Synthesis and Characterization of Multiresponsive Core-Shell Microgels

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I. Abstract

Multiresponsive microgels can be used for more precise targeted delivery of cancer treatments to reduce the impact the medication has on healthy cells. In this experiment the findings of the microgels in *Synthesis and Characterization of Multiresponsive Core-Shell Microgels* by Clinton D. Jones and L. Andrew Lyon, were tested by analyzing the behavior of poly(N-isopropylamide) (p-NIPAm) core and NIPAm-co-acrylic acid (NIPAm-AAc) shell nanoparticles (NP) in response to temperature at constant pH. The microgels were prepared through radical polymerization and characterized by scanning electron microscopy (SEM) and atomic force microscopy (AFM). Although the more concentrated 1:10 dilution showed a significant decrease in NP diameter upon heating from room temperature to 45°C, the 1:100 dilution did not show significant deswelling. This was due to the large errors in NP size, which if reduced may indicate if, or if not, the 1:100 dilution is temperature sensitive.

II. Introduction

While current methods of cancer treatment, such as radiation therapy and chemotherapy, are effective at killing cancer cells, they present a significant risk to non-cancerous cells. A potential way to circumvent this issue is with the use of melittin, a peptide that is the main component of honeybee venom. Through previous in-vitro studies, melittin has been proven to be an effective inhibitor of tumor growth.[1] However, a delivery system targeting cancerous cells must be developed for melittin. A core-shell microgel that is multi responsive can perhaps be an optimal drug delivery system for melittin.[1] Multi-responsive microgels are more sensitive to their external environment, and thus could outperform their singular-responsive counterparts and provide more selective drug delivery.

Microgels are crosslinked polymers that are responsive to the environment, which includes factors such as temperature and pH. They are gaining increasing attraction in the medical field due to their use as drug delivery systems and as well as use in processes such as catalysis and chemical separations.[1] In this context, microgels are loaded with a desired compound, and, in certain conditions, will deswell and release the compound into the surrounding environment. Core-shell microgels can be constructed as well, which allows the microgel to be

environmentally-sensitive to two distinct factors. This increases the precision in what kind of environment the microgels will deswell.

The type of microgel used was poly(N-isopropylacrylamide) or pNIPAm, which is temperature-responsive. The efficacy of pNIPAm compared to other responsive polymers is due to the significant variation in physical and chemical properties during phase transition.[1] Phase transitions occur due to a variety of environmental factors, such as temperature for pNIPAm, and can change the hydrophobicity, particle size, porosity, refractive index, colloidal stability, scattering cross section, electrophoretic mobility, and rheology of the microgel. Current work on pNIPAm microgels focuses on multi-responsivity, in other words, environmental sensitivity to both temperature and another distinct factor.

The microgels were synthesized using free radical polymerization, which is a type of chain growth polymerization reaction in which a polymer is formed through the addition of radical species. First, an initiator molecule decomposes into radical species through for example heat or light, and then attacks the monomers to form radical monomers and initiate polymerization. This polymerization reaction is very commonly used.

To measure the particle size in response to the temperature, scanning electron microscopy (SEM), and atomic force microscopy (AFM) were used. A scanning electron microscope (SEM) uses a beam of high energy electrons to get information about the external morphology of solid species.[3] AFM uses a sharp tip that moves over a sample surface to visualize materials on the nanometer scale.[4]

III. Methods

In this experiment the independent variable was the temperature of the microgels, as pNIPAm is temperature responsive, and the dependent variable was the size of the microgels. The control group consisted of the nanoparticles at room temperature, and the experimental group were the nanoparticles that were heated to 45°C.

The materials, N-Isopropylacrylamide (NIPAm), acrylic acid (AAc), N,N'-methylenebis-(acrylamide) (BIS), sodium dodecyl sulfate (SDS), and ammonium persulfate (APS) were purchased from Sigma Aldrich and used as received.

1. **Synthesis of Nanoparticles**

The NIPAm core was synthesized by dissolving NIPAm and BIS in deionized (DI) water, followed by being degassed for 15 minutes. Next, SDS was dissolved in the monomer solution and filtered using a 0.2 μm Whatman syringe filter. The solution was then heated for one hour under constant nitrogen purge at maximum stir rate. After the addition of APS, radical polymerization occurred, and the reaction proceeded for five hours at 70°C. To obtain the NIPAm-AAc shell , NIPAm, BIS, and AAc were dissolved in DI water and stirred while SDS and APs were added. This solution was filtered the same way as above.

To construct the nanoparticles, the core solution was stirred and heated to 70°C for 15 minutes before being purged with nitrogen for one hour at room temperature. Next, the shell solution was added and the reaction proceeded for 30 min at 70 °C. Over the next 22.5 minutes, the remaining shell solution was added at a rate of 3.75 mL/min, and then the reaction continued to proceed for five hours at 70 °C.

To purify the core-shell solution a dialysis was performed. Six cm of dialysis tubing was cut and soaked in DI water for 10 minutes. Then, one end of the bag was tied and filled with the core-shell solution. A weight was attached at one end to keep the bag submerged in DI water, and the dialysis proceeded for two weeks. To confirm the dialysis, the conductivity of the excess DI water was measured.

2. **SEM Imaging**

Samples of the core and core-shell solutions were vortexed, followed by sonication for five minutes before being centrifuged at 15,000 rpm for five minute. The supernatant was replaced with water. The sample was then vortexed, sonicated, and centrifuged with the same procedure. The core and core-shell samples were then added to separate carbon tape and sputter coated with gold. The SEM used was a FEI Quanta 200 3D Dual Beam Scanning Electron Microscope.

3. **AFM Imaging**

The core-shell solution was drop cast onto a silicon wafer and imaged using the tapping mode with a CT170R tip. The images collected were 5x5 µm. Four samples were analyzed: a 1:10 core-shell dilution dried at room temperature, a 1:10 core-shell dilution dried at 45 °C for 10 minutes, A 1:100 core-shell dilution dried at room temperature, and a 1:100 core-shell dilution dried at 45 °C for 10 minutes.

IV. Results

Expected pH	Actual pH
10.01	9.7
7	7.1
4.01	4.2
NIPAm Core Measured pH	**NIPAm Core Calibrated pH**
3.2	**3.5**

Table 1. pH of core solution. The pH of known buffer solutions and the core solution were measured using a pH meter. The pH of the core solution was determined using a calibration curve.

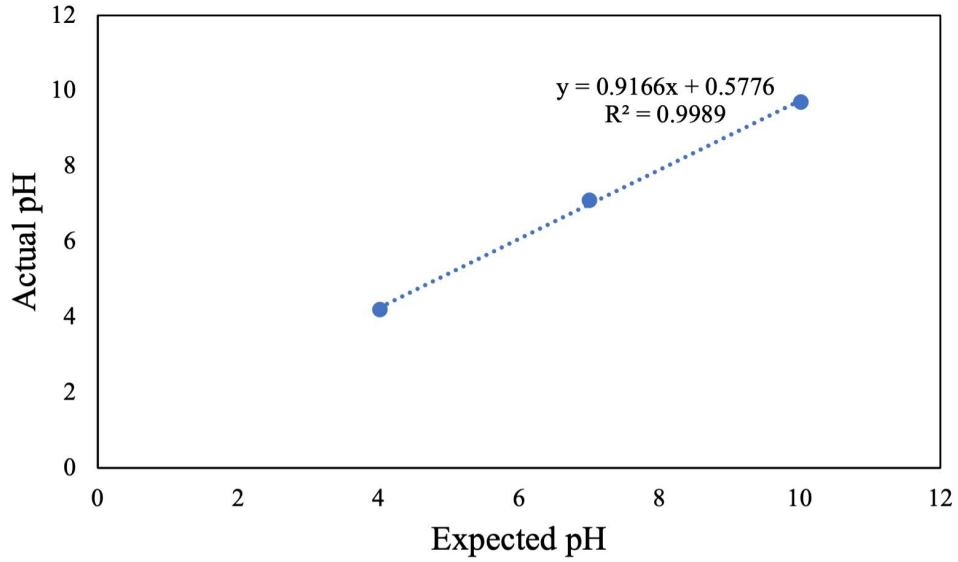
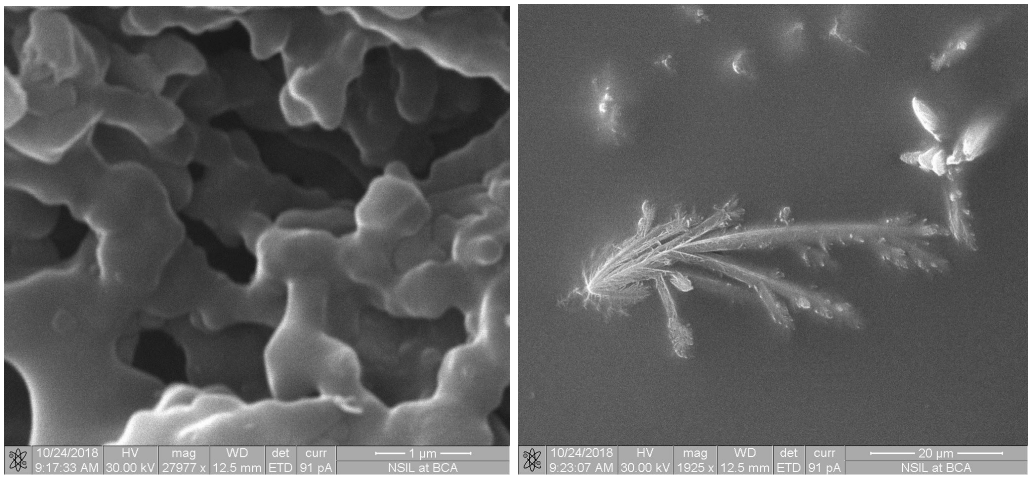


Figure 1. pH Calibration Curve. Used to determine the adjusted pH of the core solution.

Sample	DI Water (µS/cm)	Core (µS/cm)	Core-Shell (µS/cm)
10x dilution	13	30	25
1x dilution	1	3	2

Table 2. Conductivity of deionized after 13 days of dialysis. The conductivity of the water in the graduated cylinders was measured with a conductivity probe. The slight conductivity of the water indicates some particles from within the bag were able to pass through the bag membrane.



a b

Figure 3. SEM image before dialysis. (a) core at 1 µm (a) core-shell at 20 µm.

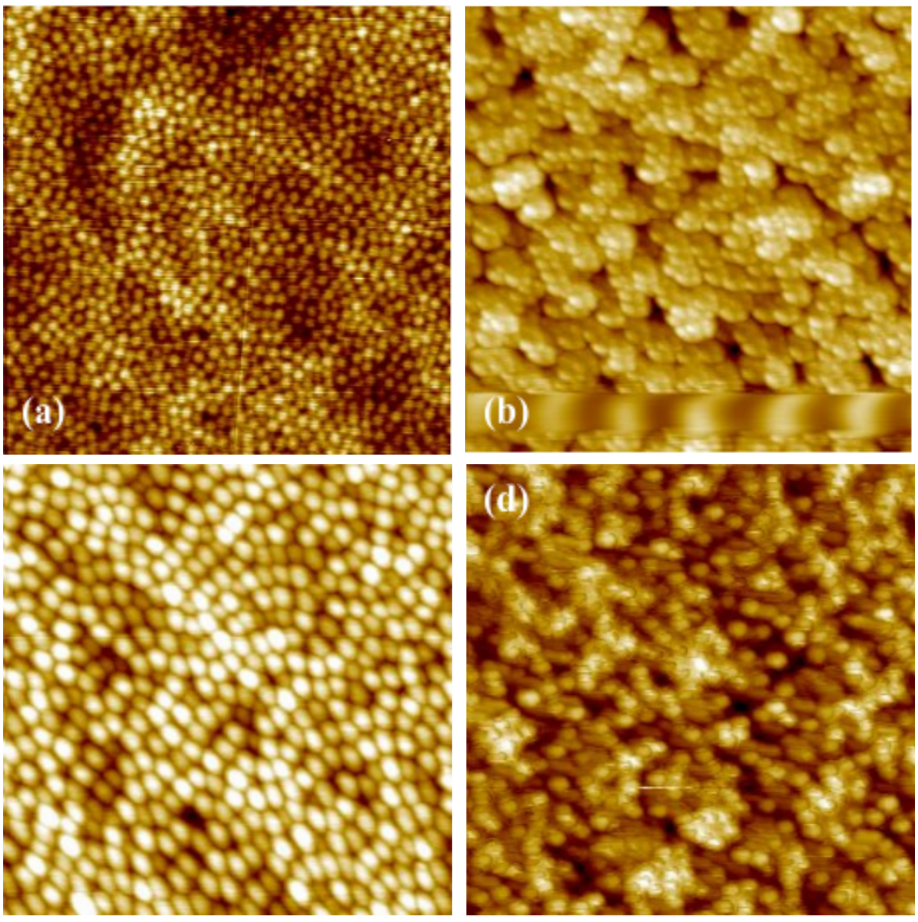


Figure 4. AFM imaging of core-shell nanoparticles. Top-down view of core-shell nanoparticles (5 μm x 5 μm) at (a) dried at room temperature, (b) 1:10 dilution dried at 45°C, (c) 1:100 dilution dried at room temperature, and (d) 1:100 dilution at 45°C. Tapping mode with CT170R tip was utilized to collect the data with TraxAFM.

	Solution 1	Solution 2
Average Deswelling (nm)	98	24
Error (nm)	33	70
% change	59	18

Table 3. Average Deswelling in the two Dilutions. Average deswelling way measured by subtracting the average particle size at 45°C from that at room temperature.

V. Discussion

Using the expected and actual pH values of the standard solutions, the calibration curve in Fig 1 was established and the actual pH of the NIPAm core solution was determined to be 3.5, as listed in Table 1. To ensure that the changes in nanoparticle size were only influenced by the temperature, all solutions were kept around pH 3.5 using buffers.

After the core nanoparticles were synthesized, a SEM image was taken as shown in Fig 3. The globular nature of the nanoparticles indicated that there were excess reagents coating the nanoparticles. To purify them dialysis was performed, and to ensure it was successful the conductivity of the DI water, core, and core-shell solutions were measured for the pure and 10x diluted solutions. The conductivity results are listed in Table 2. The conductivity of the DI water in both dilutions indicates that some particles from the dialysis bag were able to pass through the membrane and increase the conductivity of the DI water. SEM images after dialysis were attempted to be taken, however the purified NP were unable to remain on the carbon tape during the air drying process. In future experiments another drying technique, such as freeze drying, could be used to obtain SEM images of the purified NP. Therefore instead of SEM images, AFM images of the purified NP were obtained, as shown in Fig 4. From these images the particle sizes were determined by SPIP software.

Table 3 lists the average deswelling of the nanoparticles. The nanoparticles exhibited a 59% decrease in the radius of their core when diluted to a 1:10 ratio (solution 1) of their original concentration and a 18% decrease in a 1:100 dilution (solution 2). The average particle sizes at both room temperature and 45°C in solutions 1 and 2 had significant errors, and although the results from solution 1 show that there is a statistical difference in the particle size at room temperature and 45°C, the same cannot be said for solution 2. For solution 2 the large errors result in the average particle sizes at room temperature and 45°C to not be statistically different. Therefore only the results from solution 1 align with the results from previous studies, which found that the NP deswell significantly upon heating to 45°C.[1] The large errors in particle size may be the result of measuring the nanoparticle size using the images, which could lead to inaccuracies as the borders may be vague. Overall, the data shows that the 1:10 dilution of the NP was temperature responsive, but the 1:100 dilution was not.

Furthermore, this data shows there is a possible correlation between concentration of the nanoparticle solution and radius change. Nanoparticles in the more concentrated solution, 1:10, exhibited a significantly greater change in radius than the 1:100. This could be due to the increasing amount of nanoparticle interactions in the 1:10 solution, however, only two data points is not enough to fully theorize a correlation between concentration and the amount of deswelling.

VI. Conclusion

In this experiment the findings in *Synthesis and Characterization of Multiresponsive Core-Shell Microgels*, by Clinton D. Jones and L. Andrew Lyon, were evaluated by analyzing how NIPAm core and NIPAm-AAc shell nanoparticles reacted in response to temperature at a constant pH of 3.5. It was found that a 1:10 dilution of the core-shell solution was temperature sensitive and exhibited significant deswelling as the particle size was reduced by 59%. However the 1:100 dilution was found not to be temperature dependent as the particle size at room temperature and 45°C were not statistically different due to large errors in the particle sizes. These errors may have arisen in part due to the SPIP software that was used to determine particle size from AFM images. Although the findings from the 1:10 dilution match that of the previous study, the 1:100 dilution findings do not. To verify if the 1:100 dilution truly is not temperature responsive, or if

these findings are a result of the errors, further experiments at 1:100 dilution are necessary. Subsequent experiments at other dilution factors should be performed to better understand the significance of concentration.

VII. References

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Acknowledgements

We would like to thank the CURIE board (Mirai Kii, Minji Lee, Josephine Widjaja, Erin Rieckmann, and Caitlin Iswono) and our mentor, Lariana Cline. We would also like to extend our thanks to Professor Barranger, the sponsor of the CURIE decal. The data in this experiment was collected in the Chemistry & Nanotechnology Laboratory at Bergen County Academies in Hackensack, New Jersey. Research was performed under Dr. Deok-Yang Kim, with assistance from Dr. David Reeves in the Microscopy Laboratory at Bergen County Academies.