THE SYNTHESIS AND STUDY OF POLY(N-ISOPROPYLACRYLAMIDE)/

POLY(ACRYLIC ACID) INTERPENETRATING POLYMER

NETWORK NANOPARTICLE HYDROGELS

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Homogeneous hydrogels made of an interpenetrating network of poly(N-isopropylacrylamide) (PNIPAm) and poly(acrylic acid) (PAAc) are synthesized by a two-step process; first making PNIPAm hydrogels and then interpenetrating acrylic acid throughout the hydrogel through polymerization. The kinetic growth of the IPN is plotted and an equation is fitted to the data.

When diluted to certain concentrations in water, the hydrogels show reversible, inverse thermal gelation at about 34°C. This shows unique application to the medical field, as the transition is just below body temperature. A drug release experiment is performed using high molecular weight dyes, and a phase diagram is created through observation of the purified, concentrated gel at varying concentrations and temperatures.

ACKNOWLEDGMENTS

There are several people at the University of North Texas that I would like to thank. Dr. Zhibing Hu, my graduate advisor, for his support and advice, Dr. Sushama Dandekar, both for serving on my graduate committee, as well as offering the class that first piqued my interest in hydrogels, and Dr. Xiaohu Xia for his assistance and suggestions in the laboratory.

I would like to thank my parents for their constant love, as well as their support of my education.

Finally, I would like to thank my beautiful wife and best friend, Corrie, for her love, and for her encouragement of me in everything that I do.

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LIST OF ABBREVIATIONS

APS   Ammonium persulfate

BIS   Methylene-bis-acrylamide

BSA   Bovine serum albumin

DLS   Dynamic light scattering

IPN   Interpenetrating polymer networks

LLS   Laser light scattering

NIPAm   N-isopropylacrylamide

PAAc   Poly(acrylic acid)

PBS   Phosphate-buffer solution

PDI   Polydispersity index

PNIPAm   Poly(N-isopropylacrylamide)

Rh   Hydrodynamic radius

SDS   Sodium dodecyl sulfate

CHAPTER 1

INTRODUCTION

A gel consists of randomly crosslinked polymer chains and contains a large amount of solvent filling interstitial spaces of the network. A commonly recognizable gel is gelatin, where a protein is dispersed through water[1]. Any gel where the medium is water can be further characterized as a hydrogel. Hydrogels may go through reversible volume transitions based on certain external stimuli, such as temperature and pH value[2]. Because of these changes, and the compatibility of water with biological systems, hydrogels can be utilized as sensors, for tissue replacement or engineering, separation, or drug delivery systems[3, 4].

Of specific interest are hydrogels which show reversible, inverse thermal gelation. These are a flowable solution at a lower temperature, and above some transition temperature, they gel. In this thesis, the synthesis and study of poly(N-isopropylacrylamide)/ poly(acrylic acid) interpenetrating polymer network (PNIPAm/PAAc IPN) hydrogel is discussed. The PNPAM/ PAAc IPN is especially interesting because of its transition temperature and lower required concentrations. At room temperature, it behaves like a liquid, but at about 33°C, it gels. One advantage of this over commercial thermally-responsive gels is that

this can occur at concentrations as low as 2.5-3% (gels commercially used currently for bio—medical applications require concentrations above 15-20%)[4].
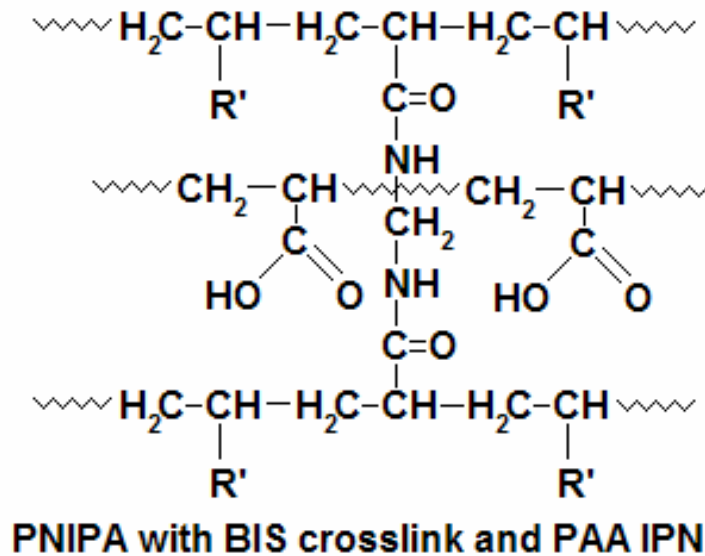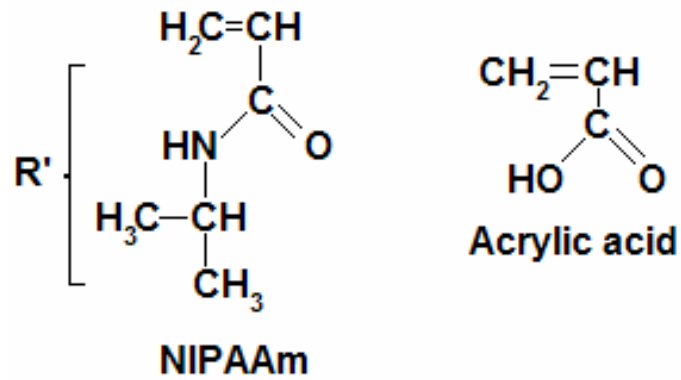
Figure 1.1: Chemical structures of NIPAm, acrylic acid, and a PNIPAm/PAAc IPN.

This makes PNIPAm/PAAc IPN's ideal for drug delivery systems, as it flows and can be mixed with a drug and injected at room temperature, but at body temperature, it gels and release a drug slowly through diffusion.

In this paper, I discuss my research in PNIPAm/PAAc IPNs. Chapter 2 discusses the theoretical background of dynamic laser light scattering (DLS), as well as the instrumental setup in the laboratory. Chapter 3 discusses the synthesis and light scattering study of the IPN nanoparticle hydrogels, as well as drug release experiments. Chapter 4 covers the development of a concentration-temperature phase diagram of the IPN particles. Chapter 5 summarizes the results of the work.

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2. Tian, Q.; Zhao, X.; Tang, X.; Zhang, Y. *J. Appl. Polym. Sci.* **2003**, 87, 2406-2413.

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CHAPTER 2

BACKGROUND OF LASER LIGHT SCATTERING

Many useful analytical techniques use some of the quantum properties of electromagnetic radiation. It is well understood that the frequency of light can be changed when it comes in contact with certain molecules. This frequency shift, with the advent of monochromatic and variable light sources as well as more advanced photo detectors, can be easily studied and valuable information can be obtained from the molecules being studied.

When photons impinge on a molecule they can either impart energy to, or gain energy from, the translational, rotational, vibrational, and electronic degrees of freedom of the molecules[1]. This causes a frequency shift on the photons. Therefore, the frequency spectrum of the scattered light exhibits resonances at the frequencies corresponding to these transitions. Light scattering therefore provides information about the energy spectra of molecules. In this paper, I discuss only the real-time dynamics of the system, so only the translational and rotational degrees of freedom need to be studied. This is called Rayleigh scattering, or more commonly, dynamic light scattering.

From the semiclassical light scattering theory[1], when light comes into contact with any medium, the light's electric field causes the electrons of the medium being studied to polarize in an oscillating manner. This causes the

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molecules to radiate light, which is released in all directions, or scattered. Any changes in frequency, polarization, intensity (measured by photon count) or angular distribution can be related to changes in the particles being studied. Therefore, it is possible, using laser light scattering, to gain information on the structure of the scattering material.
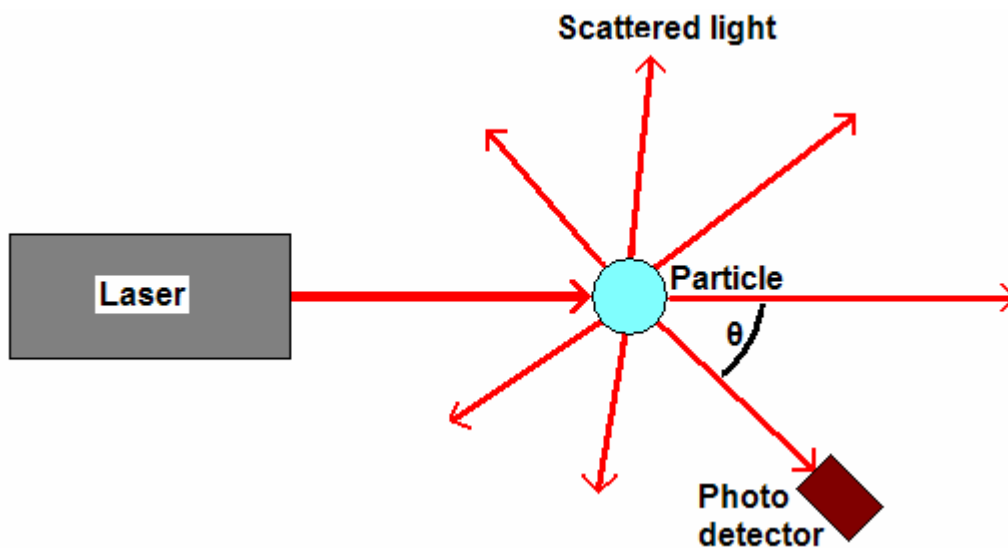
Figure 2.1: Laser light scattering.

For this experiment an ALV/DLS/SLS-5000 LLS spectrophotometer is used with its ALV-5000 digital time correlator software. It is equipped with a helium-neon laser (a Uniphase 1145P), with a wavelength of 632.8 nm and an output power of 22 mW. The incoming laser light is polarized 90° from the scattering plane, and a beam attenuator (a Newport M-925B) is used to regulate

the intensity. The scattered light, at the set angle, is conducted to an active

quenched avalanche photo diode (APD) detector by way of a thin optical fiber.
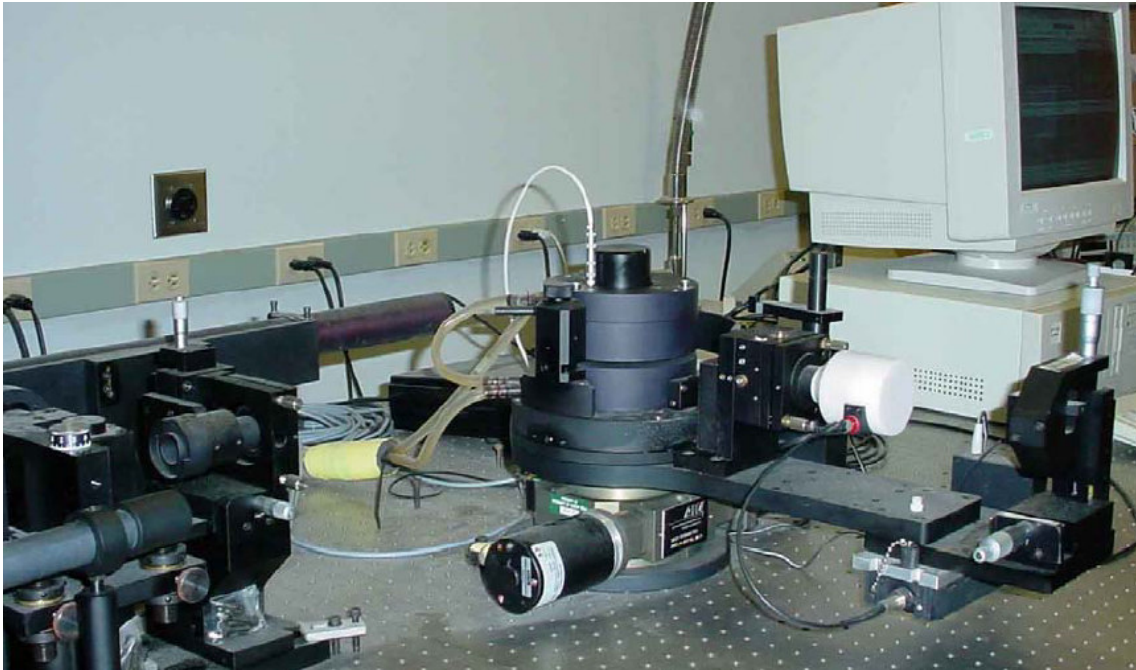
Figure 2.2: A commercial laser light scattering spectrophotometer.

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CHAPTER 3

SYNTHESIS AND LIGHT CHARACTERISTICS OF INTERPENETRATING

POLYMER NETWORKS (IPN) NANOPARTICLES

Introduction

Nanoparticles composed of poly (N-isopropylacrylamide) (PNIPAm) with an interpenetrating network of poly (acrylic acid) (PAAc) were created in the manner described below. These polymers were selected because their combination showed desirable phase transitions at temperatures near normal body temperatures. When created in solution, the particles are believed to be almost homogeneous, leaving only small interparticle spacing. This is desirable since larger spaces would not release a liquid in as controlled a manner as small spaces. This is also why a final interpenetrating polymer network (IPN) particle size of 75-85 nm is desired at a neutral pH; a smaller particle would release a mixed drug more slowly than a particle size of 150 nm or more.

Experimental Details

Materials: N-isopropylacrylamide is purchased from Polysciences, Inc. N,N'-methylenebisacrylamide is purchased from Bio-Rad Laboratories. Dodecyl sulfate, sodium salt 98%, potassium persulfate 99+%, ammonium persulfate 98+%, acrylic acid 99%, N,N,N',N'-Tetramethylethylenediamine 99%, Sodium

hydroxide 97+% were purchased from Sigma-Aldrich. Water for sample preparation is distilled and deionized to a resistance of 18.2 MΩ by a MILLIPORE system, and filtered through a 0.22μm filter to remove particulate matter.

PNIPAm microgel synthesis: The polymerization of PNIPAm is carried out in a 500 mL flask equipped with a magnetic stirrer and a nitrogen feed: 3.8 g N-isopropylacrylamide (NIPA), 0.066 g N,N'-methylenebisacrylamide (BIS) and 0.15 g sodium dodecyl sulfate (SDS) were diluted to 240 g with distilled water under continuous stirring for one hour. The solution is placed under nitrogen purging for 40 min before being placed into 70°C hot bath. Potassium persulfate (0.166 grams), which had been dissolved in 20 ml water, is then added to initiate the emulsion polymerization. The reaction last for 4 hours under nitrogen atmosphere, and temperature is kept at (70±0.5)°C throughout. Controlling the amount of surfactant regulates the final PNIPAm microgel size: a smaller nanoparticle size can be achieved by using larger amounts of the emulsifier SDS. PNIPAm nanoparticles with a hydrodynamic radius (R_h) of 50 nm where prepared for the next step, IPN synthesis.

All PNIPAm particles were purified via dialysis (Spectra/Por® 7 dialysis membrane, MWCO 10'000, VWR) against frequent changes of H_2O for 2 weeks at room temperature. The final PNIPAm concentrations were determined using the evaporation method.

IPN microgel synthesis: The preparation of IPN nanoparticles are based on the above PNIPAm microgels: A PNIPAm microgel solution containing 0.297 g polymer is diluted to 365 g with distilled water. 0.5 g N,N'-methylenebisacrylamide (BIS) and 2.3 g acrylic acid were then added. The solution is deaerated for 1 hour with nitrogen bubbling. The initiator (0.2 g potassium persulfate) and accelerator (0.2 g TEMED) were separately dissolved in water and added rapidly to the solution, making the final solution volume into approximately 375 ml. The reaction continued for 29 min under nitrogen atmosphere, and the temperature is well regulated at (21±0.5) °C with a water bath. The reaction is halted by removing the N_2 and placing the flask in an ice bath, while stirring under a normal atmosphere. The ideal particle size had an R_h of 75-85 nm. To determine kinetic information, this reaction is repeated at (19±0.5)°C and (23±0.5)°C. 2 ml samples were taken from the flask at varying times throughout the reaction for all three temperatures. These were then neutralized with sodium hydroxide to a pH of 7, and the R_h is determined using light scattering.

The IPN nanoparticle solution is purfied via dialysis (Spectra/Por® 7 dialysis membrane, MWCO 10'000, VWR) against frequent changes of distilled water for 2 weeks at room temperature. Evaporation at 70°C is used to adjust the concentration of the IPN solution. When the desired concentration is reached, an aqueous sodium hydroxide solution is used to neutralize the IPN solution.

A drug release experiment is also performed, using the following procedure:

Materials: 2 MDa Blue Dextran fluorescent dye is purchased from Sigma-Aldrich. 40 kDa Texas Red® dextran fluorescent dye and Bovine Serum Albumin (BSA) were purchased from Molecular Probes. 500 kDa Fluorescein isothiocyanate Dextran fluorescent dye is purchased from Fluka.

Procedure: 4 - 1.00 g samples of IPN solution at 8% wt were placed into 1 cm diameter cuvettes. 0.025 g of drug analog dyes (40kDa, 500kDa, and 2MDa Dextrans and BSA [67 kDa]) at a 4000 ppm concentration were added. They were well mixed, then placed into a 37°C water bath for 30 minutes. 4 resealable 20 mL vials were prepared with 10.00 g of phosphate-buffered saline (PBS) solution in each vial. These were placed in a 37°C circulating refrigerated bath for 30 minutes. The IPN samples were then forced out of the cuvettes using a syringe and heated purified water. Each gel is collected and placed into a vial containing the PBS. These were returned to the circulating refrigerated bath. Absorbance measurements (from wavelengths of 200 to 1100 nm) were made with an Agilent 8453 UV-Vis Spectrometer with a 1-cm optical path length quartz cell. The measurements were made at regular intervals, measured in minutes from when the gel is placed in solution for the samples, using the PBS solution for the background subtraction. Using Beer's law, one can determine the concentration of the dye in solution after creating a calibration curve.

Results and Discussion

The kinetic growth data is illustrated in the graph below (figure 3.1). There is a very strong correlation between temperature and particle size growth. As can be seen from the data, there is also a very rapid exponential growth associated with the kinetics. Since the particles no longer show the favorable gelling characteristics after a certain size, it is necessary to be able to quickly stop the reaction. An acceptable method is to remove the nitrogen purge and immediately place the flask into an ice bath. Equations were developed to fit with the data. The hydrodynamic radius can be described as a function of time in the following equation:
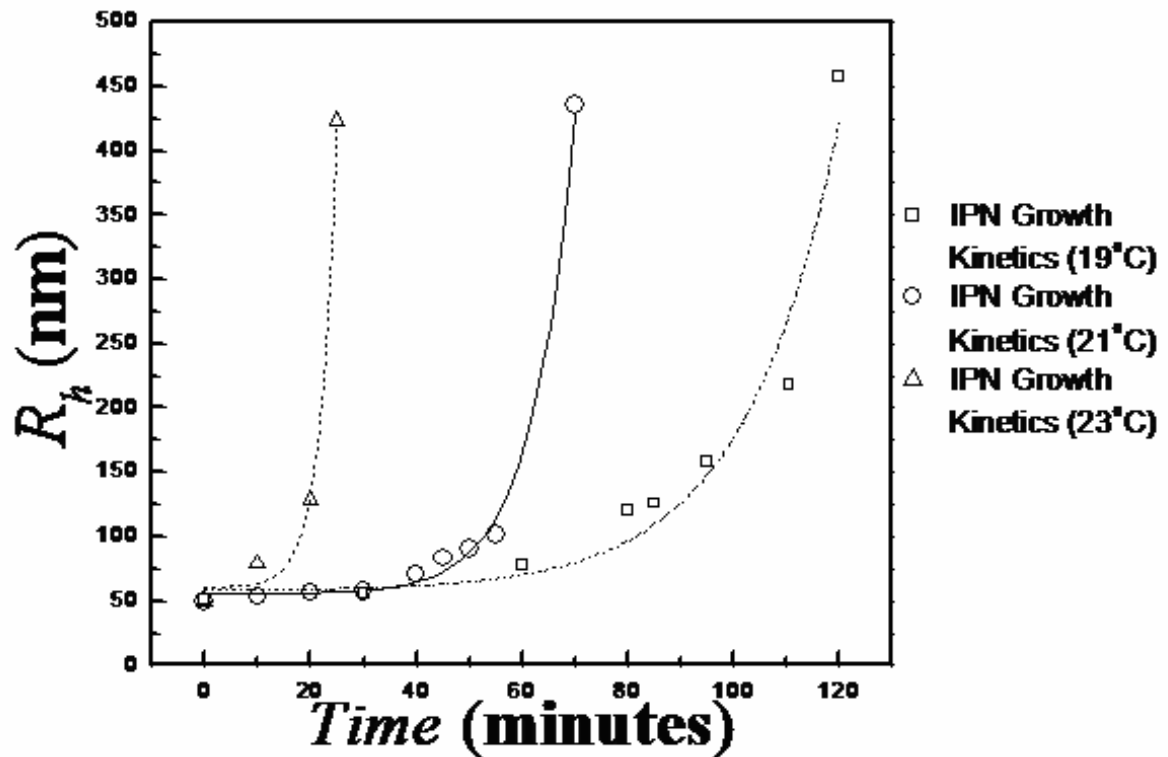
$$R_h(t) = R_0 + k*e^{t/\tau}$$

Where R_0, k, and τ can be defined as such:

Table 3.1: Exponential kinetic growth equation variables (see Fig. 3.1).

|	R_0	k	τ	R^2 (fit)
19°C	49.1	.21473	3.35075	0.99209
21°C	49.9	.10404	8.52187	0.99556
23°C	51.6	.46733	17.89698	0.95669

Figure 3.1: Kinetic growth diagram of IPN synthesis at 19°, 21°, and 23°C.

Dynamic light scattering is performed on the samples both between the two reactions, as well as after the second. Figure 3.2 shows the DLS hydrodynamic radii correlation diagram for both the 50 nm PNIPAm as well as the 83 nm IPN.

The weight ratio of PAAc to PNIPAm in the 75 nm IPN nanoparticle solutions is determined to be 0.25:1. This measurement is performed through

the evaporation method. When the synthesis reaction lasted longer, the particles

became larger, and the ratio increased.
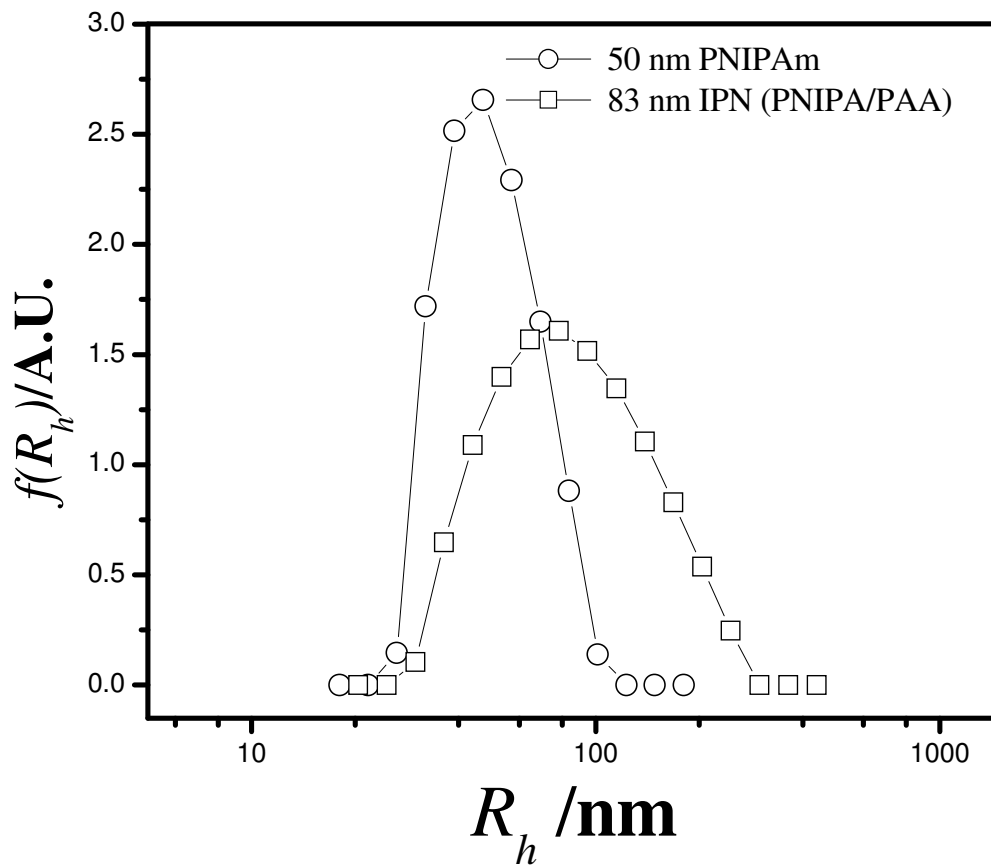
Figure 3.2: PNIPAm vs. IPN particle size distribution, measured by dynamic light scattering. This is from the first PNIPAm and IPN solutions that I synthesized; poor temperature control led to a wide particle size distribution.
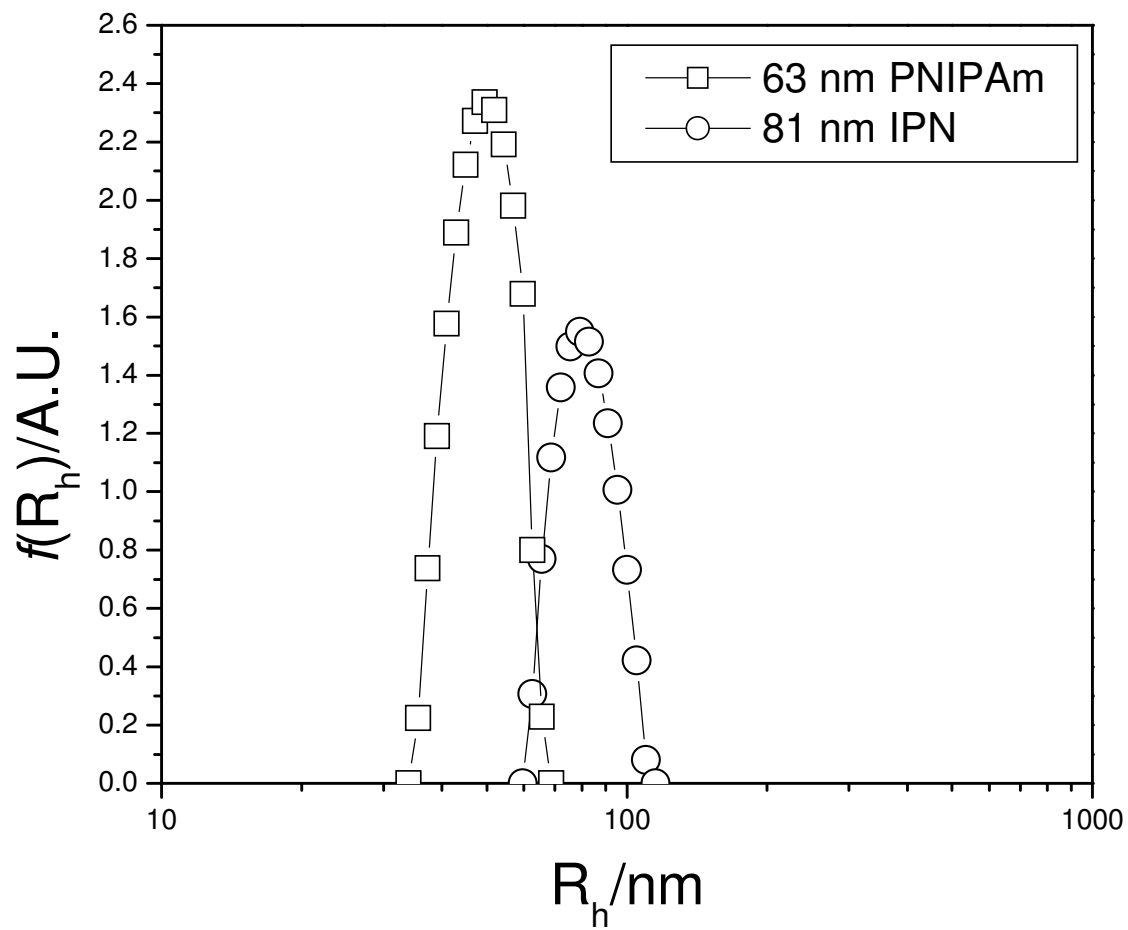
Figure 3.3: PNIPAm vs. IPN particle size distribution, measured by dynamic light scattering. These are some of the later synthesized gels, which had much better temperature control, leading to a narrow particle size distribution.

Working curves for Beers law were developed, with a linear equation fit to it. The equations for the four dyes, and their fit qualities, are listed below.

Dye	Wavelength (nm)	Concentration (ppm)	R^2
40 kDa Dextran	552	(abs-.01235)*178.2	0.99946
500 kDa Dextran	493	(abs-.01551)*239.8	0.98000
2 MDa Dextran	625	(abs+.003)*531.9	0.96824
BSA (Bovine serum albumin protein)	275	(abs-.00508)*1744	0.99992

Table 3.2: Linear fit equations Beer's law using various high-molecular weight dyes.

The drug release experiment never gave reproducible results. I believe that the gel particles slowly break away from the bulk gel that is contained in the PBS. This breakup created a concentration of gel nanoparticles then became suspended homogeneously in the PBS. The nanoparticles showed absorbance across the range on the UV-Vis where the dyes showed peaks, affecting the use of Beer's law. I had believed that the use of a control to create a baseline curve would reduce any such effects. Analysis of the data showed this to be incorrect. While Beer's law still applies, because of this interference, the calibration curves cannot be applied to the data to get a quantitative value of the concentration. Instead, each absorbance is divided by the maximum absorbance for each particular dye, and then multiplied by 100%. Data from two experiments are shown below; Figure 3.4 shows the experimental data without the use of a control.
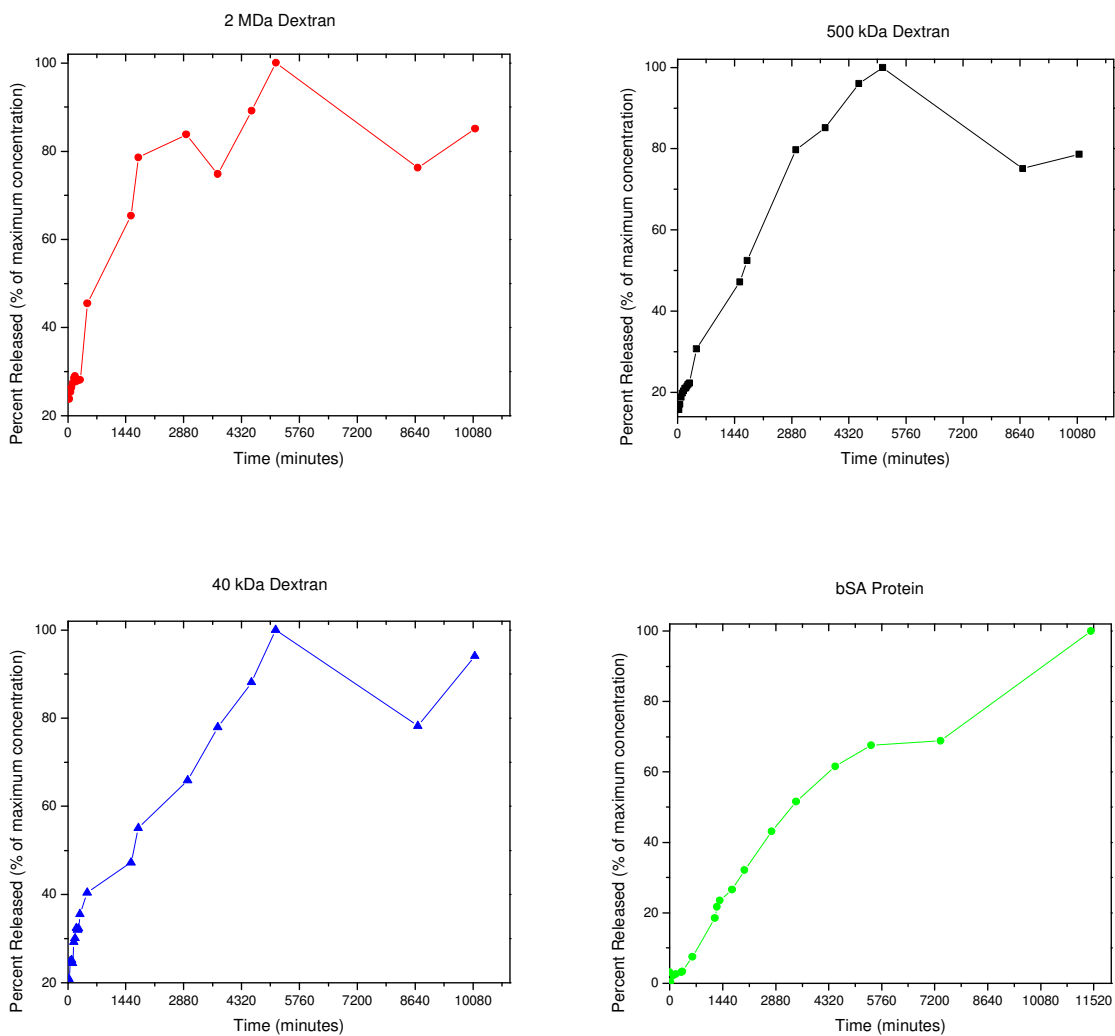
Figure 3.4: Drug release curves for (from top left, clockwise) 2MDa Dextran, 500kDa Dextran, BSA protein, and 40kDa Dextran. These are without the use of a control to set the background absorbance.

Conclusion

A final IPN particle size of 75-85 nm is desired at a neutral pH. The PNIPAm size could be easily and reproducibly controlled by the concentration of SDS in the synthesis solution. The challenge is controlling the size of the IPN. This is found to be very dependent on both temperature and time. The growth of the PAAc IPN is exponential with time and the time it took the reaction to show growth is highly dependent on the temperature. The best results were at a temperature of 21°C. At 19°C, it took too long to begin the reaction, and at 23°C, the reaction occurred too rapidly, and it is difficult to control the final size. To get the IPN final hydrodynamic radius to 75 nm, the reaction lasted 29 minutes.

The drug release experiment is not as successful as would have been liked, but the original release experiment gave acceptable results compared to the experiment using a control. I believe that the absorption interference from the IPN nanoparticles is so much higher than that of the dye that good quantitation measurements could not be collected. The release of nanoparticles into the solution would also have been somewhat random, yielding absorbance values that varied between the samples, especially at longer times.

From the experiment without a control, release profiles were not as expected over the entire range (see figure 3.4). The largest Dextran (2 MDa) is released the fastest, about 24 hours for 80% release. The next largest Dextran (500 kDa) took about 48 hours to release about 80%, and the lightest Dextran

(40 kDa) took about 72 hours to release about 80%. BSA protein (with a molecular weight of 67 kDa) indicated an even slower release, about 144 hours for 80% release. However, looking closer (for the first 5 hours; figure 3.5), with the equations for Beer's law used, and adjusting the first concentration (at about 30 minutes) to zero yields data as expected, with the lower molecular weight dextrans being released more rapidly:
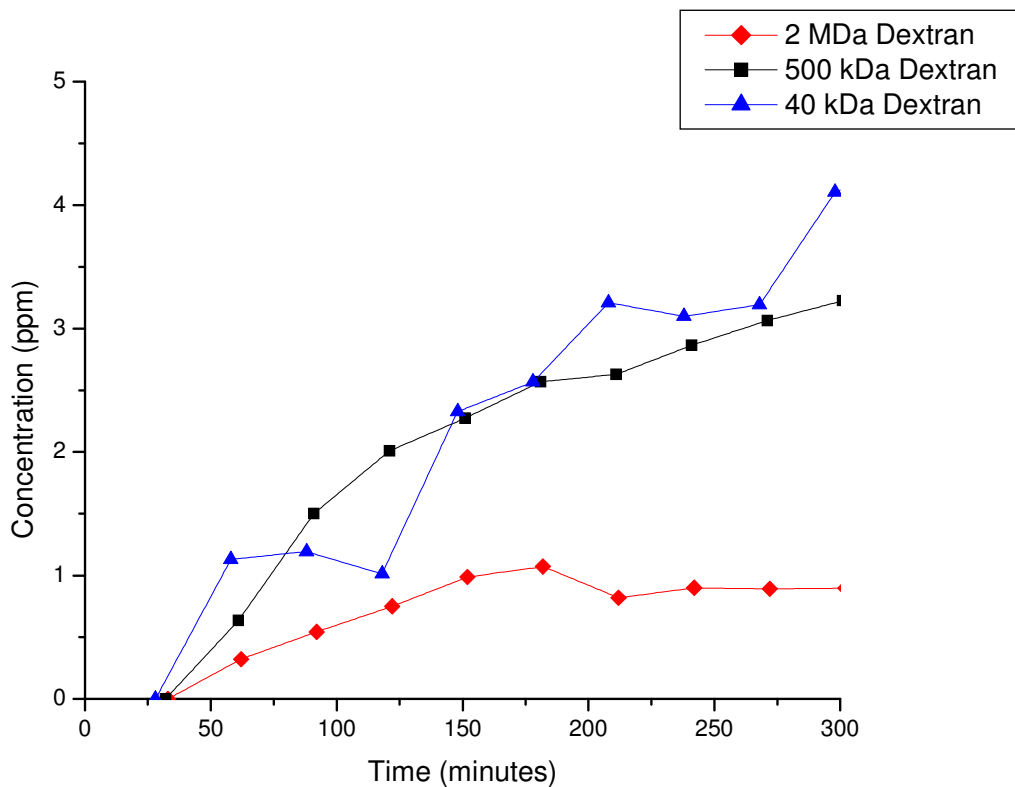
Figure 3.5: Release of 2MDa, 500kDa, and 40kDa dextrans in the first 5 hours.

The data scattering of these experiments show that the procedure needs to be modified to give more consistent results. Another experiment performed by others in my research group found that drying the gel before placing it in the buffer gave better results[1].

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       2005, 227, 275-284.

CHAPTER 4

PHASE BEHAVIOR OF IPN NANOPARTICLES

The behavior of interpenetrating polymer network (IPN) nanoparticles with respect to changes in temperature is of great interest to this thesis. An experiment is performed to determine the phase behavior of aqueous IPN solutions ranging from 1 wt % in water to 8 wt % in water, in 0.5 % intervals. These were placed in an incubator at varying temperatures from 20°C to 45°C. The samples were studied by visual inspection, and a phase diagram is developed (figure 4.1). This shows that the phase transition from liquid to gelled is around 32-35°C, depending on the IPN concentration. There is apparently some interaction that takes place between the IPN particles at a concentration between 2.5 and 3.0 wt %, to account from the loss of a slight blue color. One possible explanation for this could be due to intramolecular interactions. At the lower temperatures, the IPN is contracted, and, when at the lower concentration, each particle has little contact with the neighboring particles. As the concentration goes up, there are some intramolecular interactions that could lead to the color loss. As the temperature rises, the particle size increases, which would increase the interactions at higher temperatures. The ordered matrix that had been in place before the temperature rose is disrupted as the particles grow. This could explain the return of the blue color in the blue viscous liquid and

23
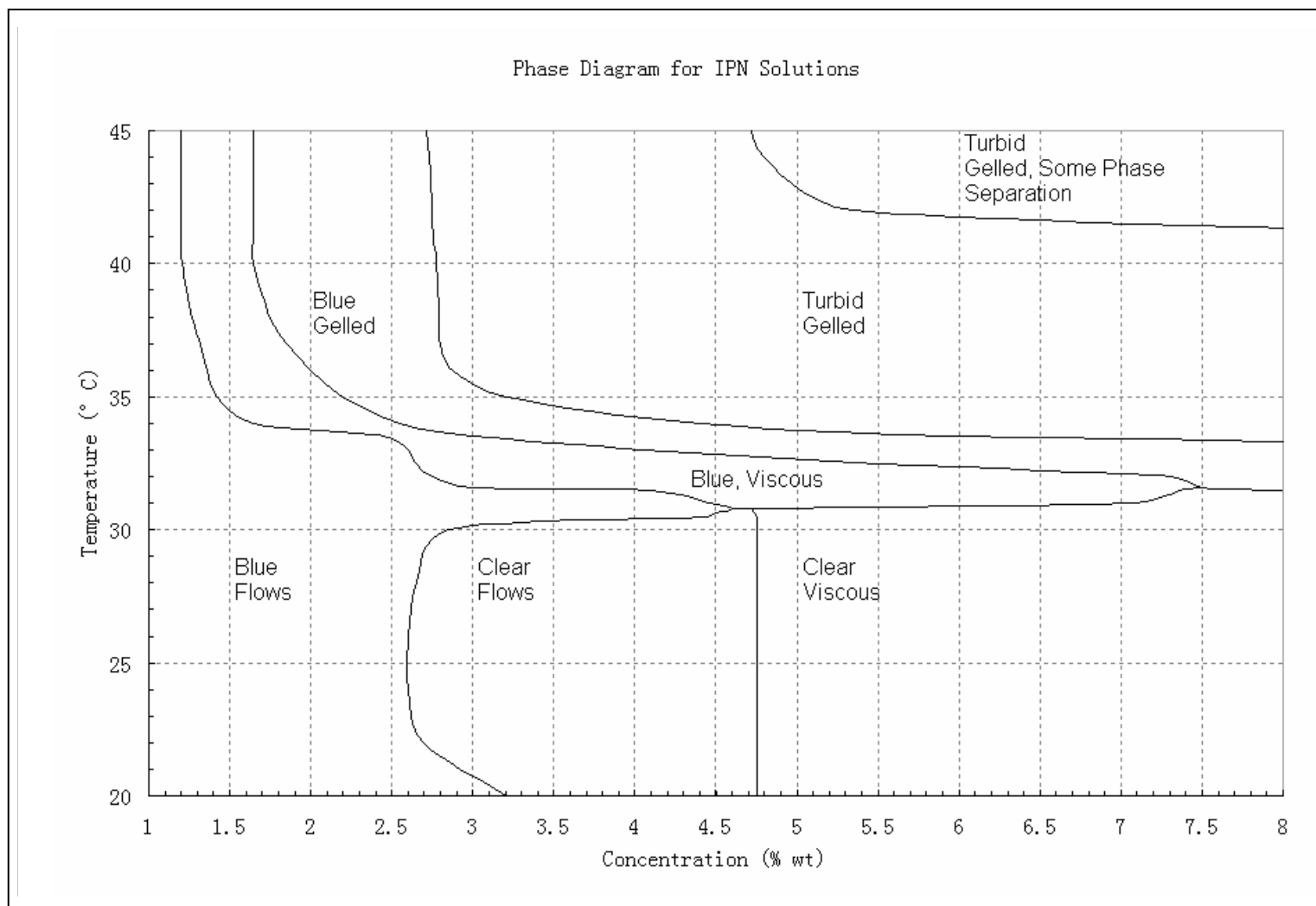
Figure 4.1: Phase diagram of IPN solutions. See Figure 4.2 for images related to diagram.

Figure 4.2: Images of IPN solution corresponding to phase diagram. From top left, clockwise, blue gelled, turbid gelled, clear flows, blue flows (~25°C for flowable images, ~37°C for gelled images).

gelled phases at the higher concentrations. At lower concentrations, the particles are suspended homogeneously, with no intramolecular interaction, allowing Bragg diffraction to cause a blue color. As the concentration increases, the particles come in contact with each other, eliminating the diffraction and causing

the light to pass through uninhibited. At the higher temperatures and a higher concentration, the particles have more interaction due to the interpenetrating poly(acrylic acid) network, causing an absorption of light and the white color.

An experiment is performed to show the increase of the particle size with temperature. Samples of poly (N-isopropylacrylamide) (PNIPAm) and IPN were diluted in purified water and placed in a test tube, which, in turn, is placed into the laser light scattering instrument. A circulating water bath is used to control the temperature of the sample holder and the sample is allowed to stabilize at the new temperature for 10 minutes. The particle size is then determined using dynamic light scattering (DLS). The results can be seen in figure 4.3.
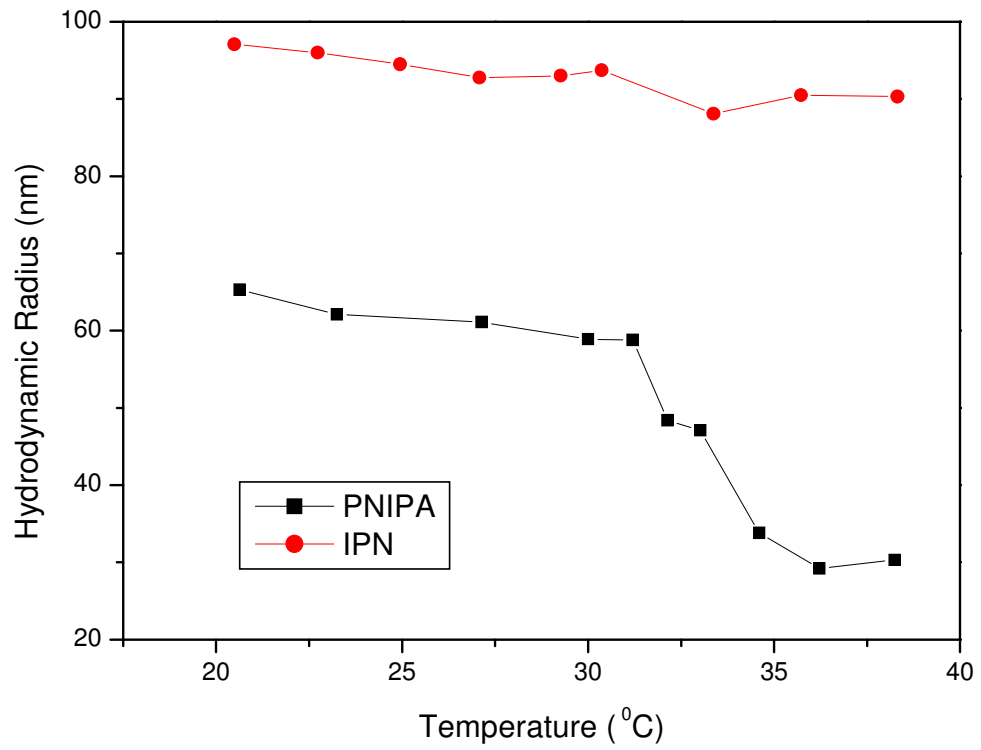
Figure 4.3: Plot of the temperature-dependent particle size of IPN and PNIPAm particles.

CHAPTER 5

SUMMARY

Interpenetrating nanoparticle hydrogels that show inverse thermal gelation have been developed with varying ranges of glass transition temperatures. These have a wide range of novel applications, some of the most exciting being in the medical field. They have shown promise as biomedical sensors, support for tissue growth, and most applicable to this paper, drug delivery. Poly (N-isopropylacrylamide) / poly (acrylic acid) interpenetrating polymer networks (PNIPAm/PAAc IPNs) synthesized for this paper show transition temperatures above room temperature and below body temperature, making them ideal for these purposes. After the gel is synthesized, purified and concentrated, it can be mixed with a drug, which would be suspended in between the nanoparticles of the gel. It could then be inserted into the body, where the drug would slowly diffuse out. Experiments summarized in this paper show that the IPN gels have the ability to hold dyes with physical characteristics similar to some drugs, and then release them in a controlled manner.

Additional studies have been performed and show that gels like this show very little immune response, and give good release characteristics for high molecular weight dyed dextrans in a water solution[1]. Products such as this could be applied to diseases that need continuous treatment, such as diabetes, Parkinson's disease and Alzheimer's disease. This is currently being used

commercially in the form of "triblocks," triblock polymers composed of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide). These solutions, however, require concentrations above 15-20%[2].

PNIPAm/PAAc IPN particles were synthesized using the seed-and-feed method. A solution of 50 nm PNIPAm particles is synthesized through precipitation polymerization. This solution is then used as the 'seeds' in the poly(acrylic acid) reaction. The advantage of this gel is that it shows inverse-thermal gelation at body temperature at concentrations as low as 2.5% to 3%.

The kinetic growth of IPN particles based off of 50 nm PNIPAm particles is studied and growth equations are determined; the most desirable reaction temperature of 21°C give a particle size kinetic equation of

$$R_H = 49.9 \, nm + 0.104 \, nm \cdot e^{\dfrac{t}{8.522 \, min}}$$

A phase diagram has also been developed that could assist in the development of sensors using IPN gels. Additionally, it could help to give a better understanding of the mechanism of the PNIPAm/PAAc IPN hydrogel. A possible explanation of the color change properties has also been suggested, and accompanied with a temperature-dependent particle size plot of PNIPAm and IPN. With a better understanding of the mechanisms involved in the phase transitions of the IPN gel, new synthesis methods could potentially be developed, producing higher purity gels, insights into successful bulk manufacturing or

perhaps yielding an IPN gel with smaller particle sizes and higher crosslinking,

giving better drug release characteristics.

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