$R(\lambda) = 6\lambda \mathcal{L}\left(\frac{1}{2\lambda}\right) \quad (7)$$

where $\mathcal{L}(x) = \coth(x) - 1/x$ is the Langevin function. Eq. (7) has been shown to be very versatile for parameterizing retention [20,21] and characterizes what has been called *normal-mode* FFF.

Although the theory presented so far has treated the solute as an ensemble of point-particles, there is an implicit size dependence through λ . For instance, in sedimentation-FFF it is generally appropriate to assume a constant solute mass density such that the gravitational force f goes as the volume r^3 of the spherical particle or $\lambda \sim 1/f \sim r^{-3}$. To consider this in a general manner, let the force scale as the particle radius r to some power α such that the retention parameter can be rewritten

$$\lambda = \frac{k_B T}{\mu r^\alpha w} = \frac{k_B T}{\mu \tilde{r}^\alpha w^{1+\alpha}} = \Lambda \tilde{r}^{-\alpha}, \quad (8)$$

where we have extracted the normalized size dependence $\tilde{r}^\alpha$ from both the force ($f \equiv \mu r^\alpha$) and the retention parameter λ . The resulting dimensionless number, Λ , characterizes the FFF apparatus itself (for this reason it is called the *device retention parameter*) and is given for a few examples in Table 1.

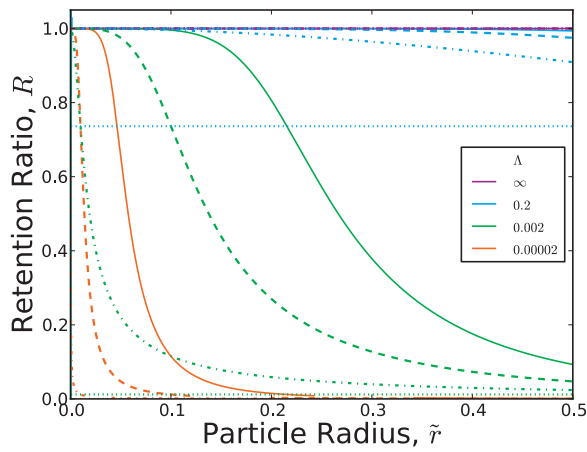


Fig. 2. Retention ratio $R(\tilde{r})$ as predicted by the standard point-particle theory for normal-mode FFF. The retention parameter λ is assumed to vary like $\lambda = \Lambda \tilde{r}^{-\alpha}$ but the particle size is otherwise not taken into account. Solid lines denote $\alpha = 3$, dashed $\alpha = 2$, dash-dot $\alpha = 1$ and dotted lines denote $\alpha = 0$ (all of which are horizontal). Small particles elute first except when $\alpha = 0$ where retention time is not a function of particle size.

Fig. 2 demonstrates that the point-particle theory of normal-mode FFF then predicts that small particles elute before large particles do. The monotonic decrease in elution time with particle size remains true for all device retention parameters Λ and all α -scaling. The magnitude of the slope increases as Λ is reduced, which is indicative of why the resolution increases with external force.

2.2. Steric-mode FFF

The steric interaction of finite-size solutes with the accumulation wall creates an excluded region as schematically shown in Fig. 3. To include the effects of steric repulsion from the wall, the integration limits on the profiles used to determine the retention ratio are altered. Steric effects are handled by stating that $c(\tilde{y})$ is the concentration profile of the centre of masses of the spherical particles of scaled radius $\tilde{r} = r/w$. The flow field is still assumed to be slow (low particle Reynolds number and insignificant inertial contributions) [39,40] and unperturbed by the presence of the solute (infinitely wide channels and dilute solutions) [41,42]. The variable c_0 is redefined as the concentration at the lowest point, i.e. a distance $\tilde{r}$ from the accumulation wall, so that the concentration profile becomes

$$c(\tilde{y}) = \begin{cases} c_0 e^{-(\tilde{y}-\tilde{r})/\lambda} & \text{for } \tilde{r} < \tilde{y} < 1 - \tilde{r} \\ 0 & \text{otherwise} \end{cases} \quad (9)$$

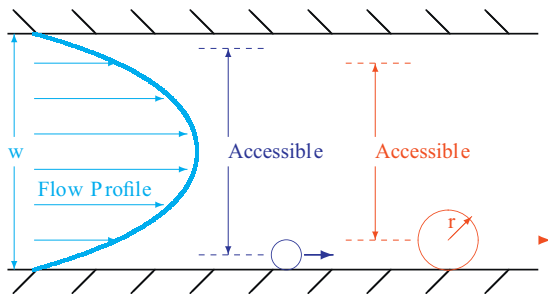


Fig. 3. Schematic representation of steric-mode FFF. When hard, finite-sized solutes undergo elution, they are excluded from a region near the wall where the fluid velocity is low. As a result, they tend to elute earlier than smaller particles.

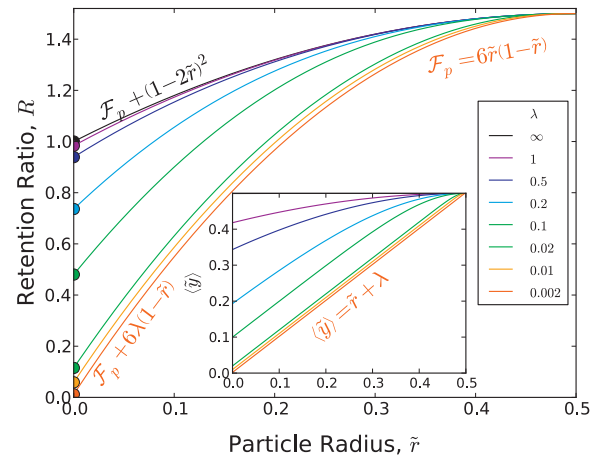


Fig. 4. Retention ratios for a variety of retention parameters varying from small forces of $\lambda = 100$ to large forces of $\lambda = 0.002$. Solid lines denote Eq. (11) and closed circles on the R -axis represent the values predicted by Eq. (7). The inset shows that the mean concentration height $\langle \tilde{y} \rangle$ grows with particle size $\tilde{r}$ when $\lambda \ll 1$.

where $\lambda = \lambda(\tilde{r})$ unless $\alpha = 0$. The concentration distribution controls the mean height

$$\langle \tilde{y} \rangle = \lambda + \frac{1}{1 - e^{-(1-2\tilde{r})/\lambda}} + \tilde{r} \coth \left(\frac{1-2\tilde{r}}{2\lambda} \right). \quad (10a)$$

The two limits are now given by

$$\langle \tilde{y} \rangle \approx \begin{cases} \tilde{r} + \lambda - e^{-(1-2\tilde{r})/\lambda} & \lambda \ll 1(b) \\ \frac{1}{2} \left[1 - \frac{(1-2\tilde{r})^2}{6\lambda} + \frac{(1-2\tilde{r})^4}{360\lambda^3} + \dots \right] \approx \frac{1}{2} & \lambda \gg 1(c). \end{cases} \quad (10)$$

We first note that Eq. (10c) agrees with Eq. (6c) when $\tilde{r} \rightarrow 0$, as it should. For strong forces (the small λ case of Eq. (10b)), solute particles are pushed right against the wall and so the mean concentration height is expected to grow linearly with the particle size $\tilde{r}$. This linear relationship is quite evident in mean concentration height for fixed, small values of λ as seen in Eq. (10b) or the inset to Fig. 4. For larger values of λ (weak forces) the solute particles are able to diffuse further from the accumulation wall and the nonlinear terms in Eq. (10a) become apparent in the inset.

As a result of shifting the mean particle height $\langle \tilde{y} \rangle$ towards the centre of the channel by increasing particle size, the mechanism for separation changes to that of *steric-mode* FFF and the retention ratio becomes

$$R(\tilde{r}, \lambda) = 6\lambda [1 - 2\tilde{r}] \mathcal{L} \left(\frac{1-2\tilde{r}}{2\lambda} \right) + \mathcal{F}_p, \quad (11)$$

which is strikingly similar to Eq. (7) for normal-mode FFF. The fraction of the channel accessible to the particle, $1 - 2\tilde{r}$, appears in Eq. (11) while in Eq. (7) it is identically unity. Beyond this effective reduction of channel height, steric effects lead to additional changes to the behaviour of the retention ratio. For convenience of comparison, these effects have been encapsulated into the new term

$$\mathcal{F}_p(\tilde{r}) = 6\tilde{r}(1 - \tilde{r}). \quad (12)$$

This result is in agreement with the commonly accepted form [5,34,43]. As seen in Fig. 4, Eq. (7) is the $\tilde{r} \rightarrow 0$ limit and so gives the points on the R -intercept of Eq. (11).

Each line in Fig. 4 corresponds to a different λ , held fixed regardless of particle size in spite of the fact that $\lambda = \lambda(\tilde{r})$ (the explicit size dependence of λ will be treated in Section 3.1). Even when λ has no size-dependence, Eq. (11) demonstrates that particles of different sizes can in fact be separated. Historically [34], λ is kept fixed and only the explicit size effects in Eq. (11) are included as is done in

Fig. 4. The present size dependence of Eq. (11) has an interesting consequence for the zero force limit of $\lambda \rightarrow \infty$.

2.3. Hydrodynamic chromatography limit

Even in the absence of a field, steric interactions lead to some fractionation as seen by the large λ curves in Fig. 4. The $\lambda \gg 1$ limit represents vanishingly small forces and corresponds to the hydrodynamic chromatography [44] limit of FFF [5,20] in which the steric-mode of separation results in retention even when the field is zero.

Since there is no force towards the accumulation wall, the particles have a uniform concentration distribution across the channel with a mean height of $\langle \tilde{y} \rangle = 1/2$. Hydrodynamic chromatography separates particles by excluding them from regions of slow moving solvent near the wall. For a fixed force (fixed λ), this leads to an elution time that decreases with increasing particle size—as opposed to normal-mode FFF as seen in Fig. 2. In Fig. 4, cases of $\lambda \geq 1$ approximate well the hydrodynamic chromatography limit (Eq. (13c)).

The hydrodynamic chromatography limit is only one of four specific limits of Eq. (11):

$$R \simeq \begin{cases} 6\lambda\mathcal{L}\left(\frac{1}{2\lambda}\right) & \tilde{r} \rightarrow 0 \text{ (a)} \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \tilde{r} \rightarrow 1/2 \text{ (b)} \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \lambda \gg 1 \text{ (c)} \\ \mathcal{F}_p + 6\lambda(1 - 2\tilde{r}) - 12\lambda^2 & \lambda \ll 1 \text{ (d)}. \end{cases} \quad (13)$$

Interestingly, the hydrodynamic chromatography result $\lambda \gg 1$ (Eq. (13c)) also appears when $\tilde{r} \rightarrow 1/2$ (Eq. (13b)) reflecting the fact that as the particle size approaches the channel height steric effects will always dominate over the external field (we will return to this limit in Section 3.4). In the limit $\tilde{r} \rightarrow 0$, Eq. (11) reduces to Eq. (13a), which is identical to the point-particle prediction of Eq. (7). Each of these limits is marked in Fig. 4. The point-particle limit agrees with the R -intercept and is marked by closed circles while the limits for hydrodynamic chromatography and strong forces are written in.

3. Regimes and transitions

3.1. Transition between normal- and steric-modes

Section 2.2 reviewed the variation of the retention ratio R in a microfluidic channel as a function of particles size $\tilde{r}$ for a fixed retention parameter λ , which is equivalent to a fixed force f regardless of size. This is the manner in which the situation is generally framed in the literature [34]. However, as was broached at the end of Section 2.1, it is generally more reasonable to allow the force to change with particle size. We now incorporate both steric effects and size dependent forces into a unified description by substituting $\lambda = \Lambda \tilde{r}^{-\alpha}$ (Eq. (8)) into the results of Section 2.2. This produces the concentration profile and the retention ratio explicitly in terms of particle size $\tilde{r}$:

$$c(\tilde{y}) = \begin{cases} c_0 e^{-\tilde{r}^\alpha (\tilde{y} - \tilde{r})/\Lambda} & \text{for } \tilde{r} < \tilde{y} < 1 - \tilde{r} \\ 0 & \text{otherwise,} \end{cases} \quad (14)$$

$$R(\tilde{r}, \Lambda) = \frac{6\Lambda}{\tilde{r}^\alpha} [1 - 2\tilde{r}] \mathcal{L}\left(\frac{[1 - 2\tilde{r}] \tilde{r}^\alpha}{2\Lambda}\right) + \mathcal{F}_p(\tilde{r}), \quad (15)$$

while the steric function $\mathcal{F}_p$ remains unchanged from Eq. (12). In these terms, $\alpha = 0$ represents a hypothetical force that is not a function of particle size (as was used in Fig. 4). Fig. 5 shows the retention ratios for a range of Λ when $\alpha = 1$ which is the appropriate scaling for many examples of FFF techniques, as seen in Table 1.

Consider first the extreme particle sizes in Fig. 5. The smallest particle sizes all have a retention ratio approaching $R \rightarrow 1$ regardless

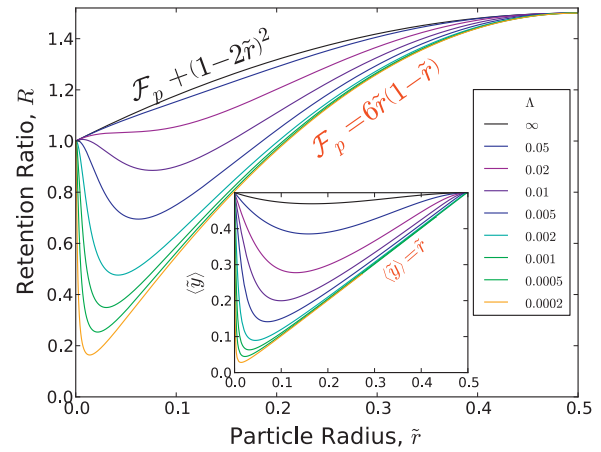


Fig. 5. Retention ratios for $\alpha = 1$. When the force varies as $\tilde{r}^\alpha$, the retention ratio is no longer a simple monotonic rise as it was in Fig. 4 for $\alpha = 0$. Below the critical device retention parameter $\Lambda_c = 1.90 \times 10^{-2}$, the retention ratio becomes nonmonotonic. The inset shows that as Λ decreases, the mean concentration height's minimum occurs at smaller and smaller particle sizes.

of Λ because Brownian motion dominates the applied force. Small $\tilde{r}$ solutes constitute tracer particles which are able to diffuse across the entire channel and sample the entire solvent velocity profile, resulting in $\langle \tilde{y} \rangle = 1/2$ and $R = 1$ for all Λ . Next, consider the largest particle sizes. The inset shows that once again the mean height has become $\langle \tilde{y} \rangle = 1/2$ for all Λ but now the $R \neq 1$. This is because the largest particles fit snugly into the channel causing the concentration distribution to be sharply distributed about $\tilde{y} = 1/2$. The solvent velocity at the centre point is $v(1/2) = 3\langle v \rangle/2$, which causes the retention ratio to approach $R = 3/2$ as seen in Fig. 5. However, $R \rightarrow 3/2$ is physically nonsensical since the speed of such large particles is not well represented by the solvent's velocity in the absence of the particle and so we shall return to this result (see Section 3.4).

Having discussed the extreme points of Fig. 5, we now turn to intermediate values. The most immediately striking property of Fig. 5 is that R is not necessarily a monotonic function of particle size. For large values of Λ , the retention ratio remains monotonic (a perturbation about the hydrodynamic chromatography limit) but when Λ decreases below some critical value Λ_c the retention ratio R becomes nonmonotonic with a minimum at some particle size $\tilde{r}_{NS}(\Lambda)$. Above $\tilde{r}_{NS}$, the retention ratio in Fig. 5 grows in a similar manner as it did in Fig. 4. However, the behaviour of $R(\tilde{r})$ for small particle sizes in the regime $\tilde{r} < \tilde{r}_{NS}$ is much different. At small sizes, as the particle size increases, the retention ratio falls drastically. This is the same behaviour as in Fig. 2 and it is caused by the same mechanisms: As always, the concentration distribution results from the competition between the external field, thermal energy and the hard steric interaction with the wall but at these sizes particles are too small for steric effects to dominate. Instead, in this range the behaviour is the same as that predicted by normal-mode FFF and shown in Fig. 2.

All these properties of R are reflected in the mean concentration height $\langle \tilde{y}(\tilde{r}) \rangle$. The inset of Fig. 5 shows that $\langle \tilde{y}(\tilde{r}) \rangle$ is nonmonotonic with a global minimum for all values of Λ . When Λ is small, the vast majority of particle sizes are above this minimum, and above the minimum the average concentration height increases fairly linearly, $\langle \tilde{y}(\tilde{r}) \rangle \simeq \tilde{r}$. Indeed, in this strong field limit, the external force completely dominates over thermal motion and solute particles are pinned to the accumulation wall. The hard steric repulsion ensures that the mean height of the concentration profile grows linearly with $\tilde{r}$ as it did in Fig. 4. The distribution of particle sizes below

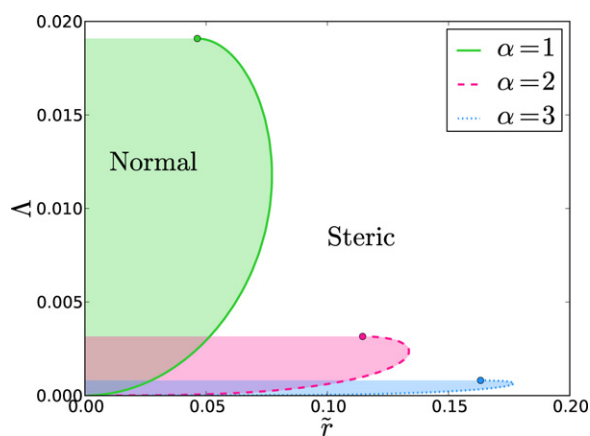


Fig. 6. A tentative operational-mode diagram for FFF (see Figs. 8 and 11). The dividing line between normal-mode FFF and large particle steric-mode FFF is the numerically determined $\tilde{r}_{NS}$. Normal-mode FFF is represented by the shaded regions and does not exist above a critical device retention parameter Δ_c . The critical device retention parameter values ($\Delta_c = 1.90 \times 10^{-2}$ for $\alpha = 1$; $\Delta_c = 3.16 \times 10^{-3}$ for $\alpha = 2$; and $\Delta_c = 8.07 \times 10^{-4}$ for $\alpha = 3$) are marked by a closed circle.

the minimum results not from the steric exclusion but from the competition between the force and the thermal noise.

It is this difference in mechanisms that suggests the importance of $\tilde{r}_{NS}$. It is a very generally stated rule in the field of FFF that smaller particles elute before larger particles in normal-mode FFF. On the other hand, in steric-mode FFF larger particles elute before smaller particles [20]. Both modes of operation are considered monotonic and from the definition of $R \propto t_R^{-1}$ in Eq. (2), this rule means that the slope $\partial R / \partial \tilde{r}$ is negative for normal- and positive for steric-mode FFF.

However, it had been intuitively obvious that there must be a transition between the two modes of operation described by Eq. (7) and Eq. (11) (sometimes called the steric-inversion point). Even before we allowed $\lambda \propto \tilde{r}^{-\alpha}$, we saw that Eq. (11) approaches Eq. (7) in the limit $\tilde{r} \rightarrow 0$. However, by extracting $\tilde{r}^{-\alpha}$ from λ , we can quantitatively predict $\tilde{r}_{NS}(\Delta)$, which is in fact the particle size for which this transition occurs as a function of Δ , by numerically finding the minima in Fig. 5 (the roots of $\partial R / \partial \tilde{r}$). The subscript NS was chosen in retrospect to signify that $\tilde{r}_{NS}$ represents the normal-to-steric transition. The transition size is traced in Fig. 6, creating an operational-mode diagram for $\alpha = [1, 2, 3]$.

A transition from normal- to steric-mode FFF exists only below a critical Δ_c . Fig. 6 marks these and the caption lists the numeric values. Above Δ_c there simply is no normal-mode and the retention ratios shown in Fig. 5 monotonically increase with particle size. This will be discussed further in Section 3.2.

3.2. Hydrodynamic chromatography-mode FFF

Section 3.1 used the $\alpha = 1$ case to show that the unified ideal retention theory Eq. (15) can map out the transition between normal- and steric-mode FFF (Fig. 6). However, a careful investigation of Fig. 5 for very small $\tilde{r}$ values when Δ is near Δ_c , (e.g. see the $\Delta = 0.01$ curve) reveals that there is a small region starting at $\tilde{r} = 0$ within which larger particles elute before smaller particles. To investigate this further, consider R for the same set of Δ but with $\alpha = 2$ as shown in Fig. 7.

The transition between normal- and steric-modes (at $\tilde{r} = \tilde{r}_{NS}$) remains; however, a local maximum at $\tilde{r} = \tilde{r}_{HN}$ (with $\tilde{r}_{HN} < \tilde{r}_{NS}$) is clearly evident. Since $\partial R / \partial \tilde{r}$ is positive in the region $\tilde{r} < \tilde{r}_{HN}$, the mode of operation has the same elution order as steric-mode FFF. However, since the thermal forces dominate over the external field in this region, this is actually a small-particle

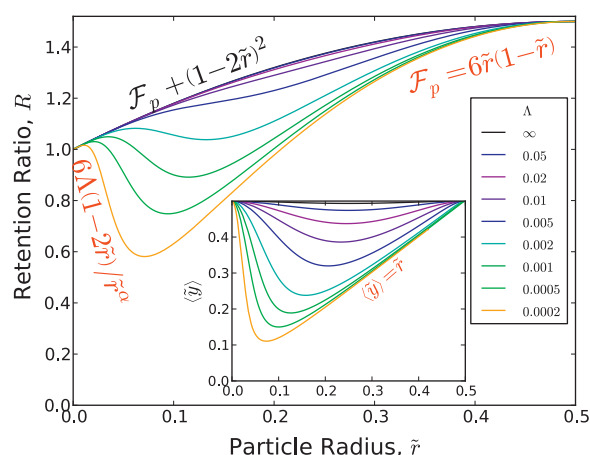


Fig. 7. Retention ratios for $\alpha = 2$. The retention ratio is monotonic above the critical device retention parameter $\Delta_c = 3.16 \times 10^{-3}$. As discussed in the text, we have three modes of operation when $\Delta < \Delta_c$: For the smallest particles the force is negligible, the slope is positive and the mode of operation is hydrodynamic chromatography. For moderately small particles the force dominates, the slope is negative and the mode of operation is normal-mode FFF. For the largest particles the effects of sterically excluded regions are most important, the slope is once again positive and the mode of operation is steric-mode FFF.

hydrodynamic chromatography regime (hence the subscript HN for the transition between hydrodynamic chromatography and normal-mode FFF).

Normal-mode operation only exists over a range of particle sizes above which steric effects dominate because of the large size of the particle and below which the external force is insignificant due to the particle's small size. This can be seen explicitly in Fig. 8. The normal-mode of operation exists as a lobe when $\Delta < \Delta_c$. Above Δ_c , this new small particle hydrodynamic chromatography regime seamlessly transitions directly to steric-mode FFF but below Δ_c , normal-mode FFF exists between the hydrodynamic chromatography- and steric-mode FFF.

A surprising result of Fig. 8 is just how far normal-mode operation can extend along $\tilde{r}$ when the force is a strong function of the particle size. When $\alpha = 3$, normal-mode can be achieved for particle radii as large as 17% the channel height.

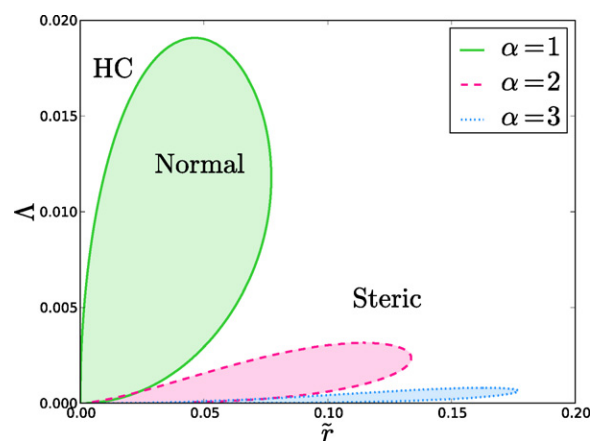


Fig. 8. Enclosed regions are the areas of normal-mode FFF in which smaller particles elute before larger particles. Outer areas are steric-mode and hydrodynamic chromatography limit regions of operation in which larger particles elute before smaller particles. Solid lines are the numerically determined roots of $\partial R / \partial \tilde{r}$ ($\tilde{r}_{HN}$ is a local maximum of R and so forms the left border while $\tilde{r}_{NS}$ is a local minimum of R and so forms the right border).

3.3. Limits

Once again it is valuable to consider the limits of the retention ratio. From Eq. (15) we obtain

$$R \simeq \begin{cases} \mathcal{F}_p + (1 - 2\tilde{r})^2 & \tilde{r} \rightarrow 0 \quad (a) \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \tilde{r} \rightarrow 1/2 \quad (b) \\ \mathcal{F}_p + (1 - 2\tilde{r})^2 & \Lambda \gg 1 \quad (c) \\ \mathcal{F}_p + \frac{6\Lambda}{\tilde{r}^\alpha} (1 - 2\tilde{r}) - \frac{12\Lambda^2}{\tilde{r}^{2\alpha}} & \Lambda \ll 1. \quad (d) \end{cases} \quad (16)$$

The hydrodynamic chromatography-mode at the smallest particle sizes that was discussed in Section 3.2 is quadratic in $\tilde{r}$ and is described by Eq. (16a) which is the same limiting behaviour as at large particle sizes (Eq. (16b)) and at large device retention parameters Λ (Eq. (16c)). This is fundamentally different from Eq. (11) in that Eq. (7) can no longer be the point-particle limit since it is impossible to have both extremely small particles and extremely large forces. However, there is no need to assume small particle sizes for the hydrodynamic chromatography. The large $\Lambda \gg 1$ limit of Eq. (16c) is the general hydrodynamic chromatography limit and its form agrees with microfluidic separation studies of hydrodynamic chromatography [45].

The three limits Eqs. (16b)–(16d) are unchanged from the forms in Eq. (13). This has the interesting consequence of making three out of four limits have the form $R \simeq \mathcal{F}_p + (1 - 2\tilde{r})^2 = 1 + 2\tilde{r} - 2\tilde{r}^2$ which is clear in Fig. 7. The $\tilde{r} \rightarrow 0$ and $\tilde{r} \rightarrow 1/2$ limits both fall on the hydrodynamic chromatography limit of $\Lambda \gg 1$. The anomalous limit is $\Lambda \ll 1$. In fact, inspecting either Fig. 5 or Fig. 7 reveals that this limit itself has two limits.

- 1 When the particle radius is large, the first term in Eq. (16d) is most important such that $R \approx \mathcal{F}_p = 6\tilde{r}(1 - \tilde{r})$. Even at the smallest Λ , as the particle size becomes large all the retention ratios grow in the same manner. The behaviour may seem fairly universal at this point in the discussion but this limit will be improved upon in Section 3.4.
- 2 When the particle radius is small but the field is extremely strong, (i.e. $\Lambda \ll \tilde{r}^\alpha$ or equivalently $\lambda \ll 1$), then the second term in Eq. (16d) dominates meaning that $R \approx 6\Lambda(1 - \tilde{r})/\tilde{r}^\alpha \approx 6\lambda$ and the strong force limit from point-particle theory (which is often considered a reasonable approximation [21]) is recovered.

3.4. Faxén-mode FFF

The finite size of the particles has been included as a steric exclusion from the wall but it has a secondary effect as well. Until now, we have considered the velocity of particles whose centres of mass are at some height $\tilde{y}$ to have velocity $\mathcal{V}(\tilde{y}) = v(\tilde{y})$ given by Eq. (3). However, this is not strictly true because the velocity profile is parabolic and so not symmetric about the centre of mass except at $\tilde{y} = 1/2$ [39,46].

The nonphysical consequences are most striking at large particle sizes. As the particle diameter approaches the channel height in Figs. 4, 5 and 7, steric exclusion dominates such that the centre of mass is relegated to the centre of the channel $\tilde{y} = 1/2$ and $c(\tilde{y})$ approaches a Delta-function regardless of the strength of the perpendicular force. The erroneous assumption that even in this limit Eq. (1) does an adequate job approximating particle velocity means that by Eq. (2) the retention ratio becomes

$$R_{1/2} = \lim_{\tilde{r} \rightarrow 1/2} R = \frac{\lim_{\tilde{r} \rightarrow 1/2} \langle \mathcal{V} \rangle}{\langle v \rangle} = \frac{v(\tilde{y} \rightarrow 1/2)}{\langle v \rangle} = \frac{3}{2}. \quad (17)$$

indicating that the elution time of the largest particles will be significantly shorter than the void time. Intuitively we expect particles that see the entire flow profile to move with the same average

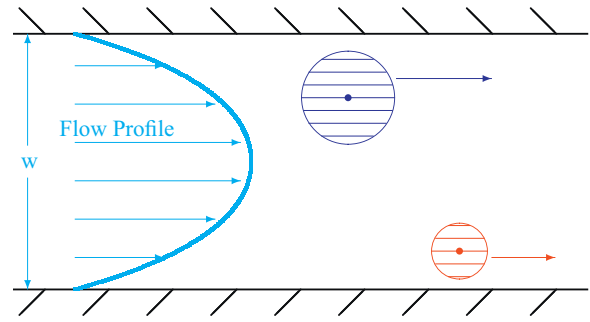


Fig. 9. Schematic of area cross sectional integration of the particle's velocity.

velocity as the solvent, i.e. $R_{1/2} = 1$. Although it is true that steric effects do dictate the retention ratio regardless of Λ in the large particle limit, continuing to estimate $\lim_{\tilde{r} \rightarrow 1/2} \langle \mathcal{V} \rangle$ by its point-particle value $v(\tilde{y} \rightarrow 1/2)$ becomes increasingly inaccurate. Let us improve upon this assumption.

At large sizes a significant portion of the particle's surface sees a much different velocity than $v(\tilde{y})$. Rather than using Eq. (1), a particle's velocity at a position $\tilde{y}$ is better estimated by

$$\langle \mathcal{V} \rangle \cong \frac{\langle \mathcal{V}c \rangle}{\langle c \rangle} \quad (18)$$

where the velocity of the large spherical particles in the presence of the confining walls is no longer estimated by the fluid speed at that point, $\mathcal{V}(\tilde{y}) \neq v(\tilde{y})$. In order to correct for the containing walls, the stress on the sphere must be integrated over its surface area as schematically shown in Fig. 9. This results in Faxén's Law and gives the velocity of the particle in steady-state to be

$$\mathcal{V}(\tilde{y}) = \left(1 + \frac{\tilde{r}^2}{6} \nabla^2 \right) v(\tilde{y}). \quad (19)$$

With this alteration, we can use Eq. (2), Eq. (3), Eq. (9) and Eq. (19) to recalculate the unified ideal retention ratio as

$$\begin{aligned} R(\tilde{r}, \Lambda) &= \frac{1}{\langle v \rangle} \frac{\int_0^w c \mathcal{V} dy}{\int_0^w c dy} \\ &= \frac{6\Lambda}{\tilde{r}^\alpha} [1 - 2\tilde{r}] \mathcal{L} \left(\frac{[1 - 2\tilde{r}] \tilde{r}^\alpha}{2\Lambda} \right) + \mathcal{F}_f(\tilde{r}), \end{aligned} \quad (20)$$

which is identical to Eq. (15) but with a steric function $\mathcal{F}_f$ that is appropriate for finite sized particles. This new steric function $\mathcal{F}_f$ differs trivially from $\mathcal{F}_p$ (given by Eq. (12)) and is

$$\mathcal{F}_f(\tilde{r}) = 6\tilde{r} \left(1 - \frac{4}{3}\tilde{r} \right). \quad (21)$$

Since the form of Eq. (20) is the same as Eq. (15), even the limits on the retention ratio given by Eq. (16) remain robust by simply replacing the steric term $\mathcal{F}_p$ with $\mathcal{F}_f$.

The tiny change from $\mathcal{F}_p$ to $\mathcal{F}_f$ is able to pull the retention ratio for large particles down from $R_{1/2} = 3/2$ to $R_{1/2} = 1$ as seen in Fig. 10 and as is more physically intuitive. Since this region of $\partial R / \partial \tilde{r} < 0$ exists for all Λ and in an ideal theory such as this one the retention ratio must go down to unity, there must always exist a region in the steric-mode regime where $R > 1$.

Fig. 11 traces out the borders between operational-modes. The enclosed, shaded regions represent the normal-mode elution order (smaller particles eluting first) while the unshaded, outer regions operate in the steric-mode elution order (larger particles eluting first). An entirely new region at large $\tilde{r} > \tilde{r}_{SF}$ with normal-mode elution order exists. We call this the Faxén-mode FFF regime and so label the transition from steric- to Faxén-mode with the subscript

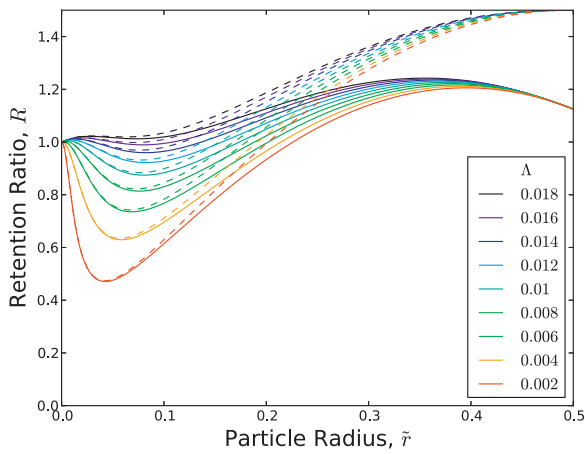


Fig. 10. The retention ratio for $\alpha = 1$ taking into account both steric effects and utilizing Faxén's Law as compared to simply assuming the centre of mass velocity as done previously. Dashed lines correspond to Eq. (15) while solid lines are from Eq. (20) and Eq. (21).

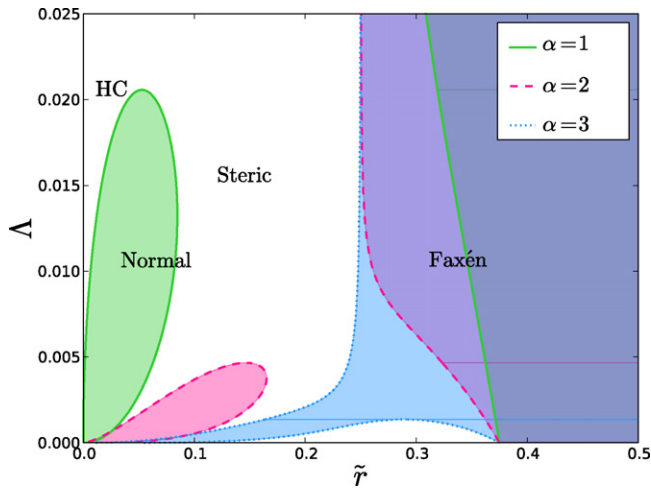


Fig. 11. Ideal FFF-modes map. The numerically determined roots of $\partial R / \partial \tilde{r}$ give the transitions between the four different modes of operation, $\tilde{r}_{HN}$, $\tilde{r}_{NS}$ and $\tilde{r}_{SF}$.

SF. Faxén-mode FFF exists for all Λ . At the largest values of Λ , $\tilde{r}_{SF} \rightarrow 0.25$ meaning that the transition occurs when the particle radius is a quarter of the channel height. At smaller Λ , the concentration is biased towards the accumulation wall and the transition doesn't occur until $\tilde{r}_{SF} = 0.375$.

For $\alpha = 1, 2$ the normal-mode and Faxén-mode form disconnected regions while the hydrodynamic chromatography-mode and the steric-mode are a connected space. On the other hand, when $\alpha = 3$ the border between the normal- and steric-modes ($\tilde{r}_{NS}$) extends to such large particle sizes that it passes $\tilde{r}_{SF}$ and so normal-mode interconnects with the Faxén-mode regime. In this case, it is the hydrodynamic chromatography-mode regime of FFF and steric-mode FFF that are disconnected regions.

4. Conclusions

The equations governing the FFF elution time of hard spherical particles in low Reynolds number flow are well known. Eq. (7) is often used for normal-mode FFF while Eq. (11) is used for steric-mode FFF. By explicitly including the external force's dependence on particle size, we are able to quantitatively predict the transition between these two modes of operation. There exists a critical device retention parameter, Λ_c , below which the transition

between normal- and steric-mode exists but above which normal-mode operation is not possible.

Furthermore, we find that a mode of operation exists at the smallest particle sizes with the same elution order as steric-mode FFF. The force pushing the particles towards the accumulation wall is size dependent and is insignificant compared to the steric force in this size regime. We quantitatively trace the transition between this small-particle hydrodynamic chromatography-mode FFF and normal-mode FFF. The domain of normal-mode operation only exists as a closed area on the $\Lambda - \tilde{r}$ mode-diagram below Λ_c . Above Λ_c there is no distinction between hydrodynamic chromatography and steric mode-FFF.

By integrating the fluid stress over the surface area of the particles, we further improve the predictions of ideal retention theory for the case of small channel heights (microfluidic devices). The steric function $\mathcal{F}_f$ in the retention ratio is altered but is no more complicated than when this improvement was ignored. The improved expression for the retention ratio predicts a new mode of operation for particles whose diameters approach the channel height. The elution order of this new Faxén-mode FFF is the same as normal-mode FFF and exists for all Λ . When the external force on the particles scales as $\alpha = 3$ (as is true for sedimentation FFF) the normal- and Faxén-mode FFF regimes intersect and form a continuous space.

Accurately predicting the transition points between the four regions can be done through two quadratic equations for the approximate rate of change of the retention ratio with respect to particle size (Eq. (22) and Eq. (24)) in the limiting cases of strong fields or small particle sizes.

FFF can act as a valuable characterization tool in microfluidic devices but the elution time is not necessarily a one-to-one function of particle size. Although this complicates the interpretation of retention profiles, four operational-modes exist, each of which can be utilized to separate samples. By utilizing Fig. 11, one may choose to construct microfluidic channels designed to specifically function in one operational-mode or another for given samples. Doing so would avoid transitions and maintain the retention ratio as a monotonic function of particle size. On the other hand, by using the nonmonotonic form of the retention ratio (Eq. (20)), a microfluidic device need not be limited to a single operational-mode but can be employed to measure all possible particle samples, from relatively tiny tracer particles to particles as large as the microfluidic channel itself.

Nomenclature

c	solute concentration
c_0	solute concentration at the accumulation wall, i.e. $c_0 = c(y = r)$
f	external force (perpendicular to flow)
$\mathcal{F}$	steric function in retention ratio
$\mathcal{F}_p$	$\mathcal{F}$ for point-particles
$\mathcal{F}_f$	$\mathcal{F}$ for finite sized particles
$k_B T$	thermal energy
r	particle radius
$\tilde{r}_{HN}$	transition from hydrodynamic chromatography to normal-mode
$\tilde{r}_{NS}$	transition from normal- to steric-mode, i.e. steric inversion point
$\tilde{r}_{SF}$	transition from steric- to Faxén-mode
R	retention ratio
$R_{1/2}$	R of largest particle, i.e. $R_{1/2} = R(r = w/2)$
t_0	void time
t_R	retention time
v	solvent velocity

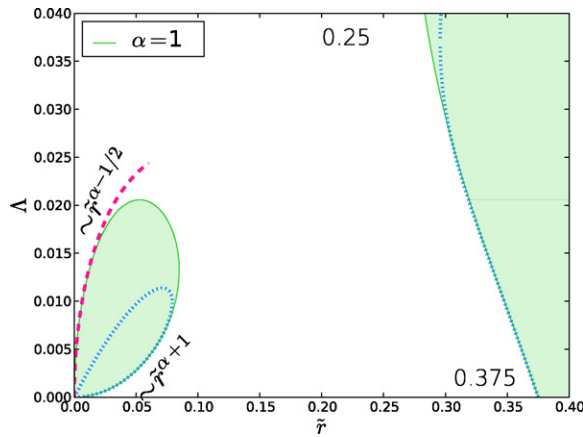


Fig. 12. Approximations to $\tilde{r}_{SF}$ and $\tilde{r}_{NS}$ work well for small device retention parameters Λ as shown by the dotted lines from Eq. (22) for the example of $\alpha = 1$. The dashed line from Eq. (24) shows the small $\tilde{r}$ approximation for the $\tilde{r}_{HN}$ transition.

v	solute velocity
w	channel height
y	distance from the accumulation wall
α	power of force with base r
λ	retention parameter
Λ	device retention parameter, i.e. $\Lambda = \lambda \tilde{r}^\alpha$
μ	external field
$\tilde{r}$	scaling by channel height
$\langle \cdot \rangle$	cross-sectional average
$\mathcal{L}(\cdot)$	Langevin function

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Appendix A. Approximations

Although the unified ideal retention ratio is given analytically by Eq. (20), the transitions $\tilde{r}_{HN}$, $\tilde{r}_{NS}$ and $\tilde{r}_{SF}$ must be found numerically from $\partial R / \partial \tilde{r} = 0$. However, in some experimentally relevant limits, the transitions can be well approximated by simple forms.

A.1. Small Λ for $\tilde{r}_{NS}$ and $\tilde{r}_{SF}$

Let us estimate the domain of the normal-mode by taking the small Λ limit of $\partial R / \partial \tilde{r}$ such that the hyperbolic cotangent terms approach unity ($\coth(x) \approx 1$). This results in the approximation

$$\frac{\partial R}{\partial \tilde{r}} \approx 6 [a + b\Lambda + c\Lambda^2] = 0 \quad (22)$$

where

$$a \equiv 1 - \frac{8}{3}\tilde{r}, \quad b \equiv \frac{2\alpha\tilde{r} - 2\tilde{r} - \alpha}{\tilde{r}^{\alpha+1}}, \quad c \equiv \frac{4\alpha}{\tilde{r}^{2\alpha+1}}.$$

This has simple, analytic roots. The two roots are plotted in Fig. 12 as dotted lines. Because it is the small Λ limit of $\partial R / \partial \tilde{r}$, Eq. (22) captures the $\tilde{r}_{NS}$ and $\tilde{r}_{SF}$ borders quite well.

To first order, we see from Eq. (22) that the value for which the transition occurs is $\Lambda \approx a/b$ which scales as

$$\Lambda \sim \tilde{r}^{\alpha+1}. \quad (23)$$

It is seen in Fig. 12 that while this small Λ approximation does a poor job estimating the total area of the normal-mode regime,

the first root does accurately estimate $\tilde{r}_{NS}$ at small Λ . The second root predicts $\tilde{r}_{SF}$ and agrees exceedingly well in the infinite force limit of $\lim_{\Lambda \rightarrow 0} \tilde{r}_{SF} = 3/8 = 0.375$ but cannot approximate the hydrodynamic chromatography limit of $\lim_{\Lambda \rightarrow \infty} \tilde{r}_{SF} = 0.25$.

A.2. Small $\tilde{r}$ for $\tilde{r}_{HN}$

The opposite approximation corresponds to the small $\tilde{r}$ values for which the expansion is $\coth(x) \approx x^{-1} + x/3 + x^3/45 + \dots$. To order $2\alpha + 1$, the rate of change of the retention ratio with particle size is

$$\frac{\partial R}{\partial \tilde{r}} = A + \frac{B}{\Lambda^2} = 0 \quad (24)$$

where

$$A \equiv 2 - 8\tilde{r}, \quad B \equiv \frac{1}{5} \left[-\frac{\alpha}{6} \tilde{r}^{2\alpha-1} + \frac{2(2\alpha+1)}{3} \tilde{r}^{2\alpha} - 4(\alpha+1) \tilde{r}^{2\alpha+1} \right].$$

Again, the roots to the approximation are simple and have been plotted in Fig. 12 as a dashed line. Despite being a small $\tilde{r}$ approximation, Eq. (24) fails as Λ approaches the critical value Λ_c .

To order $2\alpha + 1$, the value for which the transition occurs is $\Lambda \approx \sqrt{A/B}$ which scales as

$$\Lambda \sim \tilde{r}^{\alpha-1/2}. \quad (25)$$

Interestingly, this scaling means that the concavity of $\tilde{r}_{HN}$ changes with α . When $\alpha = 1$ the normal-mode regime is a “petal” as in Fig. 8 but when $\alpha > 1$ the concavity flips and the normal-mode is more “tear-drop” shaped.

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Can slip walls improve field-flow fractionation or hydrodynamic chromatography?

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ABSTRACT

One way to potentially modify the performance of field-flow fractionation (FFF) would be to move the position of the maximum flow velocity away from the mid-point of the channel, for example by using walls with non-zero slip lengths. In this short communication, we extend the ideal theory of FFF to include the effects of two slip walls. Our calculations demonstrate that while the hydrodynamic chromatography limit of FFF (weak fields) is not improved by engineering devices with slip-walls, the performance of Normal-Mode FFF can be enhanced by having slip at the depletion wall in moderate fields. We also introduce a new regime, which we call Slip-Mode FFF, where a large external field (typical of Normal-Mode FFF) and a large slip at the accumulation wall lead to sharp separations characterized by an elution order that is similar to that of hydrodynamic chromatography.

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1. Introduction

Field-flow fractionation (FFF) is a versatile size-separation method that has been used to analyze a wide variety of analytes including macromolecules such as starches [1] and tannins [2], microorganisms such as bacteria [3], erythroleukemia cells [4] and viral structures [5], environmental particulates [6] and nanoparticles [7]. By applying a transverse external field to a solution of sample particulates while simultaneously eluting the solution through a channel by a nonuniform, laminar flow profile, solutes elute through the device at different speeds and thus separation by size is achieved as shown schematically in Fig. 1 [8–14]. The flexibility of FFF results from the large assortment of transverse external fields that can be used. Gravitational [15,16], sedimentation [17,18], electrical [19,20], magnetic [21,22], dielectrophoretic [23,24], acoustic [25,26], photophoretic [27,28], cross-flow (both symmetrical [29,30] and asymmetrical [31,32]) and thermal [33,34] fields have all been used to generate non-uniform solute concentration distributions. The forces in both Thermal-FFF and Flow-FFF (the most widely used FFF fields) depend linearly on the particle's radius r and we will focus on this size dependence.

While interchanging external fields has been the subject of many investigations, it is generally taken for granted that the velocity of the carrier fluid obeys Poiseuille flow. Perhaps the exception to this is the difficulty associated with velocity skewing in Thermal-FFF when thermal gradients are large enough to produce viscosity gradients [35,36]. Yet, separation is completely dependent on the shape of the flow profile. In principle, it is possible to change the elution times and improve the performance of FFF over some range of particle sizes by changing the flow profile. This is what we examine in the present paper.

We will consider fractionation in an FFF channel that has arbitrary slip [37–39] at either wall. Slip is of interest to FFF because:

- (i) Current Flow-FFF channels are fitted with porous ceramic frits that allow the cross-flow to enter through the depletion wall (in symmetrical Flow-FFF) and exit through the accumulation wall. Although it is well known that fluid slip can occur at porous surfaces [40–45], to the best of our knowledge, slip has not been investigated in Flow-FFF apparatuses.
- (ii) Electroosmotic flow with thin Debye layers is qualitatively similar to slip flow with a Smoluchowski slip velocity [46,47]. A point-particle retention theory for the linear combination of electroosmotic flow and Poiseuille flow exists for both neutral [48] and charged [49] analytes.
- (iii) The modern ability to engineer surfaces in microfluidic devices suggests that if slip is predicted to improve FFF in certain operational regimes, then channel walls can be constructed accordingly. This ability to nanoengineer patterned,

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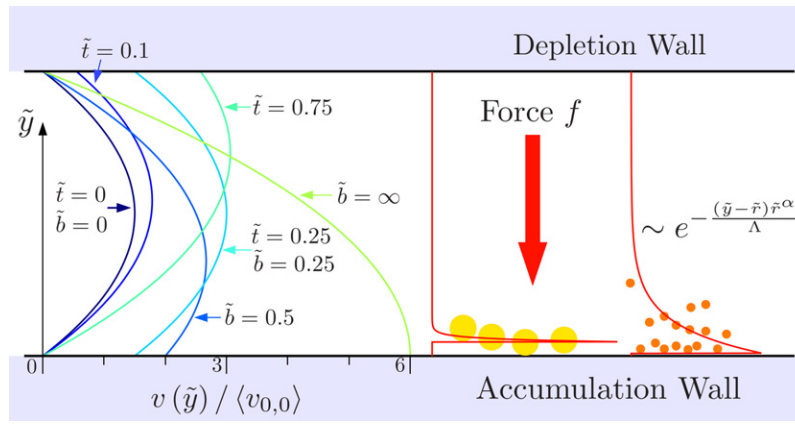


Fig. 1. Schematic of field-flow fractionation system with slip. On the left, fluid flow profiles are shown for a variety of slip lengths $\tilde{b}$ and $\tilde{t}$, for the accumulation and depletion wall respectively. Concentration distributions of large and small particles subject to a vertical external force $f(\tilde{r}) \sim \tilde{r}^\alpha$, where $\tilde{r}$ is the particle radius, are shown on the right. Larger particles have a sharper distribution but a larger excluded region than smaller particles.

ultrahydrophobic surfaces for drag reduction in microfluidic devices [50–56] is the primary motivation for the current theoretical study and although the source of the non-zero slip lengths is theoretically inconsequential for this study, the reader is referred to Refs. [38,57,58] as valuable reviews on superhydrophobic surfaces.

2. Theory

2.1. Carrier fluid flow profile

The flow profile $v(y)$ of a pressure driven flow between two plates separated by a distance w is parabolic with distance $\tilde{y} = y/w$ from the accumulation wall ($\tilde{y}$ signifies normalization by channel height w). Slip does not change this; instead, it shifts the velocity from zero to a finite value at the walls and in doing so moves the position of the maximum from the centre of the channel. A slip length $\tilde{b}$ produces a boundary condition $v(\tilde{y} = 0) = \tilde{b}(\partial v / \partial \tilde{y})|_{\tilde{y}=0}$ at the accumulation wall and a slip length $\tilde{t}$ makes the boundary condition at the depletion wall $v(\tilde{y} = 1) = -\tilde{t}(\partial v / \partial \tilde{y})|_{\tilde{y}=1}$. Solving the Navier–Stokes equation, the fluid velocity profile is given by

$$v(\tilde{y}, \tilde{b}, \tilde{t}) = 6\langle v_{0,0} \rangle \left[-\tilde{y}^2 + \left(\frac{1+2\tilde{t}}{1+\tilde{b}+\tilde{t}} \right) (\tilde{y} + \tilde{b}) \right], \quad (1)$$

where we have stated our solution in terms of the no-slip average fluid velocity $\langle v_{0,0} \rangle = \int_0^1 v(\tilde{y}, 0, 0) d\tilde{y}$ for the same pressure gradient. This equation reduces to the well-known parabolic (Poiseuille) form $v(\tilde{y}, 0, 0) = 6\langle v_{0,0} \rangle [-\tilde{y}^2 + \tilde{y}]$ in the no-slip limit. When the pressure difference is kept constant, slip moves the maximum velocity away from the centre position $\tilde{y} = 1/2$ (unless $\tilde{b} = \tilde{t}$), and increases the average flow velocity

$$\langle v(\tilde{b}, \tilde{t}) \rangle = \langle v_{0,0} \rangle \left[\left(\frac{1+2\tilde{t}}{1+\tilde{b}+\tilde{t}} \right) (6\tilde{b} + 3) - 2 \right]. \quad (2)$$

Several flow profiles are shown in Fig. 1. The equal slip such as $v(\tilde{y}, 0.25, 0.25)$ shifts the $v(\tilde{y}, 0, 0)$ parabolic profile up. The $v(\tilde{y}, 0, \infty)$ curve shows a continuous increase from zero at the no-slip wall (the depletion wall in this example) to $6\langle v_{0,0} \rangle$ at the perfect slip wall (accumulation wall).

2.2. Particle velocity

To 0th order, particles carried by the flow move at the same speed as the solvent at the centre of mass of the particle, $v(\tilde{y}, \tilde{b}, \tilde{t})$, but to correct for the curvature of the fluid profile over the surface

of a spherical particle of finite radius $\tilde{r}$, Faxén's law must be applied. The ideal velocity of the solute particle is then found to be given by

$$v(\tilde{r}, \tilde{y}, \tilde{b}, \tilde{t}) = \left[1 + \frac{\tilde{r}^2}{6} \nabla^2 \right] v(\tilde{y}, \tilde{b}, \tilde{t}) = v(\tilde{y}, \tilde{b}, \tilde{t}) - 2\tilde{r}^2 \langle v_{0,0} \rangle. \quad (3)$$

The retention theory used here is called “ideal” primarily because it neglects hydrodynamic interactions between the solute particles and the walls (although other complications such as concentration effects [59], etc. [60] are also assumed insignificant). Hydrodynamic interactions can generally be divided into inertial lift forces, which act perpendicular to the channel walls, and drag forces, which act anti-parallel to the direction of flow. The lift forces generally have components both towards (due to the shear-gradient) and also away from (due to symmetry breaking by the wall) the walls, which establish an equilibrium height [61]. Lift forces can be kept small by keeping the particle Reynolds number well below unity or be utilized to separate particles by inertial focusing [62–64] or Hyperlayer-Mode FFF [65,16]. The increased drag arises because the no-slip conditions on both the surface of the mobile particles and on the channel walls cause greater shearing of the fluid and so generate a greater effective friction coefficient than the same particulates would have in free solution, which reduces v from the value predicted by Eq. (3) [66]. This effect may become significant as the particle size approaches the channel height and so the ideal theory presented here would tend to overpredict the retention ratio (although this effect is reduced by large slip lengths [51]).

2.3. Concentration distribution

In FFF, solute particles are pushed against the accumulation wall (Fig. 1) by a force $f(\tilde{r}) \sim \tilde{r}^\alpha$ but are dispersed by diffusion. The competition between potential and thermal energy is described by the retention parameter $\lambda = k_B T / fw = \Lambda \tilde{r}^{-\alpha}$ and leads to an exponential concentration distribution

$$c(\tilde{y}) \sim \begin{cases} e^{-(\tilde{y}-\tilde{r})/\Lambda} & \text{for } \tilde{r} < \tilde{y} < 1 - \tilde{r} \\ 0 & \text{otherwise.} \end{cases} \quad (4)$$

The dimensionless device retention parameter Λ describes the FFF apparatus without implicit reference to particle size [67]. As stated earlier, because of its connection to Flow-FFF we will focus on $\alpha = 1$ (linear scaling) unless otherwise noted. Different sized solute particles have different concentration distributions dictated by their relevant Boltzmann factor and so sample the fluid velocity profile differently (Fig. 1).

2.4. Retention ratio

The retention ratio is the average solute velocity normalized by the average velocity of the carrier fluid

$$R \equiv \frac{\langle v \rangle}{\langle v \rangle} = \frac{\int_0^w c(y) v(y) dy}{\int_0^w c(y) dy \int_0^w v(y) dy}. \quad (5)$$

Experimentally, the retention ratio $R = t_0/t_R$ is found by measuring the retention time $t_R \sim 1/\langle v \rangle$ and void fluid time $t_0 \sim 1/\langle v \rangle$. The ideal retention theory [67] gives the following result for no-slip boundary conditions

$$R_{\text{no-slip}}(\tilde{r}, \Lambda) = \frac{6\Lambda}{\tilde{r}^\alpha} [1 - 2\tilde{r}] \mathcal{L}\left(\frac{[1 - 2\tilde{r}]\tilde{r}^\alpha}{2\Lambda}\right) + 6\tilde{r} \left(1 - \frac{4}{3}\tilde{r}\right), \quad (6)$$

where $\mathcal{L}(x) = \coth(x) - 1/x$ is the Langevin function. For a given device retention parameter Λ , the ideal retention ratio $R_{\text{no-slip}}(\tilde{r}, \Lambda)$ is a nonmonotonic function of particle size $\tilde{r}$ [67]. When Λ is large (a weak field), the retention ratio has one global maximum that marks the transition from hydrodynamic chromatography (HC) to Faxén-Mode FFF. As we shall see later, $R_{\text{no-slip}}$ has three extrema (and thus predicts the existence of four different regimes) when the field is strong (more precisely when Λ is smaller than a critical value Λ_c [67]), including the so-called Steric Inversion Point.

2.5. Slip retention ratio

By using the slip-velocity Eq. (3) in Eq. (5), the ideal retention ratio for finite-sized solute particles in a slit channel with slip at both the depletion and the accumulation walls can be expressed as a modification to the no-slip solution:

$$R(\tilde{r}, \Lambda, \tilde{b}, \tilde{t}) = \left[\frac{1 + \tilde{b} + \tilde{t}}{1 + 4\tilde{b} + 4\tilde{t} + 12\tilde{t}\tilde{b}} \right] \times [R_{\text{no-slip}} + R_{\text{slip}}], \quad (7)$$

where

$$R_{\text{slip}}(\tilde{r}, \Lambda, \tilde{b}, \tilde{t}) = 6 \left[\frac{\tilde{t} - \tilde{b}}{1 + \tilde{b} + \tilde{t}} \right] \left[\tilde{r} \mathcal{L}\left(\frac{[1 - 2\tilde{r}]\tilde{r}^\alpha}{2\Lambda}\right) + \frac{\Lambda}{[1 - 2\tilde{r}]\tilde{r}} + \frac{\tilde{b} + 2\tilde{t}\tilde{b}}{\tilde{t} - \tilde{b}} - \frac{1}{e^{(1-2\tilde{r})\tilde{r}^\alpha/\Lambda} - 1} \right]. \quad (8)$$

It is easy to verify that $R(\tilde{r}, \Lambda, 0, 0)$ reduces to $R_{\text{no-slip}}(\tilde{r}, \Lambda)$ in the no-slip limit since $R_{\text{slip}}(\tilde{r}, \Lambda, 0, 0) = 0$ and the prefactor in Eq. (7) is then equal to unity.

3. Results

3.1. Weak fields

One of the benefits of writing the retention ratio as the sum of the no-slip solution and a correction term is that the limits of the no-slip term have already been analyzed [67]. The limits $\tilde{r} \rightarrow 0$, $\tilde{r} \rightarrow 1/2$, and $\Lambda \gg 1$ of $R_{\text{no-slip}}$ are all identical because in each of these the force is insignificant compared to the effect of steric exclusion from the near wall regions. To first order in $\tilde{b}$, $\tilde{t}$, and $1/\Lambda$, the retention ratio is

$$R(\tilde{r}, \Lambda, \tilde{b}, \tilde{t}) \approx 1 + 2(1 - 3\tilde{b} - 3\tilde{t})(\tilde{r} - 2\tilde{r}^2) + (\tilde{b} - \tilde{t}) \frac{(1 - 2\tilde{r})^2 \tilde{r}^\alpha}{2\Lambda}. \quad (9)$$

At both the smallest and largest particle sizes ($\tilde{r} = 0$ and $\tilde{r} = 1/2$) the retention ratio goes to $R \rightarrow 1$, as it ideally should when

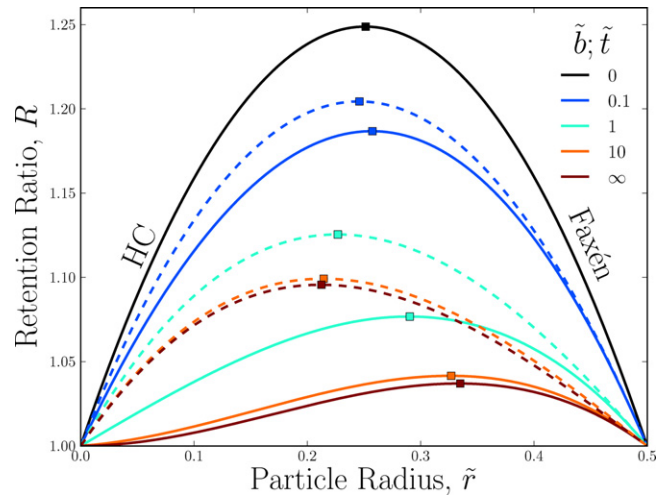


Fig. 2. The retention ratio of FFF with a large but finite device retention parameter $\Lambda = 0.25$. Only hydrodynamic chromatography (HC) and Faxén-Mode FFF exist, and the transition between the two is denoted by a square. No-slip FFF is a black (top curve) solid line. The other solid lines show slip at the depletion wall while the dashed lines show slip at the accumulation wall; the colour code for the various slip lengths $\tilde{t}$ and $\tilde{b}$ is given in the legend.

hydrodynamic complications are neglected. When $\alpha = 1$, the maximum retention ratio is found for a particle of size

$$\tilde{r}_{\text{max}}(\Lambda, \tilde{b}, \tilde{t}) \approx \frac{1}{4} \left[1 + \frac{\tilde{t}}{16\Lambda} - \frac{\tilde{b}}{16\Lambda} \right], \quad (10)$$

and the retention ratio for this particle size is:

$$R_{\text{max}}(\Lambda, \tilde{b}, \tilde{t}) \approx \frac{5}{4} \left[1 - \frac{3}{5}(\tilde{t} + \tilde{b}) + \frac{\tilde{b} - \tilde{t}}{40\Lambda} \right]. \quad (11)$$

3.1.1. True hydrodynamic chromatography limit

In the true HC limit of $\Lambda \rightarrow \infty$ (no external force), the no-slip retention ratio $R_{\text{no-slip}}(\tilde{r})$ is exactly parabolic and its maximum $R_{\text{max}} = 5/4$ at $\tilde{r}_{\text{max}} = 1/4$ (this is nearly indistinguishable from the top curve in Fig. 2) marks the transition from HC (for small particles) to Faxén-Mode FFF (for large particles). Since the retention ratio has a one-to-one correspondence with particle sizes only up to $\tilde{r} = \tilde{r}_{\text{max}} = 1/4$, this represents the useful range of HC in the no-slip limit (the non-monotonic nature of the $R(\tilde{r})$ curve would lead to confusion if the sample also included particles larger than $\tilde{r}_{\text{max}}$). Forgoing a discussion of band broadening, the ability to resolve different sizes is related to the slope of the curve, $dR(\tilde{r})/d\tilde{r}$. The symmetry of $\lim_{\Lambda \rightarrow \infty} R(\tilde{r}, \Lambda, 0, 0)$ in Fig. 2 suggests that the com-

monly used selectivity $S = |d \log R / d \log \tilde{r}| = (\tilde{r}/R) |dR/d\tilde{r}|$ is not as appropriate a metric for this study as the slope, $dR(\tilde{r})/d\tilde{r}$ itself. The question is thus whether slip can increase the range of the separation and/or the rate of change of retention ratio.

In fact, Eq. (10) tells us that the range does not change since the maximum must remain centred at $\tilde{r}_{\text{max}} = 1/4$ when there is no external force to break the symmetry. However, when $\Lambda \rightarrow \infty$ in Eq. (11), R_{max} decreases in the presence of slip at either wall. This reduces the average slope ($\partial R(\tilde{r})/\partial \tilde{r} = 1 - 3\tilde{b} - 3\tilde{t}$ in the HC regime, leading to reduced resolution over the useful range $0 < \tilde{r} < 1/4$. The resolution near $\tilde{r} = 0$, which is where it is generally the largest (see Fig. 2), is given by (to first order in both $\tilde{b}$ and $\tilde{t}$):

$$\left. \frac{\partial R(\tilde{r})}{\partial \tilde{r}} \right|_{\tilde{r}=0} = 2 \left[1 - 3(\tilde{t} + \tilde{b}) + \frac{\tilde{b} - \tilde{t}}{4\Lambda} \right]. \quad (12)$$

Again, any slip reduces the slope for small particles when $\Lambda \rightarrow \infty$. We thus conclude that slip does not improve the performance of the zero field, HC limit.

3.1.2. Weak field results

When a weak external field $1/\Lambda$ is applied, the picture is qualitatively similar but the field breaks the symmetry. In the absence of slip, the retention curve in Fig. 2 is still parabolic with both an HC and a Faxén-Mode FFF regime. Eq. (10) predicts that slip at the accumulation wall should decrease the range of the HC regime by moving the maximum from $\tilde{r}_{\max} = 1/4$ to smaller particle sizes, while slip at the depletion wall should increase the range of HC. When the full form of Eq. (7) is used in Fig. 2, we see that this is exactly what occurs for weak fields. In fact, Fig. 2 also demonstrates the second effect of the weak field: slip at the depletion wall reduces the maximum retention ratio even more than slip at the accumulation wall does, in qualitative agreement with Eq. (11). Unfortunately, the small increase in the range $\tilde{r}_{\max}$ found for depletion wall slip is accompanied by a large decrease in the slope $\partial R(\tilde{r})/\partial \tilde{r}$ over the useful range $0 < \tilde{r} < \tilde{r}_{\max}$; this is in agreement with the prediction of Eq. (12). We thus conclude that FFF does not benefit from slip at either wall in the HC or low force regimes.

3.2. Strong fields

3.2.1. No-slip conditions

When the external field Λ is below some critical value Λ_c [67], two additional regimes, called the Normal- and Steric-Modes, appear between the HC and Faxén-Modes, i.e. for intermediate particle sizes; typical cases are shown in Fig. 3a and b as solid, black lines. Let us briefly introduce each of the FFF modes:

- At the most minute particle sizes, the external force is negligible because $f \sim \tilde{r}^\alpha$ and we have a tiny HC regime in which R grows with particle size $\tilde{r}$. Since this is not visible in Fig. 3a, we verify its existence by zooming in for a weaker field in Fig. 3b.
- A local maximum (denoted by a diamond in Fig. 3a and b) represents the transition from HC to Normal-Mode FFF. For strong fields, Normal-Mode has high resolution: the retention ratio drops significantly over a short range of particles sizes (because larger particles stay closer to the accumulation wall)

and the slope $\partial R(\tilde{r})/\partial \tilde{r}$ is strongly negative. This is generally the preferred mode of operation of FFF.

- This is followed by Steric-Mode FFF (a circle marks the transition in Fig. 3a), in which steric exclusion from the accumulation wall dominates the dynamics. The Steric-Mode regime has a large range $\Delta \tilde{r}$; however, the (positive) slope $\partial R(\tilde{r})/\partial \tilde{r}$ is less favourable than in Normal-Mode. In strong fields, the Steric-Mode slowly climbs from the global minimum (circles) to the global maximum (squares in Fig. 3a).
- Finally, at the largest particle sizes, the retention ratio must return to unity and so in Faxén-Mode FFF, the retention ratio slowly declines.

In practice, one must use a single regime since the retention curve is highly non-monotonic. In the no-slip limit, both the narrow, high-resolution Normal-Mode regime and the broader, lower-resolution Steric-Mode regime can in practice be used for separating particles, although Steric-Mode is more susceptible to hydrodynamic effects which increase the particles' friction coefficients. As we shall see, the presence of slip changes the range and mean resolution of all four regimes.

3.2.2. Slip at depletion wall

Slip at the depletion wall (solid lines in Fig. 3a) improves Normal-Mode FFF by increasing the retention ratio drop for small particles (the minima (circles) are significantly lowered). Since the range of this regime remains essentially constant, there is a large gain in resolution here. This improvement can be understood from the schematic Fig. 1: slip at the depletion wall causes the slope of the fluid velocity $v(\tilde{y}, 0, \tilde{t})$ to be increased near the accumulation wall. In Normal-Mode FFF, solutes spend the majority of their time near this wall so the average velocity difference generated by different average heights is increased. However, we note that this increased fluid velocity is actually smaller as we get closer to the wall (as Fig. 1 demonstrates with the $v(\tilde{y}, 0, 0.1)$ or $v(\tilde{y}, 0, 0.75)$ flow profiles). To first order in both $\tilde{y}$ and $\tilde{t}$, the velocity of the fluid near the accumulation wall increases like $\sim \tilde{r}(1 + \tilde{t})/2$. As a consequence of this, when particles are pushed closer to the wall by stronger forces, slip has less of an impact on their velocity in the flow. The increase in resolution as a function of $\tilde{t}$ is thus less pronounced in stronger fields. Therefore, the Normal-Mode increases significantly

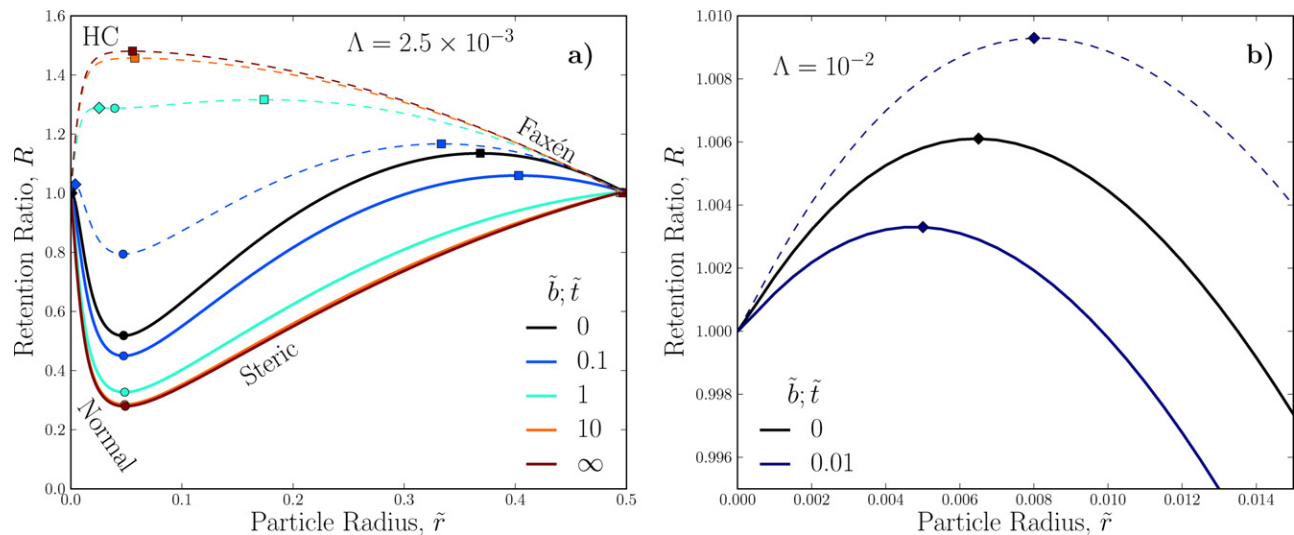


Fig. 3. Retention ratio as a function of particle size for slip at the depletion wall (solid lines) and slip at the accumulation wall (dashed lines). The no-slip solution is given by the solid black line. Diamonds denote the transition from hydrodynamic chromatography (HC) to Normal-Mode FFF, circles mark the normal- to Steric-Mode transition and squares label the transition to Faxén-Mode FFF. (a) Retention ratio as a function of particle size for $\Lambda = 2.5 \times 10^{-3}$. (b) Retention ratio of small particle sizes for $\Lambda = 10^{-2}$ showing HC and the beginning of Normal-Mode FFF.

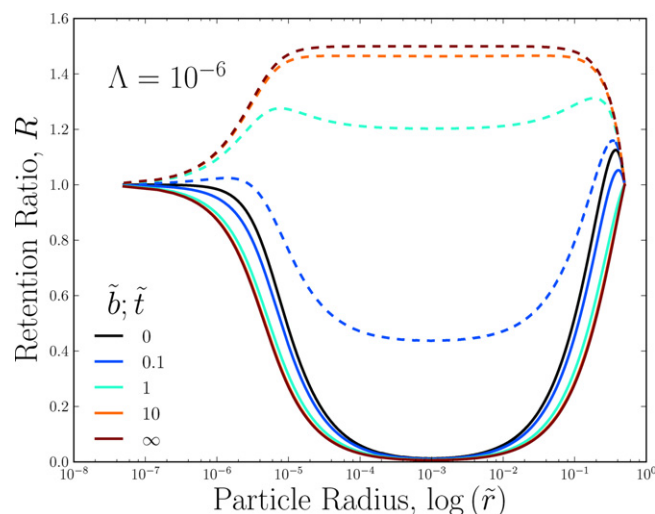


Fig. 4. Retention ratio for a device retention parameter $\Lambda = 10^{-6}$ on a logarithmic scale for slip at the depletion wall (solid lines) and slip at the accumulation wall (dashed lines). The no-slip solution is given by the solid black line.

in resolution only if the field is moderate. One should compare the significantly increased drop in retention ratio when $\Lambda = 2.5 \times 10^{-3}$ seen in Fig. 3a to the nearly identical curves over the Normal-Mode range when $\Lambda = 10^{-6}$ (a more practically realistic value for FFF) seen in Fig. 4. For strong fields, such as shown in Fig. 4, Normal-Mode FFF occurs over a tiny range of relatively small particle sizes (the same is true for HC) and slip at the depletion wall has less of an impact than it does for more moderate fields.

As can be seen in Fig. 3a, slip at the depletion wall also moves the transition point between the Steric- and Faxén-Modes (the square points) to larger particle sizes. This broadens the range over which the Steric-Mode can be used, without significantly affecting its average resolution (slope). However this does not present a benefit in practice since particles nearly the size of the channel ($\tilde{r} = 1/2$) can create problems such as clogging and are affected by non-ideal (increased) drag coefficients.

We have seen in Fig. 3a and b that slip at the depletion wall alters the range of each regime and that the change is less pronounced for stronger fields in Fig. 4, but how does this change for different device retention parameters and different slip lengths? The $\Lambda - \tilde{r}$ operational-mode diagram Fig. 5a maps out how the ranges of the four FFF-Modes change (for $\alpha = 1$). We see that slip at the depletion wall typically broadens the range of Steric-Mode FFF. Furthermore, it increases the closed area that defines the Normal-Mode regime: as we can see in Fig. 5a, the area increases both because its range of particle sizes $\Delta\tilde{r}$ is increased and also because the critical device retention parameter Λ_c above which Normal-Mode does not exist is raised. This means that when there is slip at the depletion wall, Normal- and Steric-Mode FFF will exist in weaker fields (larger Λ) than they would have for no-slip FFF. The critical value Λ_c increases because slip at the depletion wall moves the height at which the velocity of the fluid is maximum away from the accumulation wall.

Moreover, slip at the depletion wall causes the critical device retention parameter Λ_c to occur at smaller particle sizes, reducing both the range and the amplitude of the minuscule HC regime (see Fig. 3b). In fact, when $\tilde{r} \geq (1 + \sqrt{15})/14 = 0.35$, the critical device retention ratio occurs at $\tilde{r} = 0$ and HC does not exist at all below $\Lambda < \Lambda_c$: Normal-Mode extends to the smallest particle sizes and Fig. 5a has non-zero intercepts (if and only if $\alpha = 1$). The intercept then grows with slip as $\Lambda_c = \tilde{t}/4(1 + \tilde{t})$ as plotted as filled symbols in Fig. 5a. This is fundamentally different from the no-slip case in which a diminutive HC range always exists even for the strongest fields (or equivalently, the slope of the retention ratio at $\tilde{r} = 0$ is

always positive). Fig. 4 shows an example of this phenomenon: HC does not exist for these values of $\tilde{r}$ at $\Lambda = 10^{-6}$.

3.2.3. Slip at accumulation wall

If instead a no-slip condition $\tilde{r} = 0$ is maintained at the depletion wall and a slip length $\tilde{b}$ is allowed to grow at the accumulation wall, the elution behaviour is quite different (dashed lines in Fig. 3a). The Normal-Mode operational regime deflates and eventually vanishes, as seen in Fig. 5b. Simultaneously, the transition to Faxén-Mode marches to smaller and smaller particle sizes with increasing slip, and the maximum range and retention ratio of HC also increase. The result is a poor performance in all regimes, until the slip length $\tilde{b}$ is large enough to completely change the nature of the retention curve (the top three lines in Fig. 3a).

When the field is strong and the slip length is comparable to or larger than the channel height ($\tilde{b} \gtrsim w$ or equivalently $\tilde{b} \gtrsim 1$), the retention curve once again has only a single maximum (at a small value of $\tilde{r}_{\max}$) and the retention ratio goes directly from a steep HC-like regime to a slowly decreasing regime similar to Faxén-Mode FFF (squares in Fig. 3a, dashed lines). Remarkably, in Fig. 3a, the slope for $\tilde{r} < \tilde{r}_{\max}$ and $\tilde{b} \rightarrow \infty$ is even greater than it is in Normal-Mode for no-slip FFF. While increasing the field strength for large $\tilde{b}$ does not significantly increase $\tilde{r}_{\max}$, it does decrease $\tilde{r}_{\max}$ as demonstrated in Fig. 4 suggesting that high resolution can be achieved in this regime. Although technically this looks like the HC regime because it is the first region with a positive slope, the external field is large while HC implies that it is negligible. Furthermore, the particle size range and resolution are more akin to no-slip Normal-Mode FFF than to no-slip HC. Therefore, we call this limit of small Λ and large $\tilde{b}$ Slip-Mode FFF.

In many ways, the mechanism behind Slip-Mode FFF is indeed more similar to Normal-Mode FFF. In the Slip-Mode FFF regime, larger particles feel a stronger force towards the accumulation wall where the fluid velocity is much greater as shown in Fig. 1. Thus the retention ratio increases rapidly with particle size just as it dropped rapidly for no-slip Normal-Mode FFF. However, at some point steric exclusion from the near wall region dominates and the average height increases linearly with particle size. In the no-slip case, steric exclusion subjects particles to faster flowing solvent

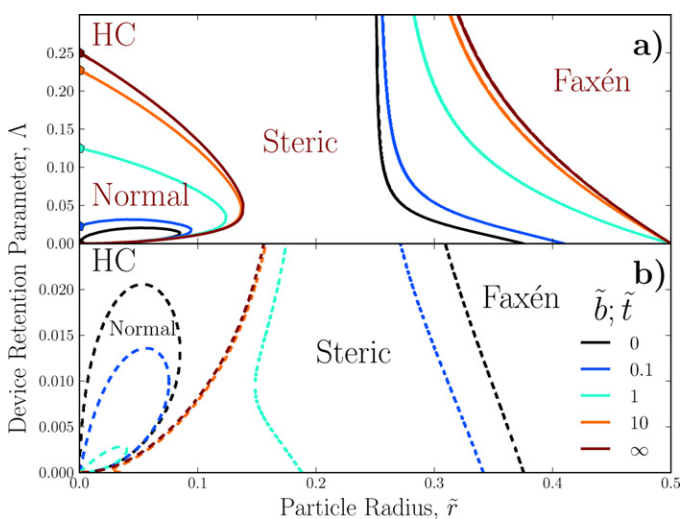


Fig. 5. The $\Lambda - \tilde{r}$ phase diagram for a number of different colour-coded slip lengths. Each line traces the position of the extrema points defined in Fig. 3a as a function of Λ and so maps the transitions between operational-modes. (a) Like in Fig. 3a, solid lines denote slip at the depletion and no-slip at the accumulation wall ($\tilde{b} > 0, \tilde{t} = 0$). The intercepts are marked with filled symbols. (b) Dashed lines denote slip at the accumulation and no-slip at the depletion wall ($\tilde{b} > 0, \tilde{t} = 0$). The intercepts are all at the origin.

Table 1

Summary of the impact of slip at either wall for the force scaling exponents $\alpha = [1, 2, 3]$. For each mode of operation (or regime) with device retention ratios above or below Λ_c , three metrics are used to characterize the impact of slip on the resulting retention ratios: range $\Delta\tilde{r}$, retention disparity ΔR over that range, and ratio $\Delta R/\Delta\tilde{r}$ as a rough measure of resolution. Increases (decreases) in performance with slip are marked by + (–). When there is no clear increase or decrease for the range of Λ the symbol $\emptyset$ is used. Normal- and Steric-Mode FFF do not exist for $\Lambda > \Lambda_c$ and so are labeled NA (not applicable). These conclusions are based on solutions of the retention ratio as a function of particle radius for slips $0 \leq (\tilde{t}; \tilde{b}) \leq 10,000$ and device retention parameters $10^{-4} < \Lambda < 10^{-1}$.

Slip	Regime	Range, $\Delta\tilde{r}$						Retention disparity, ΔR						Ratio, $\Delta R/\Delta\tilde{r}$					
		$\alpha=1$		$\alpha=2$		$\alpha=3$		$\alpha=1$		$\alpha=2$		$\alpha=3$		$\alpha=1$		$\alpha=2$		$\alpha=3$	
		$\Lambda < \Lambda_c$		$\Lambda > \Lambda_c$		$\Lambda < \Lambda_c$		$\Lambda < \Lambda_c$		$\Lambda > \Lambda_c$		$\Lambda < \Lambda_c$		$\Lambda < \Lambda_c$		$\Lambda < \Lambda_c$		$\Lambda > \Lambda_c$	
		$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$	$\Lambda < \Lambda_c$	$\Lambda > \Lambda_c$
$\tilde{t} > 0$	HC	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Normal	+	NA	+	NA	$\emptyset$	NA	+	NA	+	NA	+	NA	+	NA	+	NA	+	NA
	Steric	$\emptyset$	NA	+	NA	+	NA	$\emptyset$	NA	$\emptyset$	NA	+	NA	$\emptyset$	NA	$\emptyset$	NA	$\emptyset$	NA
	Faxén	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
$\tilde{b} > 0$	HC	+	–	+	–	+	+	+	$\emptyset$	+	$\emptyset$	+	$\emptyset$	+	+	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$
	Normal	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA
	Steric	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA	–	NA
	Faxén	+	+	+	+	+	–	+	$\emptyset$	+	$\emptyset$	+	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset$

and causes the retention ratio to grow with particle size. However, when there is large slip at the accumulation wall, steric exclusion exposes the particles to slower velocities and so the retention ratio slowly decreases with particle size as if it were in the Faxén regime.

In Slip-Mode FFF, the external field must be strong ($\Lambda \lesssim 10^{-2}$) for large slip at the accumulation wall to significantly improve the resolution. When $\tilde{b} \rightarrow \infty$, the maximum in the retention ratio approaches $R_{\max} = 3/2$ and $\tilde{r}_{\max}$ moves to smaller and smaller sizes. The slope is greatest at the smallest particle sizes and increases with field as

$$\lim_{b \rightarrow \infty} \frac{\partial R(\tilde{r})}{\partial \tilde{r}} \bigg|_{\tilde{r}=0} = \frac{1}{2} + \frac{1}{8\Lambda}. \quad (13)$$

The resolution can thus be extremely high in this novel FFF method.

3.2.4. Slip at both walls

If the slip length at the depletion wall and at the accumulation wall are increased together such that the solvent flow profile maintains its symmetry, the range of each operational mode remains unchanged. Only the difference between the two slip lengths matters for transitions and inversion points. Therefore, there is no reason to have slip at both walls from an optimization point of view.

4. Conclusion

We have shown that slip at the channel walls of field-flow fractionation systems can modify the retention ratio both quantitatively and qualitatively. These theoretical predictions are the product of generalizing the ideal retention theory to account for arbitrary slip lengths at both or either wall. Since Flow-FFF channels are likely to have some slip, we chose to focus on linear dependence of the transverse force on the particle size (i.e., $\alpha = 1$). However, the metrics used to judge the effect of slip on FFF for all possible scaling exponents $\alpha = [1, 2, 3]$ have been condensed into Table 1.

Our investigation into the impact of slip on FFF leaves us with the following practical conclusions:

- Hydrodynamic chromatography does not benefit from slip.
- The resolution of Normal-Mode FFF in a moderate external field can be improved by slip at the depletion wall.
- Slip at the depletion wall typically increases the range of Steric-Mode FFF with little impact on its resolution.
- Normal- and Steric-Mode FFF persist for weaker fields when there is slip at the depletion wall.

- A new high-resolution mode, Slip-Mode FFF, is predicted to exist when the field is strong and slip at the accumulation wall is comparable to or larger than the channel height.

This set of conclusions suggests that experimentalists utilizing Flow-FFF should characterize the slip length of their apparatuses to determine whether or not slip plays a negligible role. Furthermore, we suggest that it would be interesting to build a microfluidic, Thermal-FFF device with a large slip length at the accumulation wall to test the existence and advantages of the new Slip-FFF mode of operation described in this paper.

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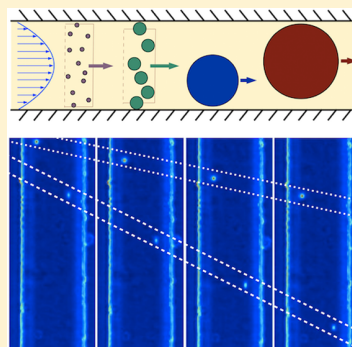
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Field-Flow Fractionation and Hydrodynamic Chromatography on a Microfluidic Chip

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ABSTRACT: We present gravitational field-flow fractionation and hydrodynamic chromatography of colloids eluting through 18 μm microchannels. Using video microscopy and mesoscopic simulations, we investigate the average retention ratio of colloids with both a large specific weight and neutral buoyancy. We consider the entire range of colloid sizes, including particles that barely fit in the microchannel and nanoscopic particles. Ideal theory predicts four operational modes, from hydrodynamic chromatography to Faxén-mode field-flow fractionation. We experimentally demonstrate, for the first time, the existence of the Faxén-mode field-flow fractionation and the transition from hydrodynamic chromatography to normal-mode field-flow fractionation. Furthermore, video microscopy and simulations show that the retention ratios are largely reduced above the steric-inversion point, causing the variation of the retention ratio in the steric- and Faxén-mode regimes to be suppressed due to increased drag. We demonstrate that theory can accurately predict retention ratios if hydrodynamic interactions with the microchannel walls (wall drag) are added to the ideal theory. Rather than limiting the applicability, these effects allow the microfluidic channel size to be tuned to ensure high selectivity. Our findings indicate that particle velocimetry methods must account for the wall-induced lag when determining flow rates in highly confining systems.



Field-flow fractionation (FFF),^{1,2} a broad class of separation techniques, is achieved by imposing a transverse force f across a channel of height h to a solution of solutes such as colloids,³ macromolecules,⁴ or cells.⁵ A concentration gradient is established in response to the competition between the thermal energy, $k_B T$, and the potential energy drop across the channel, fh . The system can thus be described in terms of the dimensionless retention parameter, $\lambda = k_B T / fh$. This solute distribution is carried through a channel by a Poiseuille flow with a characteristic retention ratio (average colloid velocity normalized by average fluid velocity, $R = \langle V \rangle / \langle v \rangle$). Colloids of different sizes r have different concentration distributions and therefore different retention ratios, R .

This simple system possesses surprisingly rich elution behavior. Previously, we considered the ideal retention theory (ignoring complications such as nonparabolic flow,⁶ nondilute concentration effects,⁷ slip,⁸ or hydrodynamic/lift interactions⁹), and predicted the existence of four distinct operational modes.¹⁰ The transitions between them were mapped; however, only three of the four modes and only a single transition had ever been previously observed experimentally.

For large λ , thermal energy dominates and solutes diffuse across the entire channel. Only steric interactions with the walls limit their distribution, as shown in Figure 1. This is the hydrodynamic chromatography limit of FFF (HC),¹¹ where larger particles elute before smaller particles. Ideally, density-matched tracer particles used for micro-particle image velocimetry¹² elute in HC, in the absence of any net external body forces.

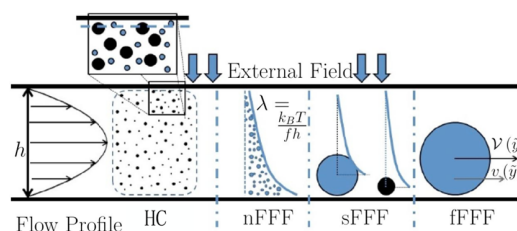


Figure 1. Schematic of ideal field-flow fractionation operational modes.

If the potential energy dominates over the thermal energy, the concentration profile across the channel height becomes exponential. In this normal-mode field-flow fractionation (nFFF), ensembles of small solutes can loosely be thought of as point particles subject to an external force that increases with particle size r (nFFF in Figure 1). Therefore, larger particles stay close to the accumulation wall where the lower flow velocity causes them to move more slowly than smaller particles.¹ Experimental observation of the transition between HC and nFFF has not previously been reported.

However, nFFF can only continue to exist for a certain size range before the size dependence changes once again. Larger (heavier) particles are pushed against the wall and steric interactions exclude the particles from sampling the slow

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moving velocity so larger particles elute faster in this steric-mode field-flow fractionation (sFFF), as seen in Figure 1.¹³

At the largest sizes, a significant portion of each particle's cross section is subject to varying fluid velocities. Ideally, even large particles in flow profiles with no curvature, such as Couette flow, would possess the same velocity as the fluid at the particles' centers of mass. However, when the flow profile possesses curvature and the particles' size is comparable to that curvature, such as large particles in Poiseuille flows, then the velocities can differ significantly from the velocity of the fluid at the particles' centers of mass. In such situations, a correction to the Stokes' law is required that causes larger particles to elute more slowly than they would otherwise. Faxén's law describes this phenomenon. We have recently considered Faxén's law in the context of FFF and predicted the existence of the Faxén-mode field-flow fractionation (fFFF),¹⁰ but this new separation mode remains unverified by experiments or simulations. In this work, we experimentally and computationally demonstrate the existence of fFFF but show that Faxén's law is relatively negligible at all particle sizes. We show that this is because increased hydrodynamic friction due to interactions with the channel walls play a more significant role at large particle-to-channel height ratios. We show that this is particularly important in microfluidic systems.

In field-flow fractionation, an external force is applied perpendicular to the flow direction to create a solute concentration distribution. In a general sense, the external force scales with normalized particle size like $f \sim \tilde{r}^\alpha$, where $\tilde{r} = r/h$ and the power α differs for different types of FFF external fields. For weight-based subtechniques, $\alpha = 3$, while $\alpha = 1$ for flow- or thermal-based FFF. Therefore, we can write $\lambda = \Lambda \tilde{r}^{-\alpha}$, where the device retention parameter Λ characterizes the FFF apparatus.¹⁰ In the case of gravitational field-flow fractionation (GrFFF), $\Lambda = (3k_B T)/(4\pi G \Delta \rho h^4)$ results from gravitational acceleration, G , acting on colloids with a density difference $\Delta \rho$ between the carrier fluid and particles. We have previously predicted that all four ideal operational modes can be described by a single unified, ideal retention theory formula,¹⁰ namely

$$R(\tilde{r}, \Lambda) = \frac{6\Lambda}{\tilde{r}^\alpha} [1 - 2\tilde{r}] \mathcal{L}\left(\frac{[1 - 2\tilde{r}]\tilde{r}^\alpha}{2\Lambda}\right) + \mathcal{F}(\tilde{r}) \quad (1)$$

In eq 1, the Langevin function is given by $\mathcal{L}(x) = \coth(x) - 1/x$ and the second term is

$$\mathcal{F}(\tilde{r}) = \begin{cases} 6\tilde{r}(1 - \tilde{r}) & \text{if Faxén's law is neglected} \\ 6\tilde{r}\left(1 - \frac{4}{3}\tilde{r}\right) & \text{if Faxén's law is accounted for} \end{cases} \quad (2)$$

As mentioned, such an ideal theory neglects complications to the flow profile (such as nonparabolic flow⁶ or slip⁸) and to the mobility of the eluting particles (such as nonspherical particles¹⁴). Paramount among these, it does not take into account hydrodynamic interactions that lead to wall-induced increased drag (wall drag).

Experiments utilizing FFF can only avoid ambiguity in determining solute size due to the nonmonotonicity of eq 1, by working in only one of the four modes (usually nFFF for its high selectivity). So far, the only mode transition observed experimentally is the steric-inversion point between nFFF and sFFF.^{15–21} To highlight the validity of our theoretical treatment (eq 1), the results of five different nonmicrofluidic

subtechniques of FFF (thermal FFF,¹⁵ symmetric-flow FFF,¹⁶ asymmetric-flow FFF,¹⁷ hollow-fiber FFF,¹⁸ and GrFFF¹⁹) around the steric-inversion point are shown in Figure 2. A least-

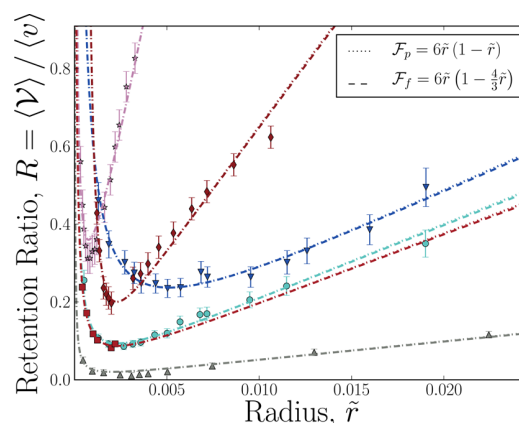


Figure 2. Fit of eq 1 to a variety of FFF systems. Dotted lines ignore Faxén's law, while dashed lines account for it. In this range, the dotted and dashed lines overlap perfectly. Thermal FFF utilizes a temperature gradient and $\alpha = 1$. The data is for $\Delta T = 40$ K ($\bullet$ ¹⁵) and $\Delta T = 5$ K ($\blacktriangledown$ ¹⁵). Symmetric ($\blacktriangle$ ¹⁶), asymmetric ($\blacksquare$ ¹⁷), and hollow-fiber ($\star$ ¹⁸) FFF use a cross flow and have $\alpha = 1$. For GrFFF, $\alpha = 3$ ($\blacklozenge$ ¹⁹). Data reproduced with permission from ref 15. Copyright 2004 Elsevier BV. Data reproduced with permission from ref 16. Copyright 1995 Wiley-VCH. Data reproduced with permission from ref 17. Copyright 2006 Elsevier BV. Data reproduced with permission from ref 18. Copyright 2002 Elsevier BV. Data reproduced from ref 19. Copyright 1992 American Chemical Society.

squares fitting procedure that varies the device retention parameter and the void time were used to determine the best fits shown in Figure 2. Even for multiple scalings as shown ($\alpha = 1$ and 3), in all conditions, the ideal retention theory fits the transition from nFFF to sFFF quite well regardless of Faxén's law: Dotted lines neglect Faxén's law, while dashed lines include it. For the given normalized sizes the two forms of $\mathcal{F}(\tilde{r})$ are essentially indistinguishable. We conclude that in such nonmicrofluidic situations, the correction due to Faxén's law is trivial to include but of negligible importance.

The ideal retention theory fits the data in Figure 2 well because the particles are small relative to the channel height ($\tilde{r} < 0.025$, in all cases). When the particle sizes are larger, the theories differ greatly for the different forms of $\mathcal{F}(\tilde{r})$ in eq 2.

The theoretically predicted retention curves for $\alpha = 3$ and $\Lambda = 1.6 \times 10^{-5}$ (strong field) and $\Lambda = 10^{-3}$ (weak field) across the entire range of possible colloid sizes are shown in Figure 3 and Figure 4, respectively. Such theoretical curves have been discussed in detail¹⁰ and only a few important features must be noted: (1) When Faxén's law is neglected (dotted lines), the sFFF regime extends to particles with a diameter equal to the channel height and $R(\tilde{r} \rightarrow 0.5, \Lambda) \rightarrow 1.5$. However, when Faxén's law is properly included (dashed lines), fFFF appears, causing the retention ratio to peak and then slowly decrease to $R(\tilde{r} = 0.5, \Lambda) = 1$. (2) All four FFF modes are expected for $\Lambda = 1.6 \times 10^{-5}$, but when $\Lambda = 10^{-3}$, only HC and fFFF are predicted. The ideal theory for $\Lambda = 1.6 \times 10^{-5}$ (dashed line in Figure 3) predicts that HC exists in the range of $0 < \tilde{r} < 0.023$. After reaching a maximum, the slope of $R(\tilde{r}, \Lambda)$ then changes to negative through the range of $0.023 < \tilde{r} < 0.083$. This represents nFFF, which exists until the steric-inversion point

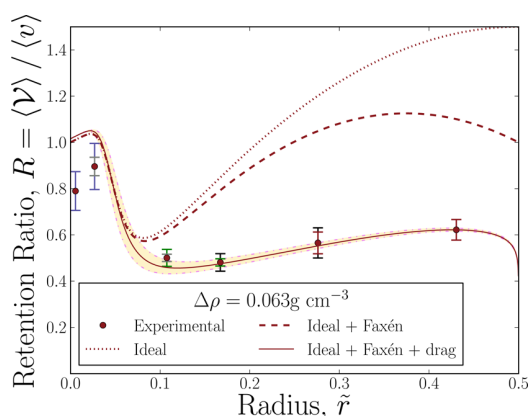


Figure 3. Experimentally determined retention ratios in a channel of height $h = 18 \pm 1 \mu\text{m}$ in a gravitational field ($\alpha = 3$) are presented as closed circles. Density difference between colloids and the carrier fluid is $\Delta\rho = 0.063 \text{ g cm}^{-3}$. This density difference is equivalent to a device retention parameter of $\Lambda = 1.6 \times 10^{-5}$. Error bars represent the statistical uncertainty on the mean, while the shaded area (bracketed by purple dashed–dotted lines) shows the uncertainty on the experimental parameters about the theoretical prediction. Data points possess multiple colored error bars associated with each pair of coeluted colloids. The dotted line shows the ideal retention ratio when Faxén’s law is neglected. The dashed line includes Faxén’s law. The retention ratio that numerically accounts for increased drag coefficients (and Faxén’s law)²² is shown as a solid line.

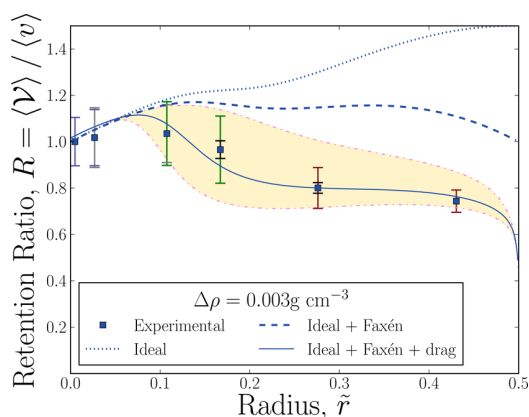


Figure 4. Same as Figure 3 but for a small density difference between colloids and the carrier fluid of $\Delta\rho = 0.003 \text{ g cm}^{-3}$ or equivalently a device retention parameter of $\Lambda = 10^{-3}$.

(the global minimum at $\tilde{r} = 0.083$). The sFFF range is found for $0.083 < \tilde{r} < 0.37$, and it possesses a relatively small positive slope. Beyond $\tilde{r} = 0.37$, an fFFF regime exists. On the other hand, for $\Lambda = 10^{-3}$ (dashed line in Figure 4), HC occurs for a much larger range ($0 < \tilde{r} < 0.14$). There is then a large regime with a relatively flat plateau (with only small variations in slope representing negligible nFFF and sFFF) and an eventual reduction of the retention ratio R back down to unity. The details of this behavior are discussed in ref 10.

In this manuscript, we will use a microfluidic device to look at the traditionally less accessible large particle/small channel regime. In microfluidic channels, separation of both particles that are minute compared to the channel height but also particles that are comparable to the channel height are possible and indeed routine.

EXPERIMENTAL SECTION

Experimental Methods. In order to explore the less accessible modes of field flow fractionation, our system must elute ensembles of colloids that are significantly smaller than h , as well as others with diameters comparable to h . A microfluidic apparatus allows us to investigate the entire range of relative particle sizes. Miniaturized FFF systems have been used previously,^{23–25} but never with such a relatively broad range of particle radii or such small channel heights. A section of our microfluidic channel and two eluting colloids can be seen in Figure 5.

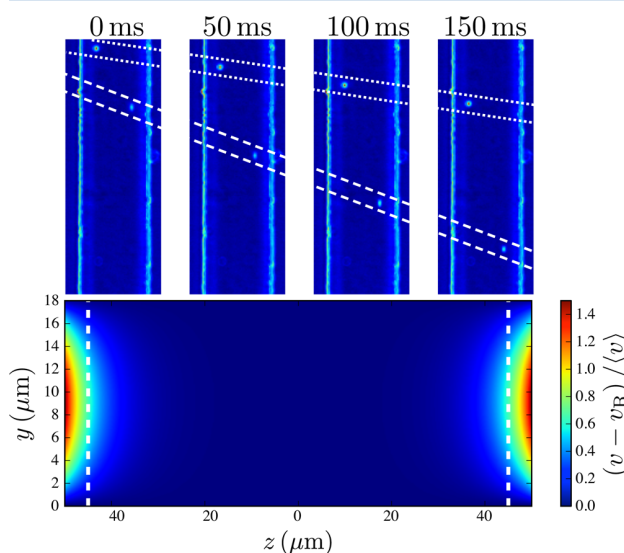


Figure 5. Top row: Sequential video microscopy frames of two colloids eluting through a $18 \mu\text{m}$ high and $100 \mu\text{m}$ wide microfluidic channel. The two colloids have a density difference of $\Delta\rho = 0.063 \text{ g cm}^{-3}$, which is equivalent to $\Lambda = 1.6 \times 10^{-5}$ in dimensionless units. Each frame shows a larger $3.0 \mu\text{m}$ colloid moving with a slower velocity represented by white dotted lines connecting its position between frames and a smaller, faster $1.9 \mu\text{m}$ colloid. The time between shown frames is 50 ms. Bottom: Difference between laminar flow profile in a rectangle channel, v_R , that is $18 \mu\text{m}$ high and $100 \mu\text{m}$ wide and the ideal Poiseuille (parabolic) velocity, v , normalized by the average $\langle v \rangle$. The flow only varies substantially from parabolic in the vicinity of the lateral walls. Dashed lines indicate the region beyond which events were disregarded.

The benefits of performing FFF on a microfluidic chip (especially thermal FFF) have been well-documented,²⁶ and it has been suggested that microfluidic channels may help in purifying, characterizing, and generally processing nanomaterials.^{27,28} This work utilizes microfluidic devices to study the elution of particles that encompass the full range of sizes; however, we can envision FFF in future nanofluidic systems.^{29,30} Current systems commonly utilize electrokinetic³¹ or confinement-based techniques,³² which have been particularly successful in studying DNA,^{33–36} but nanofluidic HC^{37–40} and electric double layer methods^{41–45} have also proven viable. In such systems, one expects nonideal effects to be even more pronounced. Indeed the importance of increased hindrance has long been recognized in the nanofiltration literature.^{46–48} As we shall show, although they do not limit the applicability of microfluidic or nanofluidic technology to FFF, nonideal effects of hydrodynamic interactions with channel

walls must be accounted for when considering the elution of particles of sizes comparable to the channel height.

Our microfluidic devices consisted of 40 mm long polydimethylsiloxane (PDMS) elastomer (Sylgrad 184 from Dow Corning) channels fabricated using soft lithography based on replica molding.⁴⁹ The PDMS was replicated from a master mold (SU8-10 MicroChem), created on silicon wafers. Each silicon wafer was cleaned using piranha etch then baked at 200 °C for 30 min and let cool for 5 min. Spin speed and time were chosen based on final thickness requirements. The wafer was then prebaked at 65 °C for 2 min followed by 95 °C for 6 min and allowed to cool at least 5 min. It was then exposed to UV, with the maximum intensity of 16.18 mW cm⁻² for 15 s and postbaked at 65 °C for 1 min and 95 °C for 2 min. Finally, SU-8 developer removed the photoresist. All wafers were coated with aminosilane to facilitate PDMS removal.

The PDMS with curing agent (10:1 mixing ratio) was poured on the master mold and baked at 80 °C for 2 h. The cured PDMS was peeled off the mold to create the microchannel structures. They were cut out, inlet holes were punched, and devices were rinsed for 5 min in each of these solutions; soapy water, deionized water, ethanol, and isopropanol and treated in oxygen plasma (Glow Research AutoGlow) at 30 W for 30 s and bonded to the glass slides. They were then baked at 80 °C for at least 2 h afterward.⁵⁰

Each microchannel has a width of $100 \pm 10 \mu\text{m}$ and a height of $h = 18 \pm 1 \mu\text{m}$. All dimensions were confirmed by atomic force microscopy and optical profilometry (MicroXAM-100). Pressure-driven flow was established through the channel, using pressure regulators (Marsh Bellofram Type 10) and Solenoid valves (SMC S070C-SDG-32), which were controlled by LabView (National Instruments). Experimental data was only recorded once the flow rate stabilized. Stabilization against pressure drift was verified by checking that the difference in mean velocities of a randomly selected sample of particles from the first quarter of a video set and sample from the final quarter were significantly smaller than the standard deviation within either set (typically differences varied by about 2% and were only a tenth of the standard deviation of either set).

Polystyrene microparticles with nominal radii of $r = \{0.100 \pm 0.005; 0.485 \pm 0.005; 1.9 \pm 0.1; 3.0 \pm 0.2; 5.0 \pm 0.4; 7.8 \pm 0.8\} \mu\text{m}$ and density of $\rho_p = 1.060 \pm 0.002 \text{ g cm}^{-3}$ (Bangs Laboratories, Inc.) in 1% suspensions were diluted with the carrier solution before injection. The density difference between that of the colloids and of the carrier fluid could be adjusted to control Λ . The base carrier fluid was an aqueous solution containing 0.1% Contrex Alkaline⁵¹ (Decon Laboratories, Inc., PA, USA), to prevent nonspecific sticking and aggregation, with a density of $\rho_c = 0.997 \pm 0.001 \text{ g cm}^{-3}$.

In some experiments, approximately 23% glycerol was added to the carrier fluid to match the carrier density ($\rho_{cG} = 1.063 \pm 0.002 \text{ g cm}^{-3}$) to that of the colloids. The densities of all carrier fluids were measured using a suspended microchannel resonator (SMR).⁵² All experiments were conducted at room temperature ($25 \pm 1 \text{ }^\circ\text{C}$). In the base carrier fluid, $\Lambda = (1.6 \pm 0.4) \times 10^{-5}$. When the particles are neutrally buoyant in the glycerol solution, $\Lambda = (3 \pm 2) \times 10^{-3}$.

Experiments were performed using pairs of colloids in order to minimize uncertainty related to variability in carrier fluid velocity between experiments, and relative retention ratios were measured. The movement of colloids through the channels was captured using video microscopy, and particle velocities were

obtained using custom analysis software. A brief series of representative frames are shown in Figure 5.

A tracking program identified individual particles and generated velocity distribution profiles for each particle size based on the Fourier transform of the position versus time data. Both programs, for capturing and tracking, were controlled by Labview (National Instruments). Between 150 and 200 events were considered in determining each data point. The smallest particles ($r = 0.1 \mu\text{m}$) were analyzed semimanually due to automatic tracking difficulties. In this case, captured videos were analyzed frame by frame, by ImageJ (Image Processing and Analysis in Java), and the mean velocity was calculated from the change in position along the channel versus time (Figure 5).

Error bars in Figures 3 and 4 represent the statistical uncertainty on the mean, while the shaded regions represent the uncertainty associated with the experimental parameters about the numerically determined prediction (which will be introduced in Results and Discussion). Because experimental runs were performed using pairs of coeluting colloids, each data point (except the smallest and largest particles) has two error bars representing the statistical uncertainty for each run. The error bar colors identify pairs in Figures 3 and 4.

The aspect ratio, width to height, of the channel is approximately 5:1. Although theory and simulations assume an infinite aspect ratio, as can be seen in Figure 5, the expected flow profile of the carrier fluid in a rectangular microchannel only differs from the Poiseuille flow profile near the lateral walls. Therefore, the instances for which colloids were recorded within $5 \mu\text{m}$ of the lateral walls were disregarded, in case they interacted with the lateral walls. This near-lateral wall region is marked in Figure 5 by dashed lines. If the entire rectangular channel were considered, the percent difference between the average carrier fluid velocity and the Poiseuille flow assumption would be 11.6%. By disregarding flow within $5 \mu\text{m}$ of each lateral wall, the percent difference is reduced to nearly half and 84.3% of all points on the cross section of interest are within 10% of the Poiseuille flow profile of the theory and simulations. Increasing the size of this region beyond $5 \mu\text{m}$ would require more events to be disregarded, resulting in a reduced statistical certainty.

Computational Methods. We have performed simulations of spherical colloids embedded in a coarse-grained multiparticle collision dynamics (MPCD) fluid.^{53,54} MPCD is ideal for studying FFF in microfluidic devices because both hydrodynamic interactions and thermal motion are intrinsically present (i.e., MPCD is well-suited for situations involving moderate Péclet numbers⁵⁵). A nonphysical, multiparticle collision operator, which locally conserves mass, momentum, and energy, replaces a detailed description of the interactions between fluid molecules. This operator ensures a Maxwell–Boltzmann velocity distribution and that the hydrodynamic equations of motion are obeyed over sufficiently long lengths and times.⁵⁶

Multiparticle collision dynamics simulations integrate the motion of fluid particles in two steps. The position $\vec{x}_i(t)$ of each MPCD fluid particle i is updated ballistically over a time step δt to become $\vec{x}_i(t + \delta t) = \vec{x}_i(t) + \vec{v}_i(t)\delta t$. Second, momentum is transferred between MPCD fluid particles through a multiparticle collision. The system is partitioned into cubic cells of volume $a^3 = 1$, with each cell j containing on average $\langle n_j \rangle = \rho^{\text{MPCD}} a^3 = 5$ particles. Each cell has a center of mass velocity $\vec{v}_j^{\text{CM}}(t)$ corresponding to the local mesoscopic fluid velocity

(each MPCD fluid particle has a mass m). To ensure Galilean invariance is respected, a random vector shifts the cells each time step.^{57,58} The collision operator implements an Andersen thermostat such that the iterative scheme is

$$\vec{v}_i(t + \delta t) = \vec{g}\delta t + \vec{v}_i^{\text{CM}}(t) + \vec{v}_i^{\text{RAN}} - \frac{1}{n_j} \sum_{n_j} \vec{v}_k^{\text{RAN}} \quad (3)$$

where $\vec{v}_i^{\text{RAN}}$ is a randomly generated velocity drawn from a Maxwell–Boltzmann distribution and g is a constant acceleration to generate the flow profile.⁵⁹ Simple bounce-back rules govern collisions between MPCD particles and walls, although phantom fluid particles must be included in the collision operator of cells intersecting walls to ensure that artificial viscous thinning does not occur and that the no-slip boundary conditions are respected.^{60,61} The system is periodic in $\hat{x}$ and $\hat{z}$ with the mean fluid flow in the $\hat{x}$ direction. Planar, channel walls are in the $\pm\hat{y}$ direction.

The hard bead, representing the colloid, is a spherical mobile wall, which also obeys bounce-back rules to create steric interactions with the channel walls. The colloids of density $\rho = (1000m)/(\pi a^3)$ are subject to an external force in the $\hat{y}$ direction to simulate gravitational acceleration $G\hat{y}$. In order to efficiently simulate each operational mode, the box size is varied, requiring that $G\hat{y}$ and $g\hat{x}$ are varied to keep Λ strictly constant and Re_p roughly constant. In all cases, the length of the system varies as $10 \times r$ and the breadth as $5 \times r$. Four channel heights, $h = \{210, 90, 26, 15\}[a]$ are used with the four device retention parameters $\Lambda = \{\infty, 1.87 \times 10^{-4}, 1.87 \times 10^{-5}, \text{ and } 1.87 \times 10^{-7}\}$. Because the channel height changes, the fluid acceleration must change in order to keep Re_p small and roughly constant. For each channel height, we use the fluid accelerations $\vec{g} = \{10^{-4}, 5.44 \times 10^{-4}, 6.52 \times 10^{-3}, 1.96 \times 10^{-2}\}[\delta t^{-2}a\hat{x}]$, respectively.

However, we found that for the strongest forces used the bead could become pinned to the wall with no fluid between the particle surface and the plane. When this occurs, the bead did not translate because bounce-back conditions ensure a no-slip boundary condition between the two surfaces. This unphysical situation is due to the discrete particle nature of MPCD and demonstrates a limitation of the method. For this reason, large beads in strong fields that had a null average velocity were rejected from the analysis.

RESULTS AND DISCUSSION

Experimental Results. The experimentally determined retention ratios for colloids with $\Delta\rho = 0.063 \text{ g cm}^{-3}$ are shown as circles in Figure 3. Since the absolute average carrier velocity is irrelevant and only relative changes in retention ratio are significant, the experimental values between size pairs were normalized. To compare these data to the theoretical predictions, we chose one data point and normalized it to the theoretical value. In this case, R ($r = 7.8 \text{ }\mu\text{m}$, $\Delta\rho = 0.063 \text{ g cm}^{-3} = 0.622$) was chosen in order to produce the minimum χ^2 . In scaling the retention curve in this way, the retention ratio values for $\tilde{r} = 0.0056$ and $\tilde{r} = 0.0269$ appear to be less than expected. The measured velocity difference between $\tilde{r} = 0.0269$ and $\tilde{r} = 0.106$ is likely the most indefinite value because the fastest of the smaller particles may go unrecorded due to the experimental challenges in tracking small, fast colloids. If the measured difference is systematically less than the actual

difference, then the $\tilde{r} = 0.0056$ retention ratio will be artificially shifted downward.

The data in Figure 3 is quite different from the ideal theory, using either $\mathcal{F}(\tilde{r})$ from eq 2 (dotted and dashed lines), especially in the case of large colloids. The experimentally observed retention ratio initially rises for HC then drops rapidly for nFFF. This is the first published observation of the theoretically expected transition between HC and nFFF. The minimum occurs at a larger particle size compared to the expectations of ideal theory. Past nFFF and the steric-inversion point, the retention ratio is significantly less than predicted by eq 1 (dashed lines). In fact, the retention ratio remains relatively constant such that it is almost impossible to discern sFFF, and fFFF is not experimentally observed.

As shown in Figure 4, when $\Delta\rho = 0.003 \text{ g cm}^{-3}$ and the transverse field is all but removed, the retention behavior is much different. In this weak-field case, the values of the experimental retention ratio (squares) are set by normalizing the $\tilde{r} = 0.2761$ value to $R = 0.800$. Without a strong transverse field, neither nFFF nor sFFF were predicted to exist, and this is indeed what is qualitatively observed in Figure 4. In fact, the retention ratio rises slightly to the global maximum at $\tilde{r} \approx 0.1075$ through the HC regime and slowly decreases, thus qualitatively verifying the previously predicted fFFF operational mode. However, the ideal, theoretical curves (dashed lines) fail to predict the measured retention ratios in any quantitative sense. Just as in Figure 3, at all but the smallest particle sizes, eq 1 severely overpredicts the retention ratio.

Interestingly, eq 1 represents the literature data for FFF channels with larger channel heights extremely well, as we saw in Figure 2, but fails for large colloids eluting through microfluidic channels. This inadequacy arises because hydrodynamic effects that are neglected in eq 1 must be considered when the channel heights and particle diameters are comparable. Accounting for wall-drag will be considered in detail in the Discussion and Interpretation of Results.

Obviously, microfluidic systems include further nonideal factors that can complicate the retention behavior, such as wall roughness or sticking events, the finite width of the channel, varying flow rates, nonspherical or nonmonodisperse particles, and variation in PDMS channel heights. Therefore, at this point we cannot be positively certain that the observed suppression of the retention ratio is definitively due to hydrodynamically mediated colloid–wall interactions. To corroborate our experimental findings, we now consider our mesoscopic simulations that quite naturally encompass complications due to the confinement of mobile colloids in a flowing, thermal fluid but yet are ideal in the sense that they do not possess the experimental issues mentioned above.

Computational Results. Simulations of neutrally buoyant colloids ($\Lambda = \infty$, blue circles in Figure 6) demonstrate that the retention ratio is indeed greater than unity for the vast majority of particles (i.e., $R > 1$ in the hydrodynamic chromatography limit). Simulations show two regions: for small particles, R increases, while for large particles, R decreases. MPCD simulations agree with experimental findings and substantiate the existence of fFFF in the weak fields. Furthermore, simulations of elution in weak external fields ($\Lambda = 1.87 \times 10^{-4}$, black squares in Figure 6) corroborate the experimental observation of the transition from HC to nFFF.

When the field is nonnegligible, the retention curves possess an nFFF region of steep descent after a brief HC climb. The simulation data for both $\Lambda = 1.87 \times 10^{-7}$ and 1.87×10^{-5} (red

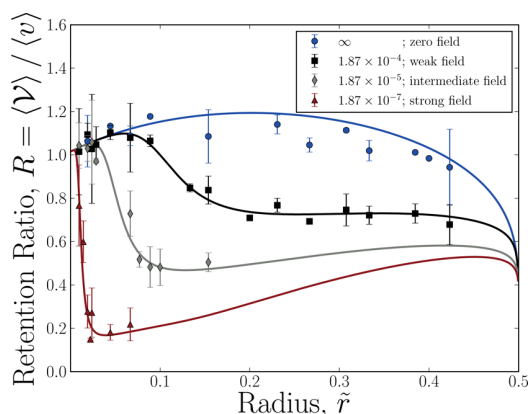


Figure 6. Simulation results for $\alpha = 3$ and $\Lambda = \{\infty, 1.87 \times 10^{-4}, 1.87 \times 10^{-5}, 1.87 \times 10^{-7}\}$ are shown as blue circles ($\bullet$), black squares ($\blacksquare$), gray diamonds ($\blacklozenge$) and red triangles ($\blacktriangle$), respectively. The solid lines show the predicted retention curve for each Λ , numerically taking into account Faxén's law and more significantly hydrodynamic interactions with both channel walls.²²

$\blacktriangle$ and gray $\blacklozenge$ in Figure 6) show a steric-inversion point at a lower retention ratio and larger particle size than predicted by the ideal theory (not shown). For strong fields and large colloids, the discrete particle nature of MPCD cannot adequately model a continuous fluid, and simulations do not span the entire range of sizes. On the other hand, when $\Lambda = 1.87 \times 10^{-4}$ ($\blacksquare$), MPCD provides simulation data across the entire range of sizes. The simulations for $\Lambda = 1.87 \times 10^{-7}$ convincingly verify the conclusions of Figures 3 and 4; even under simplified prototypical conditions, sFFF and fFFF are significantly suppressed in microfluidic channels due to wall-induced hydrodynamic effects, leaving an extended range of particle sizes over which the retention ratio does not rise. There is little selectivity or resolution between particles larger than $\tilde{r} \gtrsim 0.2$ when $\Lambda = 1.87 \times 10^{-7}$.

Discussion and Interpretation of Results. Neither the video microscopy data (Figures 3 and 4) nor the MPCD simulations (Figure 6; explicit comparison not shown) of field-flow fractionation in a microfluidic channel are well-predicted by the ideal retention theory encapsulated in eq 1. The most significant complication omitted from ideal retention theory is hydrodynamic interactions with the microfluidic channel walls, leading to an increased effective friction coefficient. Hydrodynamic interactions can be divided into forces acting antiparallel (drag) and forces acting perpendicular (lift) to the wall. Inertial lift forces arise from a combination of symmetry, breaking of the wake by the wall, and the shear-gradient generated force.⁶² In our experiments and simulations, we keep the particle Reynolds number below $Re_p = 6\tilde{r}^2\tilde{h}/\nu < 0.01$, such that lift forces remain negligible.⁶² Increased drag forces, however, can no longer be neglected. Drag forces are generated because the no-slip condition on the surface of the mobile colloid and on the channel wall causes greater shearing and greater friction than in the absence of boundaries.

Correcting the friction coefficients accurately enough over all heights requires the multipole expansion method utilized by Pasol et al. to include the effects of both walls.²² Only by numerically calculating the retention ratios using the results of Pasol et al., while also allowing the retention parameter to vary as a function of radius ($\lambda = \Lambda\tilde{r}^{-\alpha}$), do we find agreement between theory and measured experiments and simulations, as

discussed below. Many simple forms for the friction coefficient of a sphere moving near a planar surface exist in the literature, notably, the expansion solution of Faxén,⁶³ the near and far wall approximations of Goldman et al.,⁶⁴ and the empirical form of Williams et al.⁶⁵ While each of these forms slows the particles and reduces the retention ratio for large particle sizes, none adequately resembles simulations or experiments. This is because each is accurate only over a limited range of distances from a single wall, while the retention ratio results from weighted integration over all possible heights. The form given by Pasol et al. includes the effects of hydrodynamic interactions with both walls, as well as the effects of Faxén's law, and so is accurate at large $\tilde{r}$ values.²²

For all device retention parameters, Λ , the modified retention ratios numerically calculated using the friction coefficients found by Pasol et al.²² quantitatively agree with both the microfluidic video microscopy and MPCD simulation data. In Figures 3, 4, and 6, solid lines represent the numerically predicted retention ratios that take into account both Faxén's law and more importantly wall-drag effects. Retention ratios for negligible fields are the least affected by frictional effects and remain relatively parabolic, as seen in the solid line in Figure 4 and the blue line in Figure 6 ($\Lambda = 10^{-3}$ and $\Lambda = \infty$, respectively). Even in strong fields as in Figure 6 (red line; $\Lambda = 1.87 \times 10^{-7}$), HC is predicted to still exist for a brief size range and is relatively unaffected by wall-induced drag effects. Likewise, qualitatively, nFFF remains a region with a steep negative slope of the retention ratio. In weak and intermediate fields ($\Lambda = 1.87 \times 10^{-4}$ and $\Lambda = 1.87 \times 10^{-5}$ in Figure 6), the slope is nearly zero for all larger colloids. In extremely high fields ($\Lambda = 1.87 \times 10^{-7}$ in Figure 6), a slow climb remains such that sFFF is predicted to exist but with a significantly reduced slope compared to the ideal theory. Evidently, fFFF only exists for weak fields and is suppressed in larger fields. Thus, in microfluidic devices, frictional effects due to the walls are predicted to reduce resolution above the steric-inversion point compared to what would otherwise be predicted in the absence of increased drag. Finally, as $\tilde{r} \rightarrow 0.5$, the retention ratio drops suddenly, indicating that the effective friction coefficient has increased considerably.

Solid lines in Figures 3 and 4 represent the numerically predicted $R(\tilde{r}, \Lambda)$ based on the experimental parameters, while the shaded areas represent the experimental uncertainty on the curves (error bars on the data points represent the standard deviation on the mean of video microscopy data). We are able to quantitatively predict the retention ratios for microfluidic separation devices across the entire range of particle sizes investigated by accurately including the effect of hydrodynamic friction. Figures 3 and 4 show that regardless of $\Delta\rho$, wall effects only become significant once the colloid radius is increased above $\sim 5\%$ of the channel height, $\tilde{r} \gtrsim 0.05$.

CONCLUSIONS

We have experimentally and computationally investigated the utility of field-flow fractionation for fractionating the entire range of spherical particle sizes possible on a microfluidic chip. Working on a microfluidic chip allows us to experimentally observe the transition from the hydrodynamic chromatography limit of FFF to the normal-mode FFF. The existence of this transition has been hypothetical for many years, but experimental verification has not been previously reported in the literature. Our results show that normal-mode FFF remains a high-selectivity technique in microfluidic channels that can be

implemented with a simple channel design. By measuring the retention ratio of neutrally buoyant colloids in a microfluidic device, we have verified the existence of Faxén-mode FFF, though our results demonstrate that both fFFF and steric-mode FFF are substantially diminished in strong external fields. In strong fields, Faxén-mode FFF becomes an inappropriate eponym because hydrodynamic interactions with the channels produce wall drag that increases the friction coefficients of the colloids and dominates over the effect of Faxén's law. While the ideal retention theory works well for samples with hydrodynamic radii significantly smaller than the separation between plates, video microscopy demonstrates that the ideal theory fails for large colloids in microfluidic devices. Simulations verify that the ideal theory that neglects hydrodynamic complications overestimates colloid velocity, particularly in the near-wall region. Although including Faxén's law is a simple improvement to the ideal retention theory, hydrodynamic forces acting antiparallel to the velocity of the colloids dominate in precisely the regimes in which Faxén's law would otherwise have been significant (steric- and Faxén-mode FFF). These wall interactions increase drag and so decrease the retention ratio below the Faxén prediction. Moving in the vicinity of microchannel walls causes the colloids to lag behind their ideally predicted speeds, which may have important consequences for particle tracking velocimetry techniques in microfluidic systems. Micro-particle image velocimetry implicitly models the fluid velocity as equivalent to tracer particles' velocities; however, it is clear from the present study that this assumption holds a significant risk of underestimating fluid velocities, especially in micro- and nanoscale systems. Frictional effects must be rigorously accounted for in order to convert microbead velocities into accurate solvent velocity profiles, in particular when dealing with particles greater than ~5% of the channel height. We find that the multipole expansion method produced values that accurately predict the retention ratio, while more traditional approximations fail.

Although it seems at first glance that performing field-flow fractionation in a microfluidic channel introduces more complications, there is a very clear benefit of incorporating FFF components into microfluidic and into future nanofluidic devices. When the external transverse field is strong, normal-mode FFF drops sharply to the steric-inversion point offering an operational range with significant selectivity. However, rather than rapidly rising in steric-mode FFF and thus introducing ambiguity through pronounced nonmonotonicity, as occurs in traditional, macroscopic FFF devices, the retention ratio is suppressed and nearly flat for all larger colloidal particle sizes. This quality can give researchers a clear signal that the sample is no longer eluting in the microfluidic device's exclusive operating range.

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Notes

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ABBREVIATIONS

FFF, field-flow fractionation; HC, hydrodynamic chromatography; nFFF, normal-mode field flow fractionation; sFFF, steric-mode field flow fractionation; fFFF, Faxén-mode field flow fractionation; GrFFF, gravitational FFF; PDMS, poly dimethylsiloxane; SMR, suspended microchannel resonator; MPCD, multiparticle collision dynamics

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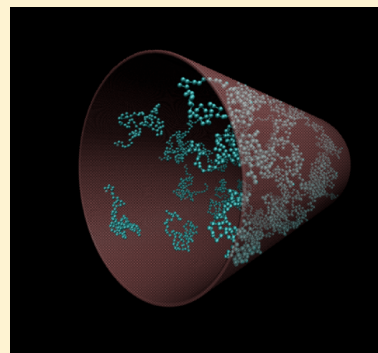
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Controlling Grafted Polymers inside Cylindrical Tubes

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ABSTRACT: Grafted polymers inside cylindrical tubes in good and Θ solvents are systematically studied by numerical self-consistent mean-field theory (SCFT) and molecular dynamics (MD) simulations. We demonstrate that within the SCFT and at low enough concentrations the properties of these systems are fully determined by the overall polymer concentration plus two reduced parameters which are derived from the SCFT. Via a suitable change of variables, the results for very different systems can thus map onto universal curves. We show that MD and SCFT results at finite concentrations nearly obey these mappings as well. These findings allow for generalized predictions of the polymer properties such as concentration profiles, chain end distributions, and when there are polymer-free “open” channels at the center of a cylinder. This provides useful guidance for the development of micro- and nanofluidic devices.



■ INTRODUCTION

As is well-known, grafted polymer layers can be used to tune surface properties and hence find applications in, for example, colloid stabilization and functional surface manufacturing.^{1,2} Recently, increasing attention has been paid to grafted chains inside cylindrical tubes or porous media, since their presence affects the flow behavior and the transport properties of these systems.^{3,4} Grafted polymer chains can also reduce the interactions between analytes and the channel walls,^{5–7} an important factor in several separation devices (for instance, proteins^{8–11} have a strong tendency to stick to glass walls). It is also possible to use reactive end-functionalized polymer chains for a variety of purposes, including the capture of specific analytes by properly chosen polymer end groups or DNA linkers,¹² and controlled protein separation¹³ and specific protein capture.^{14,15}

It is difficult to measure the polymer structure inside a tube system directly, and so experimental investigations are usually performed via indirect approaches, such as by calculating the hydrodynamic thickness of the grafted layer from hydraulic permeability measurements.¹⁶ It is even more difficult to determine the position of specific (reactive) monomers, including the end monomers, inside the brush. These difficulties enhance the potential importance of theoretical studies.¹⁷ However, although there is a rich literature describing chains tethered to flat surfaces, there is much less on tethering to convex surfaces, such as the outside of cylindrical and spherical particles,^{18–20} and systematic studies and conclusions are, so far, lacking.

Milner and Witten performed analytical calculations of the free energy of systems composed of polymers grafted onto curved surfaces, both convex and concave, and developed an expression for the bending moduli.²¹ Because their method is based on an expansion in terms of the surface curvature, it is only applicable when the thickness of the grafted layer is much

less than the radius. Manghi et al. used a self-consistent-field approach with a strong-stretching approximation to study the structure and the associated free energy of brushes in concave geometries.²² Their description was a generalization of theory for flat surfaces to weak curvature. At high curvature, the system became semidilute, and they suggested that a scaling refinement of the osmotic term should be incorporated in the expression of the free energy. Viduna et al. studied tethered chains in a cylindrical pore via Monte Carlo simulations and derived the concentration profiles and chain-end distribution functions for different parameter sets.²³ Their focus was mainly on the cases of large tube radii, and their results showed only slight differences from the cases on a flat surface.

Recently, Binder et al. made a series of studies on the properties of grafted chains inside cylindrical geometries. They identified different regimes via scaling arguments, several of which were verified by molecular dynamics (MD) simulations.²⁴ They also obtained the overall concentration profiles and chain-end distributions from both MD^{24–26} and Monte Carlo simulations.²⁷ Their results indicated that, for relatively short chains (compared to the tube radius), the concentration profiles and chain-end distributions had similar shapes as the case of flat brushes; however, when the grafted chains became long enough, overlapping chains and chain-end enrichment at the tube center were observed. Binder and co-workers also performed calculations using self-consistent mean-field theory (SCFT); the results showed good agreement with corresponding MD simulations.²⁸ In other work, Koutsoubas et al. performed a bond-fluctuation Monte Carlo investigation on the formation process and properties of polymer brushes inside

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cylinders. When uniform grafting was achieved, the structural properties were similar to those found by Binder et al.²⁹

The parameter space of polymeric systems is very rich; i.e., there are numerous parameters, and each can vary a great deal. This often limits the range of parameters that are investigated and has precluded the development of a systematic understanding of the systems of interest here. However, it can be intuitively expected that different properties of the polymer brush can, to some extent, mutually compensate; e.g., densely grafted chains in a marginally good solvent should have some similarity to less densely grafted chains in athermal solvent. In the present paper, we present an extensive study of the equilibrium structure of polymer chains tethered to the inside walls of cylindrical tubes, using both numerical SCFT and MD simulations, and we identify a few parameters, which are combinations of the underlying ones, that control these systems. This implies that many seemingly different systems, such as those investigated by Binder et al.,^{24–27} can behave in the same way. Our focus is on the polymer concentration profiles and the chain-end distributions, and we are able to show how to predict when the polymers would reach the center of the tube, and when they leave an open channel there, in terms of these few controlling parameters.

■ SELF-CONSISTENT MEAN-FIELD THEORY

We consider a polymeric system in a cylindrical tube of inner radius R and length L . Inside the tube, there are n_p polymer chains with their ends randomly anchored onto the inner wall of the tube and n_s solvent molecules. The volume of each solvent molecule (in pure solvent) is ρ_{0s}^{-1} . The fundamental properties of the polymer are its molecular weight, its unperturbed radius of gyration in bulk, R_0 , and the total volume occupied by it. The definition of the monomer is flexible but, once it is defined, its volume (in bulk), ρ_{0p}^{-1} , its degree of polymerization, N , and the corresponding statistical segment length, $b = R_0/(N/6)^{1/2}$, all follow from the fundamental properties.

The polymer grafting density, i.e., the number of polymers per unit area, is simply given by

$$\sigma = \frac{n_p}{2\pi RL} \quad (1)$$

For the SCFT, we consider the thermodynamic limit of $L \rightarrow \infty$ with fixed n_p/L and hence fixed σ . The average polymer volume fraction can be expressed as

$$\bar{\phi}_p = \frac{2\sigma N}{\rho_{0p}R} \quad (2)$$

The SCFT is formulated in terms of quantities such as N , b , and ρ_{0p} which, individually, depend on the chosen definition of the monomer. However, they always emerge in combinations which are independent of this choice, e.g., N/ρ_{0p} .

The formalism we use here has been described in detail elsewhere.^{30–32} Briefly, the equilibrium structure of the system can be calculated from the following set of mean-field equations:

$$\omega_p(r) = \frac{\rho_{0s}}{\rho_{0p}} \chi \phi_s(r) + \eta(r) \quad (3)$$

$$\frac{\rho_{0s}}{\rho_{0p}} \omega_s(r) = \frac{\rho_{0s}}{\rho_{0p}} \chi \phi_p(r) + \eta(r) \quad (4)$$

$$\phi_p(r) = \frac{\sigma R}{\rho_{0p} Q_p} \int_0^N ds q^f(r, N-s) q^a(r, s) \quad (5)$$

$$\phi_s(r) = \frac{\bar{\phi}_s}{Q_s} e^{-\omega_s(r)} \quad (6)$$

$$\phi_p(r) + \phi_s(r) = 1 \quad (7)$$

where cylindrical coordinates are used for this geometry. In these equations, χ is the Flory–Huggins parameter, which quantifies the polymer–solvent interaction, $\phi_\alpha(r)$ is the volume fraction of species α ($\alpha = P$ for the polymer and $\alpha = S$ for the solvent) at distance r from the tube axis, $\omega_\alpha(r)$ is the auxiliary field conjugate to $\phi_\alpha(r)$, $\eta(r)$ is the auxiliary field coupled to the incompressibility condition, and Q_p and Q_s are given by

$$Q_p = \int_0^R r dr q^a(r, N) \quad (8)$$

and

$$Q_s = \frac{2}{R^2} \int_0^R r dr e^{-\omega_s(r)} \quad (9)$$

The so-called end-integrated propagators $q^f(r, s)$ and $q^a(r, s)$, whose superscripts signify “free” and “anchored” ends, respectively, can be obtained from the following differential equation

$$\frac{\partial q^a(r, s)}{\partial s} = \frac{b^2}{6} \frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial}{\partial r} \right) q^a(r, s) - \omega_p(r) q^a(r, s) \quad (10)$$

with corresponding initial conditions

$$q^f(r, 0) = 1 \text{ and } q^a(r, 0) = \frac{1}{r} \delta(r - R + \varepsilon) \quad (11)$$

where ε is a infinitesimal distance from the inner wall of the tube. In addition, Dirichlet boundary conditions are imposed at the wall, which corresponds to an impermeable grafting surface, and Neumann boundary conditions are applied at the tube center reflecting the symmetry requirement of the system.

The SCFT equations are solved iteratively with the convergence criterion $|\phi_s^n(r) - \phi_s^{n-1}(r)| \leq 10^{-5}$ for all values of the radial position r , where $\phi_s^n(r)$ denotes the solvent concentration profile obtained at the n th iteration.

■ MOLECULAR DYNAMICS SIMULATIONS

In this approach, we consider a finite L , constrained as we describe below. The simulations use a simple coarse-grained model that is widely used in the literature and has previously been compared to SCFT for polymers tethered to a flat surface. Our method follows the same strategy as in refs 33 and 34 in which only polymer chains are explicitly included, and the effects of the solvent are introduced by changing temperature T .

The monomers are represented as beads with a Lennard-Jones potential between them

$$\frac{U_{WCA}(d)}{\varepsilon_{MD}} = \begin{cases} 4 \left[\left(\frac{\sigma_{MD}}{d} \right)^{12} - \left(\frac{\sigma_{MD}}{d} \right)^6 - \left(\frac{\sigma_{MD}}{d_0} \right)^{12} + \left(\frac{\sigma_{MD}}{d_0} \right)^6 \right] & d \leq d_0 \\ 0 & d > d_0 \end{cases} \quad (12)$$

where d is the distance between the beads' centers, $d_0 \equiv 2.5\sigma_{\text{MD}}$ is a cutoff length, and σ_{MD} and ϵ_{MD} are the MD length and energy scales, respectively. In addition, monomers in each chain are linked together via the finitely extensible nonlinear elastic (FENE) potential

$$U_{\text{FENE}}(d) = -\frac{k d_m^2}{2} \ln \left[1 - \left(\frac{d}{d_m} \right)^2 \right] \quad (13)$$

where the spring constant is $k \equiv 2Sk_{\text{B}}T/\sigma_{\text{MD}}^2$ with k_{B} the Boltzmann constant and $d_m \equiv 1.5\sigma_{\text{MD}}$. The temperature dependence of the spring constant makes the average bond length relatively independent of the temperature of the system.

The terminal monomers are anchored just inside the tube wall by a harmonic potential with a spring constant $k_{\text{wall}} = 300 \epsilon_{\text{MD}}/\sigma_{\text{MD}}^2$, with the minimum of this potential located a distance σ_{MD} from the wall. All monomers, including the end ones, experience a repulsive potential from the wall which is described by eq 12, except that $d_0 \equiv 2^{1/6}\sigma_{\text{MD}}$, which is what makes it purely repulsive. These choices ensure that the monomers remain within the cylinder at all times.

The anchoring positions are distributed randomly over the inner surface, except that they are all at least $0.6(A/n_p)^{1/2}$ apart, where A is the tube's total surface area. The system is always large enough that (1) there are at least 50 polymer chains tethered to the cylinder, (2) the length of the cylinder is at least as long as the contour length of the polymers to eliminate spurious self-interactions through the periodic boundary along the cylinder's axis, and (3) the length of the cylinder is also at least 1.5 times its radius.

In order to maintain a constant value of $k_{\text{B}}T$, a Langevin thermostat is used with a friction coefficient $\Gamma = 0.5m/\tau$, where m and τ are the MD mass and time scales.^{34,35} The integration time step is $\Delta\tau = 0.0025\tau$ so that integration is stable even for the highest temperatures considered here. The Θ -temperature for this manifestation of the MD algorithm is $k_{\text{B}}T = 3.0 \pm 0.1$ for values of N from 32 to 400, which covers the range used in this paper.³⁴

■ DERIVATION OF THE DOMINANT CONTROLLING FACTORS FROM THE SCFT

In principle, fully specifying one of our systems requires numerous parameters: for example, even for Gaussian chains interacting via simple contact interactions, the SCFT involves seven independent parameters, which can be chosen to be R , χ , σ , N , ρ_{OS} , ρ_{OP} , and b . However, the situation greatly simplifies if the polymer concentration is low enough, in a way defined in what follows.

To see this, one must first take the difference between eq 3 and eq 4 in order to eliminate $\eta(r)$. Then, using eq 6 and eq 7, $\omega_{\text{S}}(r)$ and $\phi_{\text{S}}(r)$ can be expressed in terms of $\phi_{\text{P}}(r)$, which leads to

$$\omega_{\text{P}}(r) = -\frac{\rho_{\text{OS}}}{\rho_{\text{OP}}} \{ \ln[1 - \phi_{\text{P}}(r)] + 2\chi\phi_{\text{P}}(r) \} \quad (14)$$

where a constant term has been absorbed into $\omega_{\text{P}}(r)$. Because the volume fraction $\phi_{\text{P}}(r)$ is always smaller than unity, the logarithm can be expanded and $\omega_{\text{P}}(r)$ expressed as

$$\begin{aligned} \omega_{\text{P}}(r) &= \frac{\rho_{\text{OS}}}{\rho_{\text{OP}}} (1 - 2\chi)\phi_{\text{P}}(r) + \frac{1}{2} \frac{\rho_{\text{OS}}}{\rho_{\text{OP}}} [\phi_{\text{P}}(r)]^2 \\ &+ \frac{1}{3} \frac{\rho_{\text{OS}}}{\rho_{\text{OP}}} [\phi_{\text{P}}(r)]^3 + \dots \end{aligned} \quad (15)$$

In this paper, "low concentration" means that it is low enough that a single term, i.e., the lowest order nonzero one, dominates the potential in eq 15.

Good Solvent. In good solvent, $\chi < 0.5$, and the first term of eq 15 dominates if

$$\phi_{\text{P}} \ll 1 - 2\chi \quad (16)$$

This defines "low concentration" for the good solvent for present purposes, in which case the potential reduces to

$$\omega_{\text{P}}(r) \simeq \frac{\rho_{\text{OS}}}{\rho_{\text{OP}}} (1 - 2\chi)\phi_{\text{P}}(r) \quad (17)$$

By introducing the reduced variables

$$s^* = \frac{s}{N}, \quad r^* = \frac{r}{R}, \quad \omega_{\text{P}}^* = N\omega_{\text{P}}, \quad q^{a*} = \frac{R}{\sigma} q^a, \quad \phi_{\text{P}}^* = \frac{\phi_{\text{P}}}{\phi_{\text{P}}} \quad (18)$$

Equations 3, 5 and 10 can be converted to

$$\omega_{\text{P}}^*(r^*) = \frac{\beta}{R^*} \phi_{\text{P}}^*(r^*) \quad (19)$$

$$\phi_{\text{P}}^*(r^*) = \frac{\int_0^1 ds^* q^{f*}(r^*, 1 - s^*) q^{a*}(r^*, s^*)}{2 \int_0^1 r^* dr^* q^{a*}(r^*, 1)} \quad (20)$$

and

$$\begin{aligned} \frac{\partial q^*(r^*, s^*)}{\partial s^*} &= \frac{1}{R^{*2}} \frac{1}{r^*} \frac{\partial}{\partial r^*} \left(r^* \frac{\partial}{\partial r^*} \right) q^*(r^*, s^*) \\ &- \omega_{\text{P}}^*(r^*) q^*(r^*, s^*) \end{aligned} \quad (21)$$

where

$$R^* = R/R_0 \quad (22)$$

(as noted above, R_0 is the unperturbed radius of gyration of the chain) and

$$\beta = 6\rho_{\text{OS}}\sigma R_0(1 - 2\chi) \left(\frac{N}{\rho_{\text{OP}}} \right) \left(\frac{1}{\rho_{\text{OP}} b^2} \right) \quad (23)$$

By expressing β this way, it can be seen that this parameter is independent of the detailed definition of the monomer, as expected.

Θ Solvent. For Θ solvent, $\chi = 0.5$ and the first nonzero term in eq 15 is the second one. It will dominate if

$$\phi_{\text{P}} \ll 1 \quad (24)$$

which defines low concentration for the Θ solvent case. In this limit, the reduced potential becomes

$$\omega_{\text{P}}^*(r^*) = \frac{\gamma}{R^{*2}} \phi_{\text{P}}^{*2}(r^*) \quad (25)$$

where

$$\gamma = \frac{108\rho_{\text{OS}}R_0^4\sigma^2}{(\rho_{\text{OP}}b^2)^3} \quad (26)$$

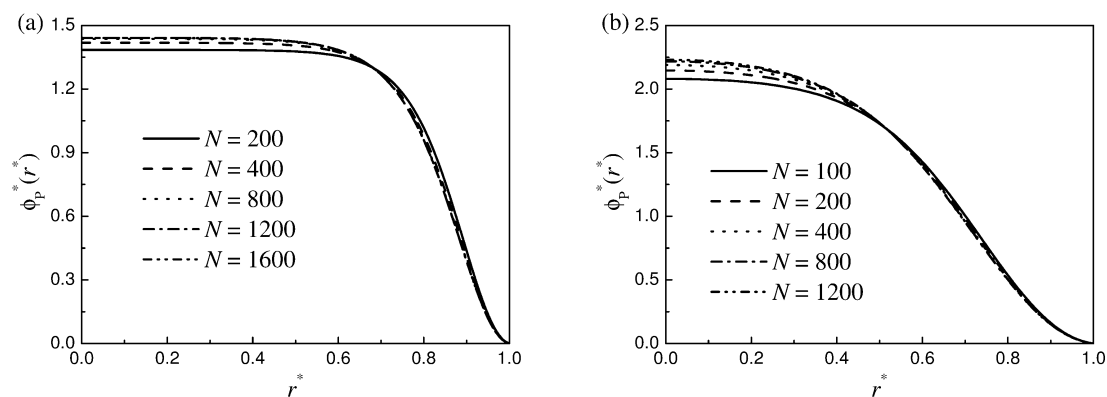


Figure 1. Polymer volume fractions plotted using reduced variables $\phi_p^*(r^*)$ and r^* , for the parameter sets listed in Table 1. Panel a is for good solvent cases and uses $R^* = 0.9$ and $\beta = 25$. Panel b is for Θ solvent and uses $R^* = 0.9$ and $\gamma = 0.5$.

Table 1. The Polymer–Solvent Systems Used in Figure 1^a

good solvent						Θ solvent			
N	σ	R	χ	$\phi_{\max}$	$\phi_{\max}/(1 - 2\chi)$	N	σ	R	$\phi_{\max}$
200	0.003 6	5.2	0	0.385	0.39	100	0.004 1	3.7	0.46
400	0.001 4	7.3	0.05	0.219	0.24	200	0.002 0	5.2	0.34
800	0.000 56	10.4	0.1	0.125	0.16	400	0.001 0	7.3	0.24
1200	0.000 35	12.7	0.15	0.095	0.14	800	0.000 51	10.4	0.17
1600	0.000 27	14.7	0.2	0.083	0.14	1200	0.000 34	12.7	0.14

^aFor all good solvent systems, $\beta = 25$ and $R^* = 0.9$. For all Θ solvent systems, $\chi = 0.5$, $\gamma = 0.5$, and $R^* = 0.9$. The values of R and σ are calculated from those parameters, and their approximate values are shown here. In each system, $\phi_{\max}$ is the maximum value in the volume fraction profile.

which is also independent of the monomer definition.

Implications and Physical Interpretation. These parameters imply that, at low enough concentration, the concentration profiles are fully determined by the average volume fraction, $\bar{\phi}_p$, the reduced cylinder radius, R^* , and either β or γ . In other words, when expressed in terms of r^* and normalized by $\bar{\phi}_p$, the concentration profiles for all systems corresponding to a common value of β or γ are expected to be identical, at least in the low concentration limit.

Returning to eq 23, we have

$$\beta \propto \frac{\rho_{0S}}{\rho_{0P}^2 b} \sigma (1 - 2\chi) N^{3/2} \quad (27)$$

which, although perhaps not obvious, is still independent of the monomer definition. The implications are that any systems with combinations of all the underlying parameters leading to the same values of R^* and β would have concentration profiles with identical shapes. For example, a doubling of the grafting density σ would have exactly the same effect as changing χ from 0.25 to 0. Changes to $N^{3/2}$ could also be compensated by changes to the other parameters such that β remains constant, but in order to maintain the same profile, the cylinder radius would then need to change in order to keep the same value of R^* .

Similarly, eq 26 implies

$$\gamma \propto \frac{\rho_{0S}}{\rho_{0P}^3 b^2} (N\sigma)^2 \quad (28)$$

In this case, changes to N and σ would have identical effects, when expressed in terms of the reduced profiles, and relative to R^* .

To gain some physical insight into β and γ , recall the Flory-type argument for a uniform planar brush, in which the free

energy per chain can be approximated by two terms, the effective interaction energy and the elastic free energy. In good solvent, this is given by

$$\frac{F}{n_p k_B T} = w_2 N \bar{\rho}_p + F_{el}(h/R_0) \quad (29)$$

where $\bar{\rho}_p$ is the polymer density throughout the uniform layer, h is the layer thickness, $F_{el}(h/R_0)$ is the elastic free energy, and w_2 is the excluded volume parameter

$$w_2 = \frac{\rho_{0S}}{\rho_{0P}} (1 - 2\chi) \quad (30)$$

At high stretching, $F_{el}(h/R_0) \propto (h/R_0)^2$, but all that is needed here is that it is a function of h/R_0 . Since $\bar{\rho}_p = N\sigma/h$ in this model, substituting eq 30 into eq 29 and simplifying, gives

$$\frac{F}{n_p k_B T} = A_1 \frac{\beta}{h^*} + F_{el}(h^*) \quad (31)$$

where A_1 is a numerical factor and $h^* = h/R_0$. For the case of Θ solvent, the interaction term needs to be replaced by the ternary interaction, i.e.

$$\frac{F}{n_p k_B T} = w_3 N \bar{\rho}_p^2 + F_{el}(h/R_0) \quad (32)$$

where

$$w_3 = \frac{\rho_{0S}}{3\rho_{0P}^3} \quad (33)$$

Together, these mean

$$\frac{F}{n_p k_B T} = A_2 \frac{\gamma}{h^*} + F_{el}(h^*) \quad (34)$$

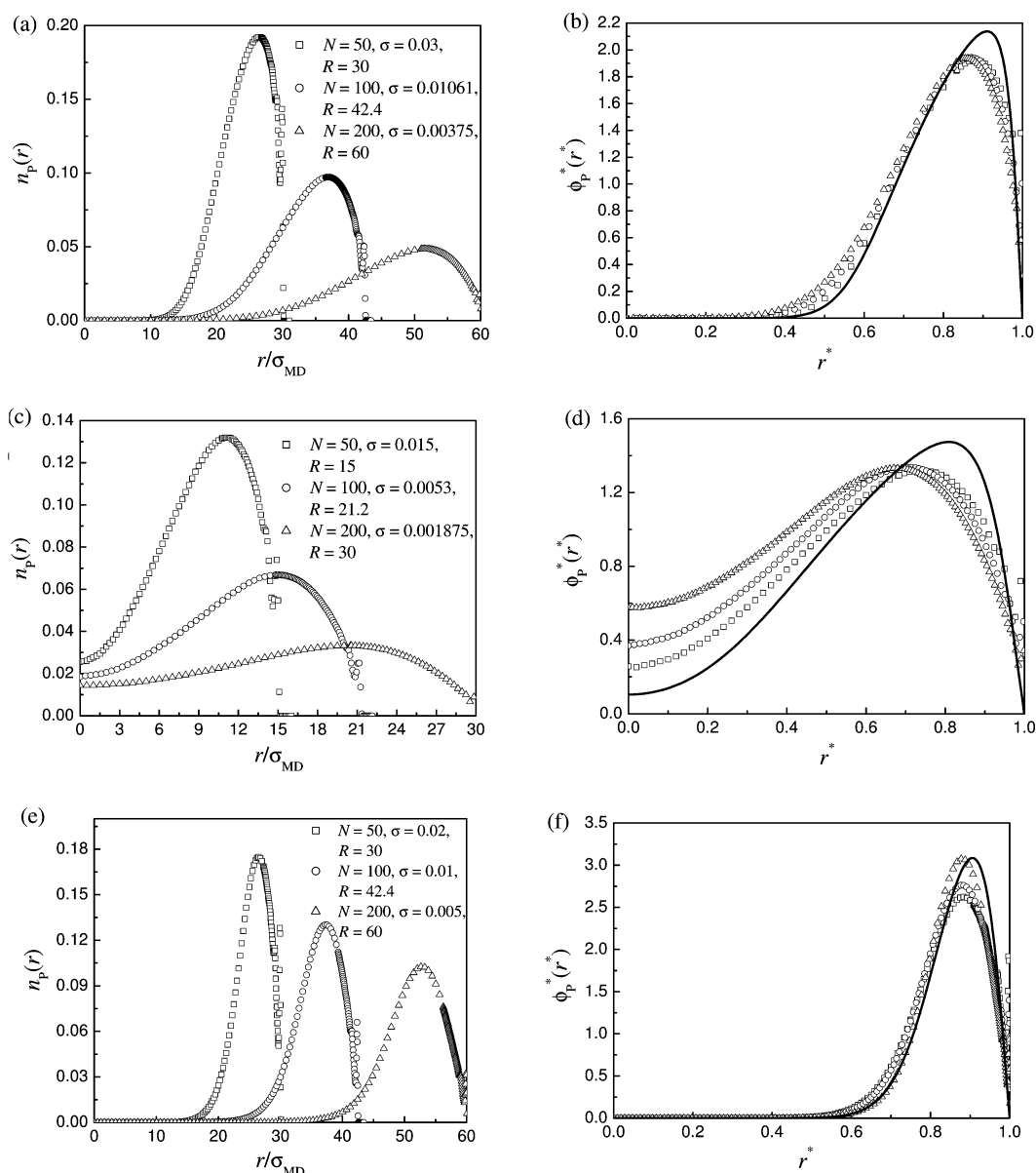


Figure 2. MD number density profiles shown with unreduced (left column) and reduced (right column) variables for different parameter sets. In each panel, $r = 0$ corresponds to the cylinder axis, and the profiles terminate at different distances, each corresponding to the radius of the particular cylinder. The SCFT results (black solid lines) are also given for comparison. σ is in units of ϕ_{MD}^{-2} , and R is in units of σ_{MD} . In panels a–d, the MD results are for $k_B T = 8$ and the SCFT are for $\chi = 0$. In panels e and f, the MD results are for $k_B T = 3$ and the SCFT are for $\chi = 0.5$. Panels a, b, e, and f are for $R^* = 10.4$, and panels c and d are for $R^* = 5.2$.

where A_2 is another numerical factor.

The first terms in each of eq 31 and eq 34 are the interaction energy per chain in a uniform layer of thickness h , in good and Θ solvents, respectively. To within numerical factors, the parameters themselves, β and γ , are the interaction energy in the hypothetical case when $h = R_0$.

Numerical Test of the Controlling Parameters. In this section, we test the validity of the two controlling parameters that we introduced when studying the variables used in the SCFT. We examine whether these parameters still provide good guidance beyond the low concentration limit, using both SCFT and MD data.

SCFT. The formalism above strictly applies only in the limits of very low concentration. Figure 1 and Table 1 explore the

degree to which these parameters control the behavior at finite concentrations, when deviations develop, and how large these deviations are. It is important to note that all the profiles presented hereafter were calculated using the full SCFT, not the approximate potentials of eq 19 or eq 25.

Figure 1 shows illustrative reduced concentration profiles for cylinders with radii comparable to R_0 , specifically $R = 0.9R_0$, which means a common value of $R^* = 0.9$. Figure 1a is for good solvents and Figure 1b for Θ solvents. There are five different systems shown in each panel. The input parameters for these calculations, as well as the maximum polymer volume fractions in the resulting profiles, are shown in Table 1. For the good solvent, the curves are for different values of χ , σ , R , and N that all correspond to the same value of β . Similarly, for the Θ

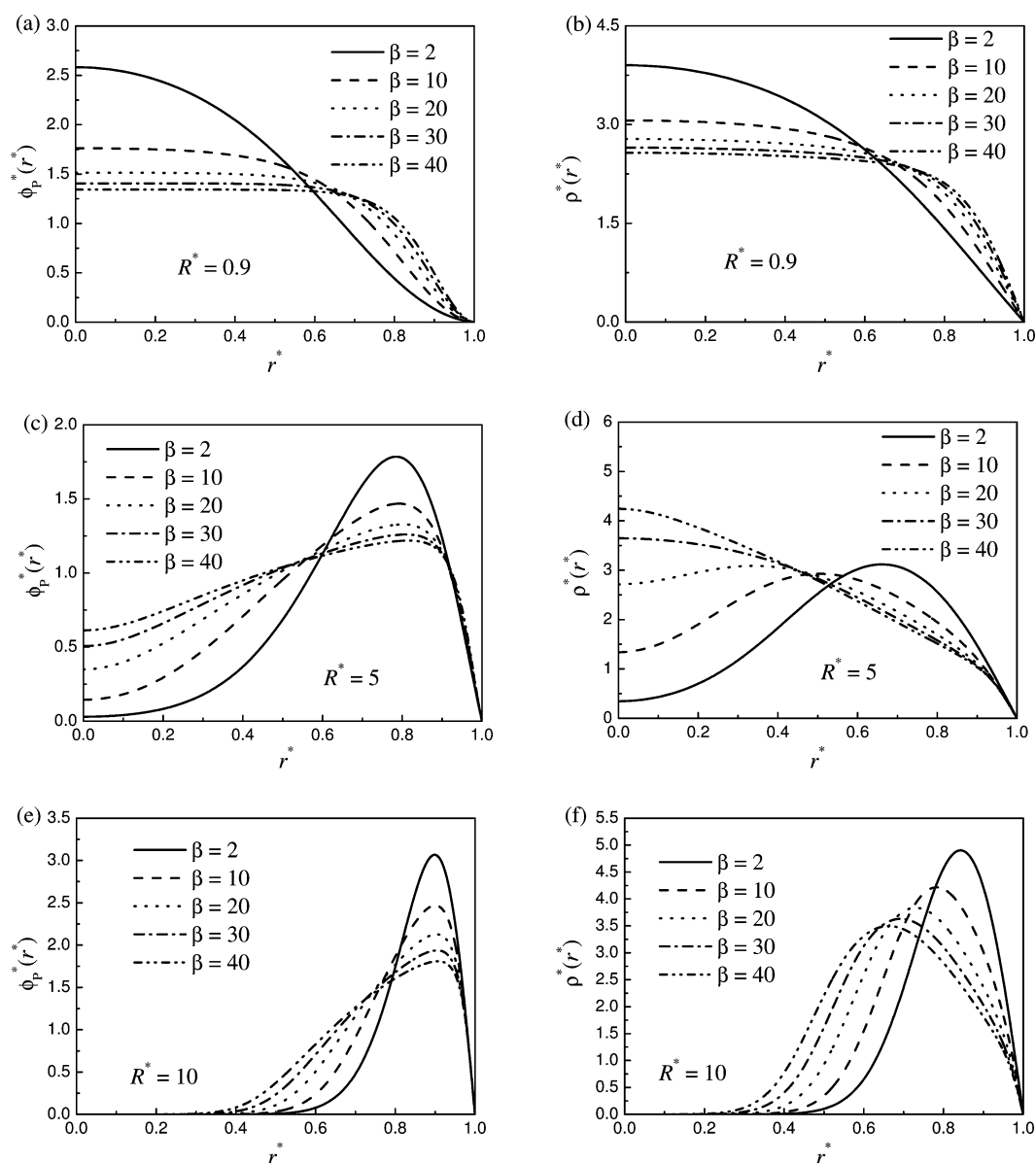


Figure 3. Reduced polymer volume fractions, $\phi_p^*(r^*)$, and chain-end distribution functions, $\rho^*(r^*)$, in good solvents as functions of r^* and different β values with $R^* = 0.9, 5$, and 10 . The actual volume fraction profiles can be obtained by multiplying each $\phi_p^*(r^*)$ by the corresponding $\bar{\phi}_p$.

solvent, the curves are for different values of σ , R , and N , but a common value of γ . In all systems, the actual polymer volume fraction is obtained by multiplying by $\bar{\phi}_p$. For the cases shown, they vary by a factor of 5 for the good solvent cases, from about $\bar{\phi}_p = 0.06$ to 0.3 , and by about a factor of 3 for the Θ solvent cases, from $\bar{\phi}_p = 0.06$ to 0.22 . Similarly, the cylinder radii vary by factors of about 3 and 4 for the good and Θ solvent cases, respectively. This implies, of course, that the actual volume fraction profiles for the five different cases in each panel differ widely from each other, but as seen in these panels, they all very nearly converge to one curve when expressed in terms of the reduced variables derived in the previous section.

For the good solvent systems, the profiles for $N = 1600$, 1200 , and 800 are virtually indistinguishable from one another, but differences emerge for the smaller N cases. For the Θ solvent, the $N = 1200$ and 800 curves are very similar, with small differences appearing for the other cases. These

observations can be related back to the parameters identified in the last section. For the good solvent, we expect a master curve if the polymer volume fraction is small compared with $(1 - 2\chi)$ and for the Θ solvent if it is small relative to unity. For this purpose, we need a measure of the polymer volume fraction, which is spatially inhomogeneous. Two obvious possibilities would be the maximum and the average of each profile. There is no compelling reason to choose one over the other, but since we are interested in probing the limitations of the applicability of the “low concentration” limit, we use the maximum. With this choice, Table 1 shows the value of the relevant parameter in each system. In all cases, the curves are nearly the same if this parameter is ~ 0.2 or smaller, and differences appear and grow when it exceeds this value. However, particularly when viewed against the overall variations in scales of the concentration profiles, the convergence of these reduced profiles is remarkably robust.

The overall conclusion of this section is that the reduced concentration profiles all converge very closely to a single curve in each solvent, with visible differences developing only when the relevant parameter defining “low concentration” reaches a value of about 0.2. Even then, given that the overall profiles vary by factors of as much as five, these are very small differences.

MD Simulations. We turn now to our MD simulations to explore if this reduction to common curves is limited to SCFT results. Our MD data are shown in Figure 2. Figure 2a,c,e shows the calculated number density profiles, $n_p(r)$, calculated in the MD simulations, for a variety of chain lengths, surface densities, and tube radii, and for both athermal and Θ solvents. For these calculations, unreduced distances are in units of σ_{MD} , and the statistical segment length is $b = 0.97\sigma_{MD}$. Within each panel (a, c, e), the different systems would all correspond to the same set of reduced variables as predicted, at least approximately, by the SCFT. The corresponding reduced concentration profiles are shown in Figure 2b,d,f along with SCFT reduced profiles obtained using the same parameters.

There is an important caveat to be made with respect to comparing the MD and SCFT profiles in Figure 2b,d,f: although all the systems in each panel should correspond to the same values of β or γ (note that this requires that the solvent quality depend only on temperature, and not on N or concentration), the actual values would depend on the precise correspondence between the temperature in the MD simulations and the χ parameter in the SCFT. Accordingly, we had to estimate this correspondence as follows. The simulations in panels a and c are for $k_B T = 8$, which corresponds approximately to an athermal solvent; to estimate β for these cases, we use $\chi = 0$, which implies $\beta = 24.4$ for the systems in panels a and b, and $\beta = 12.2$ for those in panels c and d. The simulations in panel e are for $k_B T = 3$, which corresponds approximately to Θ solvent, at least over the range of values of N used here; for these we thus use $\chi = 0.5$, which implies $\gamma = 2.7$. We have also chosen to exhibit results for much wider cylinders than we chose for the SCFT, simply to show profiles of a different shape; in particular, we chose $R^* = 10.4$ for the upmost and lowest pairs of panels and $R^* = 5.2$ for the center ones.

Figure 2a shows the MD profiles for three radii and values of N , at $k_B T = 8$. For these examples, the overall magnitudes, e.g. maximum densities, vary by a factor of about 5 and the layer thicknesses by about a factor of 3. However, as seen in Figure 2b, when scaled according to the reduced variables, the profiles are very similar and agree rather well with the SCFT curve. The profiles shown for narrower tubes in Figure 2c and the same temperature also vary in overall magnitude by about a factor of 5, and their shapes appear to be quite different. However, when scaled according to the reduced variables, i.e., Figure 2d, they appear far more similar, with very nearly the same maxima, but with discrepancies near the cylinder centers. In evaluating the differences near the centers, it is worth remembering that the number of monomers scales as $r\phi_p(r)$, and so the differences in this quantity are much smaller than in $\phi_p(r)$. Finally, the curves in Figure 2e for the Θ solvent case also differ substantially from each other, but the reduced curves agree rather well with each other and with the SCFT prediction.

In summary, the reduction to common curves we found for the SCFT results also occurs in the MD simulations, at least to a very good approximation, and the curves are very similar to the SCFT ones. This is consistent with the general level of

SCFT–MD agreement found earlier by Binder et al.²⁸ It is possible that some of the remaining differences between the SCFT and MD results could be eliminated by fine-tuning the values of the aggregate parameters β and γ , but this would provide little or no additional insight.

■ SCFT CONCENTRATION PROFILES AND END DISTRIBUTIONS

With this illustration of the usefulness of SCFT and its three controlling factors (and their limitations) in hand, we can carry out a more systematic study of these systems, in terms of these parameters. We will do so for both good and Θ solvents.

Good Solvent. We begin this with Figure 3, which shows reduced volume fraction profiles and chain end distributions in good solvents as functions of β . We consider relatively narrow tubes with $R^* = 0.9$, wider tubes with $R^* = 5$, and even wider ones with $R^* = 10$. The reduced chain end distributions are calculated using the expression

$$\rho^*(r^*) = \frac{q^{a^*}(r^*, 1)}{\int_0^1 r^* dr^* q^{a^*}(r^*, 1)} \quad (35)$$

which satisfies the normalization condition: $\int_0^1 r^* dr^* \rho^*(r^*) = 1$.

First, we examine the case of relatively narrow tubes $R^* = 0.9$ (panels a and b). For all values of β , the volume fraction profiles and the chain-end distributions start from zero at the cylinder wall and initially increase. For small β , they rise to a distinct maximum at the center. For larger β , the initial rise is faster but, away from the wall, the profiles become flatter, and the maxima at the center decrease and become less prominent. Such a decrease is due to the fact that this is the reduced profile, ϕ_p^* ; the actual volume fraction could increase or decrease.

Figure 3c,d shows the corresponding results for wider cylinders, with $R = 5R_0$. In these systems, the concentration profiles have maxima at a distance approximately R_0 from the surface (or at 0.8 in these scaled units). For small values of β , the polymers just reach to the center. With increased β , they reach farther into and beyond the center, and the concentration at the center increases. In a manner similar to what we just saw for the narrower tubes, the volume fraction profiles become flatter. The end-distribution functions also exhibit interesting behavior. For small β , they have maxima at a distance on the order of $1.5R_0$ from the surfaces, and tails extending to the center. As β increases, the maxima move away from the surface, and the value at the center rises. At large enough β , the maximum is at the center.

Finally, Figure 3e,f shows the results for much wider cylinders, with $R = 10R_0$. This is wide enough that the polymers do not reach the center for any of the values of β used. The profiles and end-distributions are qualitatively similar to those for polymers anchored to flat surfaces, and they stretch farther toward the center with increasing β .

The flattening of the profiles and the suggested narrowing of the depletion region near the surface with increasing β seen in panels a and b could seem counterintuitive, since increased β could correspond to improved solvent quantity, and one might expect the chain ends to cluster more at the cylinder center as a result. Instead, however, they are distributed more uniformly throughout the cylinder as β increases. This can be understood by the fact that a Gaussian chain in good solvent is actually much larger than R_0 . If one uses the end-to-end distance of a single chain in a good solvent as a measure of this size, it is

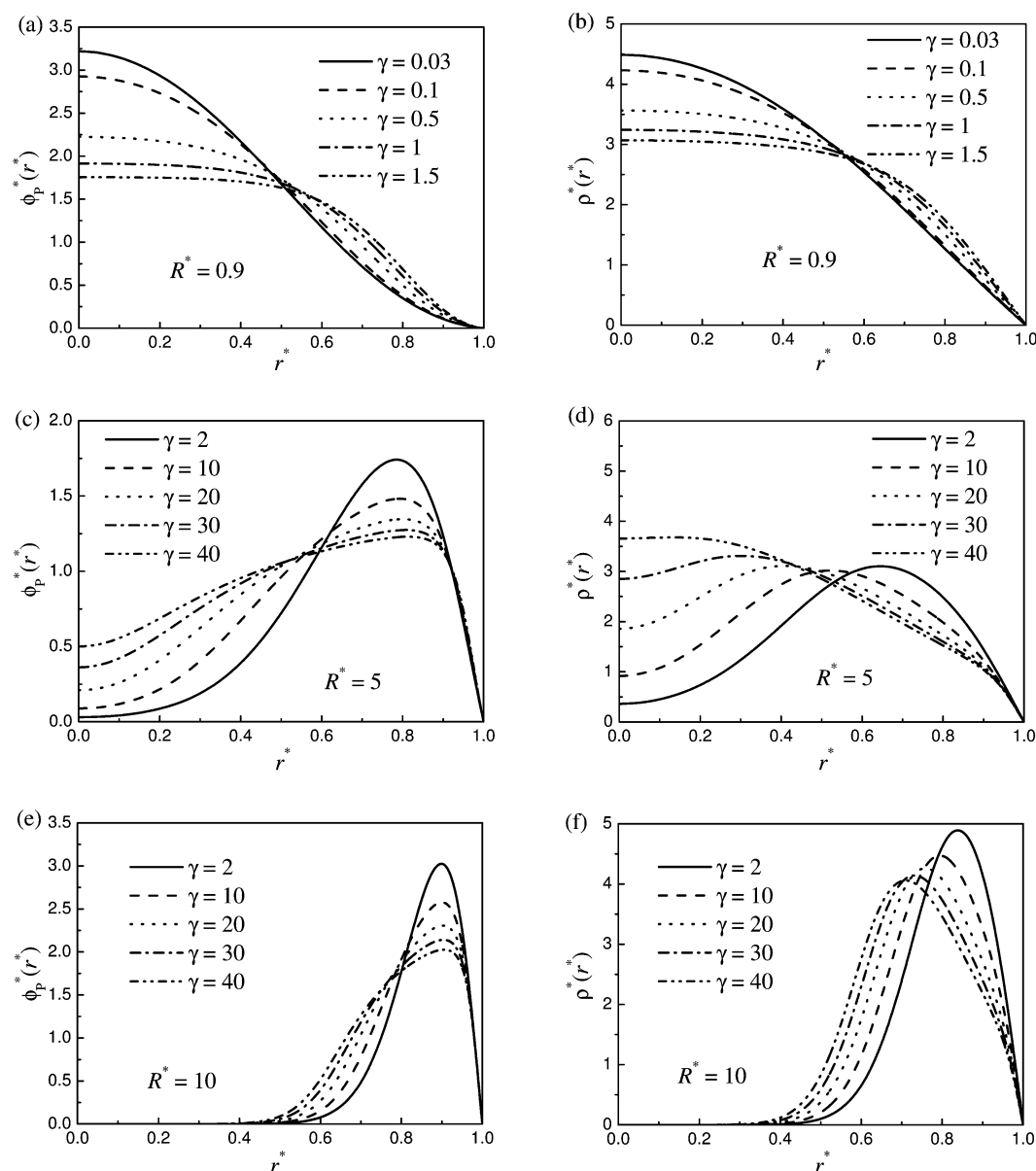


Figure 4. Reduced volume fractions, $\phi_p^*(r^*)$, and chain-end distribution functions, $\rho^*(r^*)$, in Θ solvents as functions of r^* and different reduced grafting densities, γ , with $R^* = 0.9, 5$, and 10 . The actual volume fraction profiles can be obtained by multiplying each $\phi_p^*(r^*)$ by the corresponding ϕ_p .

more like $N^{3/5}b \sim \sqrt{6N^{1/10}R_0}$. This depends weakly on N but would typically be on the order of $4R_0$. Furthermore, the overall extent of a polymer is even larger. These length scales are greater than the diameter of these narrow tubes; i.e., each chain reaches completely across to the far side of the tube even without stretching and further leads to additional “filling in” of the regions near the surface. These considerations also explain why, even in panels c and d where the cylinder radius is $5R_0$, the chain concentration is nonzero at the center.

Θ Solvent. Figure 4 shows volume fraction profiles and chain-end distributions for polymers in Θ solvent, for the same cylinder radii used for the good solvent cases just discussed. In this solvent, the second controlling parameter is γ rather than β . Qualitatively, the behavior in these systems is the same in each case as in the good solvent. In fact, the limiting cases of $\beta = 0$ for the good solvent and $\gamma = 0$ for the Θ solvent are identical,

since these cases can only be reasonably reached by taking $\sigma = 0$. We see from these figures that increases in each of the parameters have similar effects, which can be expected because β and γ have the same physical origin. Perhaps the most interesting feature of these results is how similar they are to the good solvent case.

■ CLOSED AND OPEN CYLINDERS

One implication of the above results is that whether or not polymers reach the tube center, i.e., its axis, can be controlled by any of N , σ , and solvent quality. For example, if N and σ are within range of this, then simply tuning χ could be a useful control. We turn to this question now, using the SCFT in particular. It should be remembered, however, that the MD and SCFT results are in good agreement with each other. In many applications, having polymers extend to the very center of the

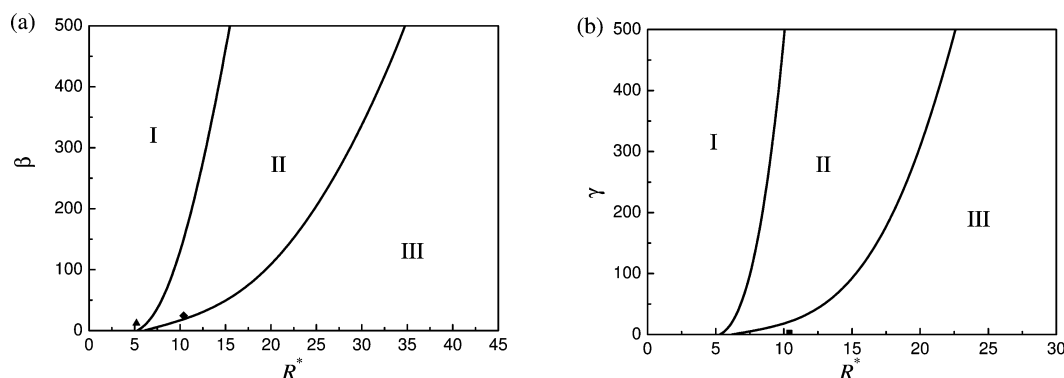


Figure 5. Dependence of the tube state on the reduced variables: (a) β and R^* for good solvent; (b) γ and R^* for Θ solvent. For each case, the solid lines between region I and II show the boundary using the criterion of $\phi_p(0) = 0.01\bar{\phi}_p$, and those between region II and III show the boundary corresponding to the states in which the inflection point of the concentration profile is right located at $r^* = 1/\sqrt{2}$. Also highlighted are the points corresponding to the calculations in Figure 2. The diamond and triangle in (a) correspond to Figures 2a,b and 2c,d, respectively, and the square in (b) corresponds to Figure 2e,f.

channel can lead to dramatic effects, especially on solvent and analyte flows.

Specifying the conditions under which polymers reach the tube center requires a definition of “reach the center”, and useful definitions may vary from one application to another. Here, we consider two definitions. The most important point of this section is that, for either one, the conditions depend only on two quantities, which are R^* and β in good solvents and R^* and γ in Θ solvents. Our results using these definitions are shown in Figure 5, which are the result of a systematic series of SCFT calculations.

The first definition is that the polymer concentration at the tube axis reach a minimum small value. For Gaussian chains, the volume fraction can be extremely small but it is always nonzero, and so using nonzero concentration at the center is not an option. Instead, we define this as reaching a small value relative to the average concentration, specifically $\phi_p(0) = 0.01\bar{\phi}_p$. In Figure 5a,b, the regions labeled “I” are all “closed” cylinders, in the sense that $\phi_p(0) > 0.01\bar{\phi}_p$. The line separating regions I and II in each panel is the locus of points where polymers just reach the axis, as defined this way. The transition from open to closed can be accomplished by changing either the cylinder radius, and hence R^* , or any of the factors included in β or γ .

It is worth noting that in each panel the boundary between region I and II intercepts the abscissa at the identical point, i.e. $R^* \approx 5$. This means that the narrowest tubes with zero polymer concentration at the center have radii $R \approx 5R_0$, and this critical value is the same for good and Θ solvent cases. Such observation is consistent with the fact that the limiting cases of $\beta = 0$ for the good solvent and $\gamma = 0$ for the Θ solvent are identical within SCFT. This critical value might be of some importance in those experimental systems where reactive polymer brushes are used to control flow. Also shown in Figure 5 are the points corresponding to the calculations in Figure 2, from which it can be seen that Figure 5 gives the correct predictions of the corresponding tube states, even for Figure 2d where some numerical differences appear in profiles.

The second definition we use is intended to approximate the conditions under which the inner half of the cylinder, by volume, is empty. The boundary between the inner and outer halves of a tube are defined by $r^* = 1/\sqrt{2}$. Again, of course, there is no distinct criterion for the polymer to be excluded

from the inner half. Instead, we approximate this criterion by the condition that the inflection point in the concentration profile is at $r^* = 1/\sqrt{2}$. This condition defines the boundaries between regions II and III in each panel of Figure 5. For all systems in region III, the inner half of the cylinder is empty, defined in this way. Figure 5 thus provides two different ways to define the important transition from open tube to closed tube.

CONCLUSIONS

By examining the low concentration limits of the self-consistent field theory, we have been able to identify a small number of factors that control the behavior of polymers grafted to the inside of microchannels. Identifying these factors provides guidance on how to tune polymers and solvent to control the opening and closure of these channels in response to external stimuli. They also allow us to systematically examine the properties of the chains, as functions of all the system parameters that enter into the few controlling parameters. For instance, we can use the SCFT to predict the distribution function of specific monomers that may be able to react with analytes in solution; we have shown some results for the end monomers, but such calculations can also be done for an arbitrary section of the chain (which could be useful, e.g., for problems involving detection via DNA hybridization).

The approximate reduction of all the results to near-universal behavior allowed us to investigate the conditions under which the polymer either reaches the tube center or leaves an open channel there, and to express the results in quite general form in terms of the few controlling parameters, thus helping us determine the “phase diagram” for this process. We showed this explicitly using two different criteria for defining what is meant by an open or closed tube; the primary result is that, for a given definition, the boundary between these two is a function of these two parameters. This general result would apply to other definitions of open and closed. We also found that polymers reach the tube center even for relatively wide tubes; the narrowest tubes with zero polymer concentration at the center have radii $R \approx 5R_0$, and this factor increases rapidly with increasing β or γ .

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Notes

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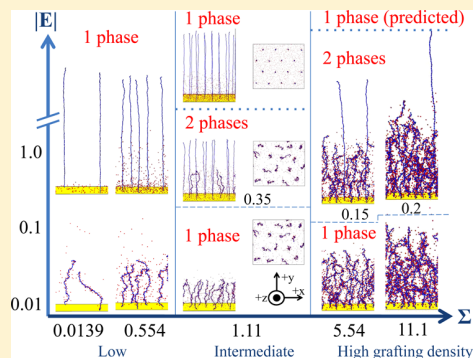
Structure of Polyelectrolyte Brushes Subject to Normal Electric Fields

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Supporting Information

ABSTRACT: Molecular dynamic simulations of salt-free polyelectrolyte brushes subject to external fields applied normal to the grafting substrate reveal the three-dimensional monomer and counterion distributions. It is found that below a critical electric field, local electroneutrality holds for densely grafted brushes and the brush height remains independent of field intensity. Above this critical field (which scales as $1/3$ with grafting density) brush height increases smoothly, and the fraction of condensed counterions decreases. The brush bifurcates into two subpopulations of stretched and collapsed chains when the grafting density is not low. At intermediate grafting densities, the majority of chains are stretched and the minority are nonstretched. At high grafting densities bifurcation and brush height growth occur consecutively. The majority of the chains are nonstretched at high grafting densities. Although not observed prior to overstretching of the chain model, it is predicted that the two subpopulations will re-merge to a single highly stretched phase when field intensity reaches a third critical value. The ability to control subpopulations of chains suggests that utilizing electric fields normal to polyelectrolyte brushes holds potential as controllable gates in microfluidic devices.



INTRODUCTION

Nanoengineered surfaces have the potential to be useful components for the design of highly efficient and sensitive lab-on-a-chip systems. In particular, the scientific community has shown immense interest in the technological applications of “smart” nanochannels (i.e., channels that would respond to stimuli such as variation in temperature, pH, or concentrations of specific chemicals). A natural and well-developed means of surface modification is grafting (or otherwise associating) polymer chains to interfaces. Polymeric brushes have been used to stabilize colloidal dispersions, to protect vulnerable surfaces, to interact with proteins or other biological molecules, and to act as lubricants. However, it is charged polyelectrolyte brushes¹ that have the greatest potential as “smart brushes”. Due to long-range electrostatic coupling between chains and free counterions, the conformational behavior of polyelectrolyte brushes depends sensitively on many factors, particularly salt concentration, grafting density, pH, and charge fraction.^{2–7} Thus, polyelectrolyte coatings can act as stimuli-responsive surfaces with environmental variations causing dynamic transitions in brush structure.

Analytical theories treating the structure of polyelectrolyte brushes face an appreciable challenge because chains and their associated counterions have both copious degrees of freedom and long-range Coulomb interactions. Scaling theories make significant simplifying assumptions in order to create governing equations for polyelectrolyte brushes that are intuitive and tractable. In high salt concentration solutions, electrostatic interactions are heavily screened and the osmotic pressure of the counterions decreases with increased salt concentration.^{8,9}

This is the so-called salted-brush regime in which brush thickness scales with the salt concentration by a power of $-1/3$ and with the grafting density by a power of $1/3$. The low salt limit itself has two regimes: (1) The Pincus-brush regime of weakly charged chains for which the diffuse counterion layer extends far beyond the brush height and (2) the osmotic-brush regime of strong polyelectrolytes for which effectively all the counterions reside within the brush.⁸ In the Pincus-brush regime, the thickness of the brush results primarily from the balance between the chains’ elastic energy and the electrostatic energy, while in the osmotic-brush regime, the chains’ elastic energy is balanced predominately by the counterions’ osmotic pressure. In early work, on the osmotic-brush regime, the brush thickness was predicted to be independent of grafting density,^{8,10} in disagreement with later experimental findings.^{11,12} More recent scaling theories that account for excluded volume effects^{13,14} and nonlinear elasticity^{12,15} have been more successful but still remain limited to an intuitive, qualitative picture of polyelectrolyte brushes.

One method of making more concrete statements and predictions about the concentration profiles of polyelectrolytes and their counterions is to numerically solve the equations of self-consistent field (SCF) theories.^{9,15–21} SCF approaches are able to satisfactorily fit the brush profiles at different grafting densities and salt concentrations of high charge fraction polyelectrolytes¹¹ and even reproduce the scaling of brush

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height with grafting density to investigate the impact of environmental variations on brush conformation such as changes in solvent quality,²² pH,²³ and protein uptake.^{24,25} However, SCF is based on the assumption of local electroneutrality and is a mean-field method. It struggles at high grafting densities, is unable to capture the dynamics of tethered polymers, and fails to predict behavior when electroneutrality does not hold locally at all points within the brush.

Molecular dynamic (MD) simulations do not have these shortcomings.²⁶ In fact, computational simulations have likely provided the most insight into polyelectrolyte brushes. Most simulation methods that have been applied to polyelectrolyte brushes have included the solvent implicitly (the exception being dissipative particle dynamics^{27–31}). Monte Carlo^{32–34} simulations have been conducted to verify SCF conformations, but MD simulations with a Brownian dynamics (BD) or a Langevin dynamics (LD) thermostat have been found to be the most successful. For example, BD simulations have looked at apposing brushes³⁵ and the interaction of brushes with charged nanoparticles³⁶ and surfactants,³⁷ while LD simulations have investigated the impact of varying many parameters, including charging fraction,^{13,38} added salt,³⁹ apposing brushes,^{40–42} chain-end modification,^{43,44} multivalent counterions,⁴² charge sequence,⁴⁵ and chain flexibility.⁴⁶

The impact of an external electric field, applied normal to the grafting surface is of particular recent interest.^{47,48} Electric voltage is the most obvious and convenient mechanism to transition between extended/collapsed states. This swelling and deswelling of the brush in response to DC voltages has been experimentally demonstrated.⁴⁹ For our purposes, electric fields are favorable because they can be switched on and off quickly and because they act not only on the polyelectrolytes but also directly on the counterions that surround them. When counterions move under the action of an external electric field, they drag the surrounding solvent actualizing an electro-osmotic flow. The resulting electro-osmotic flow is then itself stimuli-responsive.^{50–53} When the field is normal to the brush, the extension/collapse transition can act as a stimuli-responsive gate, limiting the passage of flowing fluid or ions^{48,54} and so has potential in the design of functional and controllable MEMS devices.

In this manuscript, we present LD simulations that investigate the polyelectrolyte conformations in the zero salt limit and map the counterion ion distributions about the chains in the presence of an external, normal electric field. This is done for low, intermediate, and high grafting densities.⁵⁵ We demonstrate that below a (density dependent) critical field, local electroneutrality holds only for high enough grafting densities and is particularly suspect for low grafting density. Below the critical field, the brush height remains relatively constant but above it there is significant charge redistribution due to the polarization by the electric field and the brush height increases with the electric field. At moderate and high grafting densities, our simulations suggest a novel phenomenon where the population of grafted polyelectrolytes bifurcates into two subpopulations: (1) At a moderate grafting density in the bifurcation condition, most of the chains are stretched and a minority remain nonstretched and (2) at a high grafting density, most chains remain nonstretched and only a few are highly stretched.

The two populations are not observed to recombine into a united ensemble of stretched chains before overpassing the limits of our computational models in high fields.

METHOD

Simulation Model and Setup. The polymer brush system is modeled as an ensemble of $M = 16$ flexible coarse-grained polyelectrolyte chains in a simulation box with dimensions $L_x \times L_y \times L_z$. Polyelectrolytes are grafted to the $z = 0$ wall. A corresponding wall at $z = L_z$ closes the system, while periodic boundary conditions are applied in the x and y directions. Each polyelectrolyte is composed of $N = 96$ negatively charged monomers plus a neutral grafted monomer. All chains are fully charged; each monomer dissociates one monovalent counterion (there is no added salt).

The interaction potentials consist of the following four kinds, with straightforward subscripts: $U_{LJ}(r)$, $U_{wall}(l)$, $U_{bond}(r)$ and $U_{coul}(r)$. All particles including monomers, counterions, and grafted monomers separated by a distance r are described by the purely repulsive Lennard–Jones (LJ) (or Weeks–Chandler–Andersen) potential

$$U_{LJ}(r) = \begin{cases} 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] + \epsilon & r \leq \sqrt[3]{2}\sigma \\ 0 & r > \sqrt[3]{2}\sigma \end{cases} \quad (1)$$

where σ represents the nominal diameter of each bead and ϵ is the interaction energy unit. Counterions have the same diameter as monomers. Both upper and lower walls are model as a 9–3 LJ potential

$$U_{wall}(l) = \begin{cases} \epsilon \left[\frac{2}{15} \left(\frac{\sigma}{l} \right)^9 - \left(\frac{\sigma}{l} \right)^3 \right] & l \leq \sqrt[3]{2/5}\sigma \\ 0 & l > \sqrt[3]{2/5}\sigma \end{cases} \quad (2)$$

with a cutoff at $l_c = \sqrt[3]{2/5}\sigma$ where l is the distance of a particle to a wall. These walls prevent counterions from escaping from the simulation system and the top boundary at $z = L_z$ has been chosen to be at position $L_z = 2N\sigma$, which is large enough to allow a full stretching of the chains in the z direction. The adjacent monomers along the chain are connected by finitely extensible nonlinear elastic (FENE) bonds defined by the interaction potential

$$U_{bond}(r) = -\frac{1}{2}K_b R_0^2 \ln \left[1 - \left(\frac{r}{R_0} \right)^2 \right] \quad (3)$$

with a spring constant $K_b = 5.833 \epsilon/\sigma^2$ and a maximum extension $R_0 = 2\sigma$. The parameters are adapted from the chain model proposed by Stevens and Kremer,⁵⁶ which has been used extensively in the literature. The K_b value in their study is 1.2 times our value because our energy unit ϵ is equal to $k_B T$ while theirs is $k_B T/1.2$. Since the monomers and counterions carry electric charges, they interact via the long-ranged Coulomb potentials as

$$U_{coul}(r) = \frac{k_B T \lambda_B q_i q_j}{r} \quad (4)$$

where $k_B T$ is the thermal energy and q_i is the charge valence of particle i . The value of q is -1 for each monomer and $+1$ for each counterion. Each grafted monomer is neutral. The Bjerrum length, $\lambda_B = e^2/(4\pi\epsilon_0\epsilon_r k_B T)$ (where ϵ_0 and ϵ_r are the vacuum permittivity and the relative dielectric constant of the solvent, respectively), defines the distance at which the magnitudes of the electrostatic energy and the thermal energy are equal.

We apply an electric field $\vec{E}$ in the negative z direction perpendicular to the wall. The motion of particle i with mass m_i at position $\vec{r}_i(t)$ is described by Langevin equation:

$$m_i \frac{d^2 \vec{r}_i}{dt^2} = -\zeta_i \frac{d\vec{r}_i}{dt} - \nabla_i U + q_i e \vec{E} + \vec{\eta}_i \quad (5)$$

The first term on the right-hand side of the equation is the friction force (with the friction coefficient ζ_i); the second term is the “conservative” force where U is the total interaction potential, and the

third term is the force due to the external electric field $\vec{E}$. The last term is a random force, which is due to the temperature of the system and follows the fluctuation–dissipation theorem.

All particle masses and diameters are m and σ , respectively. The temperature is $k_B T = 1.0 \epsilon$, and the friction coefficient is $\zeta = 1.0 m \tau^{-1}$ with the time unit, $\tau = \sigma(m/\epsilon)^{1/2}$. We set the Bjerrum length to $\lambda_B = 3\sigma$. The unit length is mapped to physical units through $\lambda_B = 0.71$ nm for water, such that $\sigma \approx 0.24$ nm. In this way, the LD unit of the electric field is $\epsilon/(e\sigma) \approx 10^8$ V/m. If our LJ spheres were to be mapped to a polyelectrolyte monomer with a mass of 200 Da, the resulting time unit, τ , would be 2.1 ps. These values suggest that the friction coefficient used in our simulations is approximately 1 order of magnitude smaller than in a typical aqueous solution of viscosity $\mu = 0.89$ mPa s (water at 25 °C), which would yield $\zeta = 12.8 m \tau^{-1}$ through Stokes' law. The smaller ζ value used here allows a faster exploration rate over system configurations, which imposes no effect on the static properties studied.

An implementation of the particle–particle particle-mesh (PPPM) Ewald algorithm for slab geometry with an approximate accuracy of 10^{-3} is used for calculations of the electrostatic interactions.⁵⁷ The calculation is done in an extended system which periodically repeats the original slab system in the z direction, separated by a large empty space between them that is twice the volume of the original slab system in this study. Furthermore, a correction term of $2\pi M_z^2/V'$ is added to the Coulomb potential to cancel the interslab interaction due to the z component of the net dipole moment $\vec{M}$ of the repeating slabs, where $V' = (3L_x L_y L_z)$ is the volume of a unit cell in the extended system. The Verlet algorithm is applied with the integration time step of $\Delta t = 0.005\tau$. A prerun phase takes about 5×10^6 time steps to bring the system into an equilibrium state, followed by a production-run phase. All data presented are obtained from an average of at least 2×10^7 time steps. To shorten the description, we will state all the physical quantities in the standard LD (ϵ, σ, m, e)-unit system.

In this work, the field strength is explored up to $|E| = 2.0$. However, such strong fields result in overstretched chains (in which the chain length exceeds the zero-field contour length). In reality, when a chain is stretched close to the contour length, several intramolecular interactions such as bond-angle potential, dihedral-angle potential, etc. become important and should ideally be taken into account in simulations. Therefore, as these details are currently ignored in our coarse-grained model, we do not present such overstretched data here. These data are given in the Supporting Information. They do qualitatively inform our conclusions.

Grafting Geometry. The grafting chain density is given by $\sigma_g = M/(L_x L_y)$. At low grafting density, each chain is isolated and occupies a half-spherical volume that can be characterized by $R_{g,0}$, the radius of gyration of a single grafted chain in zero electric field. This low density regime is generally called the “mushroom regime”. As σ_g increases such that it comes close to $(\pi R_{g,0}^2)^{-1}$, the chains start to overlap; to reduce the resulting steric interactions, the chains stretch away from the substrate surface and form a brush. Therefore, the regime $\sigma_g \gg (\pi R_{g,0}^2)^{-1}$ is called the “brush regime”. To facilitate the discussion, a dimensionless grafting density $\Sigma = \pi R_{g,0}^2 \sigma_g$ is defined. The value of $R_{g,0}$ is 21.0(1) in this work. Five grafting densities are studied: $\sigma_g = \{10^{-5}; 4 \times 10^{-4}; 8 \times 10^{-4}; 4 \times 10^{-3}; 8 \times 10^{-3}\}$, or equivalently $\Sigma = \{0.0139; 0.554; 1.11; 5.54; 11.1\}$, respectively, and we focus our attention on $\Sigma = \{0.0139; 1.11; 11.1\}$. These densities cover a broad range of regimes, including the mushroom regime ($\Sigma \ll 1$), the crossover regime ($\Sigma \approx 1$), and the true brush regime ($\Sigma \gg 1$). Unless otherwise stated, the grafting points are fixed and arranged in a hexagonal lattice of 4 rows with 4 lattice points on each row. Explicitly, the system area is $L_x \times L_y = \{1359.2 \times 1177.1; 214.9 \times 186.1; 152.0 \times 131.6; 67.9 \times 58.8; 48.1 \times 41.6\}$, respectively, for each grafting density. Although the contour length of the grafted polyelectrolytes is longer than L_x or L_y at high grafting densities, it is expected that interactions with neighboring chains will ensure that finite size effects remain insignificant. Simulations of larger and smaller systems that verify this can be found in the Supporting Information.

RESULTS AND DISCUSSIONS

Brush Height and Bifurcation. To understand the height of the polyelectrolyte brush and the chain structure inside it, we present four quantities to characterize the chains: the root-mean-square end-to-end distance, $\langle R_e^2 \rangle^{1/2}$, the root-mean-square radius of gyration, $\langle R_g^2 \rangle^{1/2}$, the mean height of the open end monomer to the substrate surface, $\langle Z_{\text{end}} \rangle$, and the mean maximum height of the monomers along the chain, $\langle Z_{\text{max}} \rangle$. The chain structure and organization can be studied from the first three quantities, while $\langle Z_{\text{max}} \rangle$ gives explicit information about the brush height. The results are shown in Figure 1 as a function of field intensity, $|E|$. The quantities are

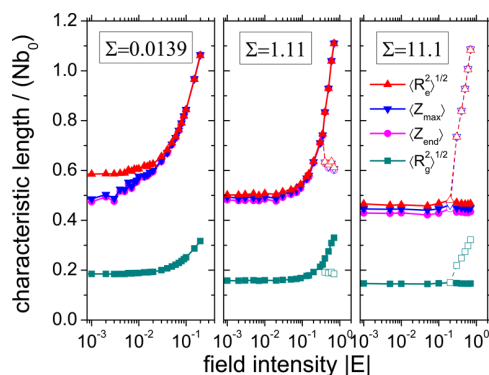


Figure 1. Characteristic lengths $\langle R_e^2 \rangle^{1/2}$, $\langle R_g^2 \rangle^{1/2}$, $\langle Z_{\text{end}} \rangle$, and $\langle Z_{\text{max}} \rangle$ as a function of field intensity $|E|$. The grafting density, Σ , is indicated in each panel. In certain conditions, the brush bifurcates into two groups with one dominating the other. The solid symbols denote the values calculated from the dominant group, while the open symbols denote the ones from the subordinate group.

normalized by the intrinsic contour length of the chain Nb_0 , where $b_0 = 1.1688(4)$ is the average bond length on a single grafted chain in zero field.

The four quantities are constant in weak electric fields for all grafting densities but as $|E|$ is increased over some critical field $|E^*|$, these quantities start to increase significantly. The value of $|E^*|$ is estimated as the field for which the relative increase of the radius of gyration is greater than 5%. The critical field $|E^*|$ depends on grafting density and equals approximately 0.02, 0.09, and 0.2 for the three Σ shown here. We note that the value of $\langle Z_{\text{max}} \rangle$ is quasi-identical to $\langle Z_{\text{end}} \rangle$, which shows that the highest monomer above the substrate is nearly always the end monomer of the chain (i.e., the ends are not coiled in the interior of the chains).

For the low grafting density $\Sigma = 0.0139$, the four quantities increase monotonically as $|E| \geq |E^*|$; however, for the other two grafting densities, the systems exhibit nontrivial behavior. For $\Sigma = 1.11$, the brush bifurcates into two groups of chains when $|E|$ is larger than a second critical field $|E^{**}| \approx 0.035$. One group is constituted of stretched chains and the other is of nonstretched chains. In Figure 1 (and Figure 4), solid symbols represent the more populated conformation, while open symbols denote the minority. For $\Sigma = 1.11$, the majority of chains are stretched. At even higher electric fields, the chains are overstretched (data shown in Figures S1 and S2 of the Supporting Information). We note that at $|E| \geq |E^{***}|$ the bifurcated brush recombined into a single phase because all the chains stretch. The values of $\langle R_e^2 \rangle^{1/2}$, $\langle Z_{\text{end}} \rangle$, and $\langle Z_{\text{max}} \rangle$ become larger than the zero-field intrinsic contour length of chain Nb_0 , showing that the chains

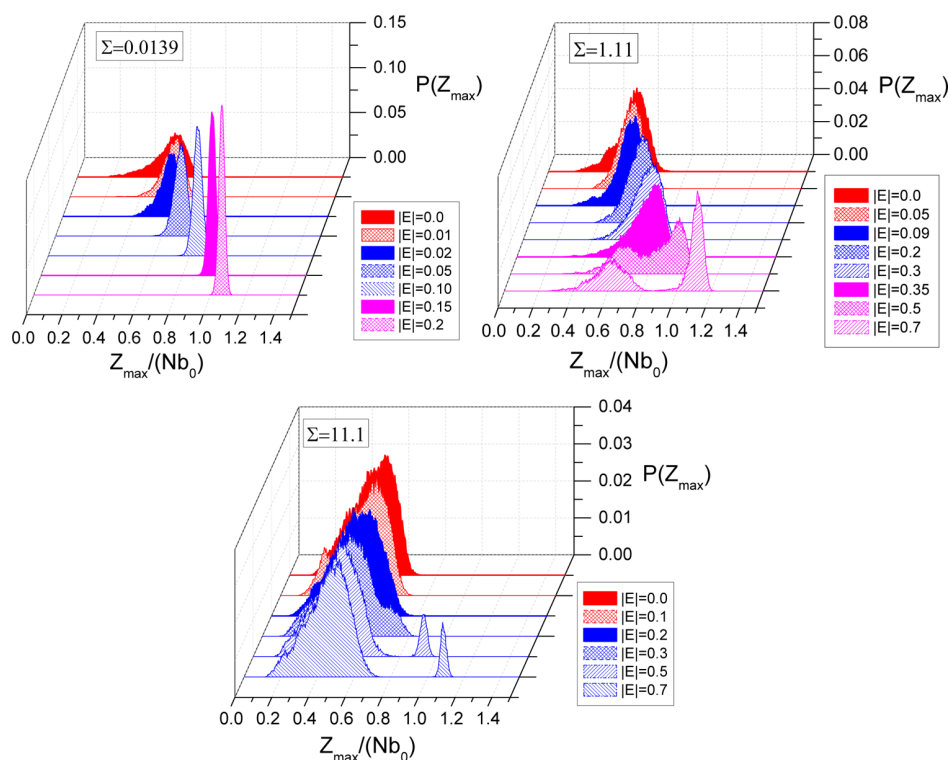


Figure 2. Probability distribution of the maximum chain height in different electric fields at surface coverages (a) $\Sigma = 0.0139$, (b) $\Sigma = 1.11$, and (c) $\Sigma = 11.1$. The field intensities $|E|$ are indicated in the figure.

are significantly stretched. For the high grafting density $\Sigma = 11.1$, the brush splits into two groups when $|E| \geq |E^*| \simeq 0.09$, which is to say $|E^*| \simeq |E^*|$, and no regime seems to exist in which the chains stretch together before bifurcation occurs. Only a few chains stretch in this case. The majority of the chain population stays in the nonstretched chain state and the four quantities are essentially unaltered by the electric field. We did not observe the recombination of the bifurcated brush into a single phase at the extremely high fields $|E| = 2.0$.

In addition to the mean values, we calculate the probability distribution $P(Z_{\max})$ to study the distribution and fluctuation of the maximum chain height in the brush (see Figure 2). The distribution is normalized by the condition $\int_0^{L_z} P(Z_{\max}) dZ_{\max} = 1$. At the lowest grafting density, $P(Z_{\max})$ displays a Gaussian-like distribution (see Figure 2a). The peak moves to larger heights as the electric field increases. Simultaneously, the width of the distribution narrows, showing a decrease in the chain-height fluctuations in the high field regions. For the intermediate density $\Sigma = 1.11$, $P(Z_{\max})$ moves to larger heights when $|E| \geq |E^*| \simeq 0.09$. For $|E| \geq |E^*| \simeq 0.35$, the distribution splits into two peaks. One peak continuously grows and narrows as the electric field increases. The other stays fixed and maintains its width, although the overall probability (surface area) decreases. The splitting of the distribution reiterates the formation of a two-layer structure in the brush: a less-dense layer formed by the stretched chains in the upper part of the brush and a denser layer at the bottom of the brush. It is observed that the peak corresponding to nonstretched chains disappears when $|E| \geq |E^*|$, and the system returns to a single-peak distribution (see Figure S2 of the Supporting Information), although the strong-field data go beyond the limits of the computational model used here.

Figure 2c presents the data for the high grafting density $\Sigma = 11.1$. The distribution bifurcates when $|E|$ is larger than $|E^*| \simeq 0.2$. The first Gaussian distribution (fixed around $Z_{\max} = 0.5Nb_0$) dominates the second peak. Thus, the majority of the chains are not stretched, and the mean thickness of the brush is approximately unchanged. It is in contrast to the case of $\Sigma = 1.11$, where the dominant Gaussian peak moves to larger heights while the minor peak is fixed. Moreover, the field region between $|E^*|$ and $|E^*|$ where the chains stretch all together with the field does not exist at $\Sigma = 11.1$, and the peaks fail to merge at the highest fields used here (even allowing overstretched chains).

We verify that the bifurcation shown here is not an artifact due to the finite-size effect of our model by performing additional simulations for different system sizes. As shown in Figure S3 of the Supporting Information, the characteristic lengths bifurcate for larger systems as well as for smaller ones. The distribution profiles are not altered significantly.

The populations of chains can be calculated by integrating the area underneath the corresponding Gaussian distribution in Figure 2; the results for the two bifurcated cases are presented in Figure 3.

For $\Sigma = 1.11$, the majority of the chains are nonstretched (● symbol in Figure 3) with nearly no stretched chains (△ symbol) until $|E| = 0.6$ where a crossover occurs. For field intensity greater than $|E| = 0.6$, the majority of chains are stretched (▲ symbol), while the minority are nonstretched (○ symbol). For the high grafting density $\Sigma = 11.1$, the majority of the chains are in the nonstretched state. At the high field $|E| = 1.0$, only about 6.25% of the chains are stretched by the field. The difference with the previous case can be attributed to the high charge density of the brush, which interacts strongly with the counterions. As a consequence, the chains and the ions

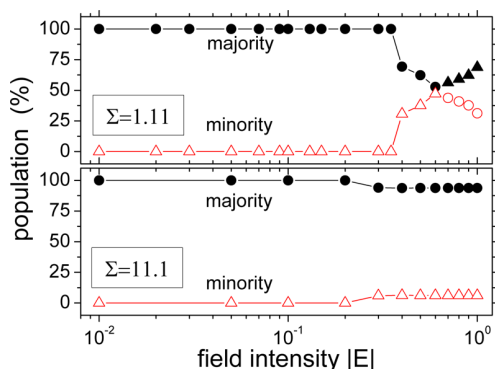


Figure 3. Monomer population (in percentage) vs field intensity, $|E|$, for surface coverages $\Sigma = 1.11$ (top) and $\Sigma = 11.1$ (bottom). The solid black symbols (● and ▲) denote the majority population. The ● represents a majority population of nonstretched chains, and the ▲ represents a stretched majority. The open red symbols, ○ and △, denote a minority population of nonstretched chains and a minority population of stretched chains, respectively.

form a stable chain–ion complex against the external electric field. Moreover, since the two chain states coexist in the brush, the system has two local minima and bifurcation could be a first-order phase transition. This topic merits a detailed investigation in the future.

Chain Structure and Orientation. The structure of chains in the brush systems can be studied by computing the ratio of $\langle R_c^2 \rangle$ and $\langle R_g^2 \rangle$ as shown in the upper panels of Figure 4. It is

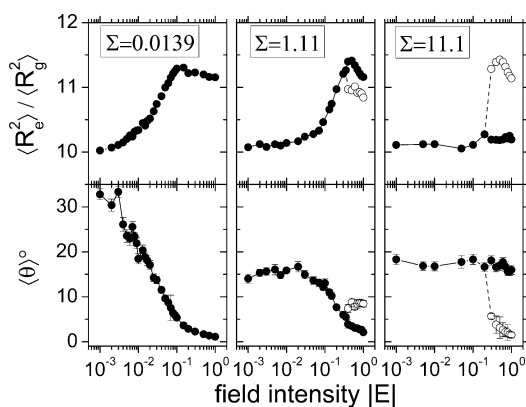


Figure 4. The conformational ratio, $\langle R_c^2 \rangle / \langle R_g^2 \rangle$, and mean polar angle, $\langle \theta \rangle$, as a function of field intensity $|E|$ for three different surface coverages, $\Sigma = \{0.0139, 1.11, 11.1\}$. The solid and the open symbols denote the values calculated from the dominant and minor groups, respectively, in the bifurcated phase region.

known that the ratio is 6 for an ideal chain, 6.3 for a self-avoiding chain, and 12 for a rodlike chain in bulk solutions.⁵⁶ It is easy to show that if a chain forms a compact spherical globule with randomly positioned ends, the ratio is 2.

In the zero field, low grafting density limit, the conformational ratio is found to be approximately 10. Higher grafting densities only perturb $\langle R_c^2 \rangle / \langle R_g^2 \rangle$ slightly. This value is significantly larger than the measured value of 7.2 for polyelectrolytes in bulk solutions,⁵⁸ showing that the brush chains are elongated even in the absence of an electric field, as expected. Increasing the electric field causes the ratio to rise to a maximum value of 11.5 (only slightly less than the rodlike

limit of 12). Thus, the structure of the grafted chains is quite rodlike.

We also examine the orientation of the grafted chains by calculating the polar angle θ of the end-to-end vector (we verified that the azimuthal angle, φ , is randomly distributed). The θ angle is calculated with respect to the surface normal (the z axis).

The probability distribution for θ is not uniform. Figure 5 presents the $P(\theta)$ distributions in different electric fields where each curve is normalized under the condition $\int_0^{\pi/2} P(\theta) \sin \theta d\theta = 1$. In the zero-field limit at the smallest grafting density $\Sigma = 0.0139$ (Figure 5a), $P(\theta)$ is approximately a constant up to $\theta \approx 20^\circ$. Increasing field intensity concentrates the distribution toward $\theta = 0^\circ$. The chain ends lie within a conical region around the grafting axis and the higher the field intensity, the smaller the cone apex angle. For $\Sigma = 1.11$ shown in Figure 5b, the range of $P(\theta)$ decreases with $|E|$ at first but then keeps roughly constant over the range of $|E| \gtrsim 0.35$. Simultaneously, the width near the grafting axis shrinks, owing to the coexisting phase of highly stretched chains. For the high grafting density (Figure 5c), no significant change in the distribution range is observed but the central peak of $P(\theta)$ starts to surge when $|E| \gtrsim 0.2$, which corresponds to the transition to the stretched-chain phases at $|E^*|$.

The mean value of the polar angle, $\langle \theta \rangle$, shown in the lower panel of Figure 4, decreases from 33° to $\sim 0^\circ$ for the case of $\Sigma = 0.0139$, showing that the chains are forced to align along the field direction. Alignment of this nature for tethered polyelectrolyte chains in electric fields has been observed in experiments⁵⁹ and simulations.^{60–62} For the intermediate grafting density $\Sigma = 1.11$, the angle is 14° in weak fields and significantly decreases when $|E| > |E^*|$. In the bifurcated phase, the nonstretched chains have a larger $\langle \theta \rangle$ than the stretched chains, as expected. For $\Sigma = 11.1$, the main population of chains explore a slightly larger angle ($\langle \theta \rangle \approx 15^\circ$). It is the subordinate, the stretched chains, which are much more perpendicular to the substrate ($\langle \theta \rangle \approx 3^\circ$).

Monomer and Counterion Distributions. It is vitally important to understand how the monomers and counterions are distributed in a brush system. Figure 6 presents the variation of the vertical concentration of these particles, $\phi_{\text{mon}}(z)$ and $\phi_{\text{ion}}(z)$, above the substrate surface for the three grafting densities. The concentrations are divided by a factor $\sigma_g b_0^{-1}$ to make them dimensionless quantities.

For the low grafting density $\Sigma = 0.0139$, $\phi_{\text{mon}}(z)$ displays a plateau structure prior to a small maximum near the surface of the brush, after which the curve rapidly decreases to zero (in Figure 6a). Viewed in this way, the shape is similar to the so-called stem-and-flower conformation.⁶³ The plateau value decreases as $|E|$ increases. At the same time, the span of the brush increases to higher values because the area under the curve is constrained by the constant number of monomers.

Similarly, the counterion distribution $\phi_{\text{ion}}(z)$ is strongly influenced by the electric field. At $|E| = 0$, $\phi_{\text{ion}}(z)$ displays a plateau value of $\approx 1.6\sigma_g b_0^{-1}$ in the region $z < 0.5Nb_0$, suggesting that about 80% of the counterions remain within the region occupied by the chains. A fraction of the counterions remain outside the layer region for entropic reasons, resulting in the tail of $\phi_{\text{ion}}(z)$ in the large z region. As $|E|$ increases, the counterions are pushed toward the substrate and the tail decreases. At $|E| = |E^*| \approx 0.02$, $\phi_{\text{ion}}(z)$ looks similar to $\phi_{\text{mon}}(z)$. When $|E| > |E^*|$, nearly all counterions reside within the chain layer (we will return to this point later) and $\phi_{\text{ion}}(z)$ develops a

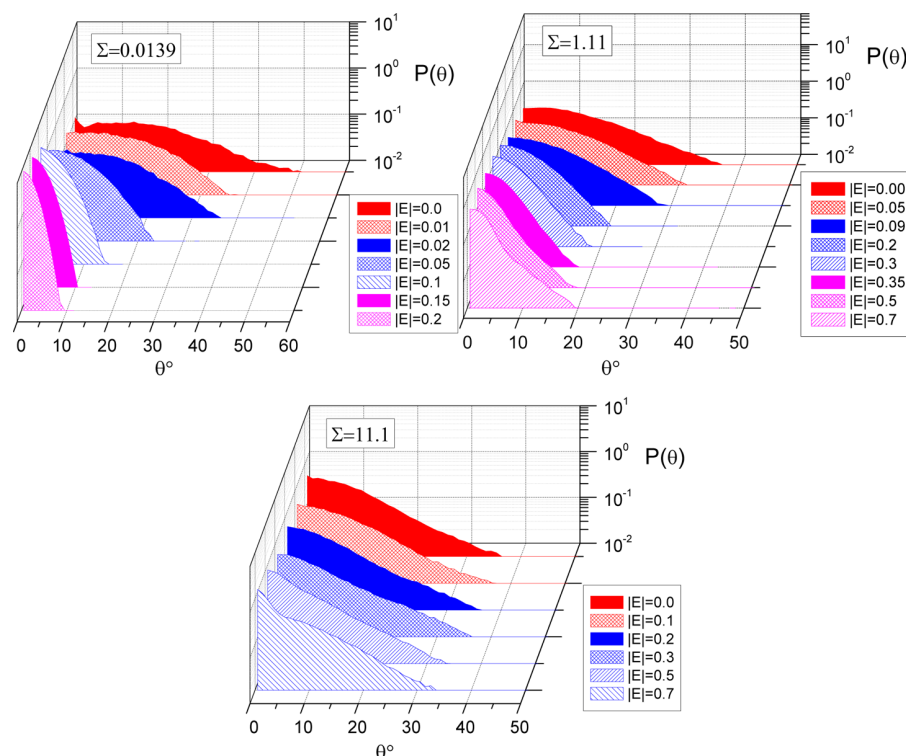


Figure 5. Probability distribution for the polar angle θ in different electric fields at surface coverages (a) $\Sigma = 0.0139$, (b) $\Sigma = 1.11$, and (c) $\Sigma = 11.1$. The field intensities $|E|$ are indicated in the figure.

peak near the substrate surface. This peak value increases with the field intensity and when $|E| \geq 0.15$, the profile of $\phi_{\text{ion}}(z)$ approaches an exponential distribution, similar to what we expect for the sedimentation of ions driven by a strong electric field.^{64,65}

At the intermediate density $\Sigma = 1.11$, the profile of $\phi_{\text{ion}}(z)$ matches well the $\phi_{\text{mon}}(z)$ profile found in weak fields $|E| < |E^*| \simeq 0.09$ (Figure 6b). Therefore, nearly all the counterions stay inside the brush region and the brush is neutralized by the counterions, unlike the previous case, $\Sigma = 0.0139$, where the net charge in the chain layer was negative in weak electric fields. In the bifurcated region above $|E^*| \simeq 0.35$, the monomer distribution $\phi_{\text{mon}}(z)$ shows two plateaus. The $z > 0.7Nb_0$ plateau is contributed by the stretched chains, whereas the plateau in $z < 0.7Nb_0$ results from both the stretched and the nonstretched chains. Since the grafting density is about 80 times larger than the previous case, the counterions need to overcome a greater osmotic pressure to enter into the brush region. Except at the strongest fields, $\phi_{\text{ion}}(z)$ displays a plateau profile over the range of $0.2 \lesssim z/(Nb_0) \lesssim 0.6$.

Noticeably, some condensed counterions are encouraged to move against the field by the oppositely charged chains. As a result, $\phi_{\text{ion}}(z)$ is broadened up to a region around $z = 0.9Nb_0$. In very strong fields $|E| \geq 1.5$ (not shown), all of the chains stretch, and the brush returns to a single phase. Due to the strong alignment for the chains, the monomers are essentially frozen in a fixed position perpendicular to the substrate surface, and consequently, $\phi_{\text{mon}}(z)$ exhibits an oscillatory behavior above the tethered points. A dense layer is formed for the counterions near the substrate surface. However, the counterions are not limited by connectivity, have more states available to them, and therefore the oscillatory behavior is smeared out. We will discuss this in more detail later.

Since the chain charge density is very high at $\Sigma = 11.1$, the counterions are trapped tightly inside the brush region. As a consequence, the concentration $\phi_{\text{ion}}(z)$ follows the profile of $\phi_{\text{mon}}(z)$, as shown in Figure 6c. Similar to the intermediate grafting density case, $\phi_{\text{mon}}(z)$ displays a protruding tail at $z \gtrsim 0.65Nb_0$ beyond bifurcation ($|E| > |E^*| \simeq 0.2$). The low concentration in the tail region suggests that only a few chains are in the stretched state. Simultaneously, we observed that a dense ion layer is established in the immediate vicinity of the substrate surface when $|E| > 0.2$. A small protruding tail remains above $z > 0.65Nb_0$ because the counterions are attracted by the stretched chains. Remarkably, the profiles of $\phi_{\text{mon}}(z)$ and $\phi_{\text{ion}}(z)$ are not significantly altered by the electric fields compared to the previous cases.

It is worth noticing that the vertical charge density of the $\Sigma = 0.0139$ and 1.11 systems exhibit two regions in strong electric fields: negative in the upper region of the brush and positive in the lower region (data not shown). For the high grafting density $\Sigma = 11.1$, this charge splitting picture is not as significant because the two charge densities are nearly identical.

The above discussion dealt only with the vertical of the mean vertical concentration for monomers and counterions. In order to elucidate the three-dimensional distributions, we calculated the top-view and side-view density distributions for both monomers and counterions. The top-view density, σ_{mon}^{xy} , shows the surface density distribution of monomers in the xy plane, integrated perpendicularly from the substrate. The side-view densities, σ_{mon}^{xz} and σ_{ion}^{xz} , show the surface densities in an xz plane that was oriented along a row of grafting points. In addition to the density distributions discussed here, further views and systems can be found in the Supporting Information.

At low grafting density ($\Sigma = 0.0139$; Figure 7), the chains are never in contact with each other, as can be clearly seen from all

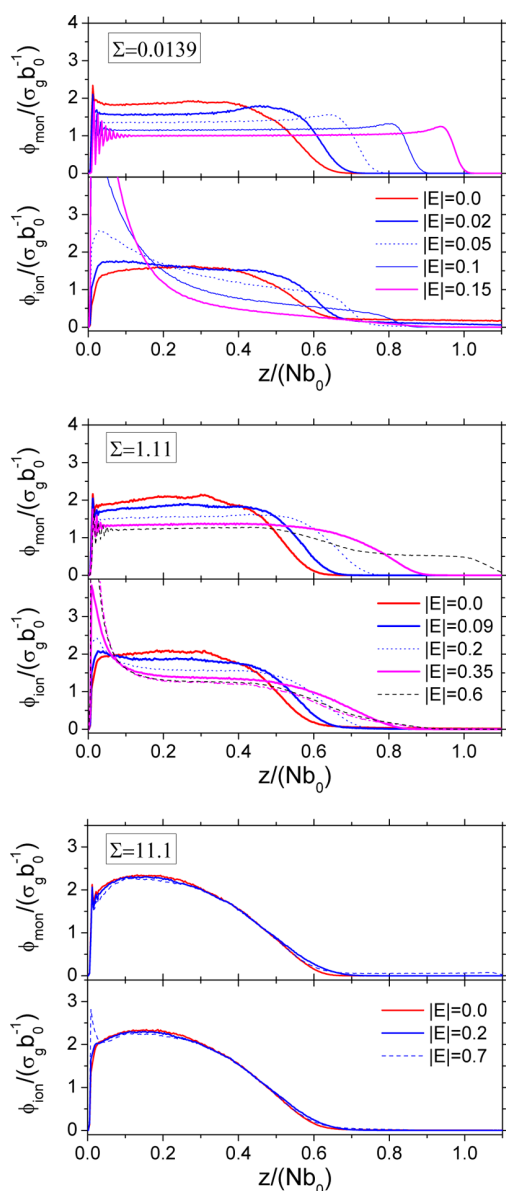


Figure 6. Vertical concentrations of monomer $\phi_{\text{mon}}(z)$ and of counterion $\phi_{\text{ion}}(z)$ in different electric fields at surface coverages (a) $\Sigma = 0.0139$, (b) $\Sigma = 1.11$, and (c) $\Sigma = 11.1$. The field intensity, $|E|$, is indicated in the figure and the vertical position, z , is rescaled by the contour length, Nb_0 .

monomer density graphs; the brush system is technically in the mushroom regime. Due to the electrostatic attraction by the chains, the counterion distribution shadows the mushroom profile of the monomers in the low field case $|E| = 0.005$. However, the counterion density is not zero above the mushrooms. As the field intensity increases and surpasses the critical value $|E^*| \simeq 0.02$, the mushroom profile elongates (becoming more of a “morel” profile than a standard mushroom) and eventually transforms to a “needle” profile, such as shown in Figure 7 for $|E| = 0.2$. Concurrently, the counterions are pushed toward the substrate. The situation is similar to a sedimentation problem, except that the needlelike chains attract surrounding counterions, which significantly perturbs the profile of the ions deviating from a simple exponential distribution.

Increasing the grafting density significantly alters these distributions. In the presence of a weak field, $|E| = 0.01$, and moderate grafting density, $\Sigma = 1.11$ (Figure 8a), the side-view monomer density shows bowl-like patterns. Each bowl sits on the tethering point with a bowl height of approximately $0.5Nb_0$. The bowl edges slightly overlap with each other, as expected for this intermediate grafting density. The top-view density $\sigma_{\text{mon}}^{\text{xy}}$ supports this picture, exhibiting a cell-like pattern. The side-view counterion density displays a similar pattern, but now a nonzero density appears both above the brush region (just like in Figure 6) and, interestingly, between the chains. At $|E| = 0.6$, shown in Figure 8b, the brush chains are split into two groups. The top-view monomer density, $\sigma_{\text{mon}}^{\text{xy}}$, displays both small and large cells, corresponding to the stretched and nonstretched chains, respectively. The stretched chains exhibit funnel-like patterns of height $\simeq 1.05Nb_0$ when viewed from the side. As a result, $\sigma_{\text{mon}}^{\text{xz}}$ displays vase-like silhouettes in which the stretched chains contribute to the necks of the vases and the nonstretched chains to the bottle bodies. The counterion density exhibits flaconlike patterns. The bottleneck is much shorter than in the monomer vase patterns because the ions are pushed toward the substrate. There is an accumulation of ions near the substrate surface and the rest stay between the flacons.

As the field intensity increases, the number of small cells increases but the size of the cell decreases as seen in Figure 8c for $|E| = 1.0$. In a strong field such as $|E| = 1.5$, the split brush recombines into one phase. Only small cells are exhibited by $\sigma_{\text{mon}}^{\text{xy}}$ and $\sigma_{\text{mon}}^{\text{xz}}$ shows tall funnel patterns at each tethering point (see Figure S5d of the Supporting Information). The flacon patterns for the counterion density transform to candlelike peaks. The monomers become very concentrated, while the counterions are spread out uniformly, except near the tethering points and the substrate.

To further verify that the bifurcation is not caused by finite size effects, top-view densities of monomers at high fields for an 8×8 system have been calculated in Figure S7 of the Supporting Information. The profile exhibits the same mixture of larger and smaller cells as seen in Figure 8.

At the high grafting density $\Sigma = 11.1$, the chains are well within the brush regime such that the monomer density is more homogeneous than in the previous cases (see Figure 9a). At $|E| = 0.7$, one single chain is stretched, while the rest remain relaxed. The top-view density displays a concentrated spot at one tethering point and a corresponding tower pattern surmounts the brush layer in the side-view density. Due to the attractive interaction, some counterions are elevated by the stretched chain and, hence, the density $\sigma_{\text{ion}}^{\text{xz}}$ shows a hump above the main brush about the stretched chain. When $|E| = 1.5$, two chains stretch (refer to Figure S6c of the Supporting Information). Because the electrostatic repulsion between the chains are not screened, the two tower patterns tilt slightly outward in the side-view density. In a single simulation run, we never observe the identity of the stretched chains to change, which is strong evidence that the system is trapped in a local minimum, so deep that a transition can hardly occur in a single MD run.

Counterion Condensation and Confinement. We have seen that the density of counterions is very high in the immediate vicinity of the chains due to electrostatic attraction. Some of these ions are presumably condensed on the chains themselves. Counterion condensation has attracted much attention over the past years.^{66–69} In order to study counterion condensation within our brushes, an ion will be defined to be

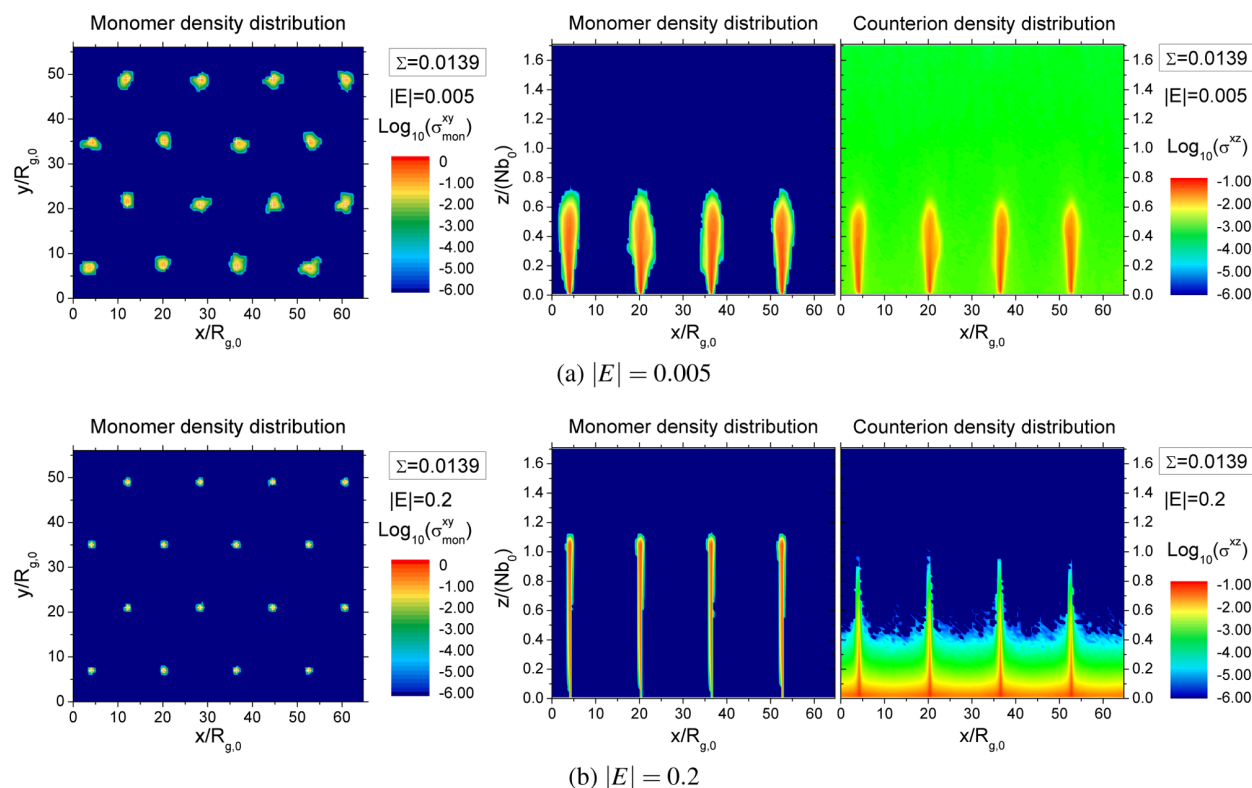


Figure 7. Top-view and side-view densities for monomers and counterions at surface coverage $\Sigma = 0.0139$. The field intensity is (a) $|E| = 0.005$ and (b) $|E| = 0.2$. The densities are presented using a log scale, which can be read from the color scale.

condensed to a chain if the distance to any chain monomer is smaller than the Bjerrum length, λ_B . A similar criterion has been used by Manning to study ion condensation.^{66,70}

Figure 10 shows the fraction of counterions condensing on the brush chains, χ_{ion} , for different grafting densities as a function of $|E|$. In weak electric fields, χ_{ion} is constant, as one might expect. At the small grafting density, $\Sigma = 0.0139$, 56% of the counterions are condensed. In accordance with Manning's theory, counterions condensing on rigid polyelectrolytes in a dilute solution should reduce the linear charge density of the chains to be effectively $-e/\lambda_B$ so that $\chi_{ion} = 1 - b_0/\lambda_B = 61\%$, a value that is slightly larger than the simulation value. This small difference is probably due to the fact that our chains are not rigid, which deviates from the hypothesis of the Manning's theory. Increasing the grafting density increases the fraction of ion condensed, but when $|E|$ is increased to be larger than the critical field, $|E^*|$, χ_{ion} decreases.

The interplay between entropy, the attraction to the chains and the applied field results in a nonmonotonic behavior of the counterion distribution inside the brush, as already discussed with respect to Figure 7, Figure 8, and Figure 9. To understand the details that impact the likelihood of counterion condensation, we also calculated the probability distribution of condensation as a function of height, $P_{ion}(z)$, for different Σ and $|E|$ (see Figure S8 of the Supporting Information). When Σ is small and there is no external field, $P_{ion}(z)$ displays a plateau, followed by a small, broad maximum. The profile is similar to the monomer distribution seen in Figure 6. With increasing grafting density, the maximum shifts toward the substrate, though the chain height $\langle Z_{max} \rangle$ remains basically unchanged (see Figure 1) suggesting that the condensed counterions are then situated deeper inside the brush. The condensed

counterions are pushed toward the substrate when an electric field is applied, but at the same time, they are dragged upward by the stretching of the chains. Consequently, $P_{ion}(z)$ deviates from the profile of the monomer distribution and the local maximum gradually disappears. For $\Sigma = 0.0139$, the condensation rather linearly decreases from the substrate when $|E| \geq 0.15$. In contrast to lower grafting densities, $P_{ion}(z)$ is not significantly affected by the electric field at $\Sigma = 11.1$. Therefore, tight counterion condensation occurs when the grafting density is high.

It is also interesting to study the confinement of ions to the "brush region" since condensation to individual chains does not provide complete information. We define the brush region to be $z \leq \langle Z_{max} \rangle$. Figure 11 presents the fraction of the counterions, κ_{ion} , located in the brush region. In the zero field about 82% of the counterions are confined to the brush for $\Sigma = 0.0139$. On the other hand, for the large grafting density $\Sigma = 11.1$ and 11.1, the confined fraction surges to the remarkably high values of 97% and 99%, respectively, in the zero field limit. We can see that applying the electric field causes κ_{ion} to monotonically approach 1.0. When the brush bifurcates, the two layers of the brush define two values for $\langle Z_{max} \rangle$, as discussed in regards to Figure 1; therefore, two fractions can be calculated. The difference between the two values gives the fraction of the counterions confined in the upper brush region. Figure 11 shows that less than 6% of the counterions are trapped in the upper region, which can be confirmed by referring to the side-view density distributions in Figures 8b and 9 (panels b and c).

Scaling Law for the Critical Electric Field $|E^*|$. As previously defined, $|E^*|$ represents the critical field beyond which the chains start to stretch. At a low grafting density, all

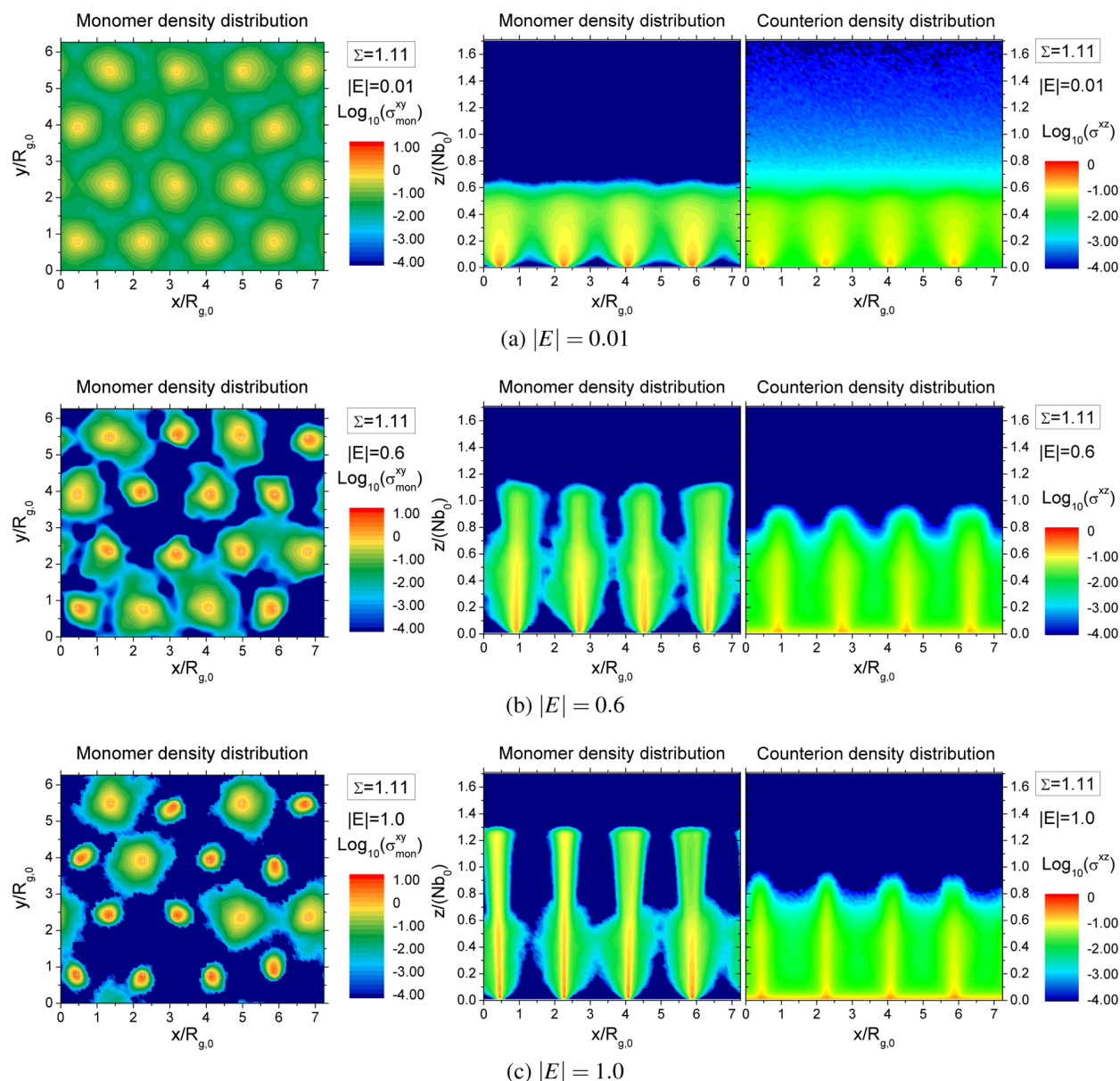


Figure 8. Top-view and side-view densities for monomers and counterions at surface coverage $\Sigma = 1.11$. The field intensity is (a) $|E| = 0.01$, (b) $|E| = 0.6$, and (c) $|E| = 1.0$. The density value can be read from the color scale.

the chains stretch together when $E > |E^*|$. At an intermediate grafting density, the chains first stretch together and then in a higher field region, $|E^{**}| < |E| < |E^{***}|$, a subset of them continue to stretch, while the rest remain nonstretched. At high grafting density, only a minor portion of them stretch when $|E| > |E^*|$. For the five studied grafting densities $\Sigma = \{0.0139; 0.554; 1.11; 5.54; 11.1\}$, our simulations found that $|E^*| \simeq \{0.02; 0.07; 0.09; 0.15; 0.2\}$, respectively. Figure 12 shows that $|E^*|$ increases with Σ following an approximate scaling law, $|E^*| \sim \Sigma^{0.342(5)}$. In our study, the characteristic brush height, $h \simeq \langle Z_{\max} \rangle$, in the zero-field limit shows a much weaker power-law dependence on Σ as seen in the inset of Figure 12. Because of the competition between the chains' elastic energy and the counterion osmotic pressure, polyelectrolyte brushes in the absence of salt do not swell significantly.^{8,10} Our simulations show only a weak scaling dependence of $\langle Z_{\max} \rangle \sim \Sigma^{-0.022(4)}$.

The origin of the exponent relating the critical field $|E^*|$ to the grafting density Σ can be understood using the following mean-field argument. We consider the brush region as a dielectric slab, in which negatively charged monomers are immersed in a sea of counterions. The slab is polarized by the external electric field. The mean volume occupied by a monomer (or a counterion, although a minority of the latter are actually found above the brush) is given by $v \sim h/(N\sigma_g)$. This defines the length scale $l \sim v^{1/3} \sim [h/(N\sigma_g)]^{1/3}$. Chain stretching occurs when the energy, $W = eEl$ gained from moving a charge over a distance, l , exceeds $k_B T$. The critical electric field is thus given by

$$E^* \sim \frac{k_B T}{el} \sim \frac{k_B T}{e} \left(\frac{N\sigma_g}{h} \right)^{1/3} \quad (6)$$

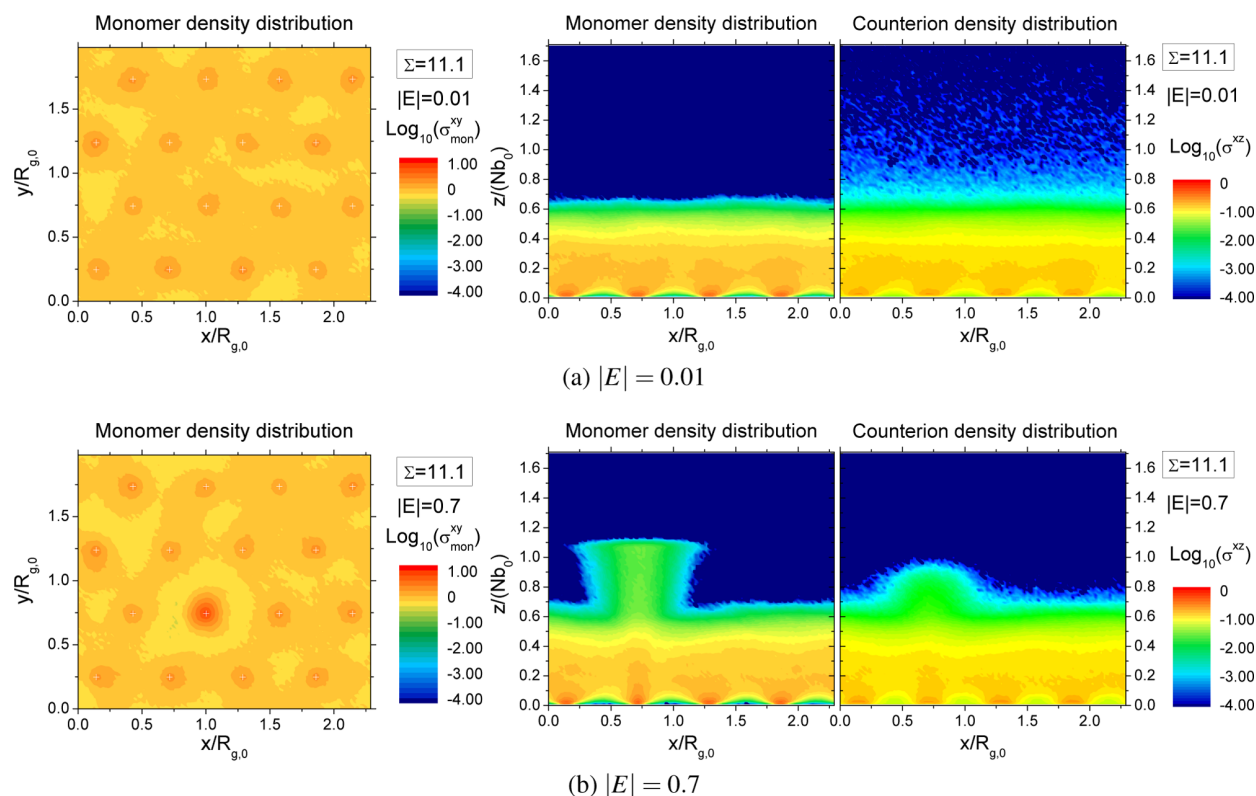


Figure 9. Top-view and side-view densities for monomers and counterions at surface coverage $\Sigma = 11.1$. The field intensity is (a) $|E| = 0.01$ and (b) $|E| = 0.7$. The density value can be read from the color scale.

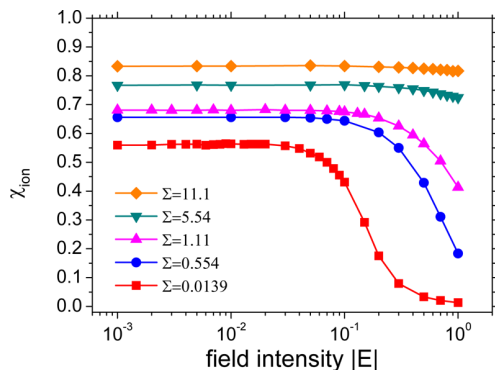


Figure 10. Fraction of condensed ions, χ_{ion} , as a function of field intensity, $|E|$, at different surface coverages, Σ .

This equation predicts a $1/3$ scaling on both the grafting density, σ_g , and the inverse brush height, $1/h$. We have plotted eq 6 in Figure 12 as a dashed, red line. The line is surprisingly close to the simulation data over several decades in grafting density, showing that the key physics has been captured in our simple mean-field theory. Note, taking into account the fact that the brush height, h , varies slightly with grafting density [as shown in the inset of Figure 12 with a weak scaling exponent of $-0.022(4)$], yields a total exponent of 0.341 , which is in excellent agreement with the results of Figure 12.

CONCLUSION

In this work, we have explored polyelectrolyte brushes subject to external fields normal to the grafting substrate and discovered that under certain conditions the ensemble of

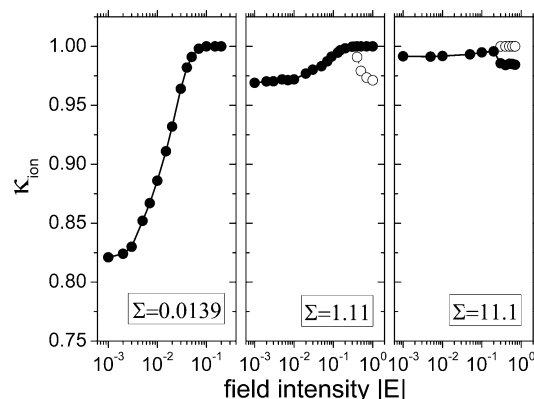


Figure 11. Fraction of counterions, κ_{ion} , in the brush region vs field intensity, $|E|$. When the brush is bifurcated, two fractions are given. The solid symbol denotes the value within the dominant chain brush layers.

chains bifurcates into two distinct populations. We summarize our results by presenting a schematic $\Sigma - |E|$ phase diagram in Figure 13.

The diagram shows snapshots of the simulations and where bifurcation occurs in the brush system. At low grafting density, all chains respond to the electric fields simultaneously: there is a unique brush height. At intermediate grafting density, the brush shows two coexisting heights in the region between two critical fields, $|E^{**}|$ and $|E^{***}|$. The majority of the chains are stretched and elongated, while the minority stay in a nonstretched state. The nonstretched chains have approximately the same height as the brush chains in weak electric

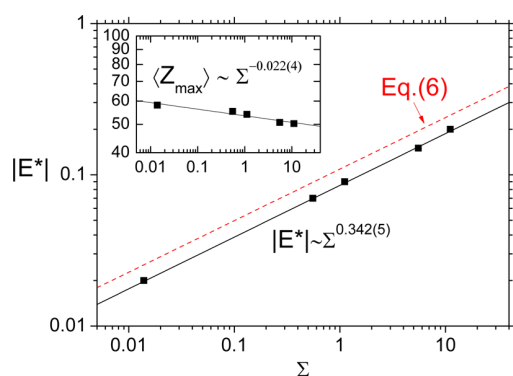


Figure 12. Critical field intensity $|E^*|$ as a function of the reduced grafting density, Σ . We show the data, best fit, and theoretical prediction. Inset: The mean maximum height of the monomers, $\langle Z_{\max} \rangle$, as a function of reduced grafting density, Σ .

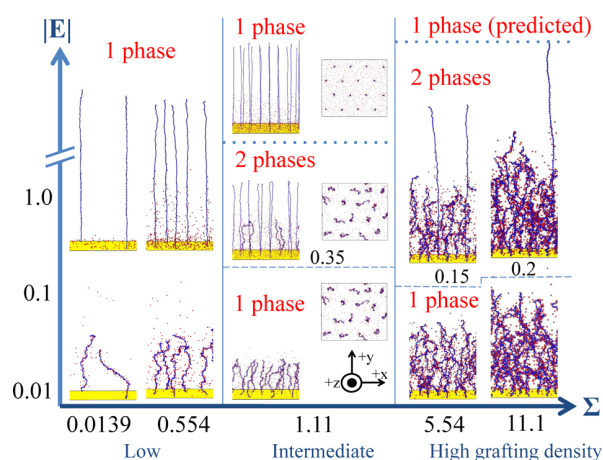


Figure 13. Schematic phase diagram for our brushes. The critical electric fields $|E^{**}|$ and/or $|E^{***}|$ are indicated in the figure in dashed lines.

fields. For electric fields stronger than $|E^{***}|$, the brush recombines into a uniform system. At high grafting density, the bifurcation occurs in a different manner. Very few chains are stretched when $|E| \geq |E^{**}|$; most chains remain in an unstretched state. These chains and the counterions form a very stable complex against the action of the external electric field.

Simultaneously to these changes to chain conformations, the counterion distributions respond to the applied electric field. In the absence of an electric field, we find a counterion distribution that corresponds to the monomer distribution when grafting densities are high (97% and 99% of ions are confined to the brush at intermediate and high grafting densities, respectively) but when grafting density is low (in the mushroom regime), only about 82% of counterions reside in the region occupied by the chains. When an electric field causes the population of chains to bifurcate, only 6% of counterions reside within the stretched region of the brush. Increasing the grafting density increases the fraction of counterions that are condensed to the backbone of the polyelectrolyte chains but above the critical field $|E^*|$ this fraction decreases, which allows for chain stretching. The tightest condensation occurs at the highest grafting densities. The critical field $|E^*|$ was found to scale with grafting density as $\sigma_g^{1/3}$. A mean-field theory suggests

that it is the confinement of the counterions in the brush which determines the critical field.

It is our hope that this detailed computational study on the structure of polyelectrolyte brushes will help guide the design of smart nanochannels. In particular, the bifurcation of polyelectrolyte chains into stretched and collapsed populations in response to electric fields may prove a valuable mechanism. For instance, utilizing polyampholytes or otherwise end-labeled charged chains may allow future researchers to bias or even choose which individual chains stretch and which remain collapsed. Another possible use is “fishing” (i.e., the tethering and subsequent trapping within the interior of the brush of analytes by alternating the electric field resulting in stretching and relaxation). The ability to have fine control over the number of rodlike objects extending from the surface may allow one to control the surface friction and flow resistance. These are only a handful of ideas on how such a stimuli responsive brush could be utilized as a component in future MEMS devices.

■ ASSOCIATED CONTENT

Supporting Information

Supporting material includes a suit of monomer and counterion distributions at different field strengths as well as simulations of larger and smaller system sizes to verify the absence of finite size effects. Phase recombination in high electric fields is also shown. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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