SWELLABLE POLY *N*-ISOPROPYLACRYLAMIDE

PARTICLES FOR PHYSIOLOGICAL pH SENSING

By

SANDHYA RANI PAMPATI

Bachelor of Science in Pharmacy

Kakatiya University

Jangaon, Telangana, India

2008

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2015

SWELLABLE POLY *N*-ISOPROPYLACRYLAMIDE

PARTICLES FOR PHYSIOLOGICAL pH SENSING

Thesis Approved:

Dr. Barry K. Lavine

Thesis Adviser

Dr. Nicholas F. Materer

Dr. Ziad El Rassi

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and deepest appreciation to my advisor, Prof. Barry K Lavine, for giving me this opportunity to finish this exciting research. Working with Dr. Lavine has been extremely gratifying. I am always thankful to him for giving absolute freedom to work on the problems and approaches of my interest.

I would also like to thank the members of my advisory committee Prof. Ziad El Rassi, Prof. Nicholas F. Materer for taking time out of their hectic schedules to read my thesis and be on my defense committee.

I want to express my gratitude to my son Sriyan. I would also like to deeply thank my beloved husband Sathish, for being on my side and taking care of me all the time. I am very grateful to my parents Vimala Devi and Thukka Rao and also my brothers Rakesh and Hari for their long-standing support. Last but not least, I wish to dedicate this thesis with love and affection to my family, and almighty for all the support and encouragement throughout my life.

Name: SANDHYA RANI PAMPATI

Date of Degree: JULY, 2015

Title of Study: SWELLABLE POLY *N*-ISOPROPYLACRYLAMIDE PARTICLES
                FOR PHYSIOLOGICAL pH SENSING

Major Field: CHEMISTRY

Abstract: Swellable polymers functionalized to respond to pH have been prepared from microgels of poly (*N*-isopropylacrylamide) copolymerized with acrylic acid, methacrylic acid, ethacrylic acid and propacrylic acid. When these *N*-isopropylacrylamide polymer particles are dispersed in a hydrogel, large changes occur in the turbidity of the membrane (measured by an absorbance spectrometer) as the pH of the solution in contact with the membrane is varied. Changes of approximately 0.3 absorbance units were observed in the swelling and shrinking of these pH sensitive copolymers of *N*-isopropylacrylamide. Swelling was nonionic as the ionic strength of the solution in contact with the copolymers was increased from 0.1M and 1.0M without a concomitant decrease in swelling. For many of the copolymers, swelling was also reversible in both low and high ionic strength pH buffered media at both ambient and physiological temperatures. Changes in the composition of the formulation used to prepare these copolymers could be correlated to their response to changes in the pH of the buffer solution in contact with them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

BACKGROUND

pH is an important parameter because the rate of many chemical and biochemical reactions are controlled by pH. The popularity of the glass electrode for pH measurements can be attributed to its accuracy, precision, and long lifetime [1]. Although glass electrodes are routinely used in the laboratory, the necessity for its continued recalibration, as a result of drift, limits its application to analysis problems in biological and environmental matrices. For example, there is considerable interest in *in-vivo* pH sensing of blood during surgery for patients suffering from tissue ischemia [2]. However, a glass electrode is simply too bulky for invasive tissue analysis. Glass electrodes, because of the lack of stability of the calibration over an extended period of time, are also not suitable to study gastroesophageal reflux disease [3]. As pH measurements are often a ubiquitous indicator of cell growth and metabolism, monitoring pH in fermentation baths [4] and bioreactors [5] over extended periods of time is crucial. Although conventional glass pH electrodes have been adapted to fermentation reactions and bioreactors, they have several drawbacks including the necessity for pressurized compensation of the electrolyte and interference by other components in the medium. In recent years, measuring pH in the ocean has become important because

of rising acidity due to the water being enriched in carbon dioxide from global warming, and in turn reducing the amount of carbonate ions available for shell building organisms [6]. Although glass electrodes have been used to measure pH in ocean and sea water, the dependence of the liquid junction potential on solution composition and concentration limits their applicability.

The use of fiber optics in pH sensing has burgeoned due to the need for continuous chemical monitoring in industrial, environmental, and biomedical fields. The term optrode has been used to describe these sensors. pH optrodes usually consist of a pH indicator bound onto a solid support or immobilized in a polymer matrix, microgel or nanogel at the distal end of an optical fiber [7, 8]. Interaction of the indicator (which is often a dye) at the end of the fiber with the solution leads to changes in the optical properties of the indicator which is detected through the optical fiber by absorbance or emission from the sensing material [9]. Optrodes have several advantages over glass electrodes including their low cost, the small size of the optical fiber and its robustness. However, the use of chromophores (i.e., dyes or indicators) has several drawbacks. First, the reagent may leach out over time. Second, chromophores photodegrade when absorption is used for sensing which limits the lifetime of the sensor, Third, chromophores are wavelength specific which limits the instrumentation that can be used. Fourth, sensors based on the fluorescence of chromophores generally exhibit very small responses.

To overcome these disadvantages, fiber optic pH sensors have been developed from optrodes that utilize swellable polymers functionalized to respond to pH [10-16]. Polymer swelling has several advantages compared to methods involving chromophores.

The analyte signal is based on turbidity and is wavelength independent. The use of polymer particles entrapped in a permeable membrane for turbidity measurements avoids the problem of photodegradation. These membranes have been shown to be stable for more than one year [17]. The polymer particles do not leach out of the membrane as compared to reagents. Near infrared wavelengths can be transmitted through an optical fiber without appreciable attenuation unlike ultraviolet or visible electromagnetic radiation. Using near infrared radiation to monitor polymer swelling enables a true coupling of the state of the art optical fiber technology with chemical sensing.

In this study, pH sensitive swellable polymer particles functionalized to respond to pH have been prepared from microgels of poly (N-isopropylacrylamide) copolymerized with acrylic acid, methacrylic acid, ethacrylic acid, and propacrylic acid to develop polymer synthates that span a large pH range including a portion of the physiological pH range. When these polymer particles are dispersed in a hydrogel, large changes occur in the turbidity of the membrane which is measured by an absorbance spectrometer as the pH of the solution in contact with the membrane is varied. Changes of approximately 0.3 absorbance units were observed in the swelling and shrinking of pH sensitive N-isopropylacrylamide polymer particles. Swelling was reversible in both low and high ionic strength pH buffered media at both ambient and at physiological temperatures. It is the goal of this study to gain an understanding of how changes in the composition of the polymer formulation influences the response of the functionalized poly (N-isopropylacrylamide) particles to changes in the pH of the solution in contact with them.

The study discussed in this thesis is a follow-up to a previous study performed by Dr. Don Nuwan Perera, who investigated pH induced swelling of copolymers of *N*-isopropylacrylamide and alkyl acrylic acids. The primary focus of Perera's study was the pH sensitive functional comonomer methacrylic acid. Changes in the formulation of copolymers of *N*-isopropylacrylamide and methacrylic acid were investigated with particular attention to the influence of N-tertbutylacrylamide and methylene-bisacrylamide on the swelling behavior of these polymer synthates. As the motivation for the study described in this thesis is physiological pH sensing, the pH sensitive functional comonomers, ethacrylic acid and propacrylic acid, were the focus of our investigation. Furthermore, the copolymers of *N*-isopropylacrylamide and alkyl acrylic acids prepared for our study were restricted to 5% crosslinking, whereas Perera investigated a wide range of crosslinking levels (5% to 20%).

CHAPTER II

THEORY

2.1. Introduction

A crosslinked polymer unlike a polymer without crosslinking will not dissolve when placed in a compatible solvent [18]. Rather, the polymer (if it is lightly crosslinked) absorbs solvent and swells until equilibrium has been reached. At equilibrium, the swelling caused by solvation or penetration of the solvent into the polymer network is counterbalanced by retractive forces due to the stretching of the polymer linkages. (In other words, swelling is a competition between two forces with solvation representing the dilution of the polymer solution by the solvent which is being opposed by an elastic retractive force arising from the stretching of the polymer due to penetration of the solvent into the three-dimensional polymer network.) The degree of swelling exhibited by the polymer is governed by the affinity of the solvent for the polymer.

Polymer swelling [19] is either ionic or nonionic. For ionic swelling, there is an additional force contributing to swelling that is absent in nonionic swelling. This force is caused by electrostatic repulsion between neighboring charges on the polymer. The

strength of this force is governed by the charge density of the polymer compared to that of the external solution (i.e., its ionic strength and dielectric constant) in contact with the polymer.

2.2 Nonionic Swelling

Flory [20] derived an equation to describe nonionic swelling (see Equation 1)

$$Q_m^{5/3} \cong \frac{(\bar{v}M_c)(\frac{1}{2} - \chi_1)}{(1 - \frac{2M_c}{M})V_1} \qquad (1)$$

where Q_m is equilibrium swelling ratio, $\bar{v}$ is the specific volume of the polymer, M_c is the molecular weight per crosslinked unit, M is the molecular weight of the polymer network, χ_1 is the polymer-solvent interaction parameter, and V_1 is the molar volume of the solvent. The equilibrium swelling ratio is the volume of the swollen polymer divided by the volume of the unswollen polymer and $(1-2M_c/M)$ is a correction factor for polymer network imperfections due to the chain extremities. This term reduced to unity when the network is perfect. χ_1 accounts for the compatibility or affinity of the polymer for the solvent. From Equation 1, the swelling ratio is dependent upon the degree of crosslinking and the affinity of the polymer for the solvent. In a good solvent, the swelling ratio decreases with increased crosslinking and with increased molecular weight of the polymer. On the other hand, increasing the polymer affinity of the solvent will

increase the swelling ratio. If $\chi_1 < 0.5$, Q_m is positive and the polymer swells. If $\chi_1 >$ 0.5, Q_m is negative and the polymer shrinks. Swelling and shrinking are governed by the polymer solvent interaction parameter when swelling is nonionic.

2.3 Ionic Swelling

Ionic swelling is caused by an accumulation of charge in the polymer. These charges give rise to electrostatic repulsion on the backbone of the polymer causing it to swell. Swelling minimizes the repulsive effect of the charges causing them to be moved further away from each other. Ionic swelling is the result of two forces: the interaction of the polymer with the solvent and the osmotic pressure difference between the bulk solvent and the polymer. Flory [20] characterized ionic swelling via Equation 2

$$Q_m^{5/3} \cong \frac{\left(\dfrac{i}{2V_u\sqrt{S}}\right)^2 + \left(\dfrac{\frac{1}{2} - \chi_1}{V_1}\right)}{\dfrac{V_e}{V_0}} \tag{2}$$

where Q_m is the equilibrium swelling ratio, i = number of electronic charges per unit polymer unit, V_u = molecular volume of polymer repeating unit, S is the ionic strength of free solvent (solution) in contact with the polymer, χ_1 is the polymer-solvent interaction parameter, V_1 is the molar volume of the solvent, v_e is the effective number of chains in the network, V_0 is the unswollen network volume.

7

From Equation 2, it is observed that increasing the amount of crosslinker within the polymer results in a subsequent decrease in total swelling. On the other hand, an increase in the ionic charge of the polymer backbone causes the equilibrium-swelling ratio to increase. The swelling of a charged polymer can be treated in terms of an osmotic pressure effect that results from differences between the charge density of the bulk polymer and that of the surrounding solvent. As for the polymer-solvent interaction parameter, it has the same effect on ionic swelling as it does on nonionic swelling. Low ionic strength solutions increase the swelling ratio because of the absence of shielding of the charges by the solvent ions or alternatively large differences exist between the charge density of the bulk polymer and that of the surrounding solution. However, shielding of the charges on the polymer backbone occurs in high ionic strength solutions or alternatively small differences exist between the charge density of the bulk polymer and that of the surrounding solution which causes a reduction in swelling. By comparison, nonionic swelling is not affected by the ionic strength of the solution in contact with the polymer. Thus, nonionic swelling can be differentiated from ionic swelling experimentally by increasing the ionic strength of the solution in contact with the polymer (e.g., 0.1M to 1.0M) and observing the degree to which the swelling is suppressed. If there is no change in swelling, the conclusion that can be drawn is that swelling is nonionic.

2.4. Polymers of *N*-isopropylacrylamide

Poly*N*-isopropylacrylamide (PolyNIPA) is a typical stimuli-sensitive polymer that has attracted considerable attention due to its unique swelling properties [21, 22]. PolyNIPA is a well-known member of a class of polymers (acrylamides) whose solubility decreases upon heating. PolyNIPA is swollen at lower temperature and exists in a shrunken state at higher temperature. The volume change that occurs over a narrow temperature range near physiological temperature corresponds to a coil-to-globule transition of the polymer chains driven by hydrophobic interactions [23]. This reversible transition, which is accompanied by expulsion of water from the polymer network, occurs at a temperature called the lower critical solution temperature or simply the transition temperature. For polyNIPA, the transition temperature is 32°C. The molecular interactions defining and maintaining the structure of the polymer network of polyNIPA in an aqueous environment include van der Waals forces, hydrogen bonding, hydrophobic interactions, and dipole-dipole interactions.

For synthates of *N*-isopropylacrylamide copolymerized with pH sensitive co-monomers, swelling can be induced by changes in the pH of the solution in contact with the polymer, which in turn alters polymer-polymer and polymer-solvent interactions. As *N*-isopropylacrylamide is present in far greater amounts than other monomers in these synthates, polymer chains containing the abundant monomer are present with intermittent inclusion of the pH sensitive functional comonomer [24]. This will be true if the relative reactivity ratio is the same for all monomers in the polymer formulation. Excess *N*-

isopropylacrylamide monomer is polymerized into chains containing well separated pH sensitive functional comonomer units. However, the three-dimensional nature of the crosslinked *N*-isopropylacrylamide polymer allows the functional comonomer units to be more closely oriented to each other than that observed for only linear chains. Furthermore, this orientation, which influences the pH response properties of these polymers, is governed by both temperature (as the volume of the polymer decreases with increasing temperature) and the composition of the formulation used to prepare the synthates.

The characterization of the unique properties of these copolymers is crucial toward advancing our understanding of polymer swelling and to the discovery of new applications for these materials. Our current understanding of polymer swelling and how the composition of the polymer relates to the swelling behavior is inadequate. Additional work needs to be undertaken to elucidate the relationships between monomer content and type and how they relate to polymer swelling. The incorporation of a pH sensitive functional comonomer into polyNIPA imparts unique properties to polyNIPA. For the reasons given above, characterization of several NIPA copolymers of acrylic acid, methacrylic acid, ethacrylic acid, and propacrylic acid was undertaken as part of the research described in this thesis.

CHAPTER III

METHODOLOGY

3.1. Introduction

In recent years, there has been considerable interest on investigating polymer swelling by embedding the swellable polymer in a suitable membrane [25-27]. Swellable polymers prepared from NIPA have been the subject of numerous reports due to their response to temperature and pH [28-30]. Measuring swelling in NIPA derived polymers, however, has been a challenging task due to the difficulties in the preparation of stable particle suspensions of NIPA polymers in aqueous solution. For swellable polyNIPA particles, the use of a secondary polymer to suspend the functionalized NIPA particles offers a potential solution to this problem. The secondary polymer used to suspend NIPA polymer particles must fulfill several requirements, which are listed in Table 3.1. Polyvinyl alcohol was chosen, since it possesses all of the attributes needed for an immobilization membrane.

Table 3.1. Ideal Characteristics of a Secondary Polymer for Membrane Preparation

Properties	Significance
Optical Transparency	The membrane should be able to prevent any attenuation of light.
Non Hazardous & Rigidity	The membrane should be convenient to work on routine basis and it should not be too rigid to limit the swelling.
Chemically Inert	The membrane should not interfere with any other components which might result in different optical properties.
Insoluble in aqueous solvent	The membrane should be insoluble in aqueous solvent as the analysis is performed in aqueous samples.

3.2. Hydrogel Membrane Preparation

The method for the preparation of the hydrogels membrane used to study polymer swelling consists of five steps: a) preparation of PVA solution, b**)** Preparation of polyNIPA particle and PVA suspension, c) PVA addition to particle dispersion and Polymerization, d**)** Membrane casting, e) Membrane storage and mounting.

a) **Preparation of PVA solution**

PVA solution was prepared by dissolving 12.5g of PVA (363154-250G, 85,000-124,000, Sigma-Aldrich) in 125 ml of DI water in a beaker, followed by stirring overnight at 70 °C.   To determine the PVA concentration, one drop of the PVA solution is weighed and heated in an oven for approximately 20 min to adjust its PVA content to 10% w/w. The formula used to determine PVA concentration is as follows:

$$\text{PVA concentration} = (M_f (PVA)*100)/ M_i (PVA) \qquad (1)$$

where, M_f (PVA) is the weight of the dried PVA after evaporation of the solvent in the oven, M_i (PVA) is the weight of the PVA drop before drying. If the PVA concentration is too low (< 10%), water is removed by heating the drop on a hot plate. If the PVA concentration is too high (> 10%), the PVA is diluted by adding the appropriate amount of water to the beaker. Once the water content of the PVA solution has been appropriately adjusted, the solution is filtered through a porous glass filter under vacuum. 3-chlorophenol is added to the filtered solution to prevent mold growth during storage. Once the preparation of the PVA solution is completed, the next step is preparation of a suspension that consists of swellable pH sensitive polyNIPA particles suspended in the PVA solution.

b) **Preparation of polyNIPA particle and PVA Suspension**

The preparation of the suspension involves determining the amount of polymer particles (stored in the methanol) added to the PVA solution. The amount of polymer particles is determined by pipetting a drop of the polymer particles onto a tared plastic dish, followed by evaporation of the methanol for approximately 30-60 min. After evaporation of the methanol, the concentration of polyNIPA particles is determined using the following equation:

$$\text{Microparticle concentration} = (M_f (m.p)*100)/ m_i (m.p) \qquad (2)$$

where M_f (m.p) is the weight of the dry microparticles after evaporation of the methanol (solvent) and M_i (m.p) is the initial weight of the microparticles. After the concentration of polymer particles is determined, the mass of the NIPA polymer is computed using the following equation:

$$\text{Mass of microparticles} = (0.02*100)/\ C\ (\text{m.p}) \qquad (3)$$

Equation 3 contains a correction factor, 0.02, which was determined experimentally (and included in the calculation) to ensure the preparation of optically transparent membranes that possess sufficient mechanical strength.

Once the weight of the particles in the suspension is determined, the corresponding mass is pipetted into a reaction vial, diluted with 1 ml of DI water and a stream of air is passed over the suspension while stirring to evaporate the all the methanol. This process is allowed to continue until a constant weight is achieved.

c) **PVA addition to particle dispersion and Polymerization**

After a constant weight of polymer particles is obtained in the reaction vial, the weight of the PVA solution required to prepare the particle suspension is determined by the following equation:

$$\text{Mass (PVA)} = (0.2*100)/\ C\ (\text{PVA}) \qquad (4)$$

2 g of 10% w/w PVA solution is added to the reaction vial. The vial is allowed to stir for about 8 hours to ensure proper mixing of its contents. 50 µL of 10% glutaraldehyde (GDA), crosslinking solution is then added to the reaction vial and stirred for 1 hour for proper mixing. Next, 100-200 µl of 0.1M HCl, which served as the initiator for the polymerization, is added to the vial. The solution is sonicated for 2 min (to remove any air bubbles) and stirred vigorously for another 2 min.   Polymerization to form the membrane is completed during this time.

d) Membrane Casting

Once the polymerization reaction to form the membrane has been completed, the entire contents of the vial are pipetted onto a glass slide for casting. The schematic for this process is shown in Figure 3.1.
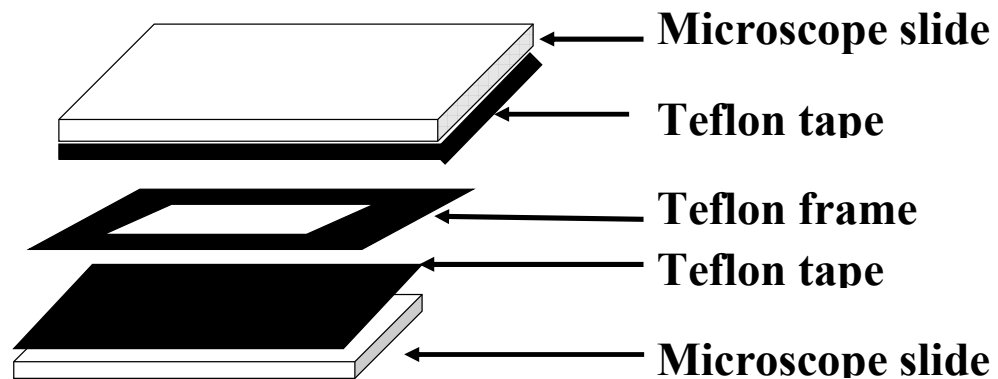

Figure 3.1. Schematic of the Molding and Casting stage

After pipetting the contents onto a glass slide with a Teflon frame, a second glass slide is placed on top of it and clamped on all edges using portfolio clips to form the mold for membrane preparation. The suitable level of hardness can be achieved by curing for

one hour. The clips are then removed and the cast is soaked in Petri-dish containing DI water for one day.

e) Membrane storage and mounting

Once the desired level of hardness for the membrane is obtained, the membrane is removed by prying at the edges of the glass slides using a razor blade. The membrane is checked for uniformity and any irregular segments and stored in a 10 ml sample vial containing DI water which is placed in a refrigerator.

The next step is mounting the hydrogel membrane onto its holder. Figure 3.2 shows a schematic of the sample holder for mounting the membrane.
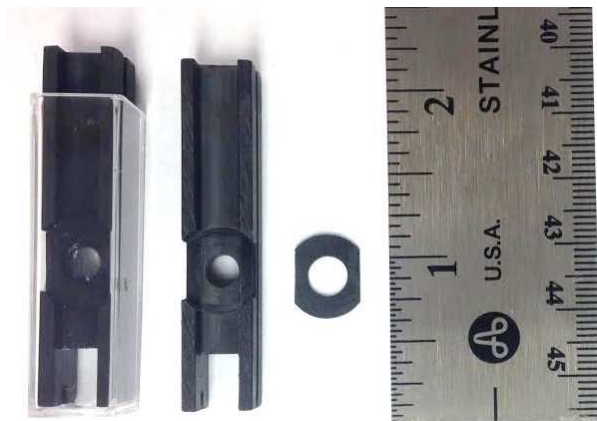
Figure 3.2. Schematic of Sample Holder for Mounting Membrane Segment

For mounting a membrane segment onto holder, a few drops of DI water are added to a microscope glass slide. The membrane is visually inspected for quality and uniformity. The segment of the hydrogel membrane that contains irregularities, tears or holes is cut away using a razor blade. The mount is then pressed against the membrane. After the membrane adheres to the mount, the sample holder slot is placed over the

membrane. The mounted membrane in the sample holder is placed in a vial containing

pH8 buffer. The unused membrane is placed back into the 10 ml sample vial containing

DI water.

3.3. Turbidity Measurements with the UV-visible Spectrometer

The swelling behavior of the hydrogel membranes which contain pH sensitive

polyNIPA particles were evaluated using a Cary 6000i UV-Visible spectrometer. The key

components of the absorbance spectrometer used for measuring turbidity include the

radiation source, monochromator, sample holder, detector and signal readout device. A

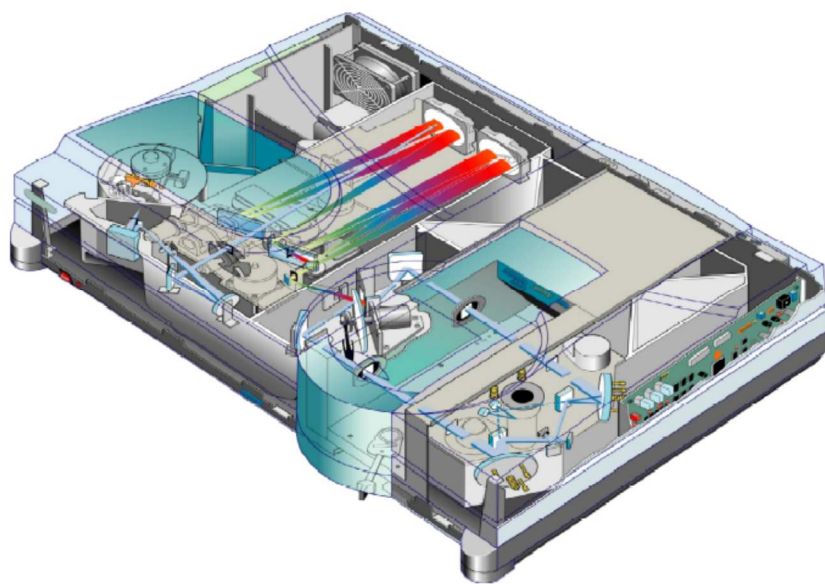schematic of an inside view of the Cary 6000i is shown in Figure 3.3.

Figure 3.3. Inside view of Cary 6000i spectrometer

*Source: http://www.varianinc.com/image/vimage/docs/products/spectr/
    uv/brochure/1942.pdf*

a. Radiation Source:

The light source for a spectrophotometer must emit a continuous spectrum of constant intensity in both the ultra-violet and visible regions. The Cary 6000i is equipped with a deuterium lamp as the continuous source for the ultra-violet region and a tungsten bulb as the continuous source for the visible region.

b. Monochromator:

The light that interacts with the sample should have a fixed wavelength to turbidity. Wavelength control is crucial and the monochromator is responsible for performing this function. The monochromator is placed between the light source and the sample to break up the incident light beam into its component wavelengths. Turbidity measurements are obtained over the wavelength range 350-700 nm.

c. Sample Stages:

The samples analyzed by the absorbance spectrometer should be positioned properly to achieve accurate and precise measurements. The Cary 6000i is equipped with a variety of sample stages including the dual cuvette stage, six-unit cell, and a single sample stage as well as a flow cell setup to transfer buffer in and out of the cuvette containing the hydrogel membrane mounted in its sample holder. The spectrometer is also equipped with a thermal control unit. These components and accessories fix the sample cuvette in the proscribed optical path of the incident beam of light to ensure reproducible measurements.

d. Detectors:

The Cary 6000i is equipped with a photomultiplier tube for detection in the UV-Visible region and an Indium Gallium Arsenide detector for the near infrared region. The technical specifications of the Cary 6000i spectrometer are listed in Table 3.2.

Table 3.2. Technical Specifications of Cary 6000i Spectrometer

Bandwidth	UV-Vis 0.01 nm to 5.00 nm,0.01 nm steps, NIR 0.02 to 10nm
Optics	Double Beam
Photometric Range	8 Abs
Sample Compartment	(W×D×H):160×433×221 mm. Access: top, Front and base
Scanning Speed	UV-Vis:2000nm/min. NIR: 4000 nm/min
Depth	710 mm
Height	380nm
Width	1020nm
Power Requirements	Mains supply of 85-264 volts AC with 300 VA power consumption. Frequency 47-63 Hz.
Weight	91 kg
Additional Specifications	**Grating:** Dual sided, 70 × 45 mm, UV-Vis: 1200 lines/mm blazed at 250 nm, NIR:600 lines/mm blazed at 1000 nm **Beam splitting system:** Chopper 30 Hz **Limiting resolution(nm):** UV-Vis: <0.05nm NIR: <0.2 **Wavelength reproducibility:** <0.025 nm **Wavelength accuracy:** UV-Vis: +/- 0.1 nm NIR: +/- 0.4 nm

Source: http://www.labwrench.com/?equipment.view/equipmentNo/4796/Varian/Cary-4000-5000-6000i.

3.4. Experimental Setup

The membrane sample holder was removed from the storage vial containing pH 8 buffer solution and was placed in a cuvette which was then inserted into the sample stage compartment of the spectrometer. A reference cuvette filled with DI water was used to compensate for fluctuations in the intensity of the light source. The sample cuvette was filled with a low pH buffer solution which served as the starting point for the pH response curve of each membrane. The pH response curves (between pH 3 and pH10) were obtained using buffers consisting of chloroacetic acid, acetic acid, 2-(N-morpholino) ethanesulfonic acid and 3-(N-morpholino) propanesulfonic acid were used to construct the pH profiles. To adjust the ionic strength of these buffers, a known and fixed amount of NaCl was added to each buffer solution. The pH ranges of the buffers used are listed in Table 3.3.

Table 3.3. pH Range of the Buffers used to Obtain the pH Response Curves

Buffers	pH range
Chloroacetic acid	3-3.8
Acetic acid	3.9-5.4
MES	5.5-7.3
MOPS	7.4-8
TRIS	7.2-9

An online buffer calculator (www.liv.ac.uk/buffers/buffercalc.html) was used to prepare each buffer solution. The polymer membrane equilibrated in each buffer for 5 min between runs for ascending pH. To measure the turbidity during the reverse process

(descending pH), the polymer membrane was equilibrated for 7 min between runs. Absorbance (turbidity) was recorded at 700 nm.

3.5. pH Response Curves

pH response curves were obtained from turbidity measurements at 700 nm. Figure 3.4 shows how a pH response curve is obtained from turbidity data.
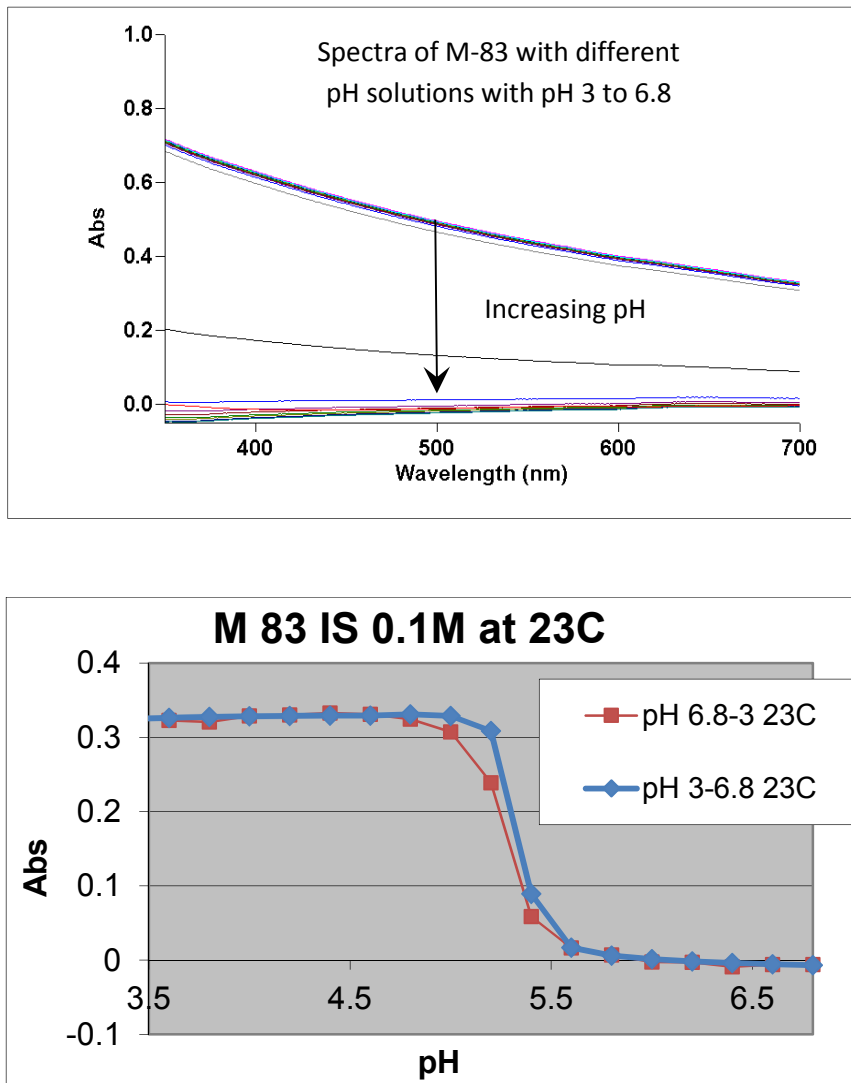

Figure 3.4. Turbidity Spectra (top) and pH response curve of the hydrogel membrane (bottom).

At lower pH, the polymer particles are in a shrunken state.   At higher pH, the particles are in a swollen state. The apparent pKa (i.e., the inflection point of the pH response curve) was computed from a first derivative plot of the data. Figure 3.5 shows the first derivative plot of the pH response curve for M-83 particles.
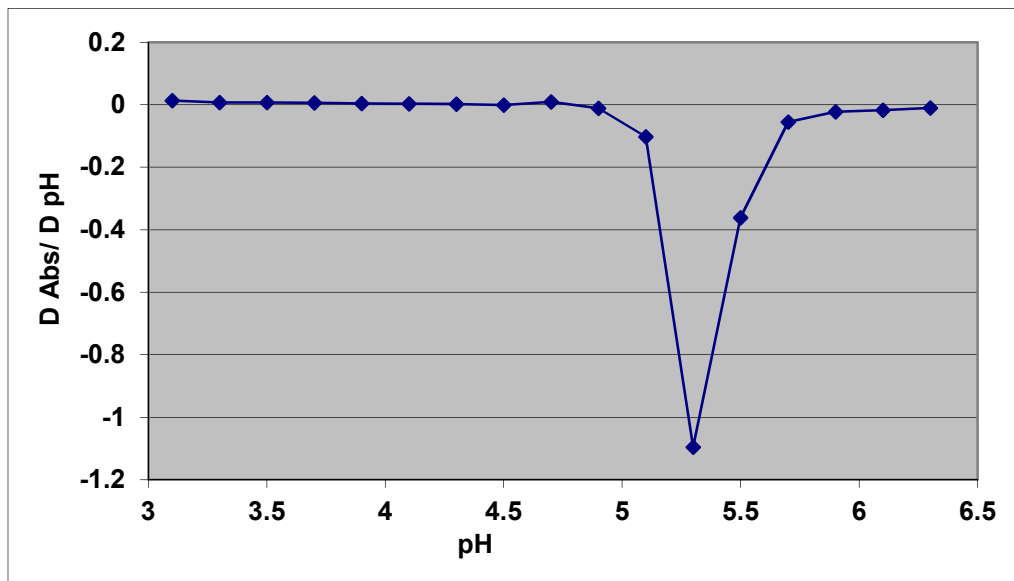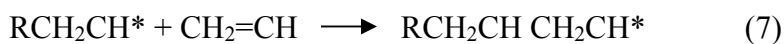

Figure 3.5. First derivative plot of the pH response curve for M-83 particle at 23 °C

3.6. Synthesis of PolyNIPA Particles
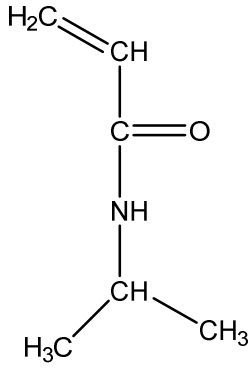
The poly NIPA particles were synthesized by free radical initiated dispersion photo-polymerization. The mechanism of free radical polymerization involves 3 steps. In initiation, a free radical is generated to start the reaction (see Equation 5).   The next step is chain growth (see Equation 6). In this step, the free radical reacts with the active part of the monomer and forms a molecule that has another reactive end. When these monomers react with other monomers continually, chain growth occurs. The last step is termination (see Equation 7). Combination and disproportionation are the two ways in which a polymer reaction is terminated. Combination is the more likely step and occurs

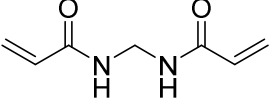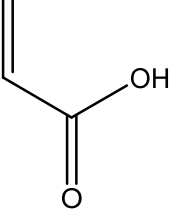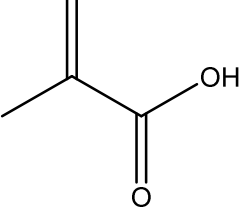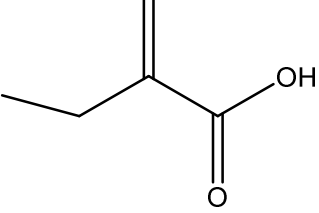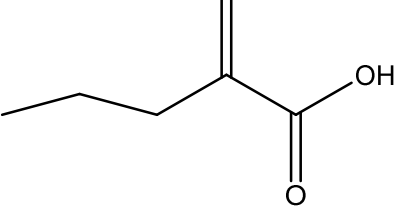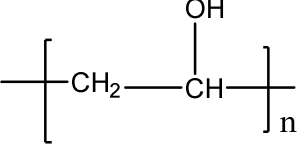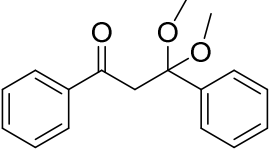when the reactive part of a chain end reacts yielding a single molecule that undergoes no further reaction.

$$\text{Initiator} \longrightarrow R* \qquad\qquad (5)$$

$$R* + CH_2=CH \longrightarrow RCH_2CH* \qquad\qquad (6)$$

$$RCH_2CH* + CH_2=CH \longrightarrow RCH_2CH\ CH_2CH* \qquad (7)$$

The components comprising the formulation used to prepare the pH sensitive PolyNIPA particles include the transduction monomer, initiator, functional co-monomer, cross linker, and solvent. *N*-isopropylacrylamide (NIPA) is the transduction monomer, 2, 2-Dimethoxy-2-phenyl-acetophenone (DMPA) was used as the initiator, acrylic acid, methacrylic acid, ethacrylic acid and propacrylic acid were used as functional co-monomers and methylene-bis-acylamide (MBA) was the crosslinker. Acetonitrile served as the solvent as all these components are soluble in it.

Table 3.4. Chemicals used in Preparation of pH Sensitive Membranes

H_2C $=$ CH $\|$ $C=O$ $\|$ NH $\|$ CH H_3C $\diagup$ $\diagdown$ CH_3	NIPA-Transduction Monomer

	MBA: Crosslinker
	Acrylic Acid Functional Co-monomer
	Methacrylic Acid Functional Co-monomer
	Ethacrylic Acid Functional Co-monomer
	Propacrylic Acid Functional Co-monomer
	Poly vinyl Alcohol
	DMPA: Photo initiator

H H—C—C≡N H	Acetonitrile: solvent
H₂C=CH C=O NH C H₃C—C—CH₃ CH₃	N-tertbutylacrylamide

The PolyNIPA formulation was prepared by adding NIPA, functional co-monomer, and MBA into a 500 ml 3-neck round bottom Pyrex flask containing 100ml of acetonitrile. When AA or MAA were used as the functional co-monomer, N-tert butylacrylamide was added to the formulation. The mixture was stirred for 30 min in a closed system to prevent oxygen from infiltrating into the reaction mixture. Once the components were dissolved, 0.2 grams DMPA and the functional co-monomer were added to the reaction mixture which was sonicated for 20 min with a Branson 1500 ultrasonicator while simultaneously being purged using dry nitrogen. After sonication and purging, the 500 ml 3-neck flask was placed in a Rayonet UV photolysis chamber equipped with G4T5 type mercury lamps and a cooling fan. The contents of the flask were stirred using a paddle to initiate polymerization. The wavelengths utilized here were from 315 nm-400 nm. (The UV cut-off of Pyrex is below 260 nm.) The reaction was carried out at ambient temperature for 12 hours and a turbid polymer suspension was formed.

After the reaction was terminated, the turbid polymer suspension was transferred into two 40 ml polypropylene centrifuge tubes and centrifuged at 3000 rpm for 10 min. The decant was separated and the particles were suspended in 25ml aliquots of acetonitrile, sonicated for 30 min and again centrifuged at 3000 rpm for 10 min. This washing procedure was repeated 4 times. The particle suspension was than washed with 25 ml aliquots of methanol (3 times). After these washings, the particles were transferred into a vial containing methanol and stored in a refrigerator.

CHAPTER IV

RESULTS

Figures 4.1 through 4.4 show the pH response profile of four poly (*N*-isopropylacrylamide) synthates prepared by copolymerization with AA, MAA, EAA, and PAA.
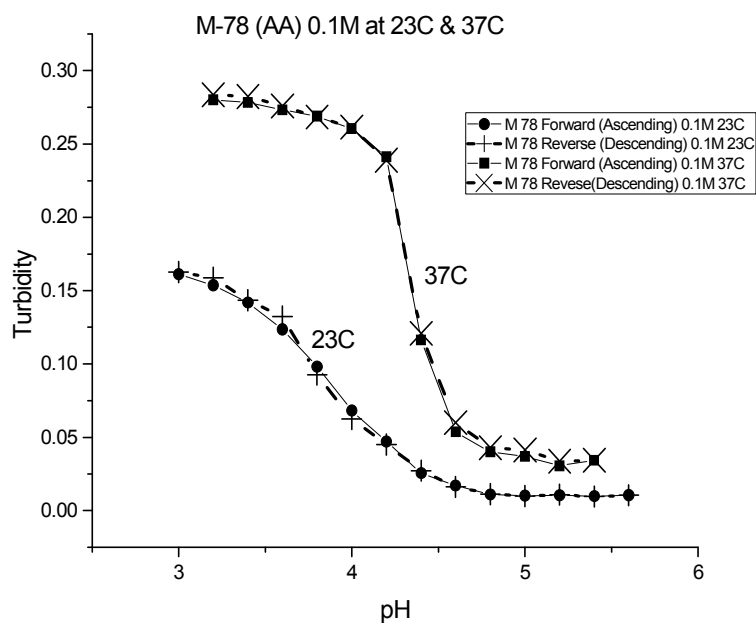
Figure 4.1. Ascending and descending pH profiles for polymer particles of *N*-isopropylacrylamide copolymerized with acrylic acid at 23 $^{\circ}$C and 37 $^{\circ}$C. Solid line = ascending pH profile. Dashed line = descending pH profile.
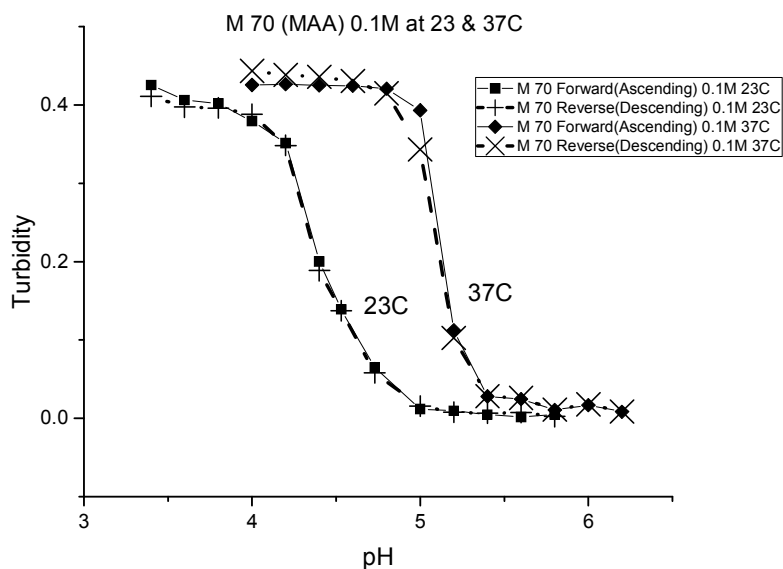
Figure 4.2. Ascending and descending pH profiles for polymer particles of *N*-isopropylacrylamide copolymerized with methacrylic acid at 23 °C and 37 °C. Solid line = ascending pH profile. Dashed line = descending pH profile.
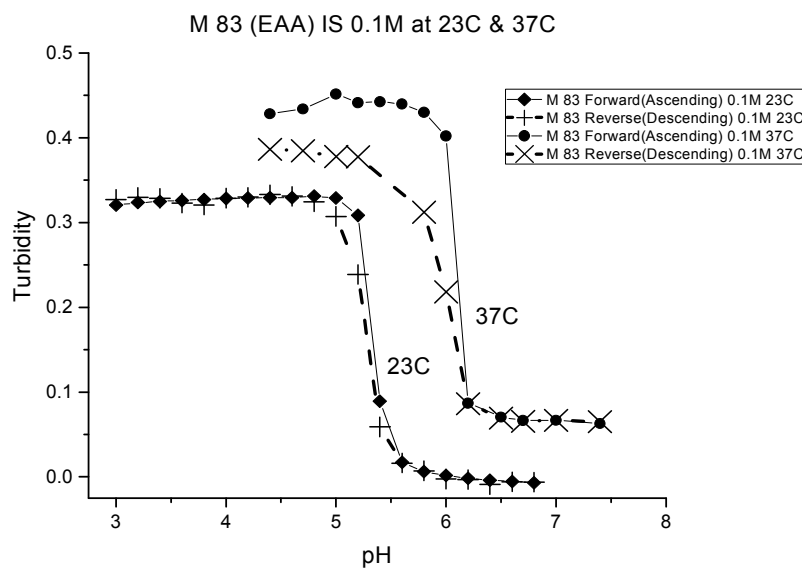
Figure 4.3. Ascending and descending pH profiles for polymer particles of *N*-isopropylacrylamide copolymerized with ethacrylic acid at 23 °C and 37 °C. Solid line = ascending pH profile. Dashed line = descending pH profile.
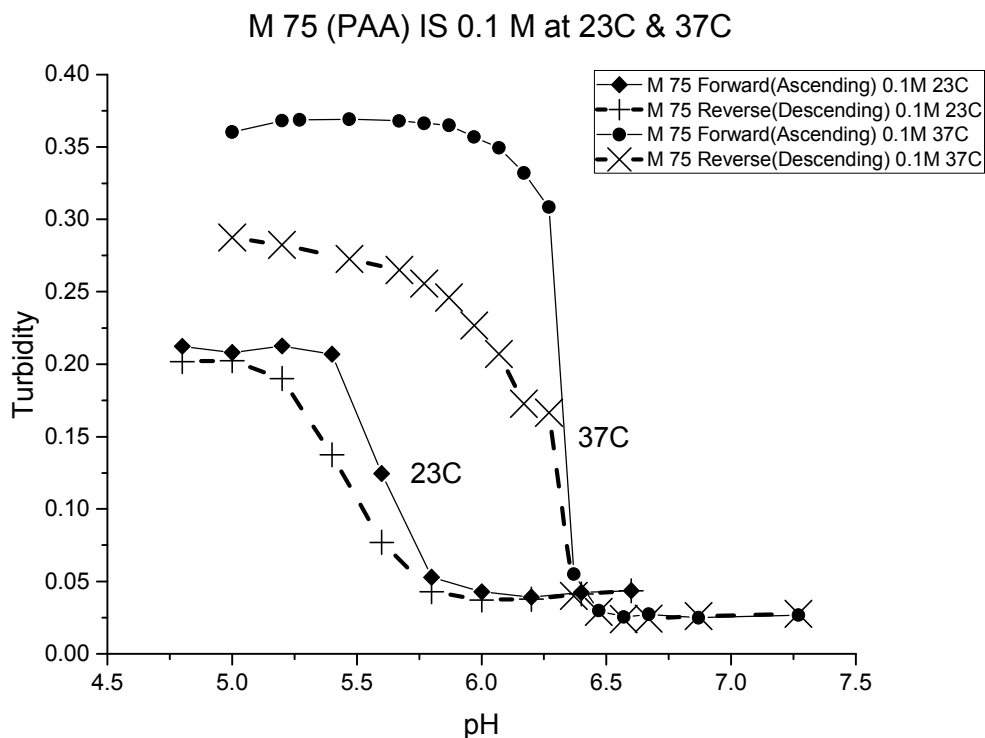
Figure 4.4. Ascending and descending pH profiles for polymer particles of *N*-isopropylacrylamide copolymerized with propacrylic acid at 23 °C and 37 °C. Solid line = ascending pH profile. Dashed line = descending pH profile.

Each synthate, whose formulation is summarized in Table 4.1, was embedded in a PVA hydrogel membrane. For each data point in Figures 4.1 through 4.4, the membrane was rinsed three times with buffer solution and allowed to equilibrate for 15 min prior to analysis by absorbance spectroscopy. Turbidity (absorbance values) collected at 700 nm was plotted against pH of the buffer solutions.

Table 4.1. Formulation of Four Poly (*N*-isopropylacrylamide) Synthates Prepared by
Copolymerization with AA, MAA, EAA, and PAA.

Component	AA(mmol)	MAA(mmol)	EAA(mmol)	PAA(mmol)
NIPA	14.6	15	15	17
Functional Co-monomer	2	2	2	2
NTBA	2	2	2	0
MBA	1.4	1	1	1

For AA and MAA, swelling is reversible at 23 $^{\circ}$C and 37 $^{\circ}$C as both the ascending

(solid line) and descending (dashed line) pH profiles are superimposable, whereas EAA

is only reversible at 23 $^{\circ}$C and PAA is irreversible at both 23 $^{\circ}$C and 37 $^{\circ}$C. At low pH,

the polymer exists in a shrunken state and the water content of the polyNIPA particles is

low. The turbidity as measured by the spectrometer is high because the refractive index

of the particles is higher than that of the PVA membrane. At higher pH values, the water

content of the polymer increases due to swelling induced by the deprotonation of the pH

sensitive comonomer.   The refractive index decreases as the particles swell and

approaches the refractive index value of PVA.   Eventually, the turbidity reaches a

limiting value that corresponds to complete deprotonation of the functional comonomer.

The inflection point in the plot of turbidity (absorbance) versus pH is the apparent

pK_a of the polymer. At the inflection point of each pH response curve shown in these

figures, there are approximately equal amounts of the protonated and deprotonated form

of the polymer. The term "apparent pK_a" is used to refer to the point where the response

is half-way between the response at low pH and at high pH as turbidity versus pH curves

have not yet been described by theory in a manner that would allow calculation of the pK_a from the observed data.

Table 4.2 lists the apparent pK_a values of the four synthates at 23 °C and at 37 °C. The apparent pKa (i.e., the inflection point), which was computed for each curve using the first derivative, can be tuned by increasing the alkyl chain length (hydrophobicity) of the pH sensitive functional co-monomer. This can be attributed to the low reactivity ratios of EAA and PAA [31]. For the copolymers in Table 4.1, NIPA is the dominant monomer with only a small amount of functional comonomer present. For AA and MAA, NIPA will be polymerized into chains containing well separated functional comonomer units since the relative reactivity ratios of all monomers comprising these copolymers are the same. Because of the lower reactivity ratios of EAA and PAA, the corresponding copolymer will not contain well separated functional comonomer units resulting in higher pK_a values, analogous to the higher pK_a values obtained for the second proton of maleic acid versus that of fumaric acid. The greater hydrophobicity of EAA and PAA compared to AA and MAA cannot be ignored and may contribute to decreasing the water content of the polymer resulting in higher pK_a values. The presence of PAA in Poly (*N*-isopropylacrylamide) also causes the lower critical solution temperature of this polymer to decrease to 23 °C compared to 36.7°C for MAA, and this has been attributed to the hydrophobic nature of the acidic form of PAA [32].

The increase in the pK_a with temperature for the four synthates is opposite to what occurs for an acid dissolved in water which is a decrease of about 0.1 – 0.3 pH units for a 10 °C increase in temperature [33]. The increase in the apparent pK_a of the polymer particles can be attributed to a decrease in the distance between functional comonomer

units in the three dimensional polymer network due to a decrease in the volume of the polymer which can be attributed to water molecules being driven out of the poly (*N*-isopropylacrylamide) copolymer as the temperature increases [34].

Table 4.2. Apparent pK$_a$ Values of the four Synthates at 23 $^\circ$C and at 37 $^\circ$C.

Apparent pK$_a$	[1]AA	[2]MAA	[3]EAA	[4]PAA
23°C	3.9	4.3	5.3	5.5
37°C	4.3	5.1	5.9	6.1

[1]pK$_a$ of AA as determined by titration with NaOH standardized by KHP is 4.25
[2]pK$_a$ of MAA as determined by titration with NaOH standardized by KHP is 4.65
[3]pK$_a$ of EAA as determined by titration with NaOH standardized by KHP is 4.65
[4]pK$_a$ of PAA as determined by titration with NaOH standardized by KHP is 4.84

To better understand the relationship between the apparent pK$_a$ of the pH sensitive poly (*N*-isopropylacrylamide) particles and temperature, pH response curves were obtained for each synthate at seven temperatures (23 $^\circ$C – 40 $^\circ$C). The apparent pK$_a$ was computed for each response curve, and the relationship between pK$_a$ and temperature was modeled using the van't Hoff relationship, see Equation 7 where K$_a$ is the acid dissociation constant of the copolymer, T is the temperature of the copolymer, ΔH^0 is the change in enthalpy associated with the pH induced swelling of the poly (*N*-isopropylacrylamide) synthate, ΔS^0 is the change in entropy associated with the pH induced swelling of the poly (*N*-isopropylacrylamide) synthate, and R is the gas constant.

$$\ln K_a = \frac{-\Delta H^O}{RT} + \frac{\Delta S^O}{R} \tag{7}$$

Table 4.3 lists the changes in enthalpy and entropy that occur due to pH induced swelling of the poly (*N*-isopropylacrylamide) synthates. For AA, MAA, and EAA, the increase in the hydrophobicity of the comonomer in poly (*N*-isopropylacrylamide) can be directly correlated to a decrease in both the enthalpy and entropy of swelling for these synthates, whereas PAA does not fit into this series. This could be attributable to the absence of NTBA in the formulation used to prepare this synthate. Alternatively, the *N*-isopropylacrylamide copolymer of PAA may undergo a conformational change as the copolymer swells which prevents it from returning to the same initial state.

Table 4.3. Changes in Enthalpy and Entropy due to pH Induced Swelling of the Poly (*N*-isopropylacrylamide) Synthates

Enthalpy & Entropy	AA	MAA	EAA	PAA
[1]ΔH (J/Mole)	-73000 ± 8580	-88700±8230	-101000±2200	-90400±9500
[1]ΔS (J/Mole-K°)	-320 ±28	-380±27	-440±7.2	-410±32

[1]Uncertainties determined from the least squares fitting of the data

As our motivation for undertaking this study is physiological pH sensing (pH 5 – pH 7.4), we decided to focus this investigations on EAA and PAA synthates. Figures 4.5 and 4.6 show pH response curves at 23 °C and 37 °C for a variety of EAA synthate formulations (see Table 4.4).
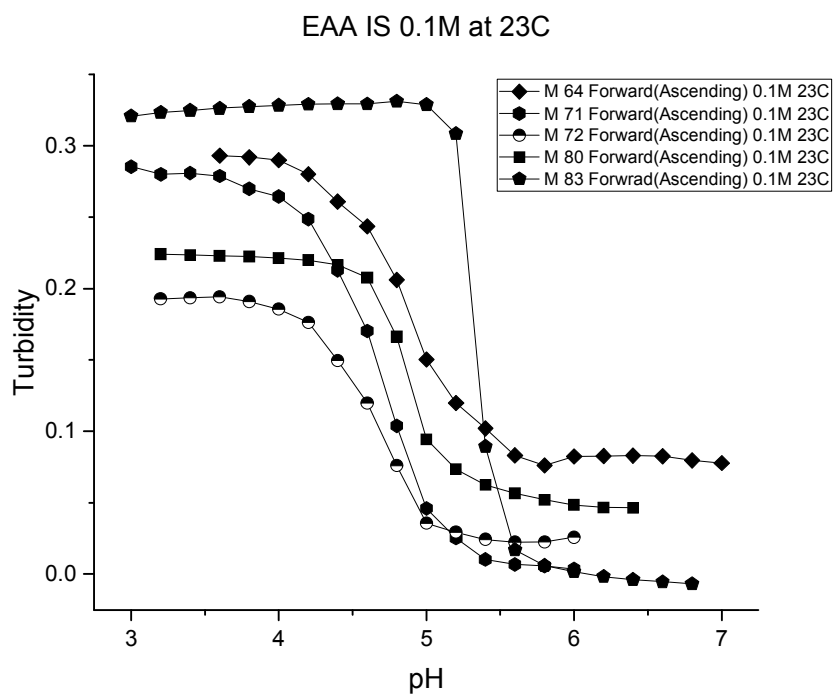
Figure 4.5. Ascending pH profiles at 23 °C for a variety of EAA synthates: M-64, M-71, M-72, M-80, and M-83 (see Table 4.4 for the formulations)
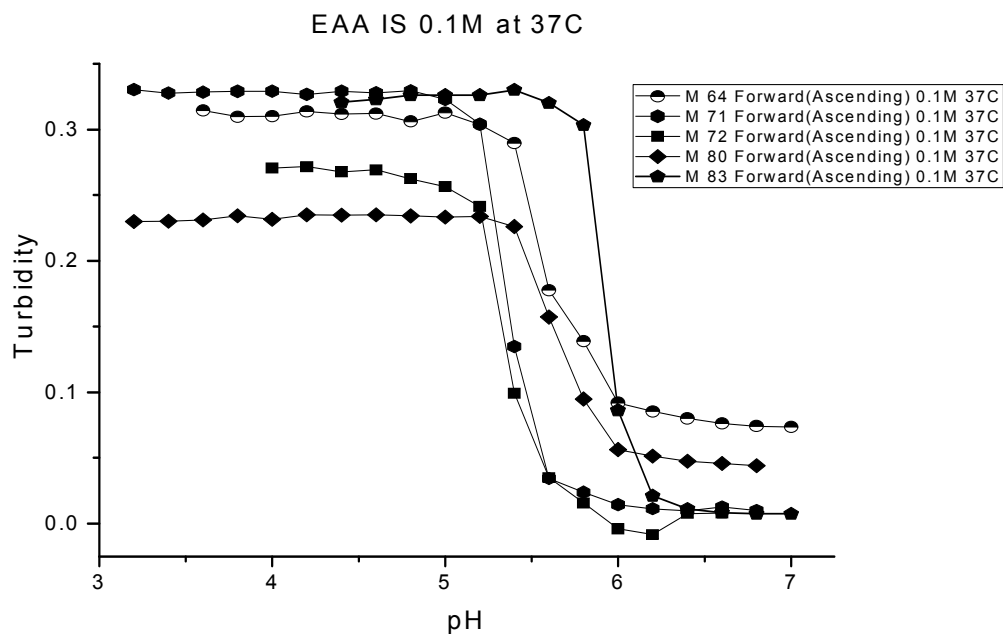

Figure 4.6. Ascending pH profiles at 37 °C for a variety of EAA synthates: M-64, M-71, M-72, M-80, and M-83 (see Table 4.4 for the formulations)

Table 4.4. Formulations of poly (*N*-isopropylacrylamide) synthates functionalized by EAA

EAA Synthate	NIPA (mmol)	EAA (mmol)	NTBA (mmol)	MBA (mmol)
M-64	16.6	2	0	1.4
M-71	16.8	2	0	1.2
M-72	17	2	0	1
M-80	17	2	0	1
M-83	15	2	2	1

From these response curves, the range of pK_a values spanned by the EAA synthates at 23 °C is 4.7 to 5.3 pH units and is 5.3 to 5.9 pH units at 37 °C. The larger pK_a value of M-83 can be attributed to the presence of NTBA which increases the hydrophobicity of the formulation. By varying the formulation of these EAA synthates (through adjustments of the amount of MBA and NTBA used in the formulation), the apparent pK_a of these particles can be tuned (see Table 4.5) and the reversibility of swelling can be enhanced (see Figures 4.3 and 4.7).

Table 4.5. pK_a values of poly (*N*-isopropylacrylamide) synthates functionalized by EAA

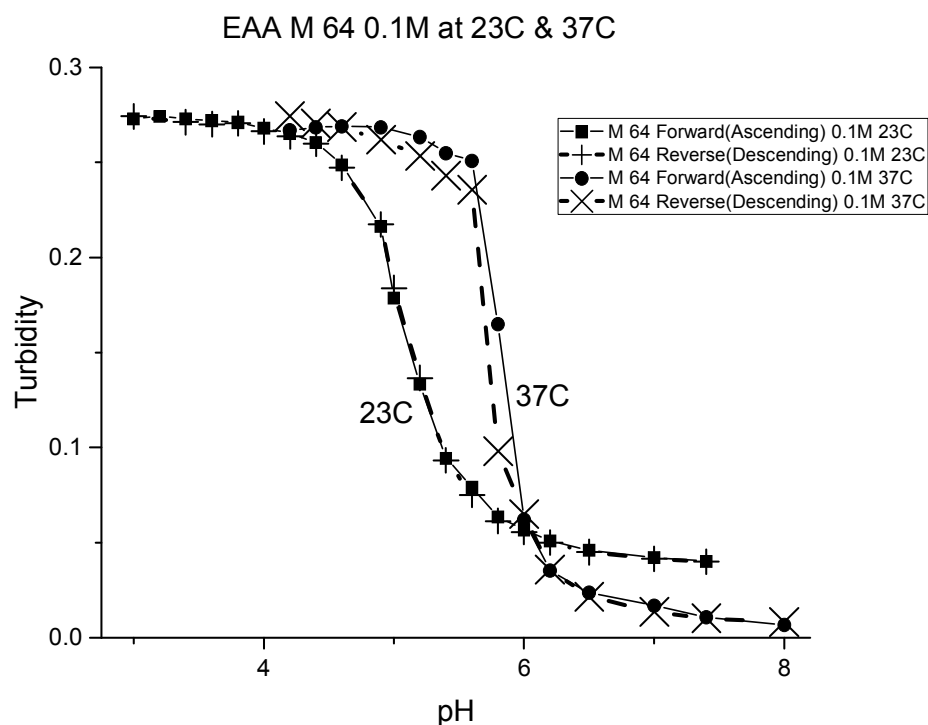| Apparent pK_a	M-64	M-71	M-72	M-80	M-83
23°C	4.9	4.7	4.7	4.9	5.3
37°C	5.5	5.3	5.3	5.5	5.9

Figure 4.7. Ascending and descending pH profiles for M-64 polymer particles (16.6mmol of NIPA, 2mmol of EAA and 1.4mmol of MBA) at 23 $^\circ$C and 37 $^\circ$C. Solid line = ascending pH profile. Dashed line = descending pH profile.

Although the formulation used for M-72 and M-80 was the same, differences in the pH response curves (see Figure 4.8) and in pK_a values (see Table 4.5) obtained for these two sets of particles indicate run to run variability in the synthesis of the pH sensitive poly (*N*-isopropylacrylamide) particles. The change in enthalpy and entropy for these particles is summarized in Table 4.6. M-64, M-71, and M-72 have similar thermodynamic values as do M-80 and M-83. We attribute this clustering to the use of fresh reagents (NIPA, NTBA, MBA, and DMPA) in the preparation of M-80 and M-83.
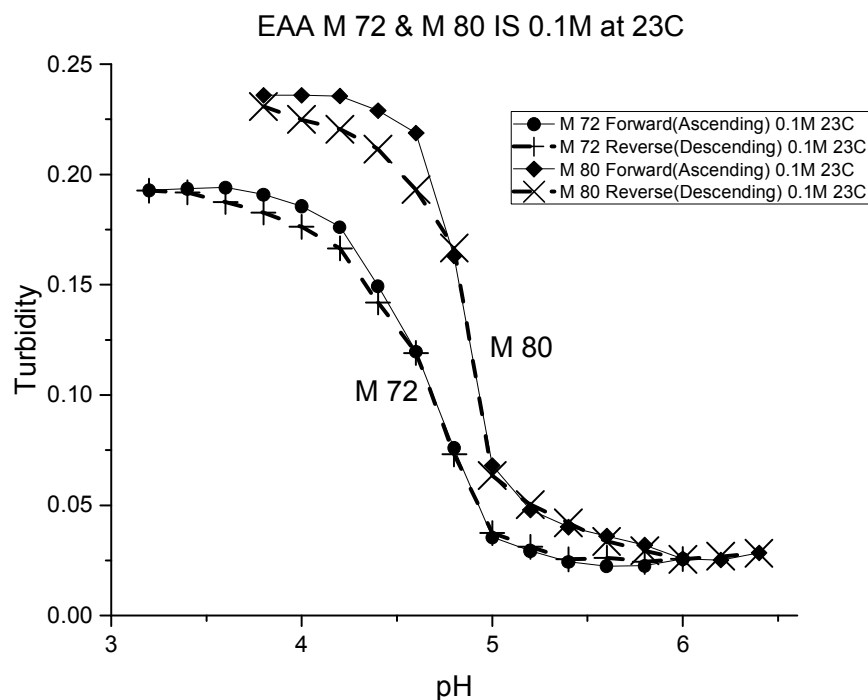
Figure 4.8. Ascending and descending pH profiles for M-72 and M-80 polymer particles (17mmol of NIPA, 2mmol of EAA and 1 mmol of MBA) at 23 °C. Solid line = ascending pH profile. Dashed line = descending pH profile.

Table 4.6. Changes in Enthalpy and Entropy due to pH Induced Swelling of Poly (*N*-isopropylacrylamide) Synthates Functionalized by EAA

Enthalpy & Entropy	M-64	M-71	M-72	M-80	M-83
[1]ΔH (J/Mole)	-81700±11600	-82000±6200	-81000±10000	-100300±3400	-101000±2200
[1]ΔS (J/Mole-K°)	-370 ± 38	-370 ± 20	-360 ± 33	-430 ± 11	-440 ± 7.2

[1]Uncertainties determined from the least squares fitting of the data

The effect of the ionic strength of the buffer solution in contact with the EAA synthates was also investigated at ambient temperature. pH response curves were generated at an ionic strength of 0.1M and 1.0M. Sodium chloride was used to control

the ionic strength of the solution. Figure 4.9 shows the pH response curve at 0.1M and

1.0M for M-72 which is representative of EAA synthates studied.
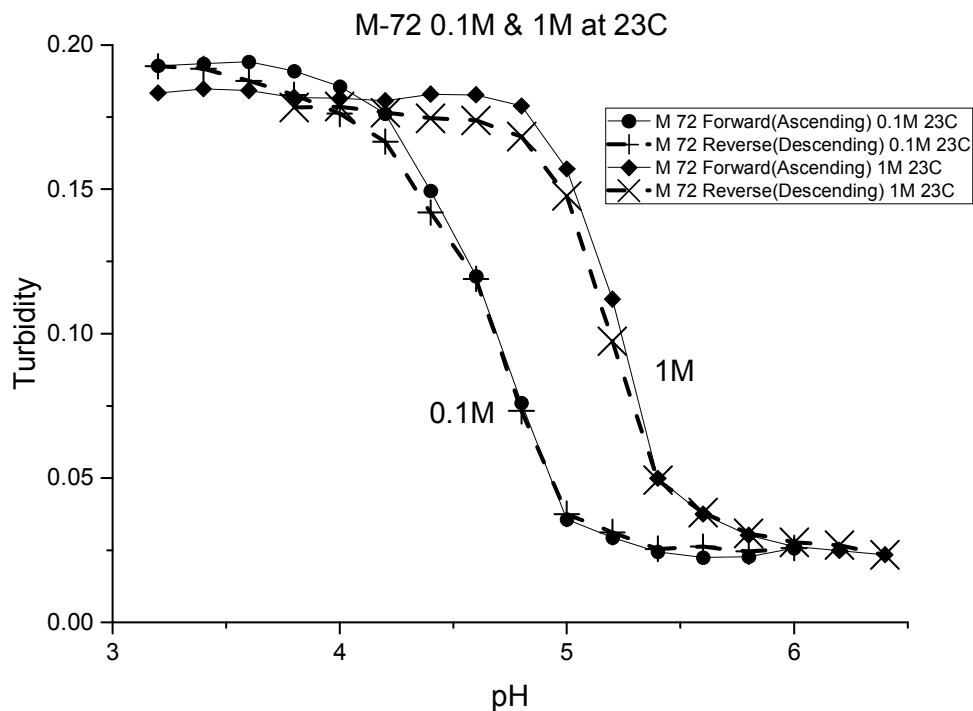
Figure 4.9. Ascending and descending pH profiles for M-72 polymer particles (17mmol of NIPA, 2mmol of EAA and 1 mmol of MBA) at 0.1M and 1M ionic strength. Temperature was ambient. Solid line = ascending pH profile. Dashed line = descending pH profile.

Swelling is both significant and reversible at both 0.1M and 1.0M ionic strength. This result indicates that swelling for these polymers is nonionic in nature. However, there is an increase in the apparent pK_a of the pH response curve of the poly (*N*-isopropylacrylamide) particle as the ionic strength of the buffer solution in contact with the particles is increased. In all likelihood, greater penetration of chloride anions into the polymer network occurs as a result of using higher ionic strength solutions. This in turn

will increase the amount of chloride anions on or in the vicinity of the polymer backbone, thereby increasing the amount of negative charge in the vicinity of the alkyl acrylic acid. The net result is a decrease in the dissociation of the proton from the carboxylic acid.

Figures 4.10 and 4.11 show ascending pH response curves at 23 $^{\circ}$C and 37 $^{\circ}$C for a variety of PAA synthate formulations (see Table 4.7).
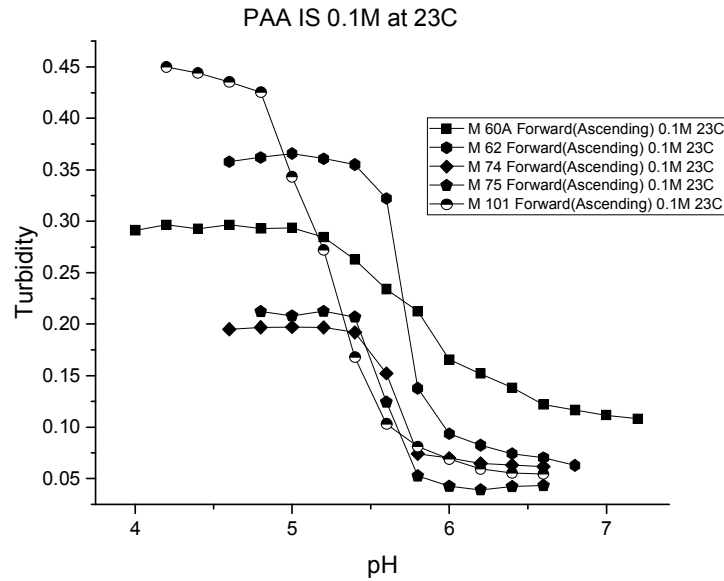
Figure 4.10. Ascending pH response curves for M-60A, M-62, M-74, M-75, and M-101 at 23 $^{\circ}$C.

PAA IS 0.1M at 37C
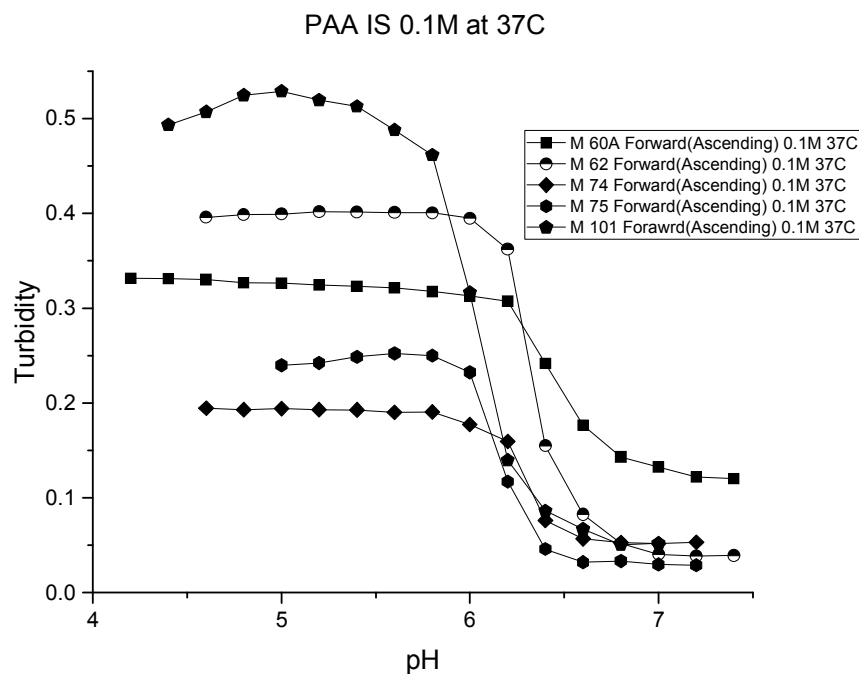
Figure 4.11. Ascending pH response curves for M-60A, M-62, M-74, M-75, and M-101 at 37 °C.

Table 4.7. Formulations of Poly (*N*-isopropylacrylamide) Synthates Functionalized by PAA

PAA Synthate	NIPA (mmol)	EAA (mmol)	NTBA (mmol)	MBA (mmol)
M-60 A	16	2	0	2
M-62	16.6	2	0	1.4
M-74	16.8	2	0	1.2
M-75	17	2	0	1
M-101	18	1	0	1

From these response curves, the range of pK_a values spanned by the PAA synthates at 23 °C is 5.3 to 5.9, and at 37 °C it is 6.1 to 6.5. By varying the formulations of these PAA synthates (through adjustments of the amount of the functional comonomer

and MBA), the apparent pK$_a$ of these particles can be tuned (see Table 4.8), and the

reversibility of the swelling can be enhanced (see Figure 4.12).

Table 4.8. pK$_a$ values of Poly (*N*-isopropylacrylamide) Synthates Functionalized by PAA

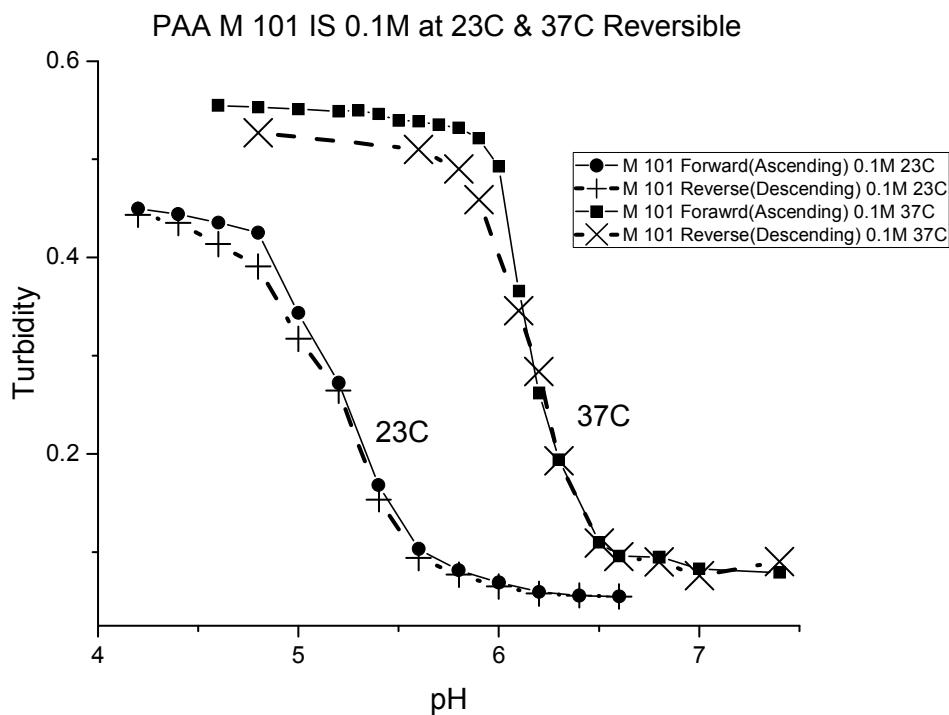| Apparent pK$_a$	M-60 A	M-62	M-74	M-75	M-101
23°C	5.9	5.7	5.7	5.5	5.3
37°C	6.5	6.3	6.3	6.1	6.1

Figure 4.12. Ascending and descending pH profiles for M-101 polymer particles (18 mmol of NIPA, 1 mmol of PAA and 1 mmol of MBA) at 23 °C and 37 °C. Solid line = ascending pH profile. Dashed line = descending pH profile.

The change in enthalpy and entropy for these particles is summarized in Table 4.9. M-60A, M-62, and M-74 have similar thermodynamic values as do M-75 and M-101. We attribute this clustering to the degree of crosslinking in the poly (N-isopropylacrylamide) PAA synthates. Both M-75 and M-101 have 5% crosslinking whereas the other three synthates utilize higher levels of crosslinking. This also explains the lower pK_a values for these particles in this series.

Table 4.9. Changes in Enthalpy and Entropy due to pH Induced Swelling of Poly (*N*-isopropylacrylamide) Synthates Functionalized by PAA

Enthalpy & Entropy	M-60 A	M-62	M-74	M-75	M-101
[1]ΔH (J/Mole)	-74000±11300	-73300±8400	-74900±8000	-90400±9500	-95400±8100
[1]ΔS (J/Mole-K°)	-360 ± 37	-360 ± 27	-370 ± 26	-410 ± 32	-420 ± 27

[1]Uncertainties determined from the least squares fitting of the data

Figure 4.13 shows the effect of ionic strength on the pH response curve of M-101. The M-101 results are similar to those obtained for M-72 (see Figure 4.9). Evidently, decreasing the amount of PAA in the formulation enhances the reversibility of pH induced swelling at both ambient and physiological temperatures and in low and high ionic strength solutions. However, decreasing the amount of PAA in the formulation also lower the apparent pK_a of the copolymer. Nevertheless, copolymers of *N*-isopropylacrylamide and PAA that reversibly swell in the physiological pH range can be synthesized using lower amounts of PAA in the formulation. Further tuning the pK_a of these copolymers may be possible by adding NTBA to the formulation as demonstrated by Perera [35] in his study of pH induced polymer swelling
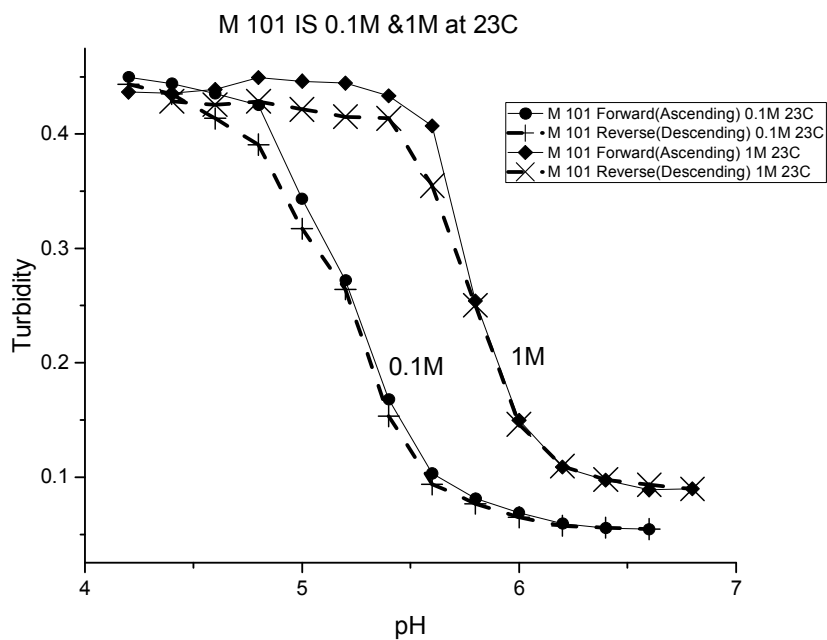
Figure 4.13. Ascending and descending pH profiles for M-101 polymer particles (18mmol of NIPA, 1mmol of PAA and 1 mmol of MBA) at 0.1M and 1M ionic strength. Temperature was ambient. Solid line = ascending pH profile. Dashed line = descending pH profile.

CHAPTER V

CONCLUSIONS

Crosslinked polymers of NIPA copolymerized with an alkyl acrylic acid when embedded in a PVA hydrogel membrane swell and shrink in response to changes in the pH of the buffer solution in contact with the membrane. Swelling is accompanied by a change in the refractive index of the copolymer. Therefore, the turbidity of the hydrogel membranes will serve as a quantitative measure of swelling and shrinking. Alkyl acrylic acids which are used as the functional comonomer impart pH sensitivity to the polymer particles.

The pH response of the polyNIPA particles is a function of the temperature and ionic strength of the medium and is dependent upon the formulation used to prepare the polyNIPA particles. To understand the effect of these factors on pH induced swelling of polyNIPA particles, a systematic study was performed. The apparent pKa of these polyNIPA particles was computed from the pH response profile for a specific formulation and set of conditions using a first derivative plot.

Polymer swelling increased as the temperature increased and the pH response of the particles occurred over a narrower range at higher temperature. The swelling that

occurs is nonionic as the ionic strength of the solution in contact with the NIPA copolymers was increased from 0.1M to 0.1M without a decrease in swelling. Four pH sensitive comonomers were investigated as part of this study: AA, MAA, EAA and PAA. At ambient temperature, the apparent pK_a increased as the alkyl chain length increased: 3.9 (AA), 4.3 (MAA), 5.3 (EAA) and 5.5 (PAA). At physiological temperature, the apparent pK_a of the particles also increased as the alkyl chain length of the functional comonomer increased: 4.3 (AA), 5.1 (MAA), 5.9 (EAA) and 6.1 (PAA). Furthermore, higher pK_a values were observed at physiological temperature.

It is evident from these results that several of these pH sensitive poly (*N*-isopropylacrylamide) particles prepared as part of this study are good candidates for optical pH sensing in the physiological pH range of 5.0 to 7.4.

REFERENCES

1. W. Vonau, U. Guth, pH monitoring: a review, J Solid State Electrochem. 10 (2006) 746–752.

2. S. Zhang, B. R. Soller, R.H. Micheels, Partial least-squares modeling of near infrared reflectance data for noninvasive in vivo determination of deep tissue pH, *Appl. Spect. 52 (1998*) 393-99.

3. E. J. Netto, J. I. Peterson, M. McShane, V. Hampshire, A fiber optic broad-range pH sensor system for gastric measurements, Sensors and Actuators B., 29 (1995) 157-163.

4. J. A. Ferguson, B. G. Healey, K. S. Bronk, S. M. Barnard, D. R. Walt, Simultaneous monitoring of pH, CO, and O using an optical imaging fiber, Anal. Chim. Acta. 340 (1997) 123-131.

5. A. S. Jeevarajan, S. Vani, T. D. Taylor, M. M. Anderson, Continuous pH Monitoring in a perfused bioreactor system using an optical pH sensor, Biotechnology and Bioengineering, 78 (2002) 467-472.

6. R. F. Service, Rising acidity brings an ocean of trouble, Science, 337 (2012) 146-148.

7. O. Korostynska, K. Arshak, E. Gill, A. Arshak, Review on state-of-the-art in polymer based pH sensors, Sensors, 7 (2007) 3027-3042.

8. K. Medlock, H. Harmer, G. Worsley, A. Horgan, J. Pritchard, pH-sensitive holograms for continuous monitoring in plasma, Anal Bioanal Chem, 389 (2007) 1533–1539.

9. M. J. P. Leiner, P. Hartmann, Theory and practice in optical pH sensing, Sensors and Actuators B 11 (1993) 281-289.

10. N. F. Sheppard, R. C. Tucker, and S. Salehi-Had, Design of a conductimetric pH microsensor swelling hydrogels, Sensors and Actuators B, 10 (1993) 73-77.

11. N. F. Sheppard, M. J. Lesho, P. McNally, and S. Francomacaro, Microfabricated conductimetric pH sensor, Sensors and Actuators B, 28 (1995) 95-102.

12. M.J. Lesho, and N.F. Sheppard, Adhesion of polymer films to oxidized silicon and its effect on performance of a conductometric pH sensor, Sensors and Actuators B, 37 (1996) 61-66.

13. Z. Shakhsher, W. R. Seitz, and K.D. Legg, Single fiber-optic pH sensor based on changes in reflection accompanying polymer swelling, Anal. Chem., 66 (1994) 1731-1735.

14. Z. Zhang, Z. Shakhser, and W. R. Seitz, Aminated polystyrene membranes for a fiber optic pH sensor based on reflectance changes accompanying polymer swelling, Mikro. Acta 121 (1995) 41-50.

15. L. Zhang, M. E. Langmuir, M. Bai, and W. R. Seitz, A sensor for pH based on an optical reflective device coupled to the swelling of an aminated polystyrene membrane, Talanta, 44 (1997) 1691-1698.

16. L. Zhang, and W. R. Seitz, A pH sensor based on force generated by pH-dependent polymer swelling, Anal. Bioanal. Chem., 373 (2002) 555-559.

17. B. K. Lavine, L. Oxenford, M. Kim, N. Kaval, M. Benjamin, and W. R. Seitz, Novel turbidimetric method to study polymer swelling, Microchemical J., 103 (2012) 97-104.

18. B. Sierra-Martin, J. J. Lietor-Santos, A. Fernadez-Barbero, T. T. Nguyen and A. Fernandez-Nieves, Swelling Thermodynamics of Microgel Particles in Microgel Suspensions – Fundamentals and Applications, H. Wyss, J. Mattsson, A. Fernandez-Nieves, and D. A. Weitz (Eds.), Wiley-VCH, Weinheim, Germany, 2011.

19. Anna Burmistrova and Regine von Klitzing, "Control of number density and swelling/shrinking behavior of P(NIPAM– AAc) particles at solid surfaces," J. Mater. Chem., 2010, 20, 3502–3507.

20. P. J. Flory, Principles of Polymer Chemistry, Cornell University Press, New York, 1953.

21. F. Meunier and A. Elaissari, Poly(N-isopropylacrylamide)-Based Particles: Preparation and Colloidal Characterization in Colloidal Polymers, A. Elaissari (Ed.), Marcel Dekker, New York, 2003.

22. K. Poschlad and S. Enders, "Thermodynamics of aqueous solutions containing poly (N-isopropylacrylamide)," J. Chem. Thermodynamics, 2011, 43, 262–269.

23. Ashutosh Chilkoti, Matthew R Dreher, Dan E Meyer, and Drazen Raucher, "Targeted Drug Delivery by Thermally Responsive Polymers," Advanced Drug Delivery Reviews, 2002, 54, 613–630.

24. H. G. Elias, An Introduction to Polymer Science, VCH, New York, 1997.

25. B. K. Lavine, D. J. Westover, N. Kaval, and L. Oxenford, "New Approaches to Chemical Sensing: Sensors Based on Polymer Swelling," Anal. Letters, 2006, 39(9) 1773-1783.

26. B. K. Lavine, L. Oxenford, M. Kim, N. Kaval, M. Benjamin, and W. R. Seitz, "Novel Turbidimetric Method to Study Polymer Swelling," Microchem. J., 2012, 103, 97-104.

27. W. R. Seitz, "Derivatized Swellable Polymer Microspheres for Chemical Transduction," Anal. Chim. Acta, 1999, 400(1-3), 55-64.

28. B. K. Lavine, G. Mwangi, N. Mirjankar, and M. Kim, "Characterization of Swellable Molecularly Imprinted Polymer Particles by Surface Plasmon Resonance Spectroscopy," Appl. Spectros., 2012, 66(4), 440-446.

29. X. Yin, A. S. Hoffman, and P. S. Stayton, Biomacromolecues "Poly (N-isopropylacrylamide-co-propylacrylic acid) Copolymers that Respond Sharply to Temperature and pH," 2006, 7(5), 1381-1385.

30. O. Oktar, P. Caglar, and W. R. Seitz, "Chemical Modulation of Thermosensitive Poly(N-isopropylacrylamide) Microsphere Swelling: A New Strategy for Chemical Sensing," Sens. Act. B., 2005, 104, 179-185.

31. N. Murthy, J. R. Robichaud, D. A. Tirrell, P. S. Slayton, and A. S. Hoffman, J. Controlled Release, 1999, 61, 137-143.

32. X. Yin, A. S. Hoffman, and P. S. Slayton, Poly(N-Isopropylacrylamide-co-propylacrylic acid) Copolymers that Respond Sharply to Temperature and pH, Biomacromolecules, 2006, 7(5), 1381-1385.

33. D. D. Perrin, The Effect of Temperature on pK Values of Organic Bases, Aust. J. Chem., 1964, 17, 484-488.

34. A. Fernandez-Nieves, H. Wyss, J. Mattsson, and D. Weitz, Microgel Suspensions, Wiley-VCH Verlag GmbH & Co, KGaA, Weinheim 2011.

35. Undugodage Don Nuwan Tharanga Perera, Computer Enhanced Spectrochemical and Chromatographic Analysis Applied to Problems in Analytical Chemistry, PhD Thesis, Oklahoma State University, 2015.

VITA

SANDHYA RANI PAMPATI

Candidate for the Degree of

Master of Science

Thesis: SWELLABLE POLY *N*-ISOPROPYLACRYLAMIDE PARTICLES FOR PHYSIOLOGICAL pH SENSING

Major Field: Chemistry

Biographical:

Personal Data:

Born in Karimnagar, Telangana, India on January 9th, 1987.
Married December 29th, 2010 to Sathish Kumar Lageshetty

Education:

Completed the requirements for the Master of Science in Chemistry at Oklahoma State University, Stillwater, Oklahoma in July, 2015

Completed the requirements for the Bachelor of Science in Pharmacy at Kakatiya University, Jangaon, Telangana, India in 2008

Recipient of the Skinner Chemistry Fellowship in July 2014

Professional:

Oklahoma State University: Teaching Assistant (2011-2015)
Research Assistant (2012-2013)