Synthesis of Thermoresponsive Poly(NIPAm) Core -Poly(NIPAm)-AAc Shell Nanoparticles

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Abstract: To demonstrate the location specificity of poly-N-Isopropylacrylamide (p-NIPAm) core with poly-N-Isopropylacrylamide-Acrylic acid (p-NIPAm-co-AAc) shell, the nanogel components have to be synthesized individually, then the shell solution has to be reacted onto the core solution, a dialysis would then be performed on the completed core-shell particle solution to remove unreacted materials, and an analysis using atomic force microscope (AFM) and a scanning electron microscope (SEM) was performed to characterize thermoresponsitivity of the nanogel. Synthesis of the nanogel components uses a free-radical polymerization reaction in which an N-Isopropylacrylamide monomer would be chained together into a polymer in the presence of a crosslinking agent which is N,N'-methylenebis(acrylamide) (BIS), a surfactant called sodium dodecyl sulfate (SDS), and a polymerization initiator called ammonium persulfate (APS). Results show how p-NIPAm core/ p-NIPAm-co-AAc shell nanogel particles were able to respond to higher temperatures by a statistically significant shrinking in diameter size. This finding suggests the possibility of applying p-NIPAm core/ p-NIPAm-co-AAc shell nanogel technology to melittin drug delivery in cancer patients.

I. Introduction

Drug therapies continue to be studied intensely in efforts to produce new medicines that can cure or mitigate symptoms of illness or diseases. Melittin is a peptide that constitutes 52% of the dry mass honeybee venom. The success of honeybee venom therapy largely relies on the properties of melittin. In-vitro studies have shown that the use of melittin can inhibit tumor growth as well as induce apoptosis in human leukaemia cells. However, the delivery of melittin can be detrimental to healthy cells; therefore, a targeted delivery system is imperative to the implementation of this chemotherapy. Existing chemotherapies are typically ingested and course through the entire body. The end

result is nonspecific leading to the extinguishing of cancer cells that have spread away from the original tumor source. This can come at the cost of healthy noncancerous cells. The use of a targeted nanoparticle delivery system can mitigate this consequence while also delivering the desired drug or chemical to unhealthy cells. Poly-N-Isopropylacrylamide nanoparticles have exhibited the possibility of being able to deliver melittin to specific cancerous locations. N-Isopropylacrylamide is a biocompatible temperature-responsive polymer that has the potential to be synthesized as a nanoparticle drug delivery system given its thermoresponsitivity and pH sensitivity. Poly-NIPAm has been studied extensively in regards to its reversible deswelling behavior: particles of this reagent are able to shrink or expand at temperatures above or below, respectively, about 32°C. In this paper, we present the synthesis and characterization of poly-N-Isopropylacrylamide nanoparticles in regards to size and thermoresponsivity with the objective of demonstrating the potential use of pNIPAm core/ pNIPAm-co-AAc shell nanoparticles as a drug delivery method by its ability to shrink at elevated temperatures.

II. Materials and Methods

Materials. Water for all reactions and procedures have been deionized as to ensure minimal foreign particulate material entering the system. N-Isopropylacrylamide (NIPAm), Acrylic acid (AAc), N,N'-methylenebis(acrylamide) (BIS), sodium dodecyl sulfate (SDS), and ammonium persulfate (APS) were purchased from Sigma Aldrich. All materials were used as received.

Preparation of Poly(NIPAm) Core. 1.4 g NIPAm and 0.10 g BIS were dissolved in deionized water and degassed for 15 minutes. 0.057 g SDS was added and dissolved in the monomer solution, then filtered through a 0.2 μm syringe filter before placing the solution into a 250 mL three-neck, round bottom flask. The solution was then heated to 70℃ under constant nitrogen purge for 15 minutes. 0.069 g APS was dissolved in 10 mL degassed deionized water and added to the heated core solution to initiate polymerization. Reaction was allowed to proceed for 5 hours at 70℃.

Preparation of Poly(NIPAm) Core. 1.3 g NIPAm, 0.10 g BIS, and 0.072 g AAc were dissolved in 150 mL deionized water. 0.057 g SDS and 0.069 g APS were added to

the shell solution while stirring. Lastly the solution was filtered before core-shell synthesis.

Core-shell particle synthesis. 75 mL of the prepared NIPAm core solution was heated to 70℃ for 15 minutes on highest stirring. The shell solution was degassed under vacuum and purged with nitrogen for 1 hour at room temperature. 15 mL of the shell solution was added to the core solution and allowed to react for 30 min. After, the remaining shell solution was added at a rate of 3.75 mL/min and the reaction was allowed to proceed for six hours at 70℃.

Dialysis. 10 cm of 6-8 kD Dialysis Membrane was soaked in deionized water and one end was tied with string. Then, 5 mL of the core-shell solution was added to the dialysis bag and the open end is secured with a string. A weight was added to the dialysis bag and the set-up was placed in a graduated cylinder with deionized water to soak for 2 weeks. After the two weeks had passed, the conductivity of the water was tested and reported to confirm successful dialysis.

Light microscopy. The presence of nanogel particles was confirmed using light microscopy by light reflection.

SEM. A scanning electron microscope was initially used in order to gain visual insight into the structures of the sample. 500 μL of core and core-shell solutions were each transferred into Eppendorf tubes, vortexed, sonicated for 5 minutes, and centrifuged for 5 min at 15000 rpm before replacement of supernatant with 200 μL of water. 5 μL of each sample was mounted on carbon tape and gold sputter-coated for SEM imaging.

AFM. Particle size and shape of nanogel particles were determined using atomic force microscopy. A 1:10 dilution and a 1:100 dilution of the prepared solution were tested under two different drying temperatures: room temperature and 45℃, after preparation on silicon. Images were collected with tapping mode with CT170R tip was utilized to collect the data with Trax AFM. Particle sizes were obtained by using SPIP software which detected particles by filters such as color threshold and spherical shape of particles. SPIP was also used to

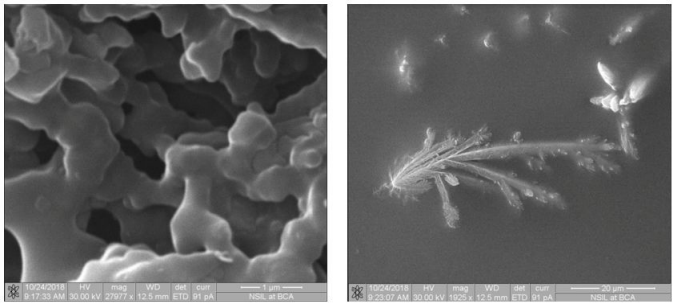

Figure 1. SEM images of Poly(NIPAm) core(top) and core-shell(bottom) solution prepared without dialysis.

calculate particle diameters in nanometers and export data in a spreadsheet.

III. Results and Discussion

The Poly(NIPAm) core-shell particles presented here were synthesized by first forming the core through polymerization of a NIPAm-BIS-SDS monomer solution at 70°C followed by the separate forming of a shell solution through the polymerization of a NIPAm-BIS-Aac- SDS-APS solution. The core and shell were integrated through the mixing consequent reaction between the two prepared solutions. The core-shell solution was then put through a 2 week dialysis wherein the measured conductivity of the water had increased, indicating that filtration by this method was beneficial. This process is fairly convenient in that it takes place at amiable conditions and requires less than 3 weeks of time to finish synthesizing. The dialysis was deemed necessary after initial trials had resulted in the formation of a mass of particles rather than easily defined individual particles with the expected dispersity as depicted in Figure 1.

Following the dialysis of the solution, the sample was unable to stay on the carbon tape required for SEM imaging. Therefore, particle sizing was done using atomic force spectroscopy as opposed to the initial scanning electron microscopy. AFM allows for the drop cast drying of the sample onto silicon which preserved the sample under the microscope.

The intensely concentrated nature of the prepared solutions made imaging and particle sizing through SEM as well as AFM difficult due to stacking of nanoparticles. In efforts to remediate this, the solution was diluted to 1:10 as well as 1:100 dilutions. The dilutions improved the resolution of the images as there were less particles; however, as seen in Figure 2.A, the 3D rendering of the first dilute solution, the particles are still very concentrated.

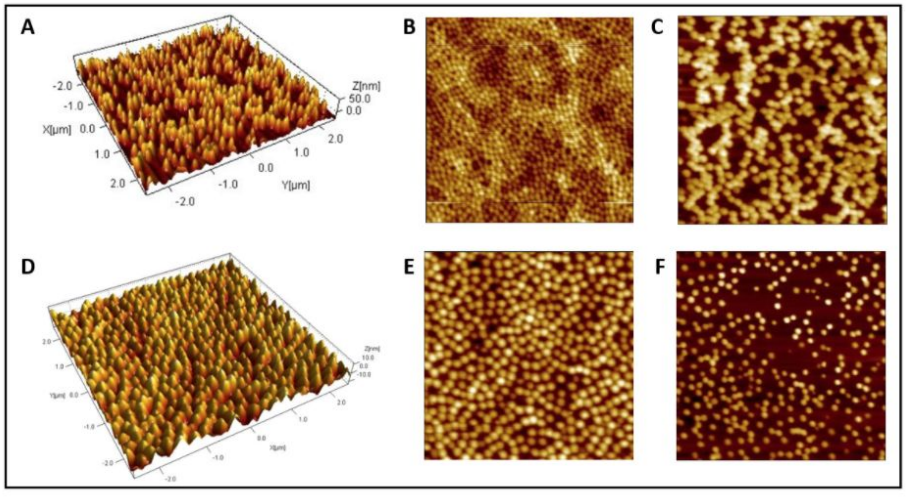

Figure 2. AFM microscopy results of dilute solution 1:10 (images A, B, and C) and dilute solution 1:10 (images D, E, and F) shown in 3D (A and D), at room temperature (B and E), and after heating to 45°C (C and F).

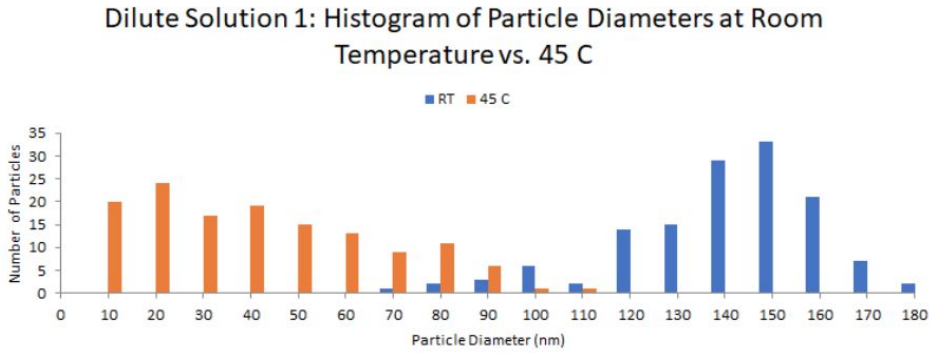

Figure 3. Comparison histogram of the distribution of particle diameters of dilute solution 1 (1:10 dilution) at room temperature (blue) and at 45°C (orange).

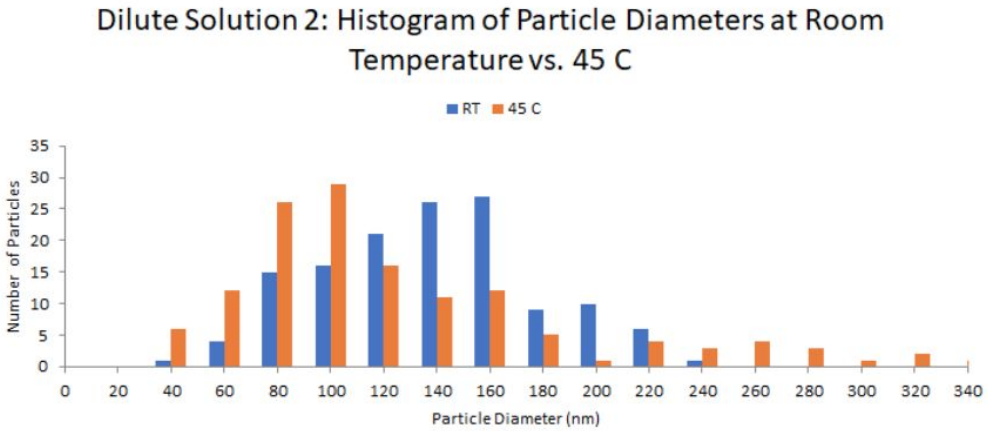

Figure 4. Comparison histogram of the distribution of particle diameters of dilute solution 2 (1:100 dilution) at room temperature (blue) and at 45°C (orange).

Core-shell nanoparticles were drop-cast dried on a silicon and imaged under the Trax Atomic Force Microscope (AFM) using tapping mode with a CT170R tip. 5x5 µm images with the AFM were collected and then analyzed using SPIP software. Figure 2 provides examples of the images taken of the 1:10 dilute solution as well as a 3D rendering of the solution at both the specified room temperature of 25°C as well as at 45°C. Figure 3 details the raw data collected from these images. The 1:10 dilute solution presents stark variations between particle sizes at Room Temperature and 45°C by a shift to lower particle diameters at higher temperature . The difference seen approaches 100 nanometers. This is indicative of the thermoresponsive properties of Poly(NIPAm) particles and its likely use as a drug delivery method by shrinking at higher temperatures. In comparison to Figure 4 with a more dilute nanoparticle solution, the difference in particle size reduction behavior is more apparent in Figure 3. At a more dilute concentration, the average particle diameter

Table 1. Statistical values of nanogel particle size distribution for 1:10 dilution and 1:100 dilution.

	Particle Size (nm)					
	1: 10 Dilution			1:100 Dilution		
	M	*SD*	*T-Test*	*M*	*SD*	*T-Test*
RT	165.2	21.8	3.8E-68	132.1	41.3	1.1E-06
45 C	67.3	25.2		108.2	56.3	

still decreases at higher temperature. However, this is a smaller reduction in size, which could be seen by the difference in mean particle diameter size of 132.1 nm to 108.2 nm in dilute solution 2 in comparison to 165.2 nm to 67.3 nm diameter reduction in dilute solution 1. This is likely due to the pH difference in the 1:100 dilute solution after the addition of more deionized water, which reduces the effectiveness of the buffer solution used. Results from the 1:10 dilution would be more accurate due to this reason. This characteristic could be useful when considering the use of these particles in temperature variant systems. For the dilute solution, to be able to maintain a fairly similar average size would allow for greater traversal since the particle's deswelling nature can also be employed with pH variation.

Analysis of the raw data provided that the average particle size of both dilute solutions decreases at a higher temperature of 45 °C as depicted in Table 1. Standard deviation analyses of both dilute solutions, however, also show a higher variance in particle diameters for more dilute solutions. This is likely due to the difference in the pH of the solutions. The buffer used proved to be ineffective at maintaining the overall pH; hence, the particles responded both to the increase in pH as well as temperature. Overall, however, T-test results on datasets of both dilute solutions show values close to zero which confirmed the statistical significance of the reduction in nanoparticle diameter after heating to 45°C and denied the null hypothesis.

IV. Conclusion

Poly-NIPAm core/ Poly-NIPAm-AAc shell nanoparticles were shown to have potential as melittin drug delivery intermediate due to its thermoresponsitivity at elevated temperature of 45°C. Although the overall result obtained verified the thermoresponsitivity of the nanoparticles, some errors weren't quantified within the parameters of the experiment, which included: deformation of nanoparticles during AFM imaging, stacking of nanoparticles affecting SPIP

identification and sizing of nanoparticles, possible interference of excess reactants in measurements with respect to the effectiveness of dialysis filtration, and errors due to dilution of buffer solution used. Further exploration into the subject could involve identification of a more effective buffer solution which could provide accurate sizing of the thermoresponsive-pH responsive particles in different dilution conditions, exploration of other nanogel structures or compositions, and trials involving the loading of the melittin drug into the nanoparticles.

References

Wedel, Bastian. *Role of Anionic Surfactants in the Synthesis of Smart Microgels Based on Different Acrylamides*; ACS Omega, Bielefeld, Germany, Jan, 2017; https://pubs.acs.org/doi/pdf/10.1021/acsomega.6b00424