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An Experiment to Introduce Temperature-responsive Polymers for Biomedical Applications: Polymer Synthesis

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An Experiment to Introduce Temperature-Responsive Hydrogels for Biomedical Applications: Polymer Synthesis

ABSTRACT

As part of an ongoing effort to introduce concepts of drug delivery into the chemical engineering core curriculum, we are developing an experiment to introduce engineering students to temperature-responsive polymers for controlled release applications. Stimulus responsive polymers experience an abrupt change in physical or chemical characteristics in response to a small external change in environment. Temperature is the most commonly exploited stimulus in responsive polymer systems, and polymers that exhibit a response in water at a temperature of about 37 °C are of particular interest in biological applications.

In this experiment, students produce a temperature responsive, acrylamide-based copolymer using free radical copolymerization. The copolymer composition is validated using NMR, IR, and elemental analysis, and the copolymer is then used to make a hydrogel which is also temperature sensitive. Students are challenged to design a water-soluble polymer with a controlled temperature response by tailoring the chemical composition of the copolymer. The physical and mechanical properties of the hydrogel such as swelling, density, and tensile strength are tested with and without drug loading, after exposure to different temperature environments. Finally, the drug release profiles will be analyzed in different temperature environments. In addition to learning about temperature-responsive drug delivery, students learn analytical techniques and concepts related to material properties, design of experiments, data analysis, and engineering design.

Keywords: Stimuli-responsive materials, methyl acrylate, acrylamide, temperature sensitive, hydrogels, NMR

INTRODUCTION

Chemical engineers play an important role in the design of drug delivery systems, which are designed to release a drug at a predetermined rate for a specific period of time. Optimal drug delivery is achieved when the administration of the drug matches physiological needs at the proper time and location (temporal modulation and site-specific targeting). A drug delivery system can be designed to respond to different environmental conditions in the body such as pH or temperature. Smart, stimuli-responsive hydrogels are used to respond to a signal caused by a disease by releasing release the appropriate amount of drug at the correct time and desired location. Temperature-sensitive hydrogels have exciting potential for drug delivery and other biomedical applications.[1]

In this paper we describe an experiment in which students synthesize a temperature-sensitive hydrogelby free radical copolymerization with the goal of optimizing the design of the hydrogel for drug delivery applications. Elemental analysis is used to confirm that the polymer is non-toxic to humans, cloud point and turbidimetry are used to determine and confirm the critical temperatures for the polymer compositions used, and NMR analysis is used to confirm the composition of the polymers used in making the sample. Subsequent experiments will focus on the characterization of the polymer and on drug release studies. In addition to learning about temperature sensitive drug delivery, students will learn concepts of polymer chemistry, materials science, and data analysis. Students will also learn about NMR, IR and elemental analyses.

BACKGROUND

Hydrogels

Hydrogels are water insoluble cross-linked three dimensional polymer networks that can absorb up to one thousand times their dry weight in water. Hydrogels have been used for a variety of biomedical applications including contact lenses, tissue scaffolds, and drug delivery systems. The cross-linking in hydrogels allows for insolubility in water and provides required mechanical strength and physical integrity thatmake hydrogels so useful in biomedical applications.[2]Hydrogels have been widely used for drug delivery systems since they allow molecules of different sizes to diffuse into or out of the network for drug loading and release, respectively.Furthermore, mechanical properties of hydrogels can be modified by making relatively simple changes to the polymer structure, such as the cross-linked density. Since the polymer chains of different hydrogels contain specific functional groups, hydrogelscan be sensitive to changesin the surrounding environment, such as the changes in pH, temperature, and pressures.[2]

Hydrogels can be classified based on the polymer's origin. Hydrogels can either be considered natural or synthetic. Natural hydrogels such as protein-based hydrogels support cellular activities and are biocompatible and biodegradable. Some of the disadvantages of natural hydrogels are they may contain biological pathogens or evoke an immune response of low mechanical strength, batch variation. Synthetic polymers are made from monomers such as acrylamide, ethylene glycol and lactic acid, and their formulation can be precisely controlled to meet the specific needs. This gives synthetic polymers a wide range of properties. Synthetic hydrogels have a low risk of biological pathogens and evoking an immune response. The disadvantages to working with Synthetic polymers are low biodegradability and possible biotoxicity.[3] Elemental analysis is a useful tool in the evaluation of potential biotoxicity of polymers for biological applications.

Temperature Sensitive Hydrogels

Temperature sensitive hydrogels are widely utilized for a variety of triggered drug delivery systems. The reason that temperature sensitive polymers/hydrogels are used for these systems is the body temperature often deviates from the physiological norm (37°C) when there is presence of pathogens or pyrogens in the body. When changes in the temperature occur hydrogels can swell or shrink, increasing or decreasing the rate of drug release. Among the temperature-sensitive hydrogels that have been investigated for drug delivery applications, Poloxamer formulations (PEG/PPO block copolymers) have been used for a wide variety of drug delivery applications including diabetes treatment, cancer therapy and gene therapy.[4]

Temperature-sensitive hydrogels are classified into 3 categories: negatively thermally sensitive, positively thermally sensitive and thermally reversible.[5] Negative temperature-sensitivity is classified as having a lower critical solution temperature (LCST) which IUPAC defines the critical temperature below which a mixture is miscible. A positive temperature-sensitive hydrogel has an upper critical solution temperature (UCST) which IUPAC defines as the critical temperature above which a mixture is miscible.[6] An example of a Negative temperature-sensitive polymer is Poly (N-isopropylacrylamide) (PNIPAAm). PNIPAAm is being tested for uses in tissue engineering and drug delivery systems. In a water solution PNIPAAm encounters it's LCST at 32ºC. Below 32 ºC the polymer is soluble as the hydrophilic interactions dominant. Above 32ºC a phase separation occurs because the hydrophobic interactionsdominate.[7]

NMR

Nuclear Magnetic Resonance (NMR) has become the most widely used technique for determining the structures of organic compounds. NMR has the most complete analysis and fullest interpretation of a compound compared to other spectroscopy methods. Though NMR requires larger amounts of sample to be tested compared to mass spectroscopy, NMR is a non-destructive method of analyzing a sample. NMR is based on the characteristic spin of nuclei and the magnetic field that is generated from that spin. The most isotopes of elements analyzed by NMR are H^{1}, C^{13}, F^{19} and P^{31}. NMR analyzes the difference in magnetic fields between atoms in a compound. This difference in energy is often less than 0.1 cal/mol, compared to infrared spectroscopy, which involves energy differences between 1 kcal/mol and 10 kcal/mol. Frequencies between 20 MHz and 900 MHz are used to irradiate specific nuclei causing the excitation of the nuclei which isrecorded.[8]

Cloud Point

The cloud point test is used to determine if the polymers synthesized are thermally sensitive. The thermodynamic cloud point is the highest temperature at which a dissolved solid phase will exist at a given pressure. Below the cloud point the second phase of the solid gives the fluid a cloudy appearance.The experimental cloud point is the temperature at which a detectable amount of solid phase forms, and is a function of the measurement technique, thermal history, time of measurement and fluid properties[9]. Cloud points are usually measured using 1% soluble solid solutions.[10]A turbidity can be conducted to determine the solid/liquid phase composition of a sample. A turbidimeter is used to determine the amount of light transmitted through thesample.[11]

Elemental Analysis

Elemental analysis is an accurate and precise measurement of the elemental composition of the sample analyzed. Elemental analysis by combustion of the sample measures carbon, sulfur, oxygen, nitrogen, and hydrogen. Elemental analysis is often used to determine elemental composition when other methods are not accurate enough. Elemental analysis can be very rapid, processing high amounts of samples. Combustion analysis is performed in either excess oxygen or some other inert gas. The combustion products are measured by infrared absorption or by changes in thermal conductivity and used to determine the composition of the sample. These measurements are compared against known certified referencematerials.[12]

EXPERIMENT

In this experiment, students produce temperature sensitive hydrogels by free radical copolymerization of a monomer solution containing the initiator ammonium persulfate, and the monomers acrylamide and methyl acrylate, with a water-ethanol solution as the solvent.

Copolymers Synthesis

Materials

- Acrylamide (AA)
- Methyl acrylate (MA)
- Ammonium persulfate (AP)
- Ethanol
- Water
- Acetone
- 50 mL Erlenmeyer flask
- Pasteur Pipette
- Micropipettes
- 200 mL Glass Jar with screw on lid
- Water bath set up at 40^0C

Procedure

In an Erlenmeyer flask, the MA monomers were copolymerized with AA at different feed compositions. The monomers composition was varying from 10% of AA to 90%. The amount of MA was set to be 1.5mL and the 50/50 water-ethanol was 13.5mL so that the solution would be a 10% monomer mixture. The amount of ammonium persulfate added to the mixture was 0.0171g. The mass of AA (M_{AA}) used in the solution was determined from the following formula:

$$M_{AA} = \frac{V_{MA} * \rho_{MA} * \%_{AA} * MW_{MA}}{\%_{MA} * MW_{AA}} \quad (1)$$

The mass of AA compared to % of AA can be seen in
Table 1. Samples below 50% AA did not become viscous and did not precipitate properly.

Table 1: Mass of AA compared to the % of AA in Solution

Composition of reaction mixture (AA/MA monomer ratio)	Mass of AA(g)	Mass of MA(mL)	Mass of AP(g)	Mass of EtOH(ml)
10/90	0.131	1.5	0.0171	6.75
20/80	0.296	1.5	0.0171	6.75
30/70	0.507	1.5	0.0171	6.75
40/60	0.788	1.5	0.0171	6.75
50/50	1.183	1.5	0.0171	6.75
60/40*	1.775	1.5	0.0171	6.75
70/30*	2.760	1.5	0.0171	6.75
80/20*	4.732	1.5	0.0171	6.75
90/10*	10.647	1.5	0.0171	6.75

*Due to finding water-soluble and thermally sensitive polymers, the rest work was done only with AA-MA (%) of 50-50, 60-40, 70-30, 80-20 and 90-10.

The radical copolymerization was performed using ammonium persulfate as the initiator and the water-ethanol solution as the solvent. The solution containing monomers, solvent and initiator was degassed by nitrogen or argon gas for fifteen to twenty minutes to remove the air from the mixture. The degassed mixture is then put into a 40°C water bath until the mixture became viscous. The time in the water bath depends on the percent of AA in the mixture. A higher percent of AA in the mixture results in a lower time required for heating. The copolymer products were isolated by precipitation in acetone from high viscosity reaction mixture. The final polymer from the high viscosity solution was precipitated in acetone solvent AA (50%) and MA (50%), AA (60%) and MA (40%), AA (70%) and MA (30%), AA (80%) and MA (20%), AA (90%) and MA (10%)) and in water (AA (40%) and MA (60%), AA (30%) and MA (70%), AA (20%) and MA (80%), AA (10%) and MA (90%)) and dried in vacuum dryer.

Cloud Point

Turbidimetry was used to measure cloud points of the aqueous solutions (1%) of the copolymers. As described before only copolymers AA (50%) and MA (50%), AA (60%) and MA (40%), AA (70%) and MA (30%), AA (80%) and MA (20%), AA (90%) and MA (10%) were tested by this method due to their solubility.

NMR

The samples were tested to see if they would dissolve in a common NMR solvent before being sent to NMR. 0.2g of dried copolymer was put into 2mL of dimethyl sulfoxide (DMSO). All samples, with the exception of the 90% AA polymer, were able to dissolve in DMSO. The 90% AA polymer was tested in deuterium oxide under the same conditions, the polymer dissolved.This polymer solution was analyzed using H-NMR. Each H under different conditions has a specific signal which appears on the NMR graph at different points or within certain chemical shift ranges.[8]

RESULTS

Optimization

All samples with below 50% acrylamide would not precipitate a useable amount of polymer, and it was concluded that all formulation should be at or above 50% acrylamide. Testing is still being run for compositions at and above 50% acrylamide to determine an optimum for that range.

NMR

Five samples were prepared for NMR testing. Of the two samples returned both samples showed a 4% error. Figures 1 and 2 show the structure of the AA and MA formulas show A,B, C hydrogens are the same. The only difference ison D Hydrogen which has threehydrogens for MA and AA has two hydrogens. This is used as to determine the quotients in equation 2 for MA and AA respectively. Table 2 shows the real composition of the copolymers as determined from 1H NMR spectra.Table 2 follows that in all the cases the content of AA units in the copolymer is somewhat lower than the content of AA in the respective reaction mixture, thus indicating that AA is less reactive than MA.

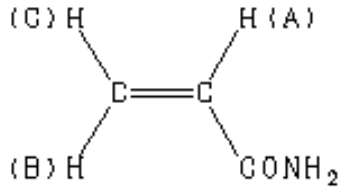

Figure 1. Acrylic Acid Polymer

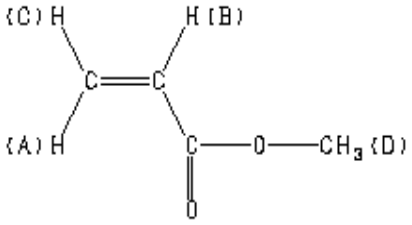

Figure 2. Methyl Acrylate Polymer

Equation 2is used to determine the real composition of the copolymers as determined from 1H NMR spectra.A_{MA} is a signal corresponding to CH_3 protons of MA units, A_{AA} is a signal corresponding to NH_2 protons of AA units, n_{hydr} is a number of protons (hydrogen).

$$\%MA = \frac{\frac{A_{MA}}{n_{hydr}}}{\frac{A_{MA}}{n_{hydr}} + \frac{A_{AA}}{n_{hydr}}} \quad (2)$$

Table 2: Characteristics of investigated samples of P(MA/AA) Copolymers

Composition of reaction mixture (AA /MA monomer ratio)	Real composition of the copolymer determined from 1H NMR spectra (AA/MA molar ratio)
50/50 Sample 1	Analysis will be done in S13
60/40 Sample 2	Analysis will be done in S13
70/30 Sample 3	Analysis will be done in S13
80/20 Sample 4	76/24
90/10 Sample 5	86/14

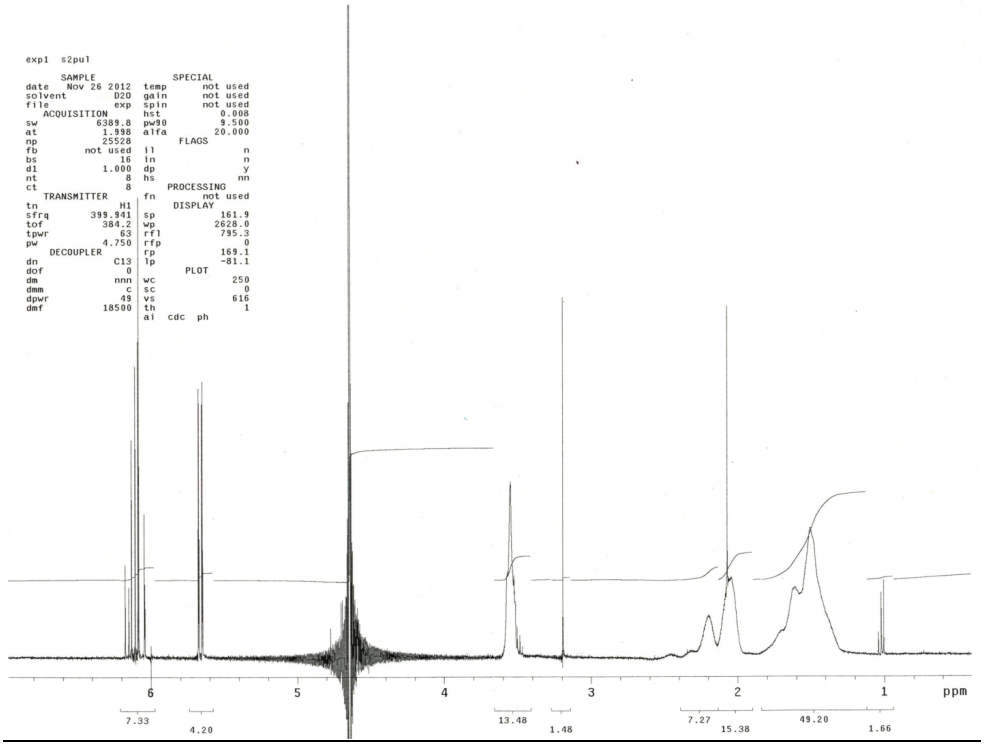

Figure 3: NMR Results from Sample 4

Figure 3 was used to determine the composition of the copolymers. Figure 1 shows high-resolution 1H NMR spectra of a D_2O solution (c = 10 wt%) of P(MA/AA) copolymer with the MA/AA molar ratio in the polymerization mixture 80/20.

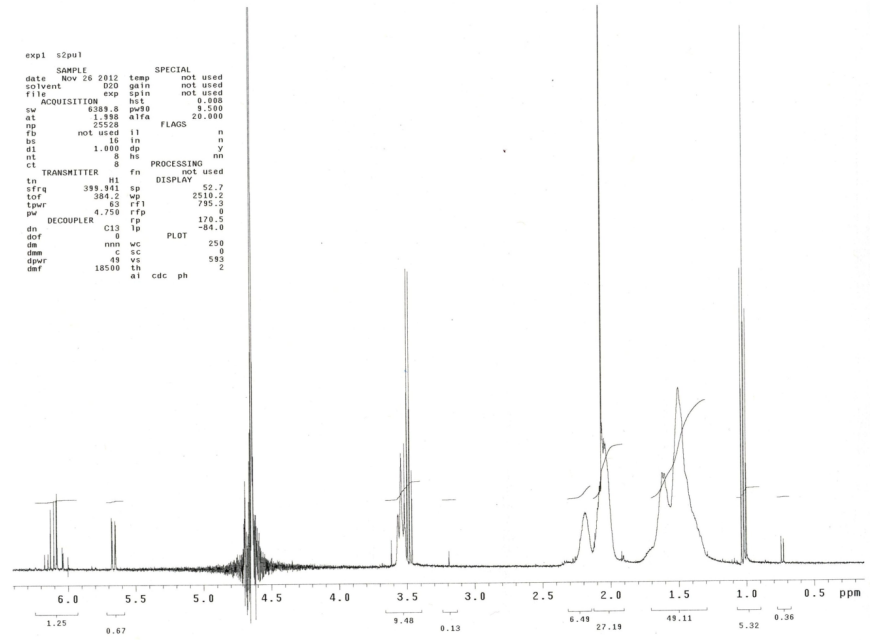

Figure 4: NMR Results from Sample 5

Figure 4 was used to determine the composition of the copolymers in sample 5. Figure 5shows high-resolution 1H NMR spectra of a D_2O solution (c = 10 wt%) of P(MA/AA) copolymer with the MA/AA molar ratio in the polymerization mixture 90/10.

Cloud Point

Figure 5 shows the results of the cloud point analysis for thermo sensitivity. Only copolymer AA (50%) and MA (50%) showed thermo sensitivity. The graph shows rapid increase from 22^0C and at approximately 50^0C reach his peak. For other copolymers we can see not much difference at all.

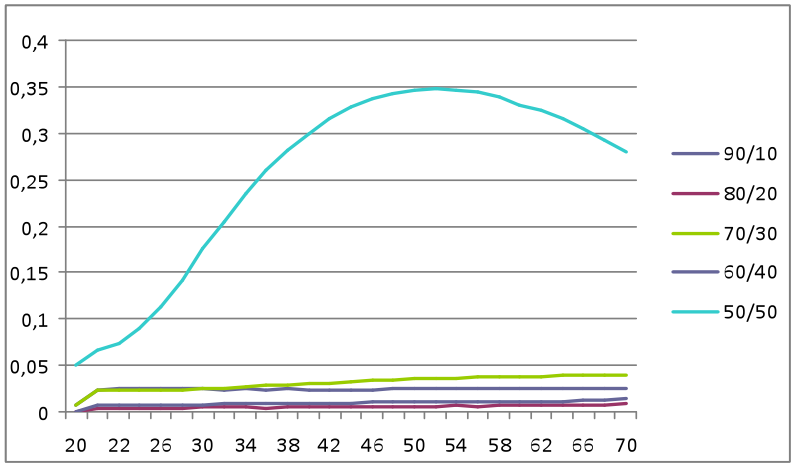

Figure 5. Evaluation of thermosensitivity using cloud point analysis: variation of turbidity with temperature for different copolymer compositions.

CONCLUSION

This paper focuses on the development and optimization of a temperature-sensitive P(MA/AA) hydrogel, and its analysis using NMR and elemental analysis. Polymersmade with less than 50% AA did not precipitate enough polymer to be useful. The 50% AA polymer did exhibit thermal sensitivity at a temperature of 50°C. NMR analysis showed that AA was less reactive than MA in the polymer synthesis. In both samples the percentage of AA in the copolymer is around 4% lower than the percentage of AA in the respective reaction mixture. The next phases of the project will focus on the characterization of the polymer and study of drug release from the polymer. This experiments being developed will be used in a materials science class to introduce students to polymer synthesis, analysis, and characterization for drug delivery applications.

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