Penn State RET in Interdisciplinary Materials

Teacher's Preparatory Guide

Exploring the Hofmeister Series with PNIPAM

Purpose
This lab is designed to help students understand how the concentration of specific anions can have an effect on the temperature at which polymers, polypeptides, and proteins will precipitate out of solution. The ordering of these ions based on their ability to precipitate proteins is known as the Hofmeister series. Students will record the lower critical solution temperatures (LCST) for PNIPAM mixed with various concentrations of three of the salts within this series. The transition temperatures will be graphed and the pattern will be analyzed to determine the overall effect that concentration has on the LCST.

Objectives

- Students within a class will collaborate in order to collect data for the LCST of three salts mixed with PNIPAM at various concentrations.
- Students will graph the LCST measurements collected by each group and analyze the results in order to determine patterns within the data.
- Students will apply problem solving skills in order to determine the concentration necessary for PNIPAM to undergo a phase transition at a specific temperature.

Time required Three 40 minute class periods

Level High school chemistry

National Science Education Standards Grades 9-12
Content Standard A. Science As Inquiry
Abilities necessary to do scientific inquiry

- Identify questions and concepts that guide scientific investigations (form a hypothesis).
- Design and conduct scientific investigations based on knowledge of major concepts, equipment, and safety precautions. Students may need to clarify parts of the experiment using evidence and logic.
- Communicate and defend a scientific argument through writing, following procedures, reviewing information, summarizing data, developing diagrams and charts and speaking clearly.

Understanding about Scientific Inquiry

- Scientists rely on technology to enhance the gathering of data

Content Standard B. Physical Science
Structure and Properties of Matter

- The physical properties of compounds reflect the nature of the interactions among its molecules.
- Carbon atoms can bond to one another in chains, rings, and branching networks to form a variety of structures, including synthetic polymers, oils, and the large molecules essential to life.

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Next Generation Science Standards
Structures and Properties of Matter HS-PS1-3

Plan and conduct an investigation to gather evidence to compare the structure of substances at the bulk scale to infer the strength of electrical forces between particles.

Teacher Background

The following three papers on the Hofmeister series were used in developing the lesson, and may be helpful to a teacher as background information. The papers are high level and may be too challenging for the average high school student, but they could be appropriate for AP students.

Jungwirth, Pavel, and Paul S. Cremer. "Beyond Hofmeister." *Nature Chemistry* 6.April 2014 (2014): 261-63. *Cremer Research Group Publications*. Web. 9 July 2015. <http://sites.psu.edu/cremer/?page_id=26>.

Zhang, Yanjie, Steven Furyk, David E. Bergbreiter, and Paul S. Cremer. "Specific Ion Effects on the Water Solubility of Macromolecules: PNIPAM and the Hofmeister Series." *Journal of the American Chemical Society* 127.41 (2005): 14505-4510. *Cremer Research Group Publications*. Web. 9 July 2015. <http://sites.psu.edu/cremer/?page_id=26>.

Zhang, Yanjie, and Paul S. Cremer. "Chemistry of Hofmeister Anions and Osmolytes." *Annual Review of Physical Chemistry* 61 (2010): 63-83.*Cremer Research Group Publications*. Web. 9 July 2015. <http://sites.psu.edu/cremer/?page_id=26>.

Teacher Materials

- 500 mg Poly(N-isopropylacrylamide)
- Deionized water
- Micropipette or plastic pipette
- 50 mL conical centrifuge tube, or sealed equivalent
- Refrigerator
- Sodium chloride
- Sodium thiocyanate
- Sodium sulfate
- Analytical or electronic balance
- Weigh boat or wax paper
- 22 vials with a volume between 4 and 5 mL
- 10 mL volumetric flask and/or 10 mL graduated cylinder
- Thermometers or temperature probes for each lab group
- Hot plates for each lab group (may be shared between groups)
- 250 mL beakers (one per group)
- Ice (crushed ice preferred)
- Tongs
- Permanent marker (Sharpie Industrial Fine Point recommended)

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Advance Preparation

The Poly(N-isopropyl acrylamide) which was used while developing this lab procedure had a molecular weight of 60,000. PNIPAM from Sigma-Aldrich with a molecular weight of 20,000-40,000 can be used as a substitute, however, a 10 gram sample is $242.50. Alternatively, you could contact Dr. Paul Cremer at Penn State (psc11@psu.edu) and request a 500 mg sample to prepare a lab kit.

Lab Kit preparation

For this experiment, you will need to prepare 22 small vials (4-5 mL is recommended) with a mixture of PNIPAM and the salt solutions of varying concentration. The solutions can be reused multiple times, so the kit only needs to be prepared once.

The first step is to prepare the PNIPAM solution for student use. 500 mg of PNIPAM should be dissolved in 50 mL of deionized water, resulting in a concentration of 10 mg/mL. An analytical balance is preferred for determining the mass, but if one is not available a less precise electronic balance and weigh boat can be used. In a research lab, a mini vortexer would be used to thoroughly mix the solution. In a high school setting, it would be best to mix in a sealed container which can be shaken until fully dissolved. The PNIPAM solution should then be placed in a refrigerator for 4-12 hours overnight.

While the PNIPAM is cooling, the salt solutions can be prepared. Each vial will eventually contain a total of 4 mL of the PNIPAM and salt solution mixture (2 mL PNIPAM solution and 2 mL salt solution). In order for the concentrations of the salt solutions to match the concentrations found in the lab data table after the 2 mL of PNIPAM solution is added, the initial solutions prepared by the teacher should be *TWICE* as concentrated. For example, the initial stock solution for the serial dilution of sodium chloride should be 2.0 M instead of 1.0 M. During the serial dilution, 1.6 M should be made instead of 0.8 M, 1.2 M should be made instead of 0.6 M, etc. 10 mL of each stock solution should be prepared using a 10 mL volumetric flask (if available), which is more than enough to produce 2 mL of each of the required concentrations. The final solutions should have a PNIPAM concentration of 5 mg/mL.

Once each of the required concentrations for the three salts are created, 2 mL of each solution should be pipetted into the small vials. If a micropipette is not available, a 10 mL graduated cylinder and disposable plastic pipettes are recommended to complete the transfer. The vials should be labeled with a permanent marker (Sharpie Industrial Fine Point is recommended) which should not dissolve or fade in warm water. In addition, it may be helpful to color code the caps so that each salt has a different color. The vials can be set aside until the PNIPAM has finished cooling.

Once the PNIPAM solution has sufficiently cooled, it should be shaken vigorously to ensure an even homogenous mixture. 2 mL should be pipetted into each of the vials. The PNIPAM and salt solution mixtures should be shaken to ensure even mixing, and then stored in a refrigerator until they are ready for use in the lab.

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***Note:** You may notice that some of the high concentration salts may turn cloudy when mixed with the PNIPAM at room temperature. This is normal as the LCST at high concentration is lower than room temperature. The solution should turn clear when cooled in the refrigerator.

Safety Information

The teacher and students should wear safety glasses and gloves at all times while handling lab materials. Tongs should be used to handle each sample in the cold or hot water bath.

Teaching Strategies

Before completing this lab, students should have an understanding of intermolecular forces (dipole-dipole, dispersion, hydrogen bonding, etc) and how they allow various molecules to attract one another. Students should also have a basic understanding of concentration with regard to molarity (moles/liter), although they do not necessarily need experience with calculating molarity.

It is suggested that the students complete some form of pre-lab activity prior to coming into lab so that they have some background information on the Hofmeister series. YouTube videos covering this topic have been created by the Cremer Research Group at Penn State, and can be used as a resource for students, and as the subject for flipped lessons or homework assignments. The videos can be found at the link below.

http://tinyurl.com/HofmeisterSeriesVideos

In order to collect all 22 of the data points required in the lab, it is recommended that students break up into groups of two, and that each group determine the LCST for no more than two vials. The data for the entire class could then be written down on the board, and copied into each lab packet.

Directions for the activities

Assuming a 40 minute period schedule, it is suggested that the Hofmeister series is introduced the class period before the lab, and that a pre-lab activity is assigned for homework. The lab should be completed during a double period, or over the course of two days. The teacher should go through a pre-lab discussion (5-10 minutes) where they explain the purpose of the lab, along with assigning each lab group two vials to test. The procedure should be demonstrated using a hot plate, ice/water bath, and a thermometer or temperature probes. Temperature probes are recommended since they are more precise, and they will lead to less error as the students throughout the class may estimate differently. The teacher should prepare three charts on a chalk or whiteboard where students can pool their data. Data collection should take approximately 15-20 minutes. Following data collection, students will spend the remaining time plotting the data points and answering the analysis questions. As students complete the lab, they should dry off the vials and return them to the teacher.

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Name:_____ Period:_____ Date:_____

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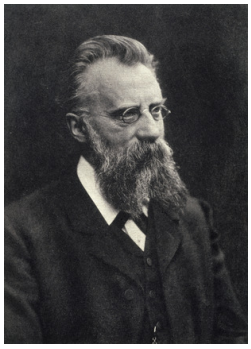
Introduction



Franz Hofmeister (1850-1922) was an early protein chemist who discovered that ions affect the solubility and stability of proteins by using purified egg whites. His research led to the ordering of anions by their ability to "salt in" or "salt out" proteins in aqueous solutions, known as the Hofmeister series. The mechanism for the Hofmeister series is still not completely understood, and it is currently the subject of research being performed at universities and research institutions around the world.

In the following lab, you will use the polymer Poly(N-isopropylacrylamide), or PNIPAM, as a substitute for protein. PNIPAM will be used since it is analogous to valine, which is one of the common amino acids. In the experiment, you will determine how salts at varying concentration have an effect on the temperature at which a protein (or in this case, a polymer) will precipitate out of solution.

Key Terms

Polymer: A substance with a molecular structure consisting of a large number of similar units bonded together.

Salting in: The phenomenon of increasing solubility of a protein with increasing salt concentration.

Salting Out: The phenomenon of decreasing solubility of a protein with increasing salt concentration.

Strongly hydrated ions: Ions that do not readily shed their hydrations shells to bind to the polymer surface.

Weakly hydrated ions: Ions that do readily shed their hydration shell to bind to the polymer surface.

Lower critical solution temperature (LCST): The transition temperature at which a polymer will fold in on itself and scatter light, causing a solution to appear cloudy.

Hydration shell: A shell of water molecules surrounding a dissolved ion.

Cold denaturation: A transition at which a decrease in temperature has caused a protein to unfold and separate, causing the solution to appear at the macro scale.

Heat denaturation: A transition at which an increase in temperature has caused a protein to unfold and separate, causing the solution to appear at the macro scale.

Materials for each lab group

- Safety glasses and gloves
- Two vials containing a PNIPAM and salt mixture
- Thermometer or temperature probe
- Hot plate (may be shared between two groups if necessary)
- 250 mL beaker
- Crushed ice
- Tongs

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Make a prediction regarding LCST and increasing salt concentration:

_____

_____

Procedure

1. Obtain each of the lab materials (including the two vials assigned to your group by your teacher) and put on safety glasses and gloves. ***Do not open the vials under any circumstances.*** They must remain sealed in order to be reused.

2. Fill the 250 mL beaker with approximately 100 mL of tap water, and a small amount of crushed ice. The total volume of water and ice should not exceed 150 mL.

3. Gently stir the ice water bath with the thermometer or temperature probe until the temperature stabilizes. The temperature should drop to 5-10 ^{0}C.

4. Place both vials into the ice water bath and continue to gently stir for approximately one minute. This will ensure that the temperature of the vials are equal to the temperature of the bath.

5. Place the beaker and thermometer onto a hot plate and turn it on to a low setting. Continue to stir every 30-60 seconds and monitor both the temperature, and the state of the solution within the vials.

6. As the solutions approach the LCST, they may appear to contain cloudy "spots" which dissipate when stirred. This is due to an uneven temperature distribution within the vial. Continue to stir gently at this point.

7. Record the LCST temperature into the data table the moment the solution appears completely cloudy, and it does not return to clear when mixed.

8. After the LCST has been recorded for both vials, remove them from the beaker with tongs and dry them off. If any of the labeling on the sides has washed off, use a permanent marker to relabel the vials before returning them to the teacher. They should be stored in a refrigerator.

9. Turn off the hot plate, dump the water from the beaker, and return all materials except for the hot plate. Allow time for the hot plate to cool before putting it away.

10. Record your lab group's data on the board. As other groups complete the data collection, fill in each of the LCST values into the chart and complete the corresponding graph using a different color for each salt. Then answer each of the analysis questions.

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Data Collection: Record the data for your group on the board and in one of the tables below. As other groups begin to finish, record their data into the tables.

Phase Transitions for PNIPAM with $NaCl_{(aq)}$

Concentration [M]	Temperature (^0C)
0.0	
0.1	
0.2	
0.4	
0.6	
0.8	
1.0	

Phase Transitions for PNIPAM with $NaSCN_{(aq)}$

Concentration [M]	Temperature (^0C)
0.0	
0.1	
0.2	
0.4	
0.6	
0.8	
1.0	

Phase Transitions for PNIPAM with $Na_2SO_{4(aq)}$

Concentration [M]	Temperature (^0C)
0.0	
0.03	
0.06	
0.09	
0.12	
0.24	
0.36	
0.42	

Analyze the Results

Use a graph to plot data points for each concentration of the three PNIPAM and salt solution mixtures. The points for each salt should be drawn using a different colored pencil. Be sure to include the color used within the legend.

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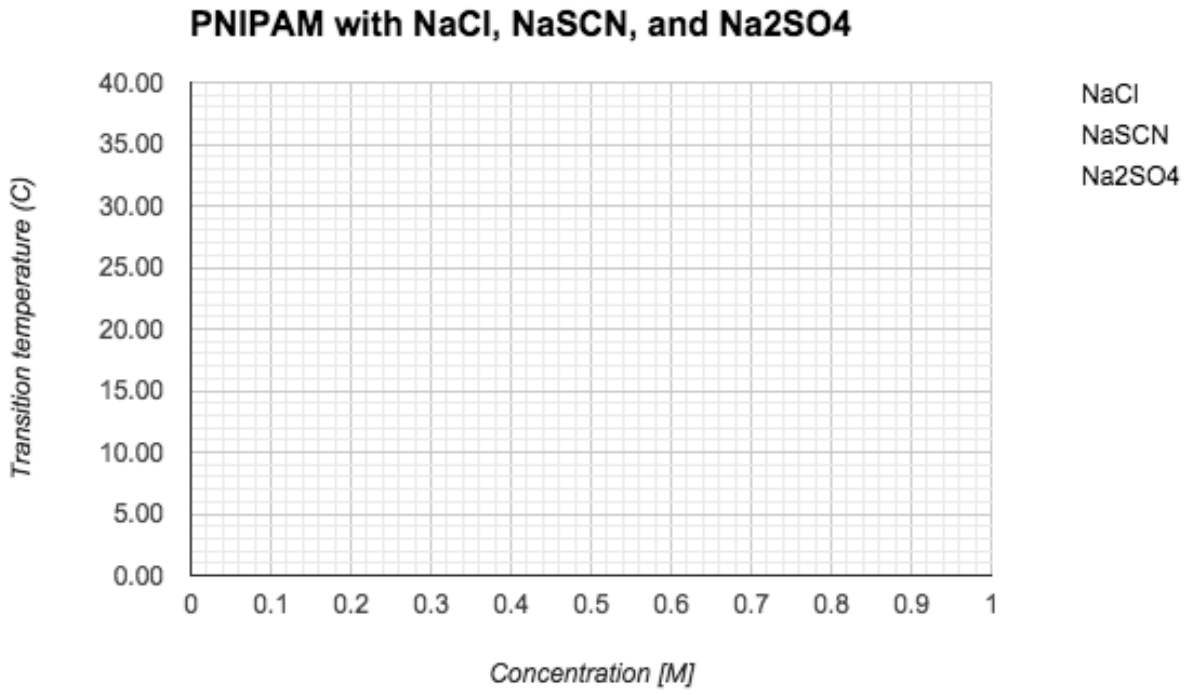

Drawing Conclusions

1. Examine the data for sodium chloride. What happened to the transition temperature as the concentration increased? Which key term properly describes this phenomenon, and why might this pattern occur?

_____

_____

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2. Examine the data for sodium sulfate. What happened to the transition temperature as the concentration increased? How does the lowest LCST compare to the lowest LCST for the other two salts?

_____

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3. How does the effect caused by the sulfate anion compare to the effect caused by the chloride anion?

_____

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4. Examine the data for sodium thiocyanate. What is the biggest difference between the grouping of these data points when compared to the other two salts? Which key term properly describes what has occurred?

_____

_____

_____

5. Rank the three salts based on their ability to affect the LCST for PNIPAM. The first salt listed should have the greatest effect, while the last salt listed should have the least effect.

_____

6. Look up the Hofmeister series for anions, and write out the complete series below. How do the experimental results compare to the ordering found in the series?

_____

_____

_____

7. A company designing thermoresponsive sensors requires a substance which will scatter light at exactly 23.5 ^{0}C. If a solution of PNIPAM is contained within the sensor, which salt could be used, and at what concentration should the salt solution be prepared?

_____

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_____

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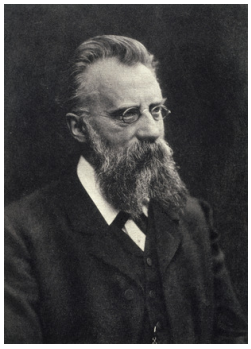
Name:_____ Period:_____ Date:_____

Exploring the Hofmeister Series with PNIPAM
(Teacher Answer Key)

Introduction



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Key Terms

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Lower critical solution temperature (LCST): The transition temperature at which a polymer will fold in on itself and scatter light, causing a solution to appear cloudy.
Hydration shell: A shell of water molecules surrounding a dissolved ion.
Cold denaturation: A transition at which a decrease in temperature has caused a protein to unfold and separate, causing the solution to appear at the macro scale.
Heat denaturation: A transition at which an increase in temperature has caused a protein to unfold and separate, causing the solution to appear at the macro scale.

Materials for each lab group

- Safety glasses and gloves
- Two vials containing a PNIPAM and salt mixture
- Thermometer or temperature probe
- Hot plate (may be shared between two groups if necessary)
- 250 mL beaker
- Crushed ice
- Tongs

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Make a prediction regarding LCST and increasing salt concentration:

As the concentration of salt increases, the LCST will decrease.

Procedure

1. Obtain each of the lab materials (including the two vials assigned to your group by your teacher) and put on safety glasses and gloves. ***Do not open the vials under any circumstances.*** They must remain sealed in order to be reused.

2. Fill the 250 mL beaker with approximately 100 mL of tap water, and a small amount of crushed ice. The total volume of water and ice should not exceed 150 mL.

3. Gently stir the ice water bath with the thermometer or temperature probe until the temperature stabilizes. The temperature should drop to 5-10 ^{0}C.

4. Place both vials into the ice water bath and continue to gently stir for approximately one minute. This will ensure that the temperature of the vials are equal to the temperature of the bath.

5. Place the beaker and thermometer onto a hot plate and turn it on to a low setting. Continue to stir every 30-60 seconds and monitor both the temperature, and the state of the solution within the vials.

6. As the solutions approach the LCST, they may appear to contain cloudy "spots" which dissipate when stirred. This is due to an uneven temperature distribution within the vial. Continue to stir gently at this point.

7. Record the LCST temperature into the data table the moment the solution appears completely cloudy, and it does not return to clear when mixed.

8. After the LCST has been recorded for both vials, remove them from the beaker with tongs and dry them off. If any of the labeling on the sides has washed off, use a permanent marker to relabel the vials before returning them to the teacher. They should be stored in a refrigerator.

9. Turn off the hot plate, dump the water from the beaker, and return all materials except for the hot plate. Allow time for the hot plate to cool before putting it away.

10. Record your lab group's data on the board. As other groups complete the data collection, fill in each of the LCST values into the chart and complete the corresponding graph using a different color for each salt. Then answer each of the analysis questions.

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Data Collection: Record the data for your group on the board and in one of the tables below. As other groups begin to finish, record their data into the tables.

***The transition temperatures may vary based on the molecular weight of the PNIPAM, and the unavoidable error associated with different lab groups visually determining the LCST.**

Phase Transitions for PNIPAM with $NaCl_{(aq)}$

Concentration [M]	Temperature (^{0}C)
0.0	**34.0**
0.1	**32.5**
0.2	**30.5**
0.4	**28.5**
0.6	**25.9**
0.8	**24.0**
1.0	**23.0**

Phase Transitions for PNIPAM with $NaSCN_{(aq)}$

Concentration [M]	Temperature (^{0}C)
0.0	**34.0**
0.1	**35.0**
0.2	**34.0**
0.4	**33.9**
0.6	**33.9**
0.8	**33.5**
1.0	**32.5**

Phase Transitions for PNIPAM with $Na_2SO_{4(aq)}$

Concentration [M]	Temperature (^{0}C)
0.0	**34.0**
0.03	**32.2**
0.06	**31.0**
0.09	**29.5**
0.12	**28.0**
0.24	**21.5**
0.36	**17.0**
0.42	**12.0**

Analyze the Results

Use a graph to plot data points for each concentration of the three PNIPAM and salt solution mixtures. The points for each salt should be drawn using a different colored pencil. Be sure to include the color used within the legend.

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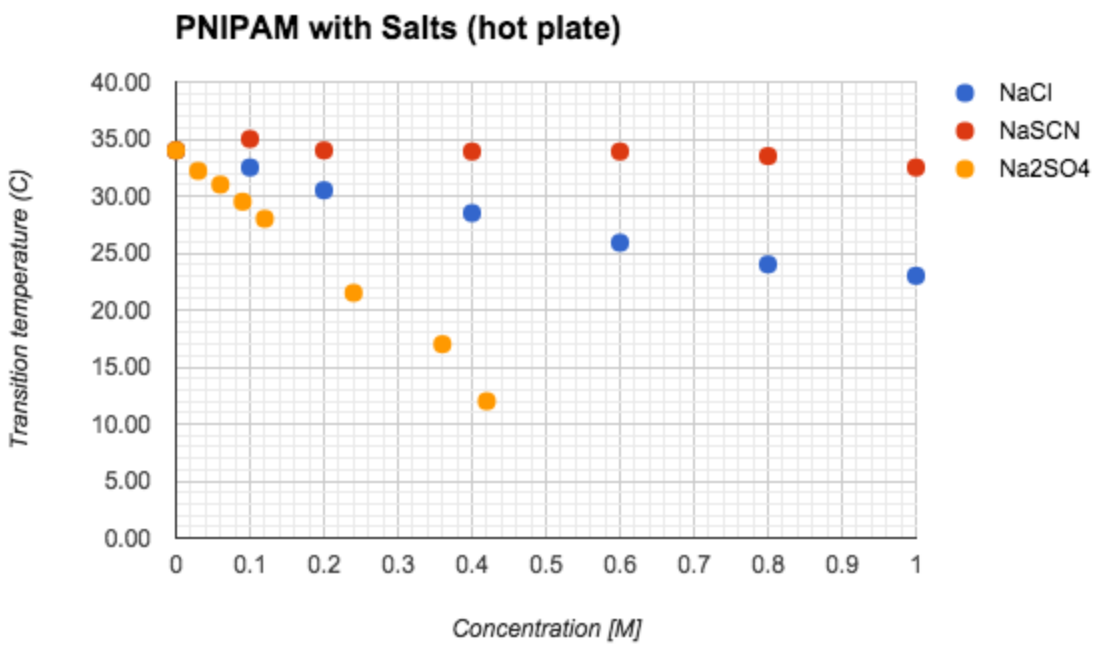

Drawing Conclusions

1. Examine the data for sodium chloride. What happened to the transition temperature as the concentration increased? Which key term properly describes this phenomenon, and why might this pattern occur?
   The transition temperature decreased as the concentration of sodium chloride increased. This is known as "salting out", and it may occur because the chloride ion makes it harder for the PNIPAM to stay in solution. The chloride disrupts the intermolecular attraction between the water and the PNIPAM.

2. Examine the data for sodium sulfate. What happened to the transition temperature as the concentration increased? How does the lowest LCST compare to the lowest LCST for the other two salts?
   The transition temperature decreased as the concentration of sodium sulfate increased. The lowest LCST for sodium sulfate is much lower than that of the other two salts, and it occurs as a lower concentration.

3. How does the effect caused by the sulfate anion compare to the effect caused by the chloride anion?
   The LCST for the sulfate solutions decreases at a much higher rate when compared to the chloride solution.

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4. Examine the data for sodium thiocyanate. What is the biggest difference between the grouping of these data points when compared to the other two salts? Which key term properly describes what has occurred?
   The data for the sodium thiocyanate shows an increase in the initial LCST at low concentration, specially the sample at 0.1 M. This is known as a "salting in" effect.

5. Rank the three salts based on their ability to affect the LCST for PNIPAM. The first salt listed should have the greatest effect, while the last salt listed should have the least effect.

   Na_2SO_4, NaCl, NaSCN

6. Look up the Hofmeister series for anions, and write out the complete series below. How do the experimental results compare to the ordering found in the series?
   CO_3^{-2} > $\mathbf{SO_4^{-2}}$ > $S_2O_3^{-2}$ > $H_2PO_4^-$ > F^- > $\mathbf{Cl^-}$ > Br^- ~ NO_3^- > I^- > ClO_4^- > $\mathbf{SCN^-}$
   The anions should be within the same order in the experiment as in the Hofmeister series.

7. A company designing thermoresponsive sensors requires a substance which will scatter light at exactly 23.5 ^{0}C. If a solution of PNIPAM is contained within the sensor, which salt could be used, and at what concentration should the salt solution be prepared.
   Results may vary based on student data, and there may be more than one salt that produces a viable solution to the problem. Based on the sample data provided in the key, NaCl with a concentration of 0.9 M would cause PNIPAM to precipitate at 23.5 ^{0}C

Suggestions for Student Assessment:

-Students should be assessed on their ability to collaborate as part of a large group in order to collect all of the data points.

-Students may be assessed on the accuracy of their data, and they should be encouraged to repeat the procedure at least once in order to ensure the results are repeatable.

-Students should be assessed based on their ability to create a visual representation of the data in the form of a neatly constructed graph complete with a key. Students may also be encouraged to create the graph using Microsoft Excel, or students could collaborate to produce the graph using Google Sheets.

-Students should be evaluated on their ability to interpret the data by answering the questions within the "drawing conclusions" section. The answers should be graded based on the quality of the writing and vocabulary within the response, and the degree to which the student touches on concepts provided within the answer key.

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