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# Effects of Hofmeister Ions on Transition Temperature of Two Thermo-Responsive Polymers

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# Effects of Hofmeister Ions on Transition Temperature of Two Thermo-Responsive Polymers

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Honors Project Report
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4200:497

**Honors Research Project**

Submitted to:

*The Honors College*

# Contents

## Honors Abstract Addendum

It was hypothesized that through the use of Hofmeister Ions, the lower critical solution temperature (LCST) of poly(vinyl methyl ether) (PVME) and poly(ethylene glycol)- poly(*N*-isopropylacrylamide) (PEG-b-pNIPAAm) block-copolymer could be controlled. Through literature searches and small lab experiments, our team found that there may be a connection between Hofmeister effects and phase transition of a thermo-responsive polymer. To try and prove this, the lab team decided to take four cations ($Mg^{2+}$,$Na^{+}$, $Cs^{+}$, $K^{+}$) in solution with the thermo-responsive polymers and compared their LCST to solutions of the thermo-responsive polymer in de-ionized (DI) water. From this study, it was found that the addition of ions in solution lowered the LCST of PVME from 32°C to 26°C-30°C (depending on the ion added) and the LCST of PEG-pNIPAAm from 31°C to 26°C -29°C (depending on the ion added). The implications of this study could aid in efforts to manufacture better drug delivery devices, cell scaffolds, and tissue growth mediums. With increased control on the temperature at which a polymer undergoes a phase transition, the more effective these products can be in practice. The work will be continued by the next group of students.

## Executive Summary

Thermo-responsive polymers are very important in the biomedical field. They are used in a variety of different applications from drug delivery to tissue scaffolding. The distinguishing properties of thermo-responsive polymers are their unique transitions based on temperature. The transition that this study focuses on is that of the lower critical solution temperature (LCST). The LCST is the temperature at which a thermo-responsive polymer will precipitate out of solution as the temperature of a solution increases.

It has been theorized and shown that for certain thermo-responsive polymers, ions in the solution would affect the surface properties of these polymers.  These ions, called Hofmeister ions, can be used to control the LCST of polymers in solution. This study is focused on evaluating how the Hofmesiter ions, mainly the cations, in solution alter the LCST of two separate thermo-responsive polymers: poly(vinyl methyl ether), PVME, and poly(ethylene glycol)- poly(*N*-isopropylacrylamide), PEG-b-pNIPAAm, block-copolymer.

The study involves taking these two polymers and measuring their LCST's in salt solutions of sodium chloride, cesium chloride, potassium chloride, and magnesium chloride at a concentration of 400 mM. For sodium chloride, the effect of ion concentration, ranging from 100 mM to 400 mM, on LCST of PVME was also examined. The LCST was determined by monitoring the clouding point (transition from a transparent solution to an opaque or a milky white solution) by increasing or lowering the solution temperature at a rate of ~ 1.80°C/min. The LCST was determined by using the change in light intensity passing through the solution. When the light intensity drastically changed from high to low while heating (or low to high while cooling), the LCST was determined. The experimental LCSTs of PVME and PEG-pNIPAAm in

DI water are 32°C and 31°C respectively. With the addition of the various ions, the LCSTs of PVME and PEG-pNIPAAm range from 26°C -30°C and 25°C to 29°C respectively, were observed. For both thermo-responsive polymers, potassium chloride had the largest effect on lowering the LCST while magnesium chloride had the least effect on lowering the LCST. From the ion concentration effect on the LCST of PVME, the concentration of 400 mM NaCl had the greatest effect on LCST at ~27°C, while the 100 mM concentration had the least effect on LCST at ~31°C. The trends that were expected for the concentration study were seen in that the low concentration did not affect the LCST comparatively to the high concentrations of sodium chloride. The different ion effect trend was opposite of what was expected. It was originally thought that the magnesium and sodium would have the greatest effect on LCST, while the cesium and potassium would not have a large effect. However, due to differences in charge, number of Hofmeister ions tested, and the difference between changing anions vs. cations for the Hofmeister effect may point to more experiments to be run before the trends derived can be confirmed.

Data and results from the work done is fine, however, the honors project also helped me on a professional level. From the experience, my ability to confidently design and run experiments that I am uncomfortable with has improved drastically. The statistics I learned in the class room could be reinforced through the data that was derived from the experiment described above. In addition to helping myself, the implications of the work done could be broader than just Dr. Newby's research group. If continued, the research that was started could help control LCST of materials. With the control of such properties, smart materials could be made for any sort of biomedical applications. Tissue engineering, cell scaffold design, and drug delivery are just a few of the biomedical applications that could be impacted by the research on Hofmeister effects on the LCST of thermo-responsive polymers[1].

To continue the efforts of this project, as stated above, more trials need to be done by students using different Hofmeister ions. Further research should be completed with Dr. Newby to find explanations for the results that were derived from the expectation. We ran the experiments with expected outcomes in mind, however, the results derived were contrary to initial thoughts. Due to the time constraints, we were unable to complete further research to explain the experimental outcomes. The next student on this project should be familiar the databases available for literature searches as well as experimental design.

## Introduction

Thermo-responsive polymers are used in several disciplines and industries. One area of interest is the use of thermo-responsive polymers in the medical field, especially in harvesting cells and cell sheets[1,2,3]. For example: (1) using thermo-responsive polymers as supports/scaffolds for culturing cells to be used in regenerative medicines or to produce biopharmaceuticals by live bacteria/mammalian cells when the bacteria/mammalian need to be harvested to collect the products inside; (2) using thermo-responsive polymers as supports for growing cell sheets that can be harvested as grafts in burn/wound repair. One issue is that cells/bacteria normally need a surface to attach in order to proliferate, to harvest these cells/bacteria after they grow, they must be detached from the surface, generally through chemical or mechanical means, which could be tedious and cause damage to the cells[2]. The easier the detachment process, the more desirable it would be. One way to make the detachment process easier is to use a thermos-responsive polymer (TRP) by simply switching the temperature to the surface where the cells are grown. The desired TRP should exhibit a lower solution critical temperature (LCST), or a temperature below which the polymer is soluble in water, but once exceeding the temperature, the polymers precipitate out. This thermal transition behavior is transferred to a polymer thin film coated on a substrate[1] that can be used as a cell/bacterial culture support. It has been known that LCST of a TRP would be affected by the ions presented in the liquid, and in some cases, it would be beneficial to turn this temperature to a desired value when detaching cells/bacteria from the TRP surface.

The effect of ion on the solubility of a polymer was first reported by Franz Hofmeister in 1888 when he studied the solubility of proteins in an aqueous solution. These ions are thus defined as Hofmeister ions. The presence of ion could shift the LCST of a TRP by up to 10°C, which is significant when one needs to consider the energy required for heating or cooling the liquid to detach cells. Also, to retain qualify and viability of cells, the exposure of cells/bacteria to a cold medium should be controlled.

Through the use of the Hofmeister series of ions, the LCST of two TRPs: poly(vinyl methyl ether) (PVME) and Poly(ethylene glycol)-b-poly(N-isopropylacrylamide) (pNIPAAm), will be evaluated to determine how the ion type and ion concentration would affect their LCST's. If the conclusion of this set of experiments points to the LCST being controllable, the knowledge will be applied to grow and detach cells/bacteria on the thin films of these two polymers and assess if they can be useful for cell/cell sheet harvesting.

## Background

### *Hofmeister Ions*

Hofmesiter Ions are known as certain salts that have the ability to precipitate certain proteins out of an aqueous solution.[4] The effects of this were first noticed, as mentioned prior, by Franz Hofmesiter in the late 1800's. These salts described above have been used to describe the denaturing of proteins, however, have been applied in many fields of science. One such fields is that of polymers. It has been found that these Hofmeister ions exhibit their effect on polymers in solution as well. Salts that are part of the Hofmeister Series are shown below.

$$CO_3^{2-} > SO_4^{2-} > S_2O_3^{2-} > H_2PO_4^{-} > F^{-} > Cl^{-} > Br^{-} > I^{-} > ClO_4^{-} > SCN^{-}$$

**Figure 1:** The series above is the Hofmeister series for anions.

$$N(CH_3)_4{}^{+} > Cs^{+} > Rb^{+} > K^{+} > Na^{+} > H^{+} > Ca^{2+} > Mg^{2+} > Al^{3+}$$

**Figure 2:** The series above is the Hofmeister series of cations

The series above are ordered left to right in ability to stabilize the structure of proteins. Often the ions to the left are called Kosmotropes and those to the right are called Chaotropes, which are described to make and break water structure respectively.[4] Kosmotropes "making" water structure gives them what is often called a "salting out" effect, which stabilize the protein in water, allowing it to stay as a particle. Chaotropes do the opposite and destabilize the proteins in water causing them to "salt in" more readily, meaning that the proteins would dissolve into water. The stability of the proteins can be attributed to how well the ions hydrate the surfaces of the molecules making them hydrophobic for Kosmotropes and hydrophilic for Chaotropes.[5] Using the logic above, it has been found that often times the Hofmeister series can be applied to polymers as well as proteins[6].

### ***LCST of Thermo-Responsive Polymers***

In order to carry out the study presented below, polymers, in particular, PVME and PEG-b-pNIPAAm copolymer were evaluated to determine how their LCST's change. pNIPAAm homo-polymer is one of the most widely used TRPs; however, pNIPAAm posts some properties, such as high glass transition temperature (~ 135 – 140°C), making it harder in certain processes. Also, studies by Zhang et al[5] on the effects of Hofmeister ions on the LCST of pNIPAAm have been conducted, but not on other thermo-responsive polymers. For example, in a 2005 study ("Specific Ion Effects on the Water Solubility of Macromolecules: pNIPAAm and the Hofmeister Series"), Zhang et al.[5] investigated the shifts of LCST of pNIPAAm by adding Hofmeister Ions to solutions of pNIPAAm. The study primarily focuses on adding various anions and varying ion concentration to observe the effect on the LCST. The authors chose to vary the anions because it has been previously established that anions tend to have larger effects on salting in and out of macromolecules. Zhang et al.[5] concludes that there is a significant difference between Chaotropes and Kosmotropes when it comes to the LCST of pNIPAAm in solution.

The LCST study shown below will focus more on the effect of changing the cation and cation concentration in solution. This will be done in order to easily maintain an environment suitable for supporting microscopic and macroscopic lives, i.e., cations of $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ are essential minerals for human health and presence in culture medium for most mammalian cells and microorganisms. Nevertheless, anions in larger concentrations tend to be toxic to cells and other living microorganisms. The information gathered from this study would help researchers for adequately tuning the culture medium to provide suitable conditions for utilizing the thermo-responsive surfaces in biomedical applications.

### *PVME and PEG- pNIPAAm*

The polymers chosen for the sets of experiments described below were chosen based on market availability, their process-ability and distinguishing characteristics. Below are the two model polymers used in this study.

*Poly (vinyl methyl ether) or PVME*: This commercially available polymer has gotten a lot of attention recently as an alternative for pNIPAAm with a similar LCST, without the presence of salt in solution, of ~ 35 ˚C.[7] The advantage of this polymer is its low glass transition temperature (~ - 18°C), which makes it much easier, as compared to pNIPAAm, for processing into various sizes and shapes. Also, the cost of PVME ($15.72/25g) is much cheaper than that of pNIPAAm (~ $250/10g). PVME’s structure can be seen below in **Figure 3.** PVME is a biocompatible, non-toxic, and thermos-responsive polymer that is used to make hydrogels and various cosmetics.[7]

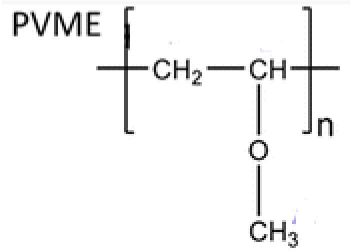

**Figure 3:** the figure above shows the basic structure of the repeat unit of PVME.[7]

*Polyethylene glycol (PEG)-b-Poly(N-isopropylacrylamide) (pNIPAAm):* This polymer is also a readily available commercially made polymer. In addition to its ease of attainability, it is also already widely used in the biomedical field. PEG-b-pNIPAAm copolymer is widely used as a hydrogel matrix to house thermo-responsive drugs. With a similar LCST (without salts in solution) to PVME, ~ 31°C, the polymer fit naturally in the study.  This block copolymer (structure shown below) is highly biocompatible and can be used for tissue engineering, making injectable hydrogels, and drug delivery.[8]

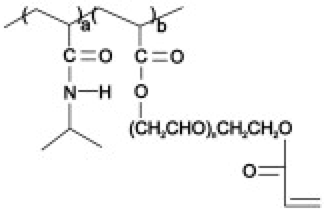

**Figure 4:** the figure above shows the basic structure of the repeat unit of PEG-b-pNIPAAm.[8]

## Experimental Methods

Experimental methods for the discovery of Hofmeister effects on various salt concentrations were realized through the use of very simple methodology. The materials required for measurement of the LCST are shown below.

## Materials and equipment

A 50% by weight solution of PVME in water was purchased from Sigma Aldrich along with powdered PEG-b-pNIPAAm. Solutions of 0.5 wt % could be made using the polymers and DI water available in the lab. Various glassware was used to mix solutions and hold liquids through experiments. A peristaltic pump was used in order to mix the water bath with the reservoir (heated and cooled). A hot plate was used to heat the reservoir while ice/cold water was used to cool the reservoir. A thermocouple was stuck into the cuvette holding the polymer solution in order to keep constant temperature throughout the experiment. The ellipsometer was used as a light intensity measurement tool. Finally a USB camera was setup to take pictures of the sample every ten seconds using a free photo taking software called YAWCAM.

## Procedure

The experiments run on the two thermos-responsive polymers (TRPs) were based on the simple idea that when a polymer in solution reaches it's LCST, the polymer "salts out" of solution and forms a cloud within the solution. From this, it was obvious that when the solution hits its cloud-point, the amount of light that passes through the solution should decrease drastically. From there, it was decided that using a light intensity measurement sensor, the LCST could be caught faster than the human eye could.

For each polymer, first a blank solution of 0.5 wt% TRP in water was made. This solution was simply made in order to confirm literature values of the LCST for PVME and the PEG-pNIPAAm blockcopolymer. PVME came in a solution of 50 wt% water/ 50 wt% PVME, so a solution of 1 wt% polymer in solution would translate to 0.5 wt% PVME in water. The blockcopolymer came as a nearly pure polymer, so a simple mass balance could determine the amount of water needed to make the correct solution.

In order to test the LCST of the polymers, first roughly three grams of the blank solution was taken and put into a clean cuvette. The cuvette was then placed in a plastic square water bath that could be set on the ellipsometer. The ellipsometer was then turned on and the laser for the ellipsometer was also powered on. The ellipsometer also had a light intensity measurement tool. This would be the primary indicator of whether or not the polymer reached its cloud-point (LCST). A simple depiction of the experiment is shown in **figure 3,** shown below. Not shown in the simple cartoon below is the heating and cooling mechanisms used to heat and cool the solution to the LCST. A peristaltic pump set at 87 mL/min pumps water in and out of the water bath shown above to a reservoir.  The reservoir was heated with a hot plate when it was desired that the solution be heated, and then cooled using cold water at about 18°C.

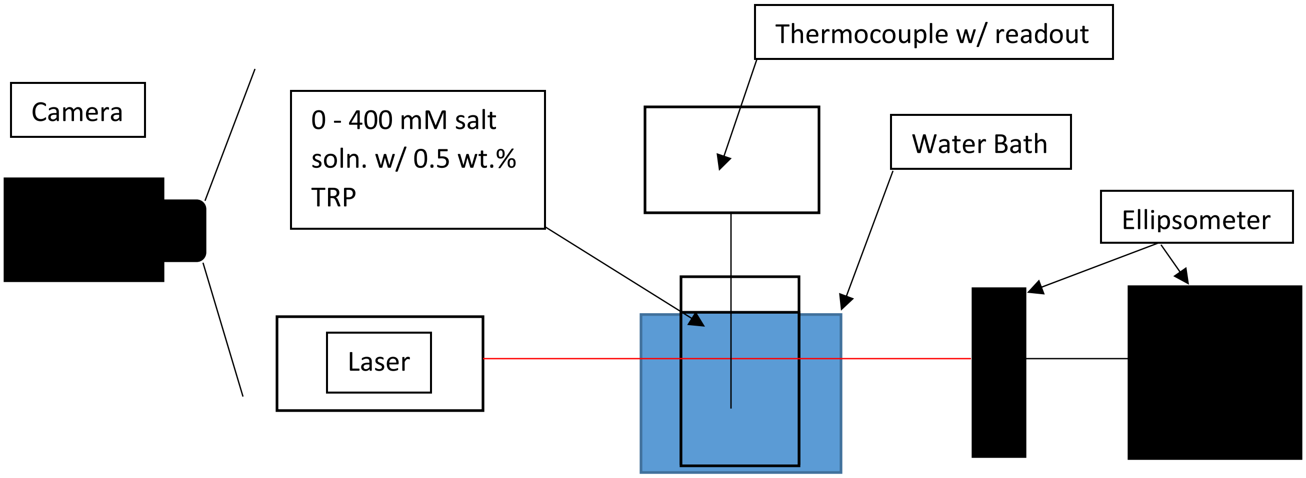

**Figure 5:** The cartoon shown is a simple setup of an LCST experimental run.

Heating and cooling was important, because the LCST was measured both during the heating process (when the solution would go from clear to clouded) and during the cooling process (when the solution would go from clouded to clear). This was performed because it is well known that a polymer can have an LCST range. Measuring during both the heating and cooling cycles produce a range of LCST if the two values are significantly different. The heating and cooling LCST was measure 3 times each for each sample.

As shown in **figure 3**, a camera is watching the light intensity readout and a thermometer throughout the experiment. The USB camera was setup with a computer and programmed to take a picture every 10 seconds in order to capture significant changes in light intensity. Using the intensity and temperature data the results below were derived.

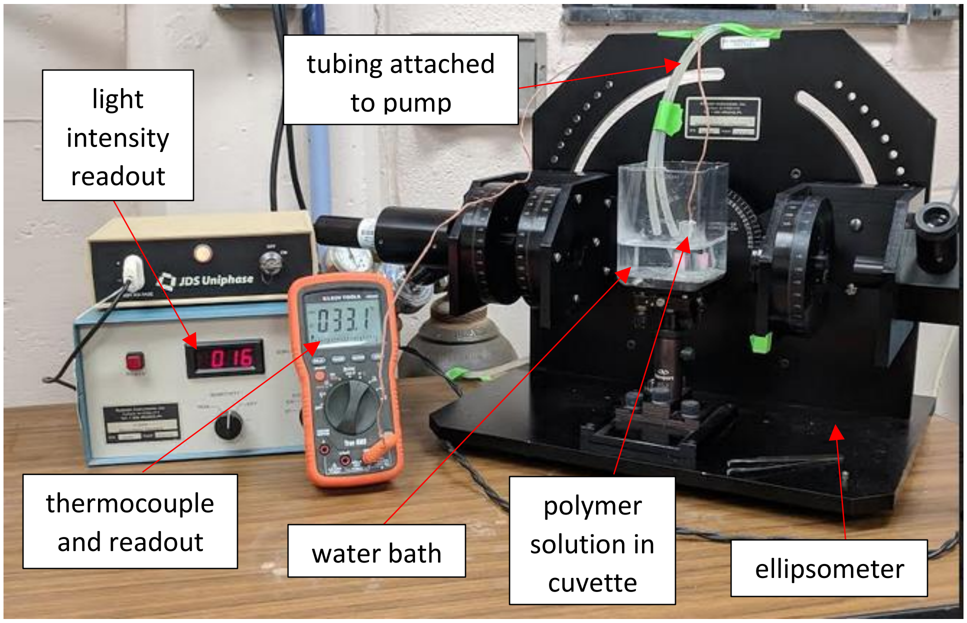

**Figure 6:** The photo shown is the experimental setup described above (sketched in Figure 5).

## Data and Results

The data presented below are the experimental results from the two sets of experiments. The first set of experiments involved PVME as the thermo-responsive polymer. With PVME, the three things were tested: a blank, a concentration study, and a various ion study. The second set of experiments is focused on PEG-b-pNIPAAm copolymer. Only two things were tested: a blank and the various ion study. Note that during each of the studies, Heat 1 to Heat 3 corresponds to heating trial, where the Cool 1 to Cool 3 correspond to the cooling trials of LCST measurement.

### *LCST of PVME in DI water:*

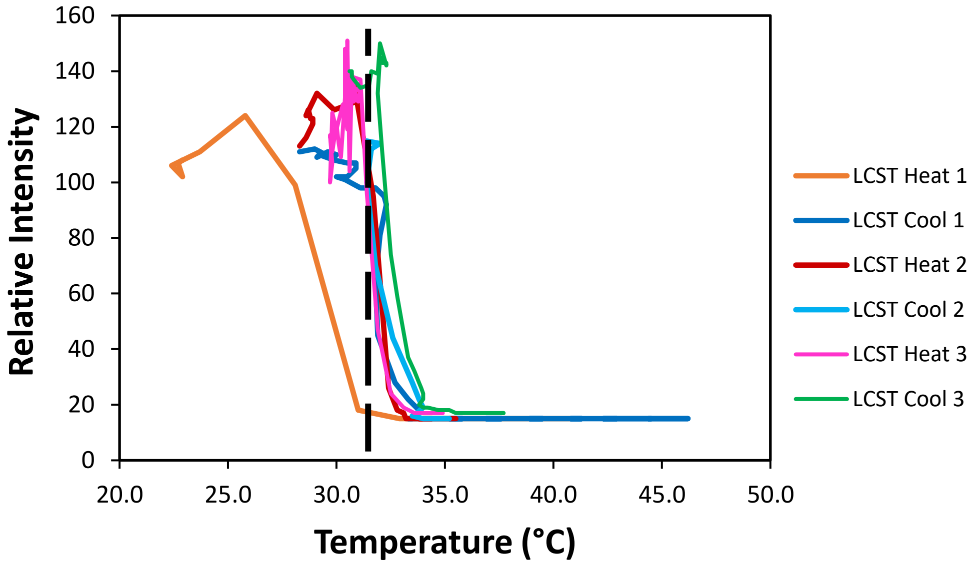

**Figure 7:** The relative intensity vs. temperature curves for the PVME blank. The dashed line shows the temperature in which the LCST was determined.

From the figure shown above, the LCST was chosen at the point at which major change in the relative laser intensity began. From the figure, the LCST of PVME was found to be roughly 32°C for both heating and cooling cycles with three runs each. The cooling cycle resulted in a slightly higher (by 0.6°C, see Table A1) LCST than the heating cycle, but the difference was insignificant. Our experiment determined LCST for PVME in water agrees with the literature values that were presented previously.[4]

### *Effects of NaCl concentration on LCST of PVME:*

The concentration study of 0.5 wt% PVME in a salt solution of PVME in NaCl. The solution concentration of NaCl from 100 mM to 400 mM. See the figure below for comparison of the various concentrations along with the blank.

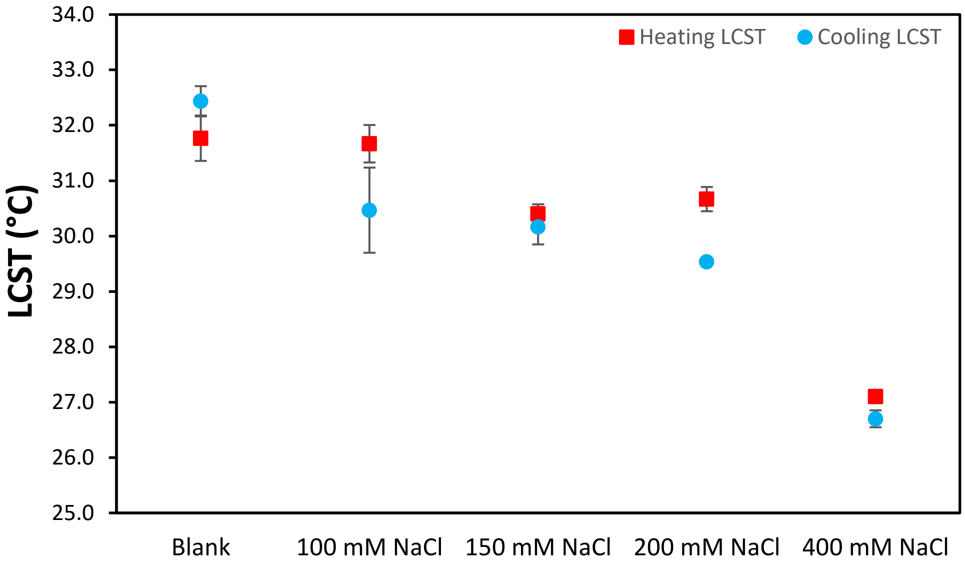

**Figure 8:** The effects of concentration of sodium chloride on the LCST of 0.5 wt% PVME in DI water are illustrated in the figure above. The red squares represent the LCST measured while heating the various solutions, while the blue circles represent the LCST of the solution LCST while cooling.

An ANOVA was run on the five groups of six measurements to observe whether or not the differences in LCST were significant. This will help identify if at least two of the groups are significantly different. The ANOVA was run with an alpha value of 0.5. The figure showed that as the concentration of the sodium cations in solution with the polymer had a significant effect on the LCST. Increasing the sodium ions in solution from 0 mM to 400 mM decreased the LCST from 32°C to the range of 26.4 – 26.7°C.

**Table 1:** The following table shows the values computed from the Anova of the five groups in excel. The p value is almost zero and Fcrit < F thus indicating that at least two of the groups are significantly different.

Anova: Single Factor

SUMMARY

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| Blank | 6 | 192.6 | 32.1 | 0.424 |
| 100 mM | 6 | 186.4 | 31.06667 | 1.278667 |
| 150 mM | 6 | 181.7 | 30.28333 | 0.173667 |
| 200 mM | 6 | 180.6 | 30.1 | 0.444 |
| 400 mM | 6 | 161.4 | 26.9 | 0.088 |

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 91.2453333 | 4 | 22.81133 | 47.35917 | 2.59E-11 | 2.75871 |
| Within Groups | 12.0416667 | 25 | 0.481667 | | | |
| | | | | | | |
| Total | 103.287 | 29 | | | | |

### *Effects of Hofmeister cation on the LCST of PVME:*

While running the experiments on PVME, originally the following cations were used in solution with 0.5 wt% PVME: $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, and $Cs^+$ with the same ionic strength (i.e., $Cl^-$ concentration). The reason these ions were chosen is because sodium is in the middle of the spectrum when it comes to Hofmeister ions, while potassium and cesium are Kosmotropes and magnesium and calcium are Chaotropes[5]. Using these ions would show how across the Hofmeister series the LCST for the thermos-responsive polymer changes. The following figure shows that addition of any of these ions discussed above will cause a statistically significant shift (see **Table A3**). The Kosmotropes seemed to have a larger effect on the LCST than that of the Chaotropes.

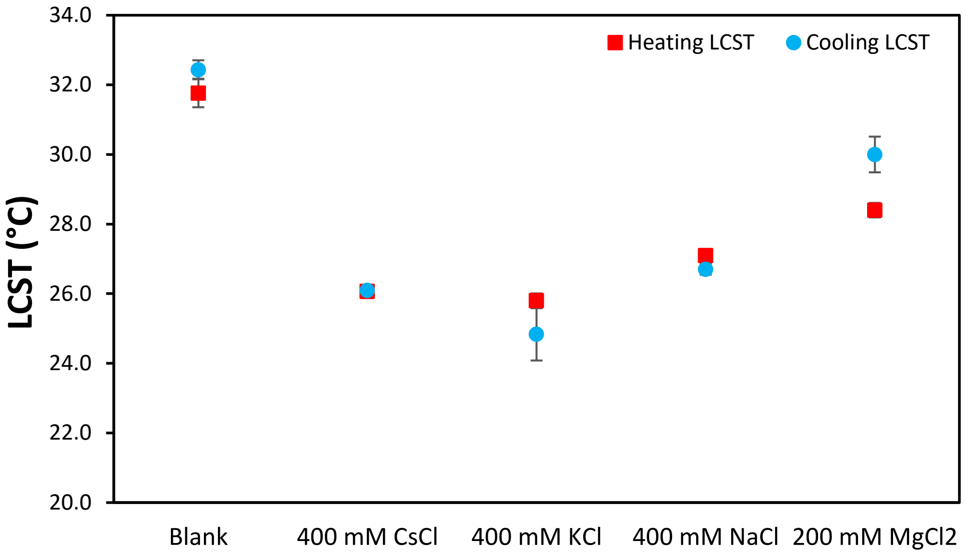

**Figure 9:** The above graph shows the trend of the LCST with various ions from left to right transitioning from Kosmotropic to Chaotropic cations. The ANOVA (found in appendix) indicates that there is a significant difference between at least two of the groups shown.

### *LCST of PEG-pNIPAAM*

Since a concentration study was done with the PVME samples, it was deemed unnecessary to complete one for the block copolymer. A blank, however, was still run to try to match the literature value to that of one produced in the lab.

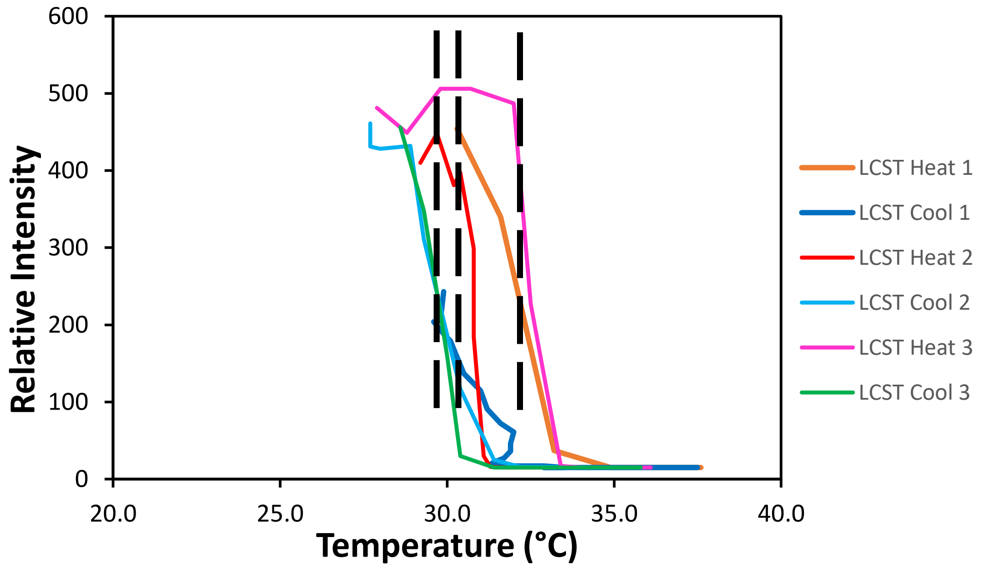

**Figure 10:** The graph above shows the relative intensity vs. temperature curves for the PEG-pNIPAAm blank. The black dotted lines used above indicate roughly where the LCST for the run was measured. The chart in the appendix has the exact values.

The values of the LCST for each trial were estimated and put into the summary **table A5** in the appendix. Take note that the values are very similar to that of the PVME samples. From the figure and the appendix chart, it is clear that the LCST of PEG-b-pNIPAAm in DI water from experiment is roughly 31°C. This will act as the basis LCST to compare to when running various cations in solution.

### *Effects of Hofmeister cations on the LCST of PEG-b-pNIPAAM*

Similarly to the PVME Ion study, the ions that were used in the PEG-pNIPAAm study are: $Na^+$, $K^+$, $Mg^{2+}$, and $Cs^+$. The following figure and **Table A6** show the results of the ion study.

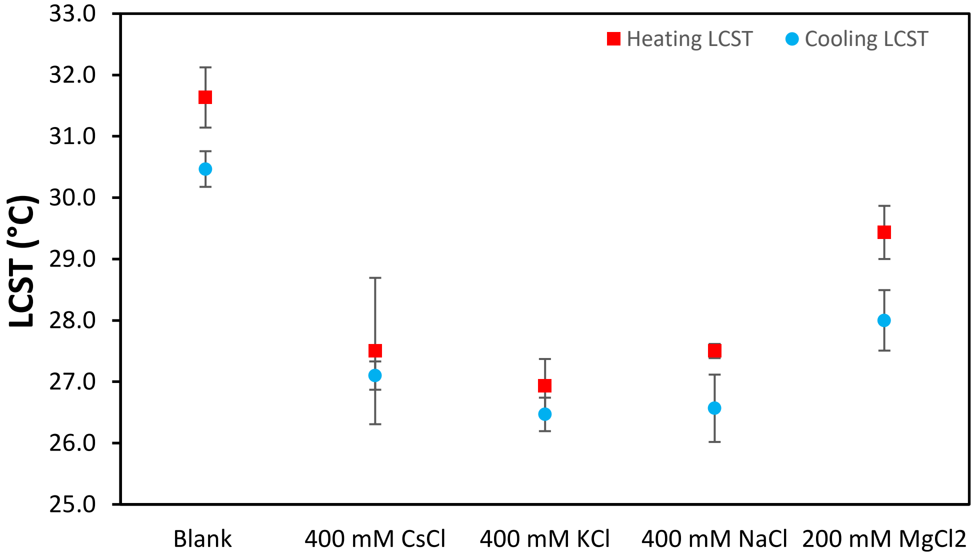

**Figure 11:** The above graph shows the trend of the LCST with various ions from left to right transitioning from Kosmotropic to Chaotropic. The values given in the appendix produce an Anova that implies that at least two of the groups shown are statistically different.

The results of this set of experiments were similar to that of the PVME experiments. Adding any of the ions produced a statistically significant change (**Table A7**) in the LCST of the PEG-pNIPAAm water solution. As it was for the PVME, the Kosmotropes appear to have a greater effect on LCST than the Chaotropes.

## Discussion

The point of running the experiments described above was to gain an understanding of how Hofmeister ions affect the LCST of thermo-responsive polymers. The results presented in this study indicated several points. First, the method of LCST measurement seems to be fairly accurate, given that the tests on the blank polymer solutions produced LCST's very close to that of the literature values. In addition, it is important to note that the alternative to this method is measuring the cloud point by eye which would prove to be much less accurate. The PVME was found to have a measured LCST of 32°C compared to the reported value of 35°C.[7] The paper referenced, by Maeda, only reported the LCST but did not provide information of concentration in water. Our result shows there is some validity to the method designed to measure the LCST of the samples throughout the duration of the experiments presented above. Acknowledging there is most certainly error when measuring the LCST, the data points measured throughout the duration of experiments are not assumed to be exactly correct, however they still provide a great indicator of the general trend. Therefore the trends that are seen can be assumed to be correct even if the values are not 100% accurate.

The NaCl concentration effects on the LCST of PVME produced the results shown in **figure 8**. A general down trend in LCST of PVME was noticed as the concentration of sodium increases. This could be proposed to be explained by the Hofmeister effect. Having the sodium and chloride ions in solution changed how the macromolecule, PVME, dealt with the water molecules at higher temperatures. It is clear that with the increased sodium chloride concentration, the PVME, became more hydrophobic at 26 – 32°C range as compared to that of the blank solution of just simply PVME in water.

The Hofmeister effects from ion to ion were the main focus of the project presented. **Figure 9** shows the results of the ion change from experiment to experiment. Note that the graphs presented show the same order of ions for the two separate thermo-responsive polymers. From left to right the ions go from Kosmotropic to Chaotropic, where $Cs^+$ and $Mg^{2+}$ cations are the extremes and the $K^+$ and $Na^+$ cations are the mild cases. It is plain to see that between the two polymers, the same general trend is shown. Note that **Figure 8**, with error shown, the data points are significantly different whereas the majority of the data presented in **Figure 11** is not significantly different. This is an issue because it shows that not all of the data is without a doubt always within the range shown. In order to correct this, more experiments would need to be run that could produce more accurate measurements. However, it is clear that some of the data is at least significantly different, meaning that there may be a direct relationship between the ions in solution and the LCST of the solution. Either way, the figures show there is a sort of parabolic trend when going from more extreme Kosmotropes to Chaotropes.

Some points to keep in mind when looking at these trends are the ion charge changed, the number of ions varied, and the magnitude of the charge of the ions. The ion charge changed is important because Zhang et al. suggests that cations do not have as large an effect on the transition properties of polymers as compared to that of the anions.[7] Cations, however, are easier to maintain biocompatibility due to the fact that many anions (in the Hofmeister Series), even in smaller concentrations, tend to be fairly toxic. Keeping that in mind, cations were tested in order to increase the likelihood of biocompatibility so the experimental results could be easily implemented in the biomedical field. Other sources of error that could have been introduced is in using only 4 different ions in the Hofmeister Series. Due to the limited time to run experiments, all cations generally presented in the Hofmeister series could not be tested to see their effects on the LCST of each of the thermo-responsive polymers. Perhaps in performing more experiments with more cations would lead to a clearer trend, or a different trend entirely. Finally the idea of using “+” charged cations vs. “2+” charged cations could have an effect that we don’t understand yet.

Considering the possibility of the results presented above, the parabolic trends seen were different from what was expected. It was expected that as the cations in solution went from Kosmotropic to Chaotropic, the LCST would continually decrease. However, the results show the more mild cations (middle of the series) have a greater effect on the LCST of both of the thermos-responsive polymers, whereas the more extreme cases have a muffled effect on the LCST.

## Conclusion

Through the simple experiments performed using a hot/cool water bath and a light intensity meter a parabolic trend of LCST was found. The two extreme cations, $Cs^+$ (Kosmotrope) and $Mg^{2+}$ (Chaotrope), were found to have higher LCST’s for both the PVME and PEG-b-pNIPAAm thermo-responsive polymers. All the while, the two mild cations, $K^+$ (Kosmotrope) and $Na^+$ (Chaotrope) showed to have the lowest LCST. The results expected were to have the Kosmotropes have higher LCST while the Chaotropes have lower LCST’s. Due to the disagreement in the hypothesis and results, it is suggested that more work should be completed. It is recommended that more ions in the cationic Hofmesiter series be run under the same experimental conditions and try to fit them in the trend established. If the new ions show a different trend than previously established, the ions tested for this experiment should be rerun. If they still do not fit this new trend, further research must be performed on the error discussion topics presented.

# Appendix A

### *PVME Blank Data*

**Table A1:** The table summarizes the results from the PVME Blank test. Data matches what is presented in **Figure 7.**

| Trial | LCST (°C) | average | stdev |
|---|---|---|---|
| Heat 1 | 31 | | |
| Heat 2 | 32.4 | | |
| Heat 3 | 31.9 | 31.8 | 0.7 |
| cool 1 | 31.9 | | |
| cool 2 | 32.6 | | |
| cool 3 | 32.8 | 32.4 | 0.5 |

### *PVME Concentration Study Data*

**Table A2:** The table summarizes the results from the PVME concentration study. Data matches what is presented in **Figure 8.**

| LCST Results | | Graph # | T °C | | | | | | T °C | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hot Trial 1 | Hot Trial 2 | Hot Trial 3 | Avg | Std Dev | Std Er | Cold Trial 1 | Cold Trial 2 | Cold Trial 3 | Avg | Std Dev | Std Er |
| - | Blank | 1 | 31 | 32.4 | 31.9 | 31.77 | 0.71 | 0.41 | 31.9 | 32.6 | 32.8 | 32.43 | 0.47 | 0.27 |
| 100 mM | NaCl | 2 | 31.9 | 31 | 32.1 | 31.67 | 0.59 | 0.34 | 29.6 | 29.8 | 32 | 30.47 | 1.33 | 0.77 |
| 150 mM | NaCl | 3 | 30.1 | 30.7 | 30.4 | 30.40 | 0.30 | 0.17 | 30.8 | 29.9 | 29.8 | 30.17 | 0.55 | 0.32 |
| 200 mM | NaCl | 4 | 31.1 | 30.5 | 30.4 | 30.67 | 0.38 | 0.22 | 29.5 | 29.6 | 29.5 | 29.53 | 0.06 | 0.03 |
| 400 mM | NaCl | 5 | 26.9 | 27.2 | 27.2 | 27.10 | 0.17 | 0.10 | 26.4 | 26.9 | 26.8 | 26.70 | 0.26 | 0.15 |

### *PVME Ion Effect Summary*

**Table A3:** The table presented below shows the results from the PVME Hofmeister Ion Effects test. Data matches what is presented in **Figure 9.**

| LCST Results | | Graph # | T °C | | | | | | T °C | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hot Trial 1 | Hot Trial 2 | Hot Trial 3 | Avg | Std Dev | Std Er | Cold Trial 1 | Cold Trial 2 | Cold Trial 3 | Avg | Std Dev | Std Er |
| - | Blank | 1 | 31 | 32.4 | 31.9 | 31.77 | 0.71 | 0.41 | 31.9 | 32.6 | 32.8 | 32.43 | 0.47 | 0.27 |
| 400 mM | CsCl | 2 | 26.2 | 25.7 | 26.3 | 26.07 | 0.32 | 0.19 | 26 | 26.1 | 26.2 | 26.10 | 0.10 | 0.06 |
| 400 mM | KCl | 3 | 25.5 | 26.2 | 25.7 | 25.80 | 0.36 | 0.21 | 26.1 | 24.9 | 23.5 | 24.83 | 1.30 | 0.75 |
| 400 mM | NaCl | 4 | 26.9 | 27.2 | 27.2 | 27.10 | 0.17 | 0.10 | 26.4 | 26.9 | 26.8 | 26.70 | 0.26 | 0.15 |
| 200 mM | MgCl2 | 5 | 28.1 | 28.8 | 28.3 | 28.40 | 0.36 | 0.21 | 29 | 30.3 | 30.7 | 30.00 | 0.89 | 0.51 |

### *PVME Ion Effect: Anova*

**Table A4:** The table presented below shows the results from the Anova on the data from **Table A3.**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Blank | 6 | 192.6 | 32.1 | 0.424 |
| 400 mM CsCl | 6 | 156.5 | 26.08333 | 0.045667 |
| 400 mM KCl | 6 | 151.9 | 25.31667 | 1.009667 |
| 400 mM NaCl | 6 | 161.4 | 26.9 | 0.088 |
| 200 mM MgCl2 | 6 | 175.2 | 29.2 | 1.136 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 181.8113 | 4 | 45.45283 | 84.06813 | 4.01E-14 | 2.75871 |
| Within Groups | 13.51667 | 25 | 0.540667 | | | |
| | | | | | | |
| Total | 195.328 | 29 | | | | |

### *PEG-b-pNIPAAm Blank Data*

**Table A5:** The table below shows the data that can be retrieved from **Figure 10.**

| Trial | LCST (°C) | average | stdev |
|---|---|---|---|
| Heat 1 | 31.6 | | |
| Heat 2 | 30.8 | | |
| Heat 3 | 32.5 | 31.6 | 0.9 |
| cool 1 | 30.4 | | |
| cool 2 | 30 | | |
| cool 3 | 31.0 | 30.5 | 0.5 |

### *PEG-b-pNIPAAm Ion Effect Summary*

**Table A6:** The table presented below shows the results from the PVME Hofmeister Ion Effects test. Data matches what is presented in **Figure 11.**

| LCST Results | | Graph # | T °C | | | | | | T °C | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hot Trial 1 | Hot Trial 2 | Hot Trial 3 | Avg | Std Dev | Std Er | Cold Trial 1 | Cold Trial 2 | Cold Trial 3 | Avg | Std Dev | Std Er |
| Blank | Blank | 1 | 31.6 | 30.8 | 32.5 | 31.63 | 0.85 | 0.49 | 30.4 | 30 | 31 | 30.47 | 0.50 | 0.29 |
| 400 mM | CsCl | 2 | 25.3 | 29.4 | 27.8 | 27.50 | 2.07 | 1.19 | 27.1 | 27.5 | 26.7 | 27.10 | 0.40 | 0.23 |
| 400 mM | KCl | 3 | 26.4 | 27.8 | 26.6 | 26.93 | 0.76 | 0.44 | 26.1 | 26.3 | 27 | 26.47 | 0.47 | 0.27 |
| 400 mM | NaCl | 4 | 27.5 | 27.7 | 27.3 | 27.50 | 0.20 | 0.12 | 25.6 | 27.5 | 26.6 | 26.57 | 0.95 | 0.55 |
| 200 mM | MgCl2 | 5 | 29 | 29 | 30.3 | 29.43 | 0.75 | 0.43 | 27.1 | 28.8 | 28.1 | 28.00 | 0.85 | 0.49 |

### *PEG-b-pNIPAAm Ion Effect Anova*

**Table A7:** The table presented below shows the results from the Anova on the data from **Table A6.**

Anova: Single Factor

SUMMARY

| *Groups* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Blank | 6 | 186.3 | 31.05 | 0.799 |
| CsCl | 6 | 163.8 | 27.3 | 1.82 |
| KCl | 6 | 160.2 | 26.7 | 0.384 |
| NaCl | 6 | 162.2 | 27.03333 | 0.638667 |
| MgCl2 | 6 | 172.3 | 28.71667 | 1.133667 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 76.8153333 | 4 | 19.20383 | 20.10732 | 1.62E-07 | 2.75871 |
| Within Groups | 23.8766667 | 25 | 0.955067 | | | |
| | | | | | | |
| Total | 100.692 | 29 | | | | |

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