# Optimization of the Synthesis Nickel Nanoparticles for the Purification of the ToxA5.1 Recombinant Protein

Kevin Nhan, Albert Parisien, Aatika Ahmed, Dr. Christopher Lan, Dr. Jules Thibault of the Chemical and Biological Engineering Department

## Introduction

Nickel nanoparticles (NNP) were synthesized from modified polyol method using ethylene glycol as a reducing agent, nickel hydroxide ($Ni(OH)_2$) as a source of oxidized nickel, polyvinylpyrrolidone (PVP) as a protective agent, and palladium chloride ($PdCl_2$) as a nucleating agent. Reaction conditions were investigated in order to determine what ideal concentrations of nickel hydroxide and PVP, pH, and time would produce NNP to best serve as a magnetic absorbent to be used to purify a hexa-histine tagged recombinant protein (ToxA5.1) from genetically engineered *E.coli.*

## Background

Tox A5.1 is an antibody used to fight against *Clostridium Difficile.* It is a single domain 15 kDa protein. In 2003, a large outbreak of *C. Difficile* in Quebec had killed over 2000 individuals, and 5 years later, 63 people in Ontario had their lives taken. Over the past summer there have been more than 20 deaths reported in the Niagara region alone. Within the United States, *C. Difficile* infections cost approximately 1.1 billion dollars are invested annually. Given rising antibiotic resistance, there is a need for new alternatives to combat this bacterium.

## Synthesis of Nickel Nanoparticles

In order to model the protein purification capability of the magnetic nickel nanoparticles six different syntheses were prepared. The four reaction conditions which varied throughout the syntheses were concentration of $Ni(OH)_2$ and PVP, pH, and the duration of the reflux reaction. The range of the suitable values for the variables were distributed into six increasing levels (**Table 1**)

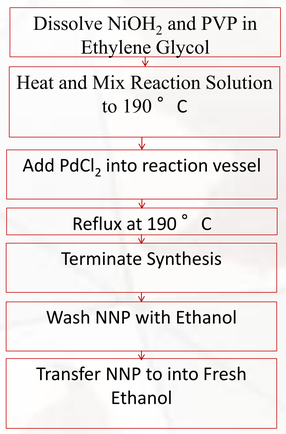
As determined by the statistical analysis of uniform design, the various levels of the four different starting reaction conditions were distributed amongst the six different syntheses (**Table 2**). These are not randomly distributed, magnitudes of the values are uniquely prescribed for the number of variables and experimental syntheses.

**Table 1**. List of reaction conditions and their varying level of intensities used for the syntheses of NNP.

| Magnitude of variable | $Ni(OH)_2$ (g/L) | PVP (g/L) | pH | Time (hours) |
|---|---|---|---|---|
| 1 | 2.0 | 0.20 | 7.0 | 0.5 |
| 2 | 5.6 | 0.76 | 7.8 | 0.9 |
| 3 | 9.2 | 1.32 | 8.6 | 1.3 |
| 4 | 12.8 | 1.88 | 9.4 | 1.7 |
| 5 | 16.4 | 2.44 | 10.2 | 2.1 |
| 6 | 20.0 | 3.00 | 11.0 | 2.5 |

**Table 2**. The reaction conditions for the modified polyol method of six unique syntheses of NNP as determined by uniform design.

| Experiment | $Ni(OH)_2$ (g/L) | PVP (g/L) | pH | Time (hours) |
|---|---|---|---|---|
| 1 | 16.4 | 1.88 | 11 | 0.9 |
| 2 | 12.8 | 3.00 | 9.4 | 2.5 |
| 3 | 9.2 | 0.2 | 8.6 | 0.5 |
| 4 | 20.0 | 1.32 | 7.0 | 1.7 |
| 5 | 2.0 | 2.44 | 7.8 | 1.3 |
| 6 | 5.6 | 0.76 | 10.2 | 2.1 |



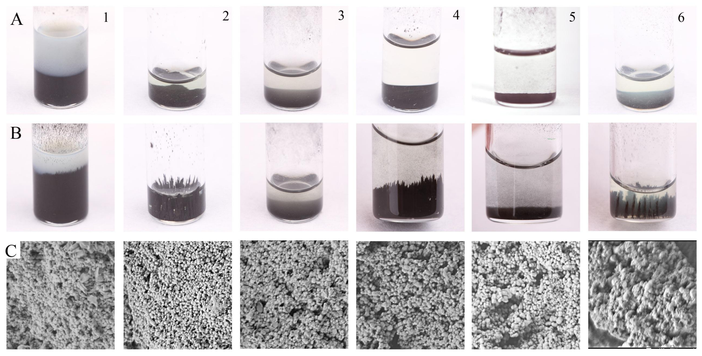




**Figure 1**. Six syntheses of nickel nanoparticles (NNP) produced under varying conditions of [Ni(OH)] , [PVP] , pH, and reaction time. Row A displays NNP without influence of a magnet. Row B shows the influence of NNP under the presence of a magnet. Row C contains pictures of SEM at 40 000 magnification.

## Protein Purification and Quantification

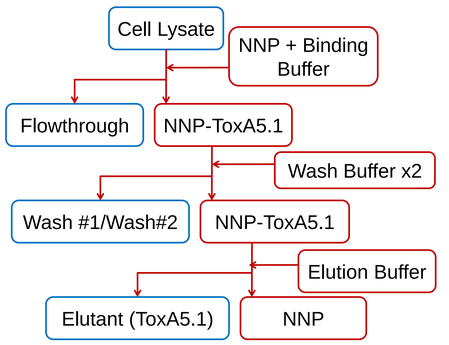



**Figure 2.** Flow chart of one cycle of the protein purification process. This cycle is repeated three times throughout the protocol. Supernatants (in blue) are analyzed by SDS-PAGE.

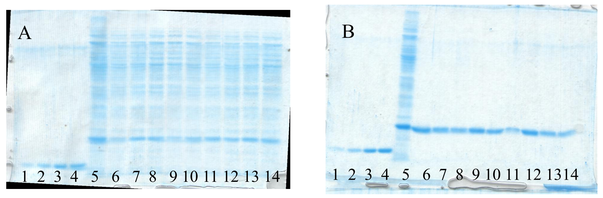



**Figure 4**. Flowthrough (A) and eluants (B) protein quantification analysis by SDS-PAGE analysis purified by Synthesis 5. Lanes 1-4 contain BSA standards, lane 5 is the initial cell lysate, and lanes 6-8, 8-11, and 12-14 are triplicates of the flowthrough from the three purification cycles.

| Synthesis | Mass of Protein Purified per Mass NNP Used (ng/mg) |
|---|---|
| 1 | 68.0 |
| 2 | 52.0 |
| 3 | 230.0 |
| 4 | 90.0 |
| 5 | 504.0 |
| 6 | 28.0 |

**Table 3**. Total mass of ToxA5.1 purified per amount of nickel nanoparticles used during the purification for three purification cycles. Mass of protein purified was quantified using SDS-PAGE analysis. Mass of NNP used in purification was determined by the concentration of Ni quantified by FAA spectroscopy multiplied by the volume of NNP used for each purification

## ANOVA and Uniform Design

There are 6 linear equations (6 syntheses) with 5 unknowns (α, β, ε, τ, Ω ). A system of equations with 5 unknowns only requires 5 equations to be solved.

$$y = \alpha x_1 + \beta x_2 + \varepsilon x_3 + \tau x_4 + \Omega$$
$$y = \alpha x_1 + \beta x_2 + \varepsilon x_3 + \tau x_4 + \Omega$$
$$y = \alpha x_1 + \beta x_2 + \varepsilon x_3 + \tau x_4 + \Omega$$
$$y = \alpha x_1 + \beta x_2 + \varepsilon x_3 + \tau x_4 + \Omega$$
$$y = \alpha x_1 + \beta x_2 + \varepsilon x_3 + \tau x_4 + \Omega$$
$$y = \alpha x_1 + \beta x_2 + \varepsilon x_3 + \tau x_4 + \Omega$$

$$y = \alpha(5) + \beta(4) + \varepsilon(6) + \tau(2) + \Omega$$
$$y = \alpha(4) + \beta(6) + \varepsilon(4) + \tau(6) + \Omega$$
$$y = \alpha(3) + \beta(1) + \varepsilon(3) + \tau(1) + \Omega$$
$$y = \alpha(6) + \beta(3) + \varepsilon(1) + \tau(4) + \Omega$$
$$y = \alpha(1) + \beta(5) + \varepsilon(2) + \tau(3) + \Omega$$
$$y = \alpha(2) + \beta(2) + \varepsilon(5) + \tau(5) + \Omega$$

$x_1$ – Concentration of $Ni(OH)_2$
$x_2$ – Concentration of PVP
$x_3$ – pH of Reaction Mixture
$x_4$ – Time of Reaction Duration
y – Mass ToxA5.1 Purified per Mass NNP used (ng/mg)

It was determined by ANOVA analysis that sole contributing factor on protein purification was dependant on pH.

## Investigating Morphology and Efficiency of NNP Protein Purification

Correlations were formed between the initial reaction conditions and the protein purification capabilities of the NNP. However, properties such as shape and size were also used to further investigate the behaviour of NNP during ToxA5.1 purification.

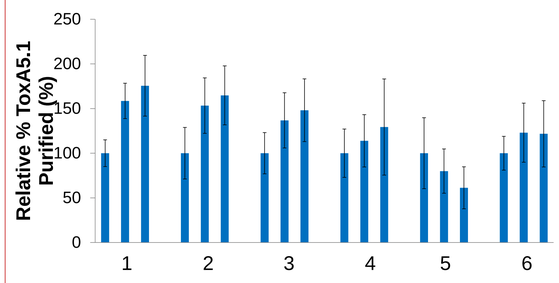



**Figure 5**. Relative % ToxA5.1 purified during three cycles of purification with NNP particles. Amounts of protein were quantified from eluants and ratios between the first cycle eluant were taken as percentages. Syntheses are represented as 1 through 6, where each bar is representative of the protein content of eluant of each purification cycle.

Efficiency of ToxA5.1 elution increased with the number of cycles. It is thought that there is remaining recombinant protein which stays adsorbed on the NNP until the next sequential purification cycle.

**Table 4**. Average particle diameter of the six syntheses of NNP. SEM images were taken (at X10000 and X40 000 magnification). Particle size was determined by pixel distance by Image J Pro, which were then scaled to length in nanometers.

| Synthesis | Average Diameter of Particle (nm) | Standard Deviation (nm) | Number of Particles Counted |
|---|---|---|---|
| 1 | 54.9 | 17.0 | 192 |
| 2 | 54.1 | 18.7 | 445 |
| 3 | 52.2 | 19.3 | 272 |
| 4 | 56.9 | 20.4 | 407 |
| 5 | 61.6 | 24.6 | 407 |

The mean of the average diameter of the six different syntheses of NNP was found to be 56 ± 3.6 nm. The lack of distribution in the diameter concludes that it is not a factor which affects protein purification.

## Conclusion

- Nickel nanoparticles were synthesized under varying reaction conditions which had produced distinct abilities in purifying protein.
- The efficiency of the NNP to purify protein ranged from 52 – 504 ng of ToxA5.1 purified/mg NNP used.
- It was predicted that the unique properties to form needles under the application of magnets was correlated to particle diameter size
- This was proven to be untrue as an average NNP diameter 56 ±3.6 nm was calculated from the six syntheses
- Anova and Uniform Design was used to find that the pH played a contributing role in the protein purifying ability.
- However the relationship between pH and purified protein is not very strong, there are other factors which play a role.
- More characterization of NNP needs to be done in order to find the properties of sphericity, surface area, porosity, and cooercivity in order to further develop the model.

Contact Information

Kevin Nhan
Third Year Undergraduate Candidate
Honour's Bachelor's of Science in Biochemistry
Bachelor's of Applied Science in Chemical Engineering (Biotechnology)
Faculty of Science and Engineering
University of Ottawa
613-744-2400

uOttawa