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Comparison of the Effects of Hofmeister Ions on Transition Temperature of Two Thermoresponsive Polymers

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Honors Project Report

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Honors Research Project

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Abstract

It has been hypothesized that poly(vinylmethylether) (PVME) could be used to replace poly(N-isopropylacrylamide) (pNIPAAM) in various thermoresponsive applications based on literature searches and previous lab experiments. PVME is cheaper and easier to process than pNIPAAM, and would make pNIPAAM applications more cost effective. In order to verify this, experimental data was gathered on the effects of four anions from the Hofmeister series (SO_4^{2-}, Cl^-, I^-, and SCN^-) on the lower critical solution temperature (LCST) of pNIPAAM and compared to previous data collected on PVME by another student in Dr. Newby's lab. If PVME and pNIPAAM have comparable LCST ranges with the various Hofmeister anions, it is an indication that PVME has the potential to replace pNIPAAM. Commercial pNIPAAM from Sigma Aldrich and Polysciences were used. The reported LCST range for pNIPAAM is 30-35°C. From this study, the pNIPAAM from Sigma Aldrich was found to be at the upper end of this range, and pNIPAAM from Polysciences at the lower end. ANOVA statistical analyses run on the three polymers for the blank DI water tests, Cl^-, and SCN^- showed a significant difference between them in DI water and Cl^-, but not in SCN^-. t Tests on the experimentally obtained LCSTs of PVME and Sigma Aldrich pNIPAAM were inconclusive with significant differences in Cl^- and SO_4^{2-}, but not in DI water or SCN^-. It is recommended that further testing to be completed by the next group of students with an increased number of tests for each anion in order to have more accurate statistical analyses. It is also recommended to use other anions in the Hofmeister series.

Executive Summary

The goal of this study is to compare how four Hofmeister anions affect the lower critical solution temperature (LCST) of poly(vinylmethylether) (PVME) and poly(N-isopropylacrylamide) (pNIPAAM) and to determine if PVME can replace pNIPAAM for various thermoresponsive applications. Thermoreponsive polymers have been attractive for various applications especially in the medical industry. Their applications have ranged from drug delivery, nanoreactors, antifouling materials, to biomimetical model systems for cells and tissue.[1,2,3,4] pNIPAAM is commonly used in the medical industry due to its reported LCST being close to the physiological temperature of the human body (30-37°C).[5] The LCST is the temperature or temperature range where the polymer will precipitate out of the solution. Hofmeister ions are known to affect the LCST of certain thermoresponsive polymers. Proving that PVME and pNIPAAM have comparable LCST values in various ion solutions will allow to employ PVME has as a replacement for pNIPAAM. PVME is cheaper and easier to process than pNIPAAM, making it a more economical choice in applications of thermoresponsive polymers.

This study involved measuring the LCST 0.5 wt% pNIPAAM solutions in DI water, 100 mM NaCl, 100 mM NaSCN, 100 mM Na_2SO_4, and 100 mM NaI. Each solution was heated and cooled three times as the intensity of a laser passed through the solution was monitored. The laser intensity decreased when polymer precipitated out of the solution during the heating and cooling cycles. Laser intensity and temperature data were collected simultaneously to determine the LCSTs of two different commerical pNIPAAMs and compared them to previous PVME data collected in Dr. Newby's lab. The LCST range was determined to be where there was a sharp decrease in laser intensity, which is also when the solution changes from clear and translucent to cloudy and opaque. The experimental LCST ranges in DI water were found to be 34.7-36.1ºC for

Sigma Aldrich pNIPAAM, 31.7-34.4ºC for Polysciences pNIPAAM, and 33.8-35.8ºC for PVME. The Hofmeister anions affected the LCST of each polymer in similar ways, with Sigma Aldrich pNIPAAM typically having a higher LCST than PVME and Polysciences pNIPAAM having a lower LCST than PVME.

Statistical analyses were conducted through ANOVA to determine any significant difference between the LCST values determined for the three polymers, and t Tests were used to determine any significant difference between the means of the LCST ranges of PVME and Sigma Aldrich pNIPAAM. The ANOVA analyses shows that in DI water and NaCl, there is a significant difference, but in NaSCN, there is no significant difference. The Sigma Aldrich pNIPAAM was the polymer initially intended to be compared to PVME as tests with all the anions were completed with this polymer and was the one used for the t Tests. The t Tests between PVME and Sigma Aldrich pNIPAAM show that in DI water and NaSCN, the two polymers' LCST values were not significantly different; but in NaCl and Na_2SO_4, their LCSTs were significantly different.

In addition to the data and results collected through this study, this Honors Research Project has also helped me on a professional level. I have gained more experience in a research lab setting allowing me to grow my confidence in running experiments and analyzing data. I have also been allowed to practice and improve my time management skills, attention to detail, communication, and technical writing; all skills that are very valuable to my professional career. The Honors Research Project has been a great way for me to strengthen and demonstrate skills taught to me in the classroom as well as during my previous co-op rotations. Furthermore, the results of this project had no clear indication of the differences between PVME and the two types of pNIPAAM as in some cases they were comparable and in others they weren't. To continue

this project's efforts, it is recommended that the next group of students conduct further testing and analysis with other anions in the Hofmeister series and potentially with various manufacturers of pNIPAAM. This would allow for a larger data pool to be used for a more accurate statistical analysis as well as potentially eliminate some of the error in testing in order to determine if PVME is a viable replacement for pNIPAAM in thermoresponsive applications.

Introduction

Thermoresponsive polymers have been employed for a wide variety of industries, but specifically the medical field is where they can be quite useful. They can be used as a carrier for drug delivery, nanoreactors, antifouling materials, and biomimetical model systems for cells and tissue.[1,2,3,4] In this study, the two thermoresponsive polymers that were evaluated are poly(vinylmethylether), or PVME, and poly(N-isopropylacrylamide), or pNIPAAM. pNIPAAM has been commonly used in the medical industry because it exhibits a lower critical solution temperature (LCST) of 30-35°C, which is close to the physiological temperature of the human body.[5] The LCST is the temperature below which the polymer is miscible in the aqueous solution and hydrophilic and above this temperature the polymer is hydrophobic and becomes immiscible in the solution.[6] The goal of this study is to compare how the Hofmeister ions affect the LCST of PVME and pNIPAAM and to determine if PVME can replace pNIPAAM for various thermoresponsive applications.

Data of the effects of four anions (SO_4^{2-}, Cl^-, I^-, and SCN^-) from the Hofmeister series on the LCST of pNIPAAM was collected and compared to previous data of PVME completed by Matthew Hlas prior to this project. There are many benefits of replacing pNIPAAM with PVME. PVME is cheaper and is easier to process comapared to pNIPAAM to process. If PVME can replace pNIPAAM, it is possible that it can also be used in many of the same medical applications as pNIPAAM while reducing costs. This can also be the stepping stone to future research on the comparability of PVME and pNIPAAM and confirm the outcomes found in this project.

Background

Hofmeister Ions and LCST

Hofmeister ions are a series of salts that have the ability to precipitate proteins for both anions and cations.[7] While they are known for their ability to precipitate proteins, they also have applications in various other areas, including thermoresponsive polymers. This study focuses on the use of four anions (SO_4^{2-}, Cl^-, I^-, and SCN^-) in the Hofmeister series because previous studies have shown that the anions have a more significant behavior on LCST than cations.[8] This behavior makes it easier to observe trends in the LCST and better compare PVME and pNIPAAM. In **Figure 1** the anions of the Hofmeister series are listed.

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

Figure 1: Depicts the Hofmeister series for anions. They are ordered from left to right based on their ability to stabilize the structure of proteins. The effects of SO_4^{2-}, Cl^-, I^-, and SCN^- are being examined in this study based on accessibility and dramatic impact on LCST compared to a blank of DI water.

The species on the left of the series are known as Kosmotropes, and the right are Chaotropes.[8] Kosmotropes have a more dramatic effect on LCST as they stabilize a molecule. In this case, allowing the polymer to "salt out" and the solution will appear cloudy. Chaotropes destabilize the molecule and allow polymers to more readily dissolve into water. This will allow for a distinct trend in the LCST of pNIPAAM and PVME to be determined. For thermoresponsive polymers, the LCST is the temperature range where above it, the polymer will "salt out" and light traveling through the solution will have a lower intensity as the solution becomes cloudy. Below the LCST range, the polymer will "salt in" and dissolve into the

solution, allowing the light traveling through it to have a higher light intensity.[6] LCST can be determined where there is a drastic change in the light intensity as the temperature of the solution is changed.

PVME and pNIPAAM

The polymers chosen for this experiment are PVME and pNIPAAM due to accessibility, process-ability, and previous research conducted. PVME is being compared to pNIPAAM because they have similar reported LCST values of 32- 35°C. Both are commercially made and easily accessible. The basic structures of PVME and pNIPAAM can be seen in **Figure 2**. However, PVME is easier to process and is much cheaper than pNIPAAM.[9,10] pNIPAAM from Sigma Aldrich costs ~ $658/10g and from Polysciences costs ~$360/10g. PVME costs ~$15.72/25g, significantly less expensive than both manufacturers of pNIPAAM.[11] pNIPAAM from Sigma Aldrich is being compared mainly to PVME, but Polysciences pNIPAAM is also being considered to determine if there is a possible difference based on manufacturer. Both PVME and pNIPAAM are biocompatible, and PVME has the potential to be used in the same medical applications as pNIPAAM. If it can be proven that PVME is comparable to and can replace pNIPAAM, it will make the processes currently using pNIPAAM much more cost effective.

Figure 2: Depicts the basic repeat structure of PVME[11] (left) and pNIPAAM[12] (right).

Experimental Methods

The experimental method utilized in this experiment was designed by two previous students in Dr. Newby's lab and allowed to simultaneously record the temperature and laser intensity of 0.5 wt% pNIPAAM salt solutions as the solution being heated or cooled in order to determine the LCST. The materials required and procedure are described below. The pNIPAAM data was collected to compare to PVME data previously obtained by Matthew Hlas.

Materials and Equipment

Powdered pNIPAAM, molecular weight of 30 – 40 kg/mol and 40 kg/mol, respectively, were purchased from Sigma Aldrich and Polysciences. Sodium chloride, sodium iodide, and sodium sulfate purchased from Sigma Aldrich. Sodium thiocyanate was purchased from Acros Organics were utilized for this experiment. Various glassware was used to mix and hold the solutions throughout the experiments. A cuvette was used to hold the polymer solution in a water bath. An ellipsometer was used to measure the intensity of the laser passing through the polymer solution, and a thermocouple was used to measure the temperature of the polymer solution throughout the experiments. A hot plate was used to heat water that was subsequently used to heat the water bath where the cuvette was placed in. A peristaltic pump set to 27 mL/min was used to interchangeably pump hot and cold water to the water bath. A microscope equipped with a camera was used to take pictures of the sample every second using YAWCAM software in order to accurately record the visual changes.

Procedure

The experimental procedure was based off previous designs used by Joshua Moser in Dr. Newby's lab. To create the polymer samples, first 400mM salt solutions with the anions (SO_4^{2-}, Cl^-, I^-, and SCN^-) from Hofmeister series were made. Then 0.5 wt% pNIPAAM solutions were

made using a simple mass balance of the salt solution, DI water, and pNIPAAM. The Sigma-Aldrich pNIPAAM solutions were made with all four of the ions, while only NaCl and NaSCN solutions were made using the Polysciences pNIPAAM.

When a polymer solution reaches its LCST, it will become cloudy as the polymer "salts out" of the solution, and the amount of laser light able to pass through will decrease. To determine the LCST of the polymers, the sample was placed in a cuvette and heated/cooled as the intensity of a laser shined through it was measured. The LCST of the polymer can present itself as a range, which is why the laser intensity was measured for both heating and cooling. The LCST was measured three times for heating and cooling to ensure accurate data was collected. The sample was heated using a flask of water on a heating plate and cooled using ice water. A peristaltic pump set to 27 mL/min was used to mix the hot or cold water with the water bath the cuvette was secured in.

The sample cuvette was attached to a 3D printed plastic stage that fit snugly into the plastic container used for the water bath, which was secured to the ellipsometer. A thermocouple was inserted into the sample solution and secured using tape to measure the temperature of the sample but without interfering with the laser light. The overall set up can be seen in **Figure 3** with the sample, water bath, ellipsometer, thermocouple, and pump. An overhead more detailed look is depicted in **Figure 4**, and the pump and hot plate are depicted in **Figure 5**. The microscope equipped with a camera was connected to a computer and recorded a picture of the experimental set every second to record the changes in laser intensity, cloudiness of the sample, and temperature reading.

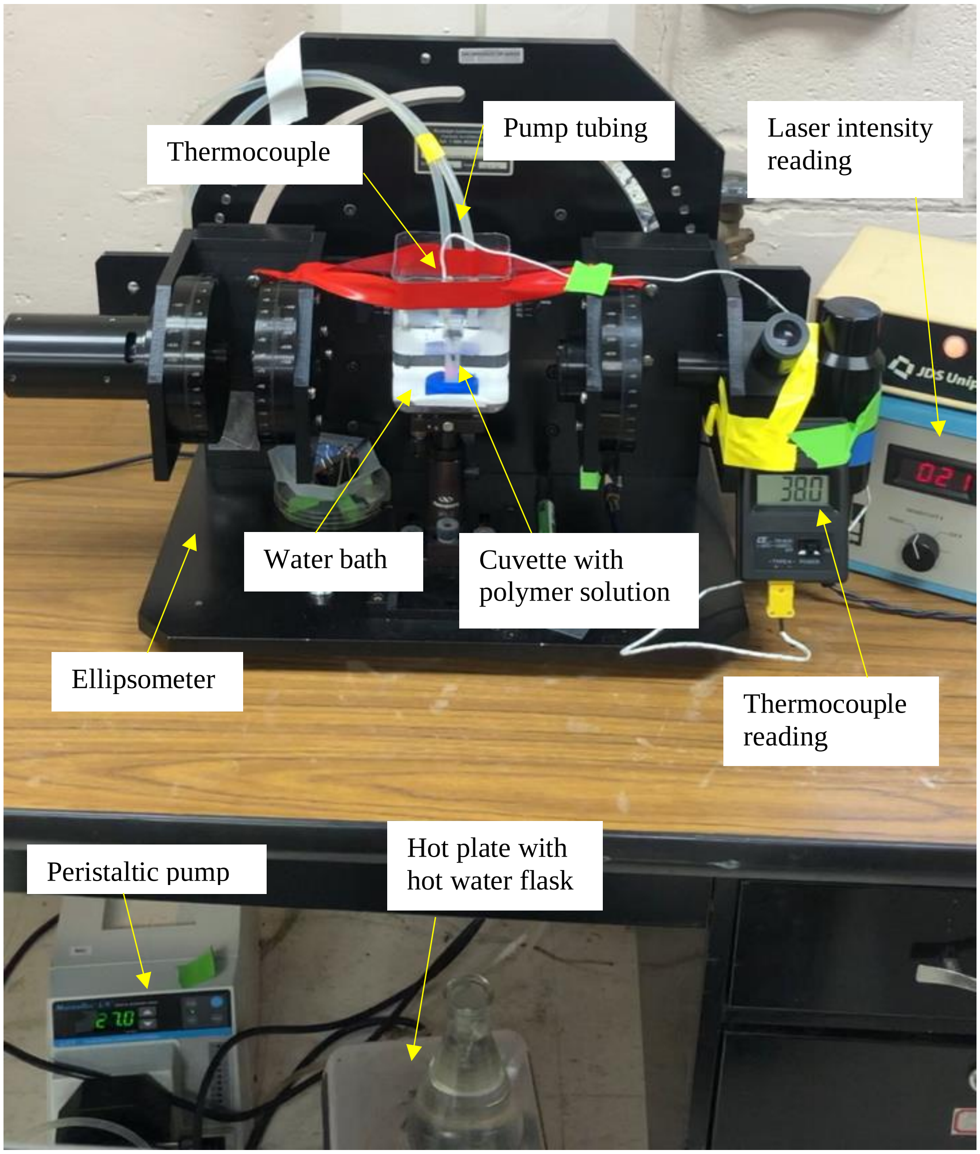

Figure 3: Depicts the experimental setup used to record the light intensity and temperature of the samples to determine LCST. The thermocouple, pump tubing, and sample water bath were secured using tape. The flask on the hot plate holds hot water that was pumped by the peristaltic pump to the water bath in order to heat the sample. The tubing was switched to a container with ice water to cool. The pump was set to 27 mL/min. The sample in this picture was cloudy as it reached its LCST. The red light in the sample was the laser hitting the cloudy polymer, making the laser intensity much lower than if it were clear.

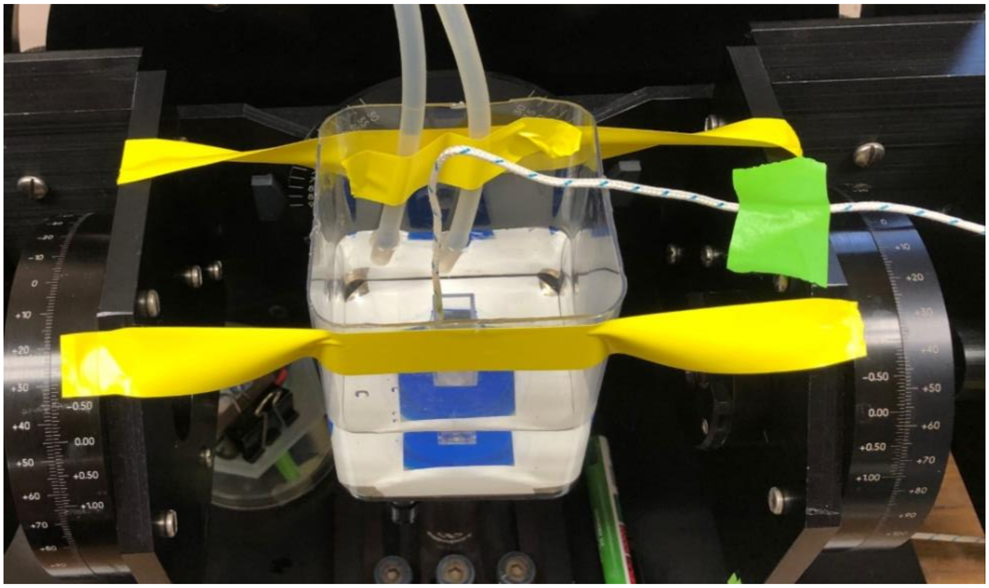

Figure 4: Depicts a closer overhead view of the water bath with the polymer sample. The thermocouple was secured using tape and gently placed out of the path of the laser. The cuvette was secured to a plastic square that fits in the water bath. The water bath was placed so the laser shines through the polymer solution. Both water bath and pump tubing were secured with tape.

Figure 5: Depicts the peristaltic pump and hot plate with hot water flask. The pump was set to 27 mL/min, and the hot plate to a setting of 4 initially and lowered if necessary. The container in the lower right of the picture held the ice water used to cool the sample.

Statistical Analysis

The LCST data was compared using ANOVA and t Test in excel with alpha values of 0.05. The alpha value indicates that there is a 5% chance that the conclusion drawn is incorrect. An ANOVA analysis was used to determine if the means of the population, in this case the three polymers, are equal. If the F value is greater than the F critical value, and the P value is less than 0.05, the populations are not equal. Those results indicate that at least one of the three polymers are different. An ANOVA cannot tell you which of the polymers is different, which is why a t Test was used. A t Test is similar to an ANOVA, but is conducted between two populations to determine if their means are different. In this case, PVME and Sigma Aldrich pNIPAAM were used. If the t Stat value is greater than the t Critical two-tail value or less than the negative t Critical two-tail value, and the P two-tail value is less than 0.05, the means of the populations are not equal. With this test, we can conclude whether or not PVME and Sigma Aldrich pNIPAAM are the same in each of the ion solutions.

Data and Results

The data presented below are the LCST results of pNIPAAM from Sigma Aldrich and Polysciences as well as PVME. The PVME data was collected by Matthew Hlas utilizing the same experimental method.

Determination of LCST

The LCST range was determined based on the drastic change in laser intensity that occurs at the LCST. Values at the top, bottom, and middle of this decrease were read off the laser intensity versus temperature graphs generated from the data. An example of the determination of these values can be seen in **Figure 6**. This method was used for both the heating and the cooling data for all of the samples, but only the heating is depicted in **Figure 6** for simplicity and ease of

reading. Each of the three tests for the pNIPAAM samples can be seen in **Appendix A** as well as the data associated with each graph.

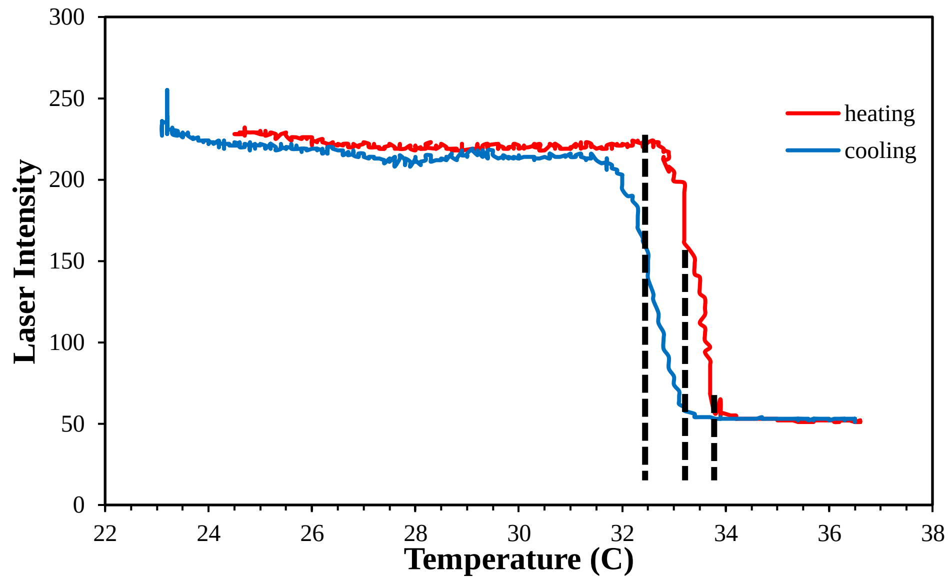

Figure 6: Graph of the relationship between laser intensity and temperature for the heating cycle and cooling cycle for the second test of DI Water and Polysciences pNIPAAM. The dashed lines down indicate the temperatures for the LCST range. The LCST occurs at the dramatic decrease in laser intensity. The first line is the top of this peak, the second line is the middle, and the last line is the bottom. The values read off this graph were roughly 32.8ºC for the top, 33.9 ºC for the bottom, and 33.5 ºC for the middle of the heating cycle; and 31.7ºC for the top, 33.1 ºC for the bottom, and 32.5 ºC for the middle of the cooling cycle.

LCST of Sigma Aldrich pNIPAAM

The Sigma Aldrich pNIPAAM ion study consisted of 0.5 wt% pNIPAAM solutions with DI water, 100 mM NaCl, 100 mM NaSCN, 100 mM Na_2SO_4, and 100 mM NaI. These ions were chosen due to accessibility and to compare to the previous PVME data already collected. The values of each of the three tests were averaged to determine an overall LCST range for each of the ions. In **Figure 7**, the average LCST ranges (top, bottom, and middle) are depicted for the heating cycles of the Sigma Aldrich pNIPAAM as well as their error, and in **Figure 8** the same for the cooling cycles. **Figure 9** shows a comparison of the middle LCST values for the heating

and cooling cycles. In three out of the five tests, the heating cycle has a higher LCST than the cooling cycle. The data associated with these graphs can be seen in **Appendix A**.

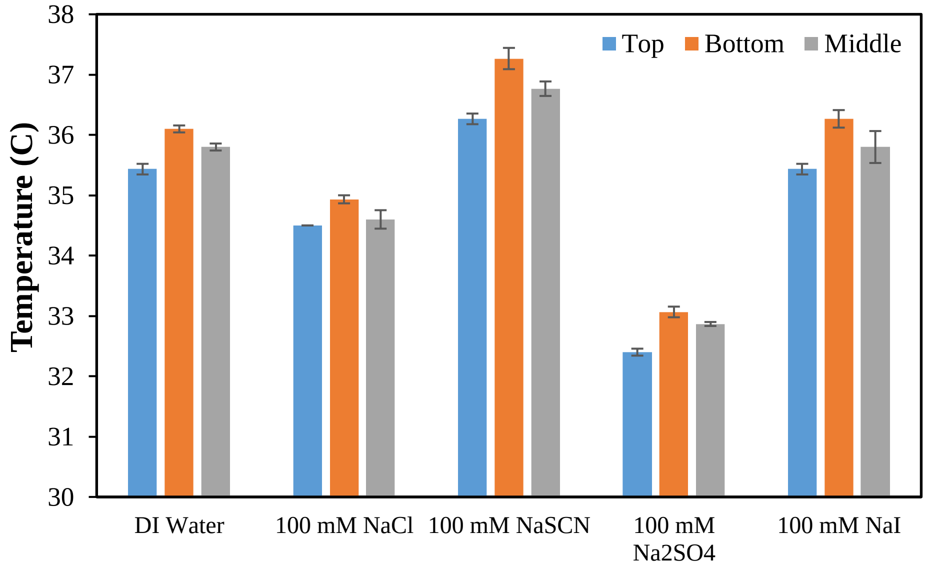

Figure 7: Graph of the top, bottom, and middle average LCST values during the heating cycle for 0.5 wt% pNIPAAM solutions of DI water, NaCl, NaSCN, Na_2SO_4, and NaI. Error for each value was calculated by dividing the standard deviation by the square root of 3 (the number of measurements). NaSCN has the highest LCST range while Na_2SO_4 has the lowest LCST range.

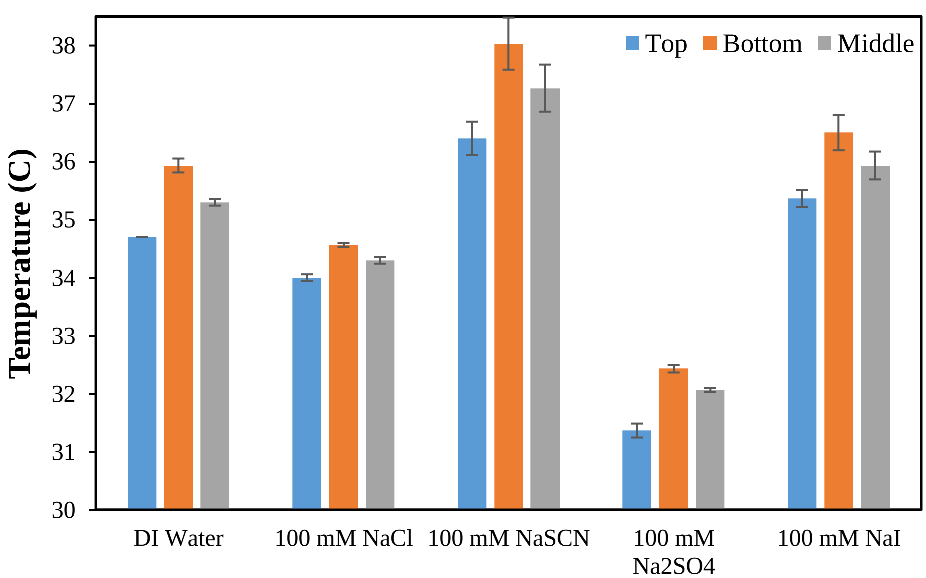

Figure 8: Graph of the top, bottom, and middle average LCST values during the cooling cycle for 0.5 wt% pNIPAAM solutions of DI water, NaCl, NaSCN, Na_2SO_4, and NaI. Error for each value was calculated by dividing the standard deviation by the square root of 3 (the number of measurements). The differences in cooling LCST range is not as dramatic as in the heating LCST.

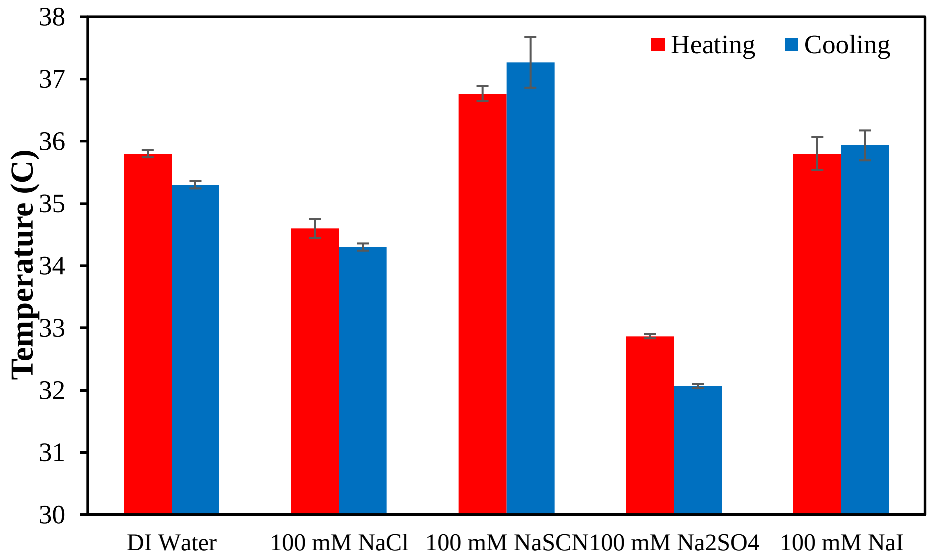

Figure 9: Graph comparing the middle average LCST values of the heating and cooling cycles for 0.5 wt% pNIPAAM solutions of DI water, NaCl, NaSCN, Na_2SO_4, and NaI. Error for each value was calculated by dividing the standard deviation by the square root of 3 (the number of measurements). For DI water, NaCl, and Na_2SO_4, the heating LCST is higher than the cooling LCST. For NaSCN and NaI, the cooling LCST is higher.

LCST of Polysciences pNIPAAM

The Polysciences pNIPAAM ion study consisted of a blank of DI water, 100 mM NaCl, and 100 mM NaSCN. Only these ions were tested. Other ions such as Na_2SO_4 and NaI were not considered because NaCl and NaSCN would provide enough information for comparing products supplied by different manufacturers. The values of each of the three tests were averaged to determine an overall LCST range. In **Figure 10**, the average LCST ranges (top, bottom, and middle) are depicted for the heating cycles of the Polysciences pNIPAAM as well as their error, and in **Figure 11** the same for the cooling cycles. **Figure 12** portrays a comparison of the middle LCST values for the heating and cooling cycles. In two out of the three tests, the cooling cycle has a higher LCST than the heating cycle. The data associated with these graphs can be seen in **Appendix A**.

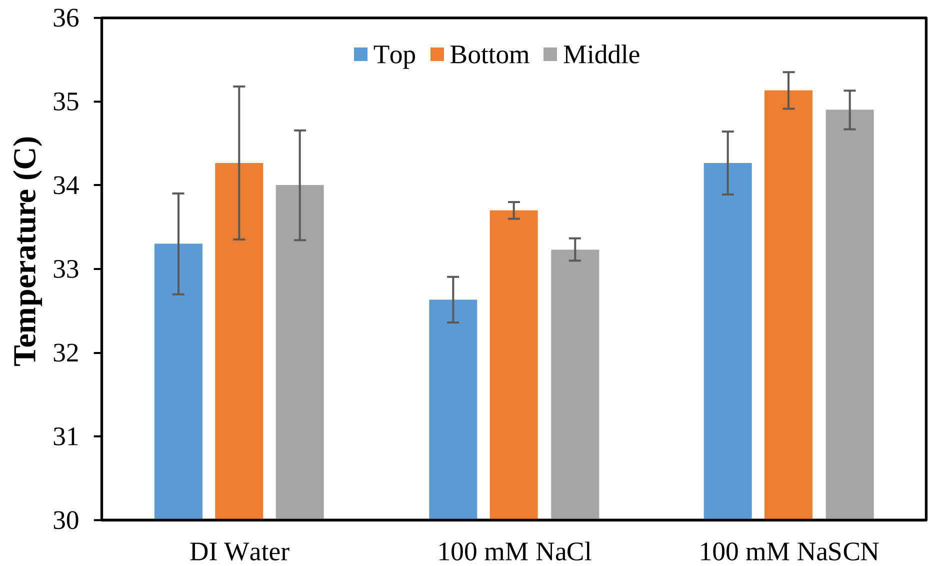

Figure 10: Graph of the top, bottom, and middle average LCST values during the heating cycle for 0.5 wt% pNIPAAM solutions of DI water, NaCl, and NaSCN. Error for each value was calculated by dividing the standard deviation by the square root of 3 (the number of measurements). NaSCN has the highest LCST range while Na_2SO_4 has the lowest LCST range. The error on the DI water is higher due to the third test having higher temperature values than the first two tests.

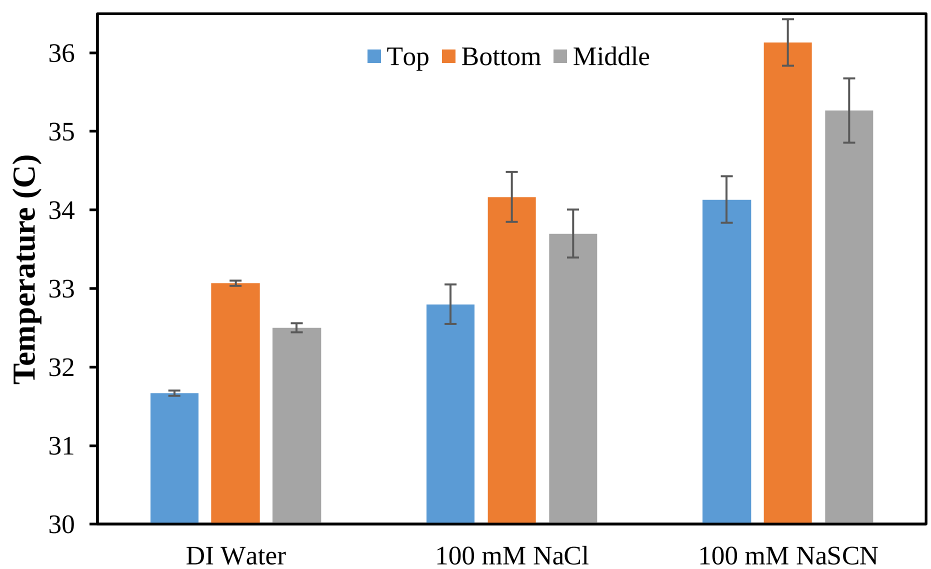

Figure 11: Graph of the top, bottom, and middle average LCST values during the cooling cycle for 0.5 wt% pNIPAAM solutions of DI water, NaCl, and NaSCN. Error for each value was calculated by dividing the standard deviation by the square root of 3 (the number of measurements).

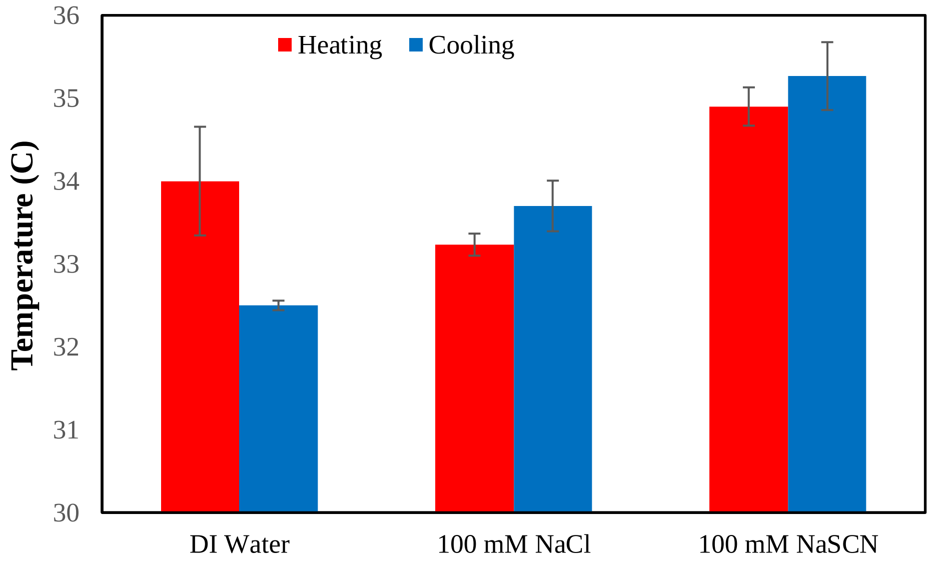

Figure 12: Graph comparing the middle average LCST values of the heating and cooling cycles for 0.5 wt% pNIPAAM solutions of DI water, NaCl, and NaSCN. Error for each value was calculated by dividing the standard deviation by the square root of 3 (the number of measurements). For DI water, the heating LCST is higher than the cooling LCST. For NaCl and NaSCN, the cooling LCST is higher. The error on the DI water is higher due to the third test having higher temperature values than the first two tests.

LCST of PVME

The PVME ion study conducted by Matthew Hlas consisted of 0.5 wt% PVME solutions with DI water, 100 mM NaCl, 100 mM NaSCN, and 100 mM Na_2SO_4. The data was obtained utilizing the same experimental procedure stated previously. The top, bottom, and middle temperature values make up LCST range for each of the ions. In **Figure 13**, the average LCST ranges (top, bottom, and middle) are depicted for the heating cycles of the PVME as well as their error, and in **Figure 14** the same for the cooling cycles. **Figure 15** shows a comparison of the middle LCST values for the heating and cooling cycles. In all four of the tests, the heating cycle has a higher LCST than the cooling cycle.

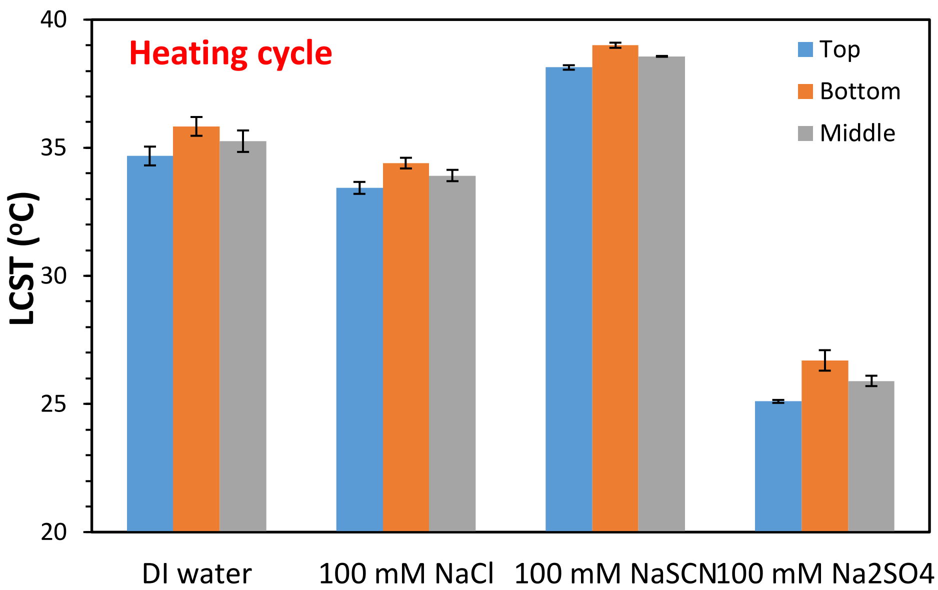

Figure 13: Graph of the top, bottom, and middle LCST values during the heating cycle for 0.5 wt% PVME solutions of DI water, NaCl, NaSCN, and Na_2SO_4. NaSCN has the highest LCST range (38 – 39°C) while Na_2SO_4 has the lowest LCST range (25 – 27°C).

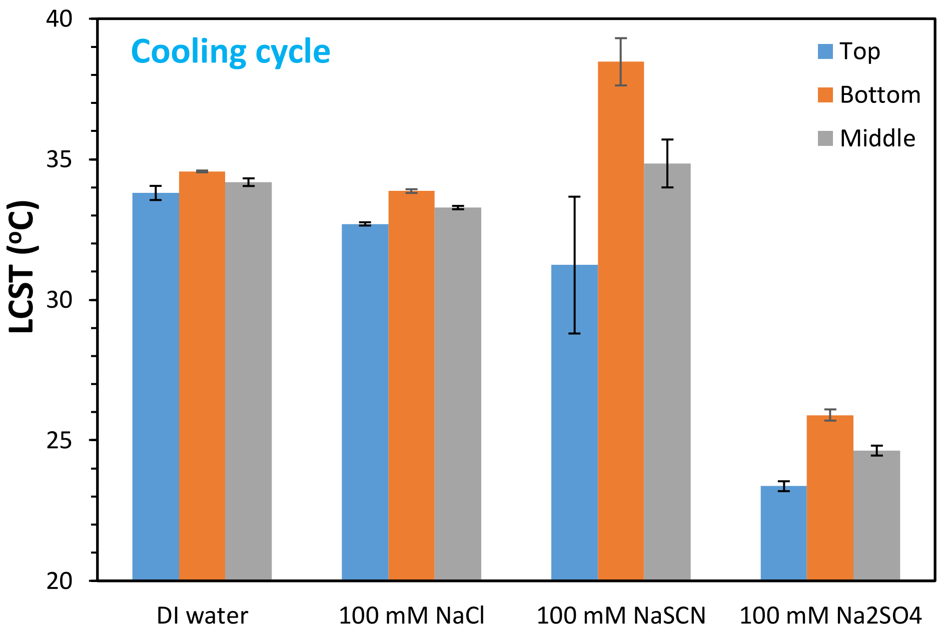

Figure 14: Graph of the top, bottom, and middle average LCST values during the cooling cycle for 0.5 wt% PVME solutions of DI water, NaCl, NaSCN, and Na_2SO_4 NaSCN has the highest LCST range (32 – 38°C) while Na_2SO_4 has the lowest LCST range (23 – 26°C).

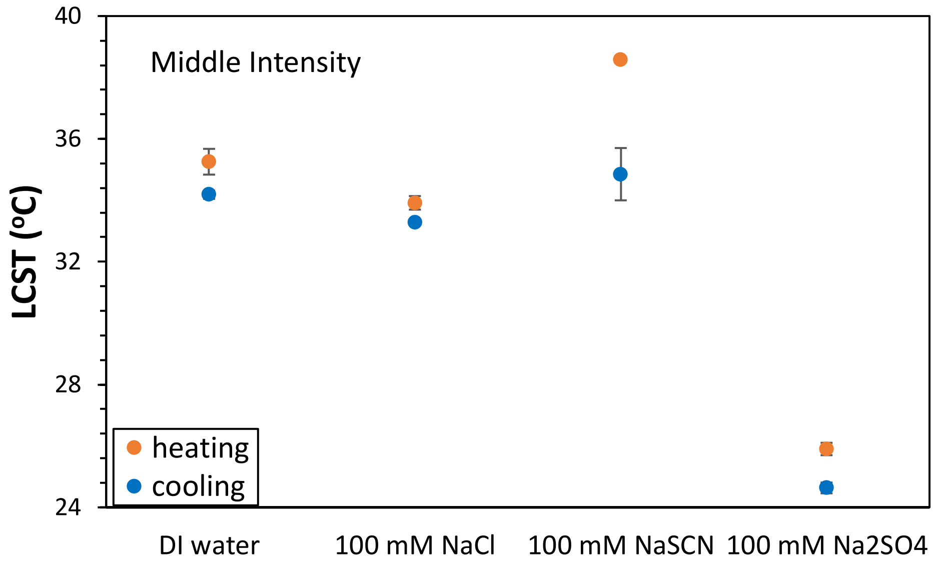

Figure 15: Graph comparing the middle average LCST values of the heating and cooling cycles for 0.5 wt% PVME solutions of DI water, NaCl, NaSCN, and Na_2SO_4. For all the tests, the heating LCST is higher than the cooling LCST. The error on the cooling NaSCN LCST is the highest.

Comparison and Statistical Analysis

To compare the ranges of LCST data for PVME, Sigma Aldrich pNIPAAM, and Polysciences pNIPAAM, an average of the top, bottom, and middle values was taken. A comparison of this rough overall LCST number is seen in **Figure 16** for the various ion solutions. Graphically, the three polymers have similar values, with $Na2_SO_4$ having the largest difference between PVME and Sigma Aldrich pNIPAAM. Worth noting is that PVME overall LCST falls right in between the LCST values of both Sigma Aldrich and Polysciences pNIPAAM.

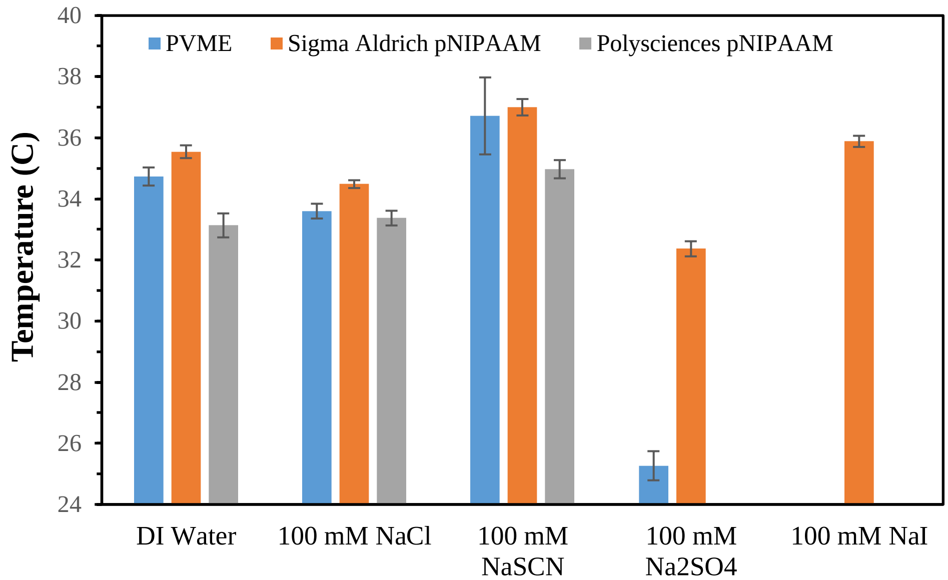

Figure 16: Graph comparing a singular averaged LCST value for 0.5 wt% solutions PVME, Sigma Aldrich pNIPAAM, and Polysciences pNIPAAM for DI water, NaCl, NaSCN, Na_2SO_4, and NaI. Sigma Aldrich pNIPAAM has higher averaged LCST values than PVME, and Polysciences has lower averaged LCST values than PVME. Error for each value was calculated by dividing the standard deviation by the square root of 6 (the number of measurements for both heating and cooling cycles).

An ANOVA was run for each of the three polymers for DI Water, NaCl, and NaSCN to determine whether the differences in the LCST range were significant. The three polymers each had six measurements; three making up the heating range, and three making up the cooling range. An alpha value of 0.05 was used in the analyses, indicating there's a 5% risk of an incorrect conclusion. An example of ANOVA results for DI water can be seen in **Table 1**, and the ANOVA summary table can be seen in **Table 2**. For DI water and NaCl, there was a significant difference between the three LCST ranges. For NaSCN, there was no significant difference.

Table 1: Table presents the values calculated from the ANOVA of the three polymers in DI water in Excel. The P-value is less than 0.05, and the F value is greater than the F-crit value. This indicates that at least two of the groups are significantly different.

Anova: Single Factor DI Water

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PVME	6	208.40	34.73	0.53
Sigma Aldrich pNIPAAM	6	213.27	35.54	0.26
Polysciences pNIPAAM	6	198.80	33.13	0.92

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18.06	2	9.03	15.82	0.000201891	3.68
Within Groups	8.56	15	0.57			
Total	26.63	17				

Table 2: Table presents the P-value, F, and F-crit values calculated from the ANOVA analyses of the three polymers in DI water, 100 mM NaCl, and 100 mM NaSCN in Excel. If the P-value is less than 0.05, and the F value is greater than the F-crit value; this indicates that at least two of the groups are significantly different. For DI water and 100 mM NaCl, there is a significant difference in the LCST ranges. For 100 mM NaSCN, there is not a significant difference.

ANOVA Summary Table

	P-value	**F**	**F crit**
DI Water	0.000201891	15.82	3.68
100 mM NaCl	0.004829836	7.77	3.68
100 mM NaSCN	0.16068292	2.07	3.68

In addition to the ANOVA tests, t Tests were run for PVME and Sigma Aldrich pNIPAAM for DI Water, NaCl, NaSCN, and Na_2SO_4 to determine if the mean of the LCST range were equal. This is because the Sigma Aldrich pNIPAAM was the primary manufacturer being examined against PVME, and the ANOVA test doesn't state which of the three polymers being compared are significantly different. An alpha value of 0.05 and a hypothesized mean difference of 0 was used in the analyses. An example of the t Test results for DI water can be

seen in **Table 3**, and the t Test summary table can be seen in **Table 4**. For DI water and NaSCN, there was no significant difference between the mean of LCST ranges. For NaCl and Na_2SO_4, there was a significant difference. The data associated with the ANOVA and t Test analyses and other results can be seen in **Appendix A**.

Table 3: Table presents the values calculated from the t Test of the PVME and Sigma Aldrich pNIPAAM in DI water in Excel. The two-tail P-value is greater than 0.05, and the t Stat value falls between the negative and positive value of the two-tail t Critical value. This indicates that there is no significant difference in the mean of the LCST ranges.

t-Test: Two-Sample Assuming Unequal Variances, DI Water

	PVME	*ma Aldrich pNIPAAM*
Mean	34.73	35.54
Variance	0.53	0.26
Observations	6	6
Hypothesized Mean Difference	0	
df	9	
t Stat	-2.24	
P(T<=t) one-tail	0.026043936	
t Critical one-tail	1.83	
P(T<=t) two-tail	0.052087872	
t Critical two-tail	2.26	

Table 4: Table presents the P-value (two tail), t Stat, and t critical (two tail) values calculated from the t Test of the PVME and Sigma Aldrich pNIPAAM in DI water, 100 mM NaCl, 100 mM NaSCN, and 100 mM Na_2SO_4 in Excel. If the two-tail P-value is greater than 0.05, and the t Stat value falls between the negative and positive value of the two-tail t Critical value; this indicates that there is no significant difference in the mean of the LCST ranges. For DI water and 100 mM NaSCN, there is no significant difference in the mean. For 100 mM NaCl and 100 mM Na_2SO_4, there is a significant difference.

t Test Summary Table			
	P-value (two tail)	**t Stat**	**t critical (two tail)**
DI Water	0.052087872	-2.24	2.26
100 mM NaCl	0.01214629	-3.23	2.31
100 mM NaSCN	0.834557616	-0.22	2.57
100 mM Na2SO4	1.01985E-06	-13.22	2.31

Discussion

The goal of the performed experiments mentioned in this study was to compare how the Hofmeister ions affect the LCST of PVME and pNIPAAM in order to determine if PVME can replace pNIPAAM for various thermoresponsive applications. Reported values of pNIPAAM LCST range between 30-35ºC, and 35ºC for PVME.[5,13] The ranges found in this study for pNIPAAM were 34.7-36.1ºC (Sigma Aldrich) and 31.7-34.4 ºC (Polysciences) in the blank test with DI water. The range for PVME was found to be 33.8-35.8ºC. These ranges align with the reported values and verify that the experimental method used was suitably accurate. However, the Sigma Aldrich pNIPAAM and Polysciences pNIPAAM differ in LCST range, and can be seen in the comparison graph of **Figure 16**. Sigma Aldrich pNIPAAM has a higher range than PVME, while Polysciences pNIPAAM has a lower range than PVME. This indicates that potentially the manufacturer of pNIPAAM can have an effect on its LCST range and not all pNIPAAM will respond the same.

The hypothesis was that each of the three polymers will follow the same trend in LCST shifts based on the various Hofmeister ions. However, the ANOVA analyses confirm that in DI water and NaCl, there is a significant difference between the three polymers tested. In NaSCN, there is no significant difference. These results mean that in two out of the three cases, at least two of the polymers were not comparable. In order to further investigate these differences, t Tests on the Sigma Aldrich pNIPAAM and PVME were conducted as the Sigma Aldrich pNIPAAM was the polymer initially intended to be compared to PVME. In DI water and NaSCN, the two polymers' LCST means were not significantly different; but in NaCl and Na_2SO_4, their means were significantly different. These results indicate that in two of the four cases, PVME and pNIPAAM were not comparable.

Ultimately, based on these statistical results, a conclusion cannot be drawn if PVME could replace pNIPAAM in thermoresponsive applications. It is possible that the manufacturer of pNIPAAM could affect the LCST range of the polymer. More tests should be run more anions in the Hofmeister series and various manufacturers of pNIPAAM. This would allow for a larger data pool to be used for a more accurate statistical analysis to determine the potential of PVME replacing pNIPAAM as well as eliminateing some of the error in testing.

Conclusion

Through the experiments conducted for this study, temperature and laser intensity data were obtained in order to determine the LCST ranges of PVME, Sigma Aldrich pNIPAAM, and Polysciences pNIPAAM. The range of PVME fell in between the ranges of Sigma Aldrich pNIPAAM and Polysciences pNIPAAM. ANOVA tests were performed in order to determine any significant differences between the LCST data of the three polymers, and t Tests were performed to determine any significant differences in the means of the LCST ranges of PVME and Sigma Aldrich pNIPAAM. These statistical analyses had no clear indication of the differences between PVME and the two types of pNIPAAM as in some cases they were comparable and in others they weren't. It is recommended that further testing and analysis should be conducted with other anions in the Hofmeister series and potentially with various manufacturers of pNIPAAM to determine if PVME is a viable replacement for pNIPAAM in thermoresponsive applications.

Appendix A

The graphs and tables in this Appendix were used to determine the LCST ranges for the two pNIPAAM polymers in this study, create the comparison graphs in the Data and Results section of this report, and complete the ANOVA and t Test statistical analyses.

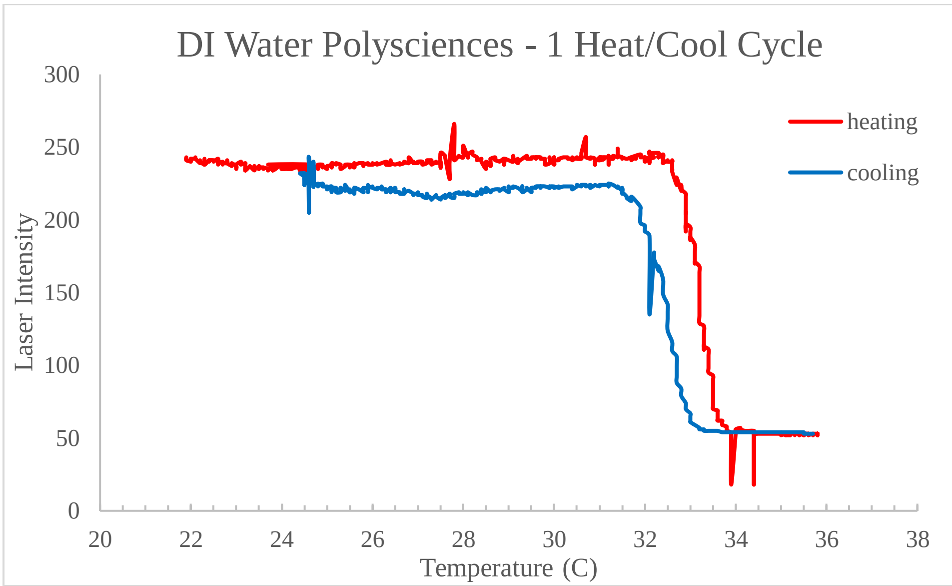

Figure A1: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in DI water. Values for the LCST range for both heating and cooling can be seen in **Table A1**.

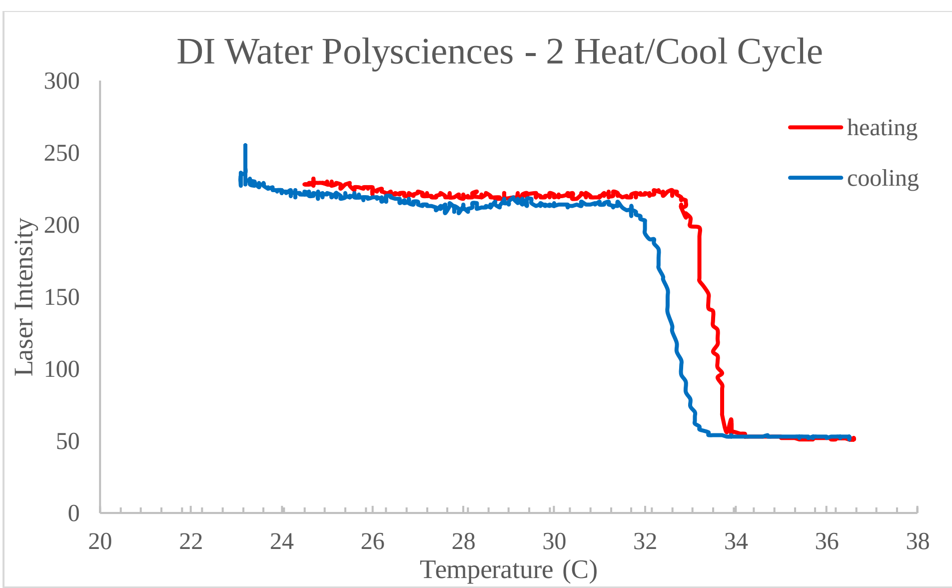

Figure A2: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in DI water. Values for the LCST range for both heating and cooling can be seen in **Table A1**.

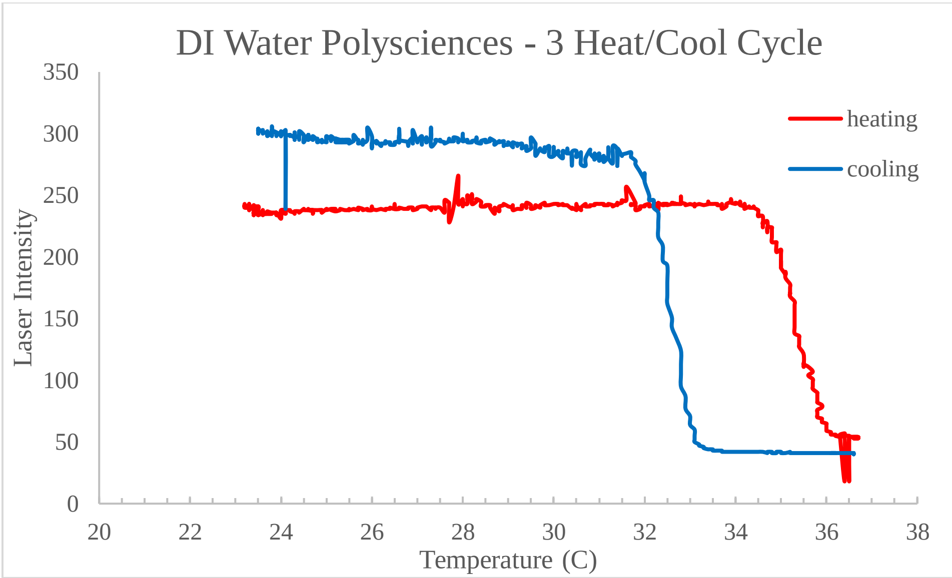

Figure A3: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in DI water. Values for the LCST range for both heating and cooling can be seen in **Table A1**.

Table A1: Lists the top, bottom, and middle LCST values read from **Figure A1**, **Figure A2**, and **Figure A3**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Polysciences pNIPAAM in DI water.

DI Water Polysciences pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	32.6	32.9	33.2
2	32.8	33.9	33.5
3	34.5	36	35.3
Average	33.3	34.3	34.0
Stdev	1.04	1.58	1.14
Error	0.60	0.91	0.66
Cooling Cycle	Top	Bottom	Middle
1	31.6	33	32.4
2	31.7	33.1	32.6
3	31.7	33.1	32.5
Average	31.7	33.1	32.5
Stdev	0.06	0.06	0.10
Error	0.03	0.03	0.06

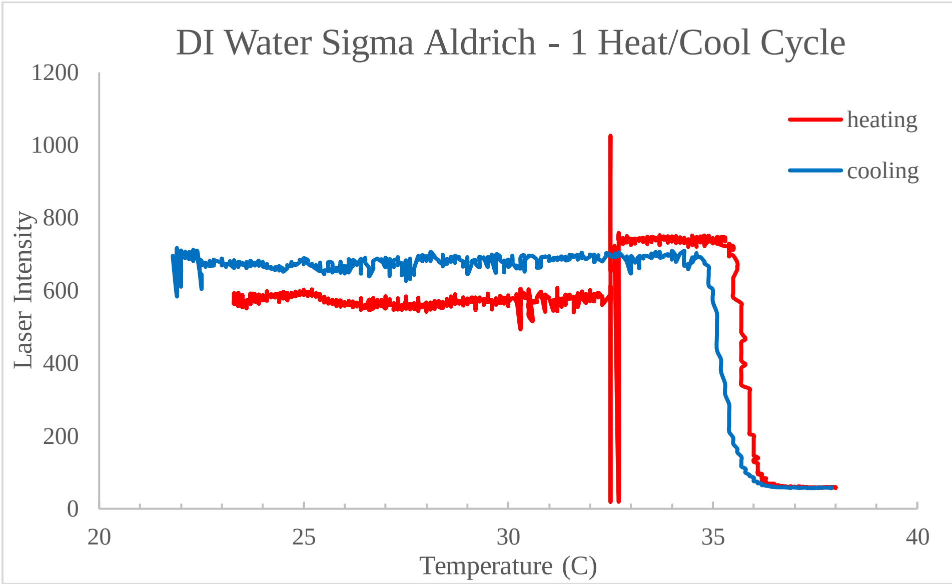

Figure A4: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in DI water. Values for the LCST range for both heating and cooling can be seen in **Table A2**. The large decreases in the heating cycle are associated with checking the laser to ensure the reading was working properly, and lowering the laser intensity for a few seconds.

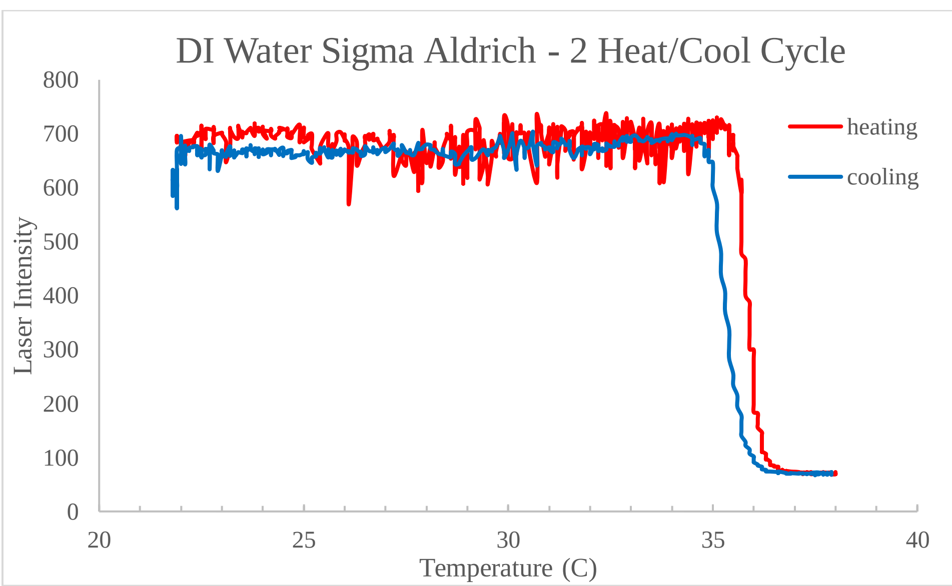

Figure A5: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in DI water. Values for the LCST range for both heating and cooling can be seen in **Table A2**.

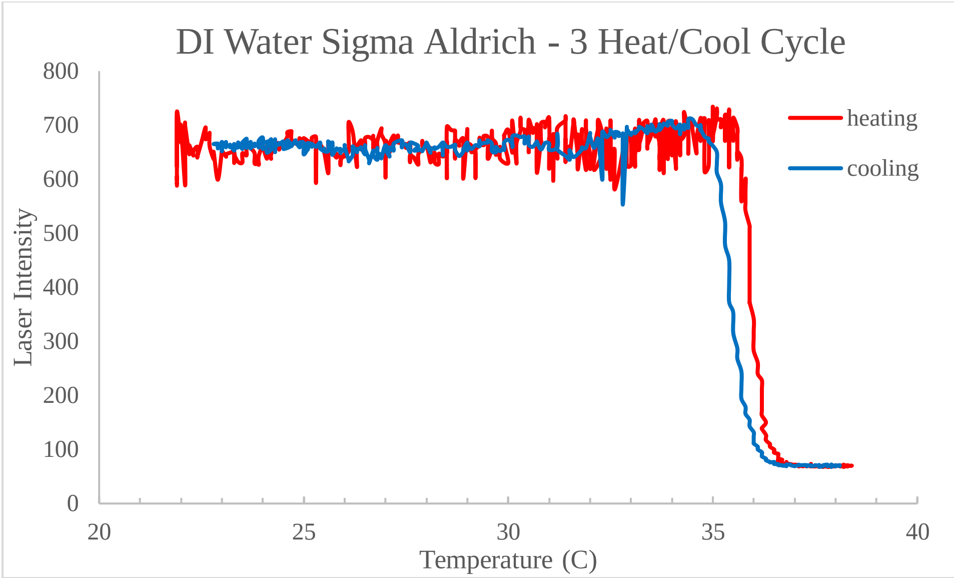

Figure A6: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in DI water. Values for the LCST range for both heating and cooling can be seen in **Table A2**.

Table A2: Lists the top, bottom, and middle LCST values read from **Figure A4**, **Figure A5**, and **Figure A6**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Sigma Aldrich pNIPAAM in DI water.

DI Water Sigma Aldrich pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	35.3	36	35.7
2	35.4	36.1	35.8
3	35.6	36.2	35.9
Average	35.4	36.1	35.8
Stdev	0.15	0.10	0.10
Error	0.09	0.06	0.06
Cooling Cycle	Top	Bottom	Middle
1	34.7	35.7	35.2
2	34.7	36	35.3
3	34.7	36.1	35.4
Average	34.7	35.9	35.3
Stdev	0	0.21	0.10
Error	0	0.12	0.06

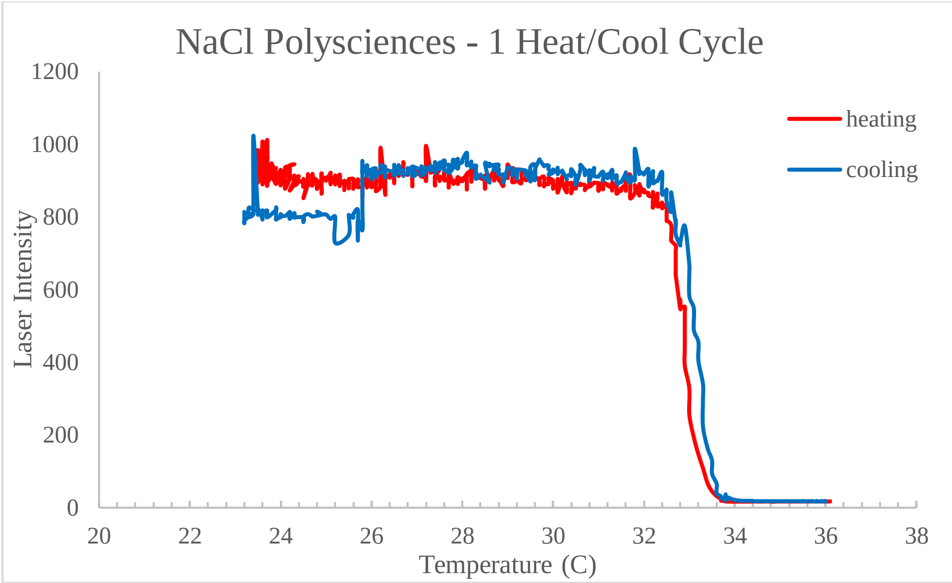

Figure A7: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in 100 mM NaCl. Values for the LCST range for both heating and cooling can be seen in **Table A3**.

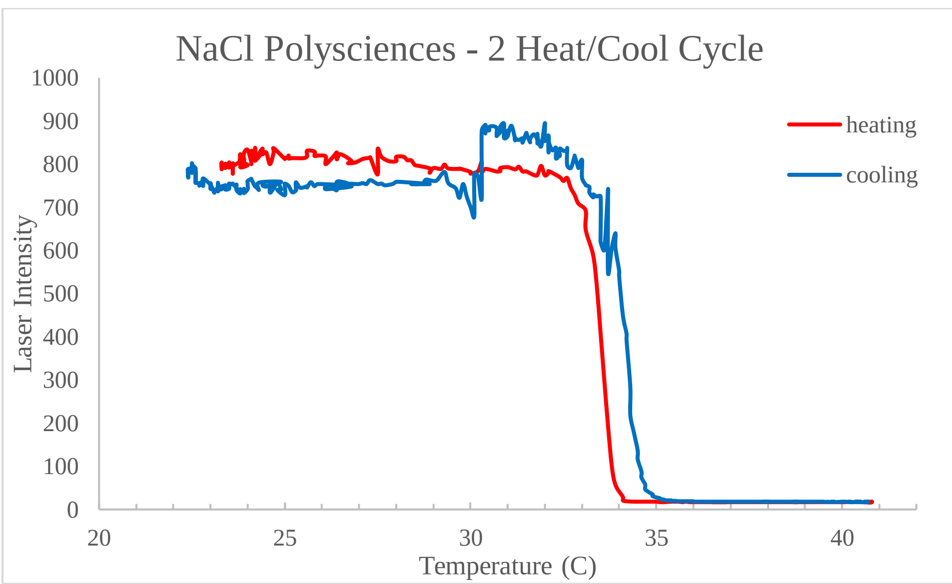

Figure A8: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in 100 mM NaCl. Values for the LCST range for both heating and cooling can be seen in **Table A3**.

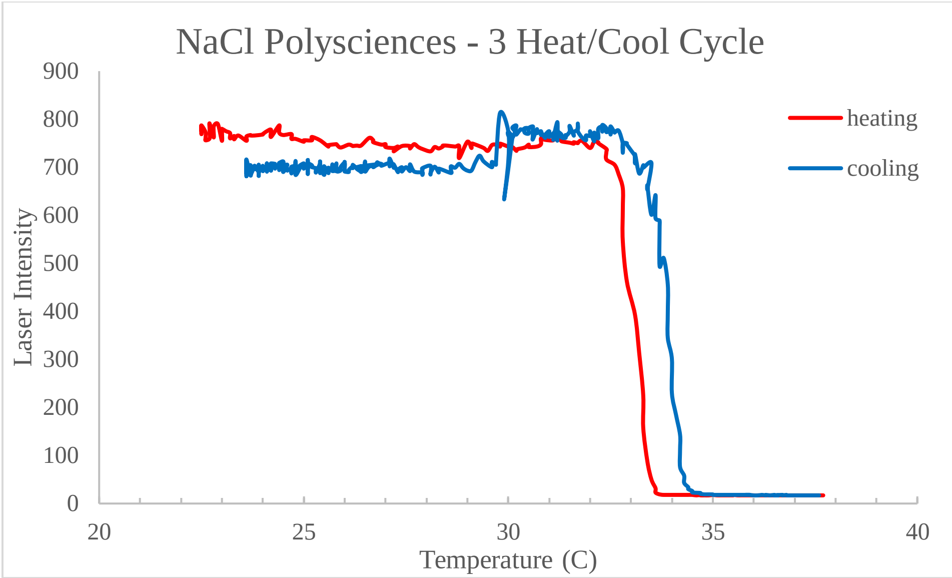

Figure A9: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in 100 mM NaCl. Values for the LCST range for both heating and cooling can be seen in **Table A3**.

Table A3: Lists the top, bottom, and middle LCST values read from **Figure A7**, **Figure A8**, and **Figure A9**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Polysciences pNIPAAM in 100 mM NaCl.

NaCl Polysciences pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	32.1	33.6	33.1
2	33	33.9	33.5
3	32.8	33.6	33.1
Average	32.6	33.7	33.2
Stdev	0.47	0.17	0.23
Error	0.27	0.10	0.13
Cooling Cycle	Top	Bottom	Middle
1	32.3	33.6	33.1
2	33	34.7	34.1
3	33.1	34.2	33.9
Average	32.8	34.2	33.7
Stdev	0.44	0.55	0.53
Error	0.25	0.32	0.31

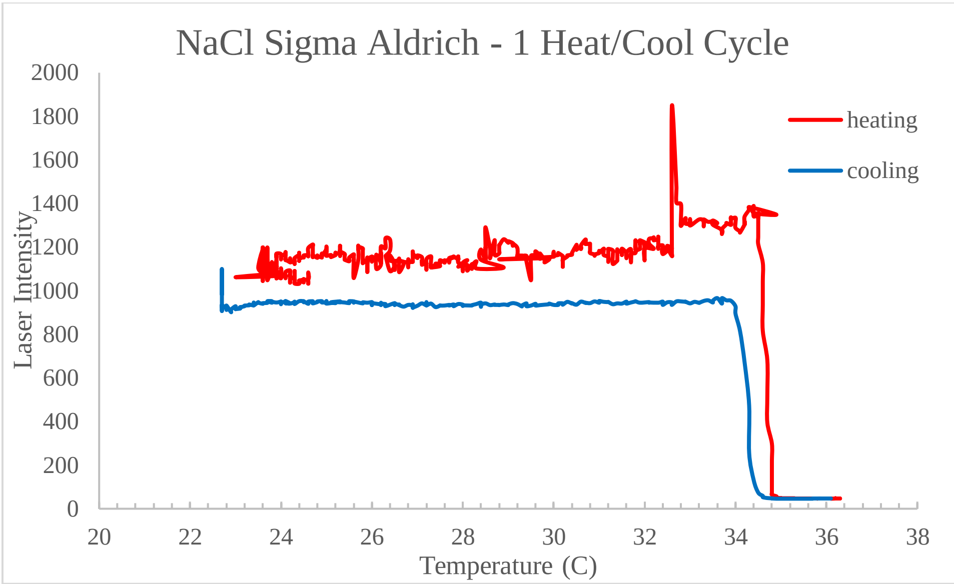

Figure A10: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaCl. Values for the LCST range for both heating and cooling can be seen in **Table A4**.

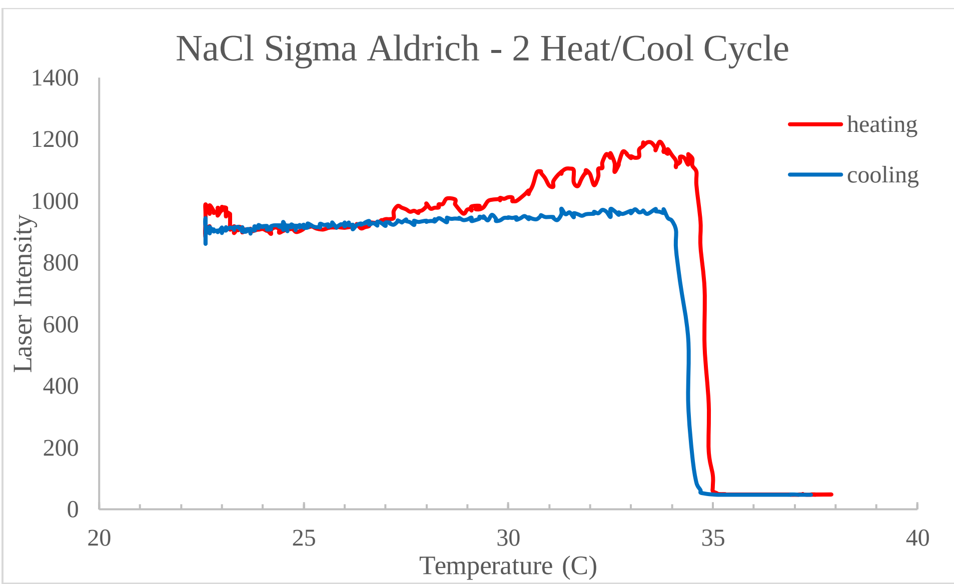

Figure A11: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaCl. Values for the LCST range for both heating and cooling can be seen in **Table A4**.

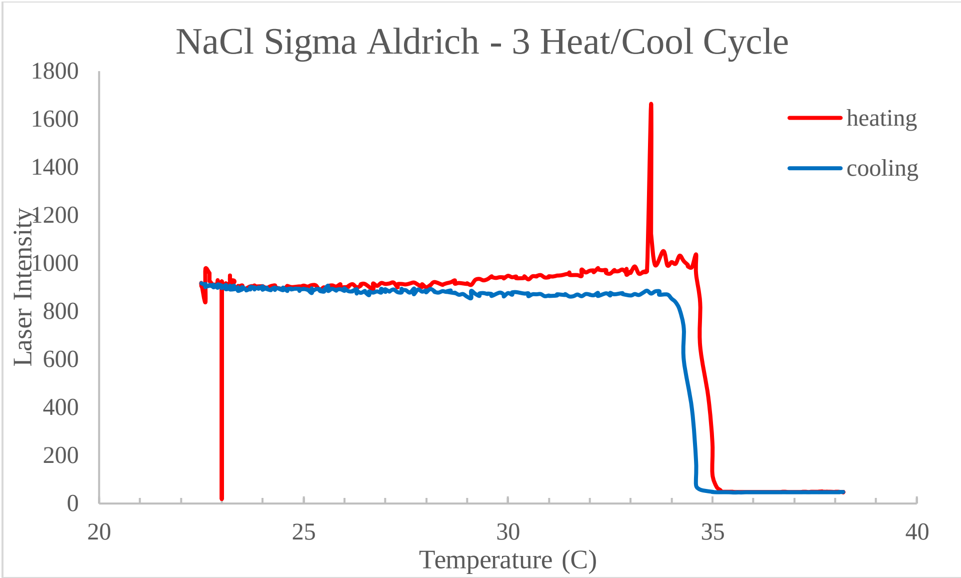

Figure A12: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaCl. Values for the LCST range for both heating and cooling can be seen in **Table A4**. The large decreases in the heating cycle are associated with checking the laser to ensure the reading was working properly, and lowering the laser intensity for a few seconds.

Table A4: Lists the top, bottom, and middle LCST values read from **Figure A10**, **Figure A11**, and **Figure A12**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaCl.

NaCl Sigma Aldrich pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	34.5	34.8	34.7
2	34.5	35	34.8
3	34.5	35	34.3
Average	34.5	34.9	34.6
Stdev	0	0.12	0.26
Error	0	0.07	0.15
Cooling Cycle	Top	Bottom	Middle
1	34	34.5	34.2
2	33.9	34.6	34.4
3	34.1	34.6	34.3
Average	34.0	34.6	34.3
Stdev	0.10	0.06	0.10
Error	0.06	0.03	0.06

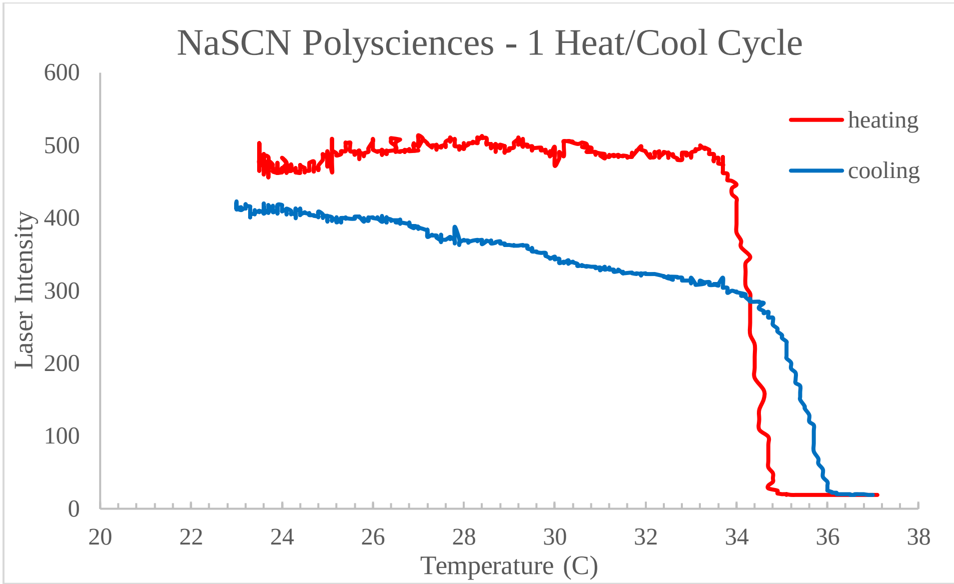

Figure A13: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in 100 mM NaSCN. Values for the LCST range for both heating and cooling can be seen in **Table A5**.

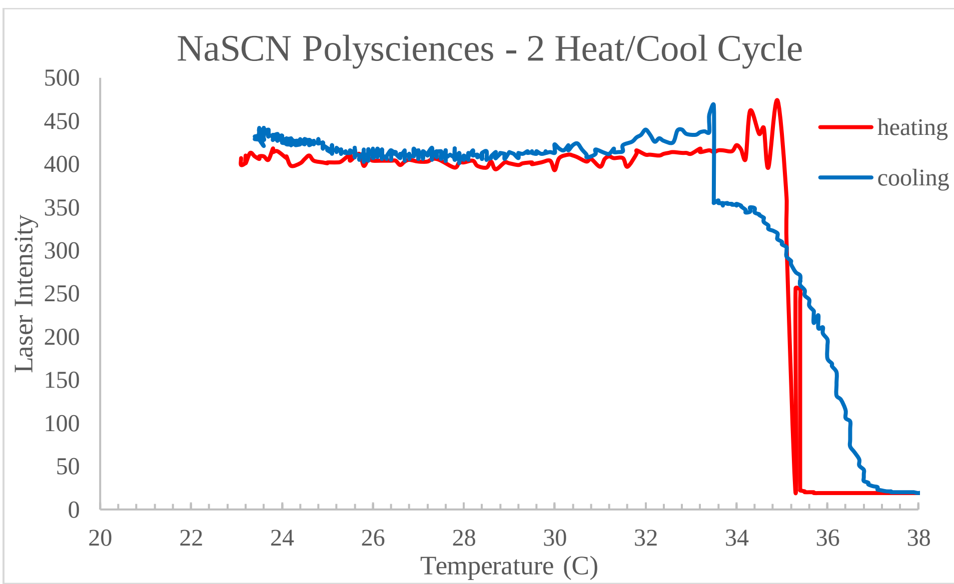

Figure A14: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in 100 mM NaSCN. Values for the LCST range for both heating and cooling can be seen in **Table A5**. The large decreases in the heating cycle are associated with checking the laser to ensure the reading was working properly, and lowering the laser intensity for a few seconds.

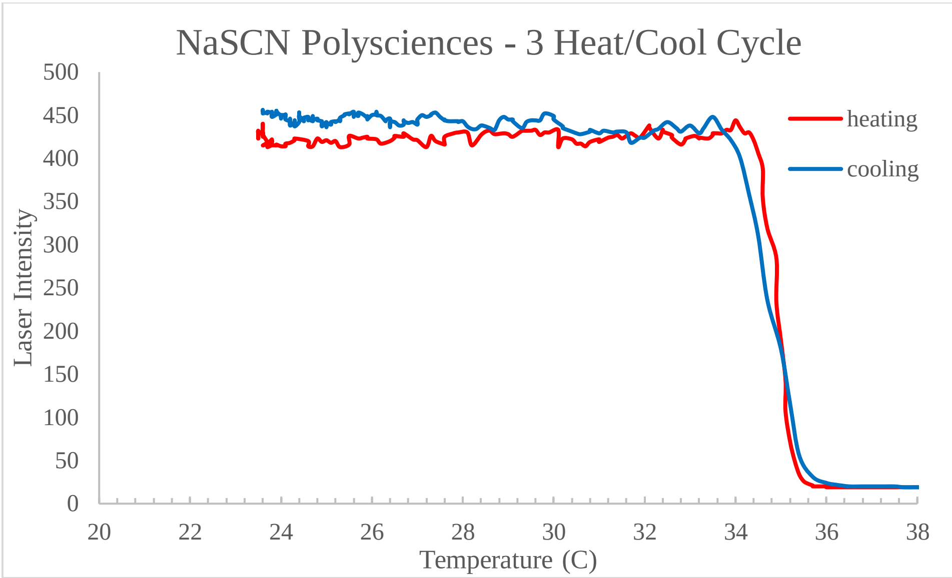

Figure A15: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Polysciences pNIPAAM in 100 mM NaSCN. Values for the LCST range for both heating and cooling can be seen in **Table A5**.

Table A5: Lists the top, bottom, and middle LCST values read from **Figure A13**, **Figure A14**, and **Figure A15**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Polysciences pNIPAAM in 100 mM NaSCN.

NaSCN Polysciences pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	33.6	34.7	34.5
2	34.9	35.3	35.3
3	34.3	35.4	34.9
Average	34.3	35.1	34.9
Stdev	0.65	0.38	0.40
Error	0.38	0.22	0.23
Cooling Cycle	Top	Bottom	Middle
1	34.7	36	35.4
2	34	36.7	35.9
3	33.7	35.7	34.5
Average	34.1	36.1	35.3
Stdev	0.51	0.51	0.71
Error	0.30	0.30	0.41

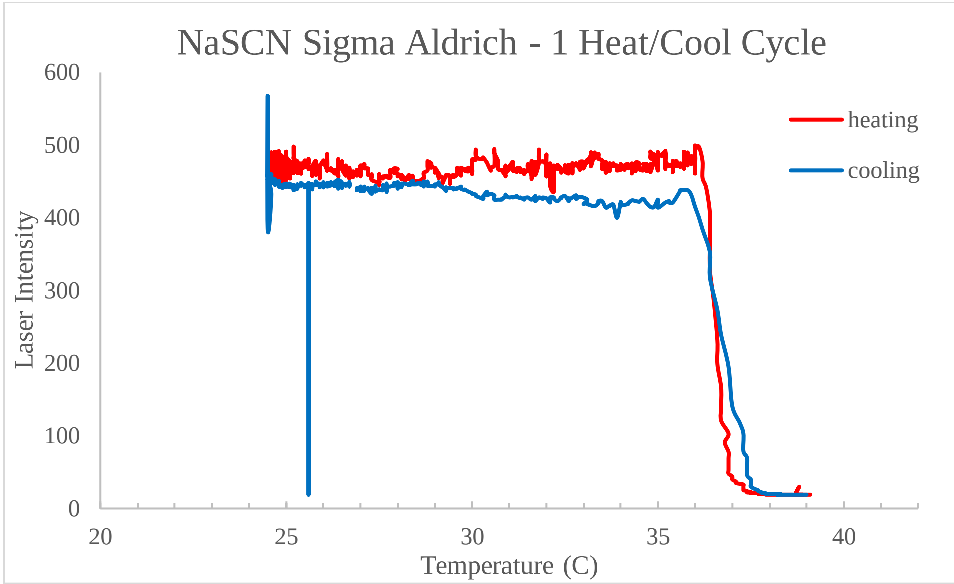

Figure A16: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaSCN. Values for the LCST range for both heating and cooling can be seen in **Table A6**. The large decrease in the cooling cycle is associated with checking the laser to ensure the reading was working properly, and lowering the laser intensity for a few seconds.

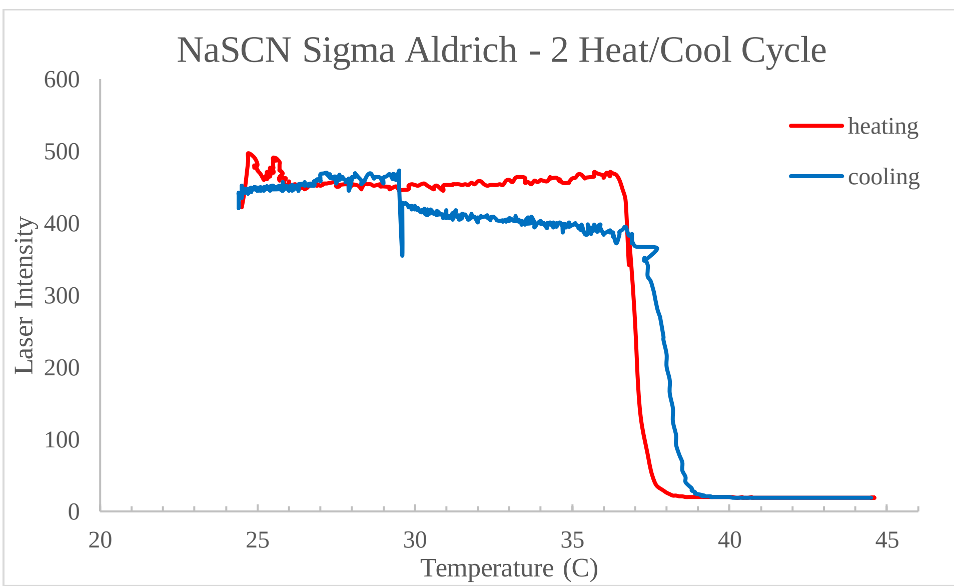

Figure A17: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaSCN. Values for the LCST range for both heating and cooling can be seen in **Table A6**.

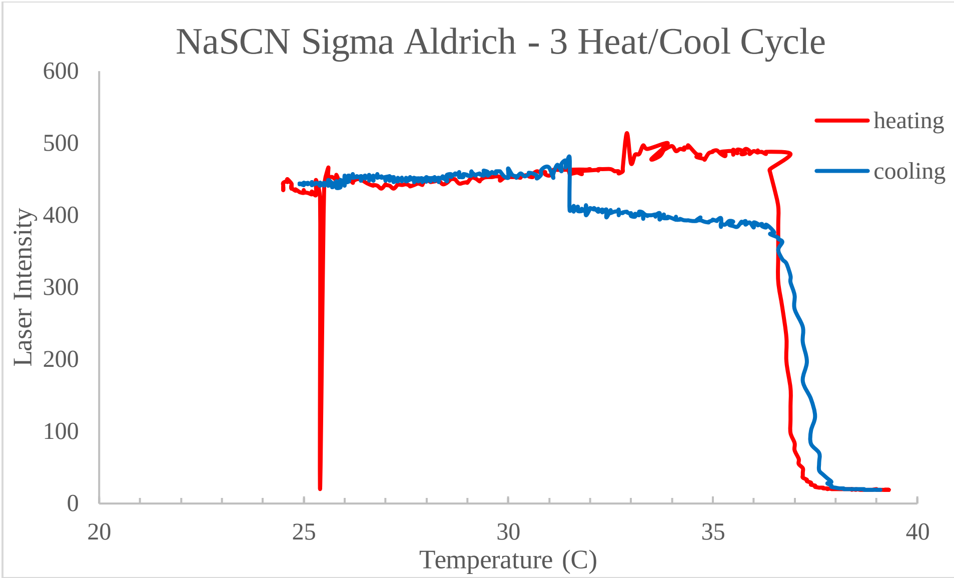

Figure A18: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaSCN. Values for the LCST range for both heating and cooling can be seen in **Table A6**. The large decrease in the heating cycle is associated with checking the laser to ensure the reading was working properly, and lowering the laser intensity for a few seconds.

Table A6: Lists the top, bottom, and middle LCST values read from **Figure A16**, **Figure A17**, and **Figure A18**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Polysciences pNIPAAM in 100 mM NaSCN.

NaSCN Sigma Aldrich pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	36.1	37	36.6
2	36.4	37.6	37
3	36.3	37.2	36.7
Average	36.3	37.3	36.8
Stdev	0.15	0.31	0.21
Error	0.09	0.18	0.12
Cooling Cycle	Top	Bottom	Middle
1	35.9	37.4	36.6
2	36.9	38.9	38
3	36.4	37.8	37.2
Average	36.4	38.0	37.3
Stdev	0.50	0.78	0.70
Error	0.29	0.45	0.41

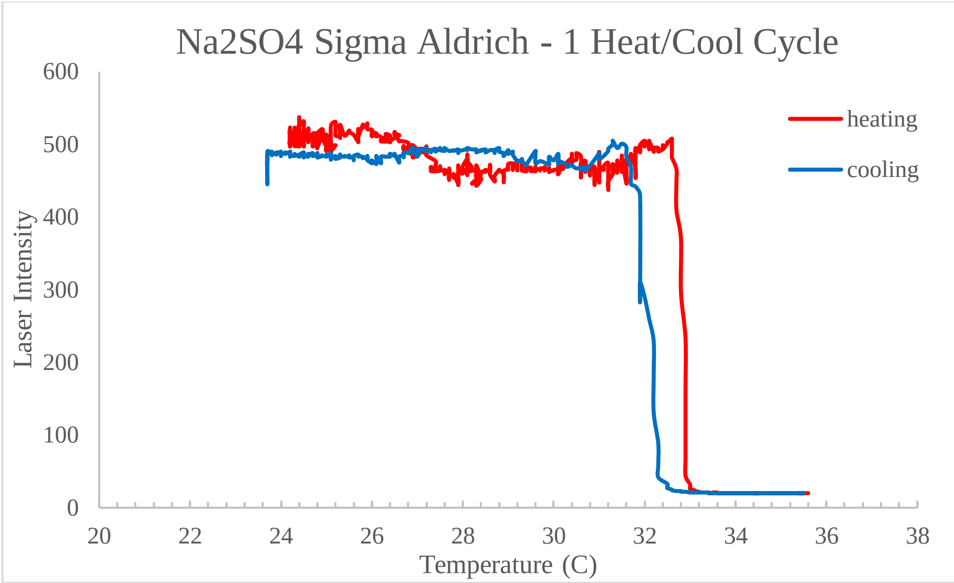

Figure A19: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM Na_2SO_4. Values for the LCST range for both heating and cooling can be seen in **Table A7**.

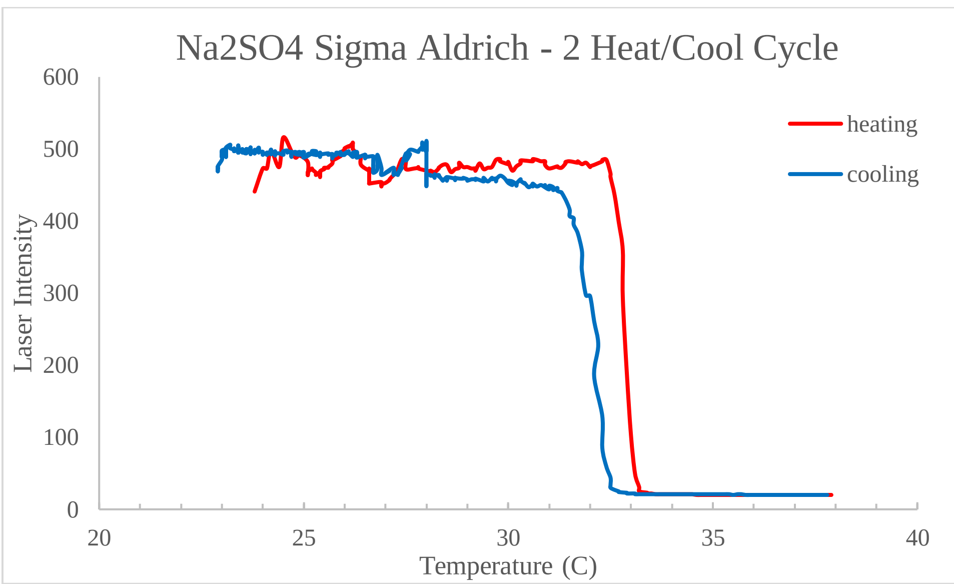

Figure A20: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM Na_2SO_4. Values for the LCST range for both heating and cooling can be seen in **Table A7**.

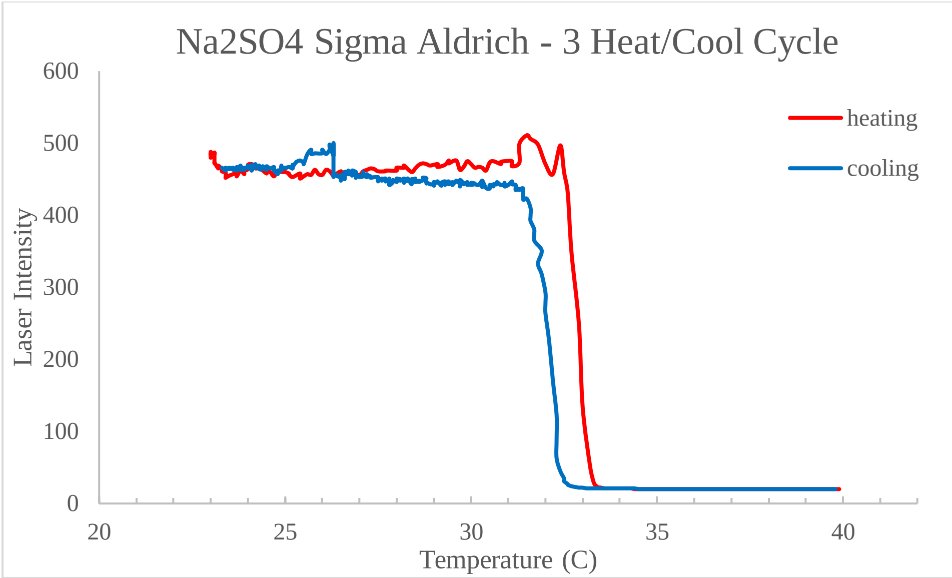

Figure A21: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM Na_2SO_4. Values for the LCST range for both heating and cooling can be seen in **Table A7**.

Table A7: Lists the top, bottom, and middle LCST values read from **Figure A19**, **Figure A20**, and **Figure A21**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Polysciences pNIPAAM in 100 mM Na_2SO_4.

Na2SO4 Sigma Aldrich pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	32.5	32.9	32.9
2	32.3	33.1	32.8
3	32.4	33.2	32.9
Average	32.4	33.1	32.9
Stdev	0.10	0.15	0.06
Error	0.06	0.09	0.03
Cooling Cycle	Top	Bottom	Middle
1	31.6	32.3	32.1
2	31.3	32.5	32.1
3	31.2	32.5	32.0
Average	31.4	32.4	32.1
Stdev	0.21	0.12	0.06
Error	0.12	0.07	0.03

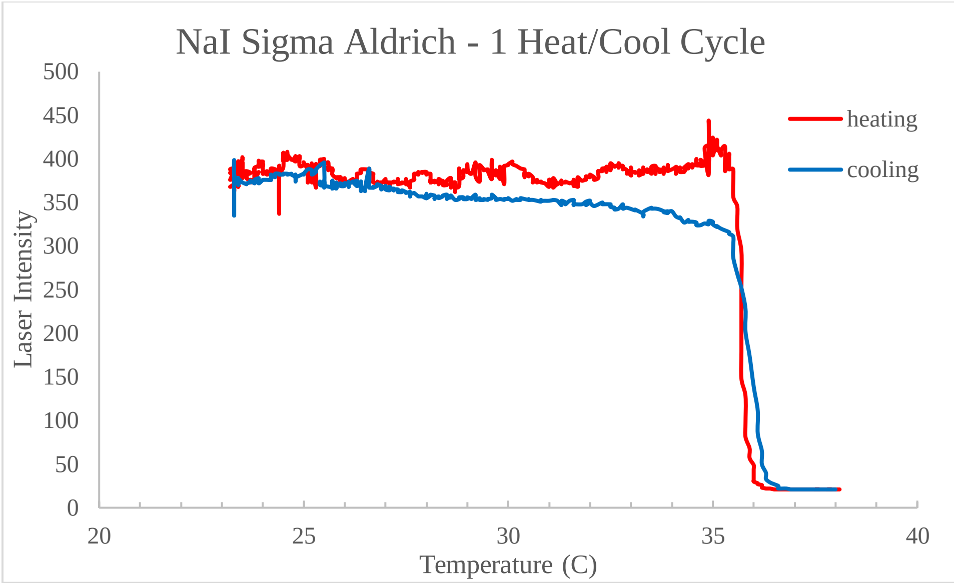

Figure A22: Depicts the relationship between temperature and laser intensity for the first heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaI. Values for the LCST range for both heating and cooling can be seen in **Table A8**.

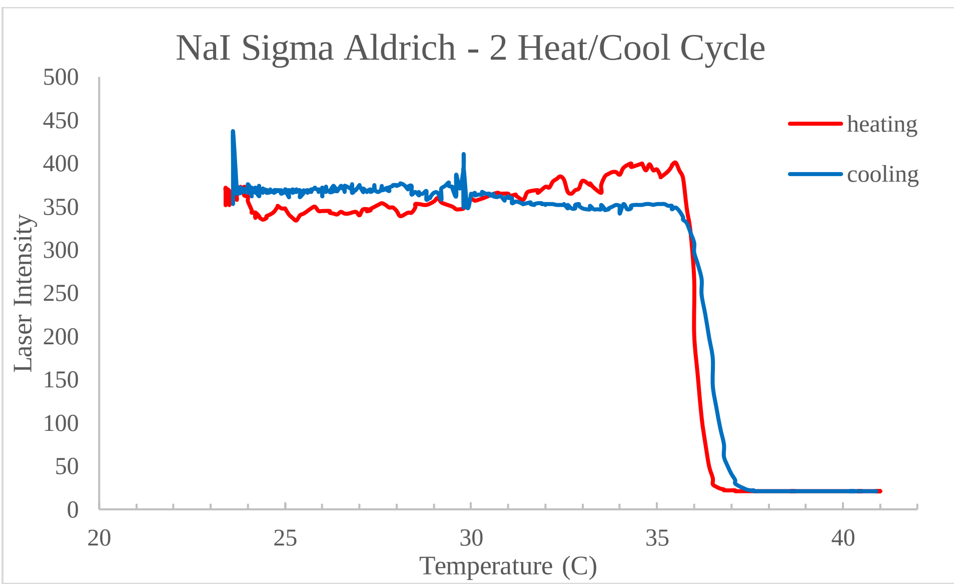

Figure A23: Depicts the relationship between temperature and laser intensity for the second heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaI. Values for the LCST range for both heating and cooling can be seen in **Table A8**.

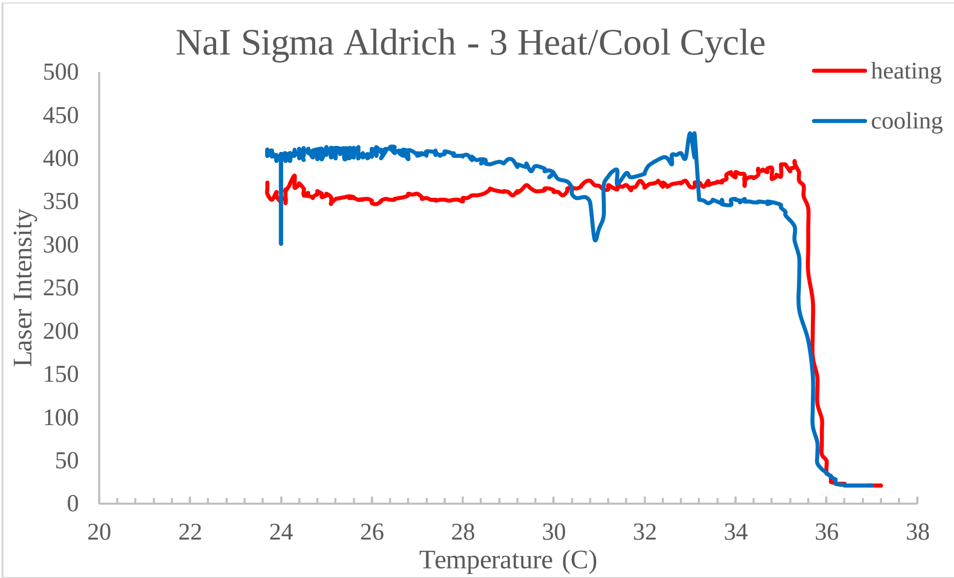

Figure A24: Depicts the relationship between temperature and laser intensity for the third heating and cooling cycle of 0.5 wt% Sigma Aldrich pNIPAAM in 100 mM NaI. Values for the LCST range for both heating and cooling can be seen in **Table A8**.

Table A8: Lists the top, bottom, and middle LCST values read from **Figure A22**, **Figure A23**, and **Figure A24**. Also presents the average, standard deviation, and error associated for the heating and cooling cycles of 0.5 wt% Polysciences pNIPAAM in 100 mM NaI.

NaI Sigma Aldrich pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
1	35.4	36.3	35.7
2	35.6	36.5	36.3
3	35.3	36	35.4
Average	35.4	36.3	35.8
Stdev	0.15	0.25	0.46
Error	0.09	0.15	0.26
Cooling Cycle	Top	Bottom	Middle
1	35.4	36.3	35.8
2	35.6	37.1	36.4
3	35.1	36.1	35.6
Average	35.4	36.5	35.9
Stdev	0.25	0.53	0.42
Error	0.15	0.31	0.24

Table A9: Lists the average top, bottom, and middle LCST values for PVME, Sigma Aldrich pNIPAAM, and Polysciences pNIPAAM that were used to make the bar graphs for each of the polymers in the Data and Results section of this report as well as complete statistical analyses. The standard deviation and error were reported in the previous tables in Appendix A.

PVME LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
DI Water	34.7	35.8	35.3
100 mM NaCl	33.4	34.4	33.9
100 mM NaSCN	38.1	39	38.6
100 mM Na2SO4	25.1	26.7	25.9
Cooling Cycle	Top	Bottom	Middle
DI Water	33.8	34.6	34.2
100 mM NaCl	32.7	33.9	33.3
100 mM NaSCN	31.2	38.5	34.9
100 mM Na2SO4	23.4	25.9	24.6
Sigma Aldrich pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
DI Water	35.4	36.1	35.8
100 mM NaCl	34.5	34.9	34.6
100 mM NaSCN	36.3	37.3	36.8
100 mM Na2SO4	32.4	33.1	32.9
100 mM NaI	35.4	36.3	35.8
Cooling Cycle	Top	Bottom	Middle
DI Water	34.7	35.9	35.3
100 mM NaCl	34.0	34.6	34.3
100 mM NaSCN	36.4	38.0	37.3
100 mM Na2SO4	31.4	32.4	32.1
100 mM NaI	35.4	36.5	35.9
Polysciences pNIPAAM LCST Data (C)			
Heating Cycle	Top	Bottom	Middle
DI Water	33.3	34.3	34.0
100 mM NaCl	32.6	33.7	33.2
100 mM NaSCN	34.3	35.1	34.9
Cooling Cycle	Top	Bottom	Middle
DI Water	31.7	33.1	32.5
100 mM NaCl	32.8	34.2	33.7
100 mM NaSCN	34.1	36.1	35.3

Table A10: Lists the overall average LCST and error values for PVME, Sigma Aldrich pNIPAAM, and Polysciences pNIPAAM that were used to make **Figure 17**.

Average LCST Values (of Heating and Cooling) (C)			
	PVME	Sigma Aldrich pNIPAAM	Polysciences pNIPAAM
DI Water	34.7	35.5	33.1
100 mM NaCl	33.6	34.5	33.4
100 mM NaSCN	36.7	37.0	35.0
100 mM Na2SO4	25.3	32.4	
100 mM NaI		35.9	
Error Bars			
DI Water	0.3	0.2	0.4
100 mM NaCl	0.2	0.1	0.2
100 mM NaSCN	1.3	0.3	0.3
100 mM Na2SO4	0.5	0.2	
100 mM NaI		0.2	

Table A11: Table presents the values calculated from the ANOVA of the three polymers in 100 mM NaCl in Excel. The P-value is less than 0.05, and the F value is greater than the F-crit value. This indicates that at least two of the groups are significantly different.

Anova: Single Factor 100 mM NaCl

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PVME	6	201.60	33.60	0.35
Sigma Aldrich pNIPAAM	6	206.90	34.48	0.10
Polysciences pNIPAAM	6	200.23	33.37	0.35

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.13	2	2.07	7.77	0.004829836	3.68
Within Groups	3.99	15	0.27			
Total	8.12	17				

Table A12: Table presents the values calculated from the ANOVA of the three polymers in DI water in Excel. The P-value is greater than 0.05, and the F value is less than the F-crit value. This indicates that there is no significant difference in the LCST ranges.

Anova: Single Factor 100 mM NaSCN

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
PVME	6	220.30	36.72	9.52
Sigma Aldrich pNIPAAM	6	222.00	37.00	0.43
Polysciences pNIPAAM	6	209.83	34.97	0.53

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.47	2	7.24	2.07	0.16068292	3.68
Within Groups	52.42	15	3.49			
Total	66.89	17				

Table A13: Table presents the values calculated from the t Test of the PVME and Sigma Aldrich pNIPAAM in 100 mM NaCl in Excel. The two-tail P-value is less than 0.05, and the t Stat value is less than the negative value of the two-tail t Critical value. This indicates that there is a significant difference in the mean of the LCST ranges.

t-Test: Two-Sample Assuming Unequal Variances, 100 mM NaCl

	PVME	*ma Aldrich pNIPAAM*
Mean	33.6	34.48
Variance	0.35	0.10
Observations	6	6
Hypothesized Mean Difference	0	
df	8	
t Stat	-3.23	
P(T<=t) one-tail	0.006073145	
t Critical one-tail	1.86	
P(T<=t) two-tail	0.01214629	
t Critical two-tail	2.31	

Table A14: Table presents the values calculated from the t Test of the PVME and Sigma Aldrich pNIPAAM in 100 mM NaSCN in Excel. The two-tail P-value is greater than 0.05, and the t Stat value falls between the negative and positive value of the two-tail t Critical value. This indicates that there is no significant difference in the mean of the LCST ranges.

t-Test: Two-Sample Assuming Unequal Variances, 100 mM NaSCN

	PVME	*ma Aldrich pNIPAAM*
Mean	36.72	37
Variance	9.52	0.43
Observations	6	6
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.22	
P(T<=t) one-tail	0.417278808	
t Critical one-tail	2.02	
P(T<=t) two-tail	0.834557616	
t Critical two-tail	2.57	

Table A15: Table presents the values calculated from the t Test of the PVME and Sigma Aldrich pNIPAAM in 100 mM Na_2SO_4 in Excel. The two-tail P-value is less than 0.05, and the t Stat value is less than the negative value of the two-tail t Critical value. This indicates that there is no significant difference in the mean of the LCST ranges.

t-Test: Two-Sample Assuming Unequal Variances, 100 mM Na2SO4

	PVME	*ma Aldrich pNIPAAM*
Mean	25.27	32.37
Variance	1.36	0.37
Observations	6	6
Hypothesized Mean Difference	0	
df	8	
t Stat	-13.22	
P(T<=t) one-tail	5.09927E-07	
t Critical one-tail	1.86	
P(T<=t) two-tail	1.01985E-06	
t Critical two-tail	2.31	

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