Penn State RET in Interdisciplinary Materials

Teacher's Preparatory Guide

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Intermolecular Forces and Phase Separation

Purpose: This lab is designed to help students understand how the chemical identity of different anions affect the temperature at which a polymer separates out in a solution.

Essential Questions:
1. What are non-covalent interactions (intermolecular forces) and why are they important in nature?
2. What is the Hofmeister series?
3. How does the identity of an anion affect the temperature at which a polymer separates out in solutions?

Time required: Three 45 minutes class periods.

Level: High school

National Science Education Standards 9-12

Science practices

Engaging in scientific investigation requires not only skill but also knowledge that is specific to each practice. The eight science practices are as follows:
1. Asking questions.
2. Developing and using models.
3. Planning and carrying out investigations.
4. Analyzing and interpreting data.
5. Using mathematics and computational thinking.
6. Constructing explanations.
7. Engaging in argument from evidence.
8. Obtaining, evaluating, and communicating information

Content Standard A. Physical Science

HS-PS1-3 (Matter and its Interactions): 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.

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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Content Standard B. Chemical Reactions

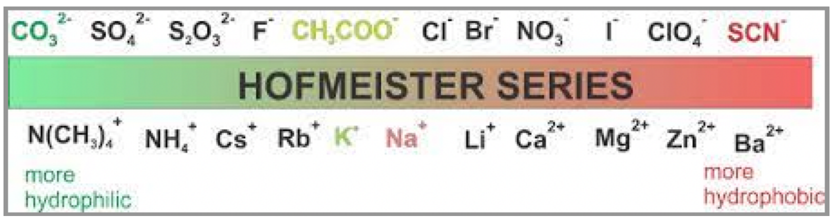
HS-PS1-5. Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the solution on the rate at which a reaction occurs.

Chemical Reactions

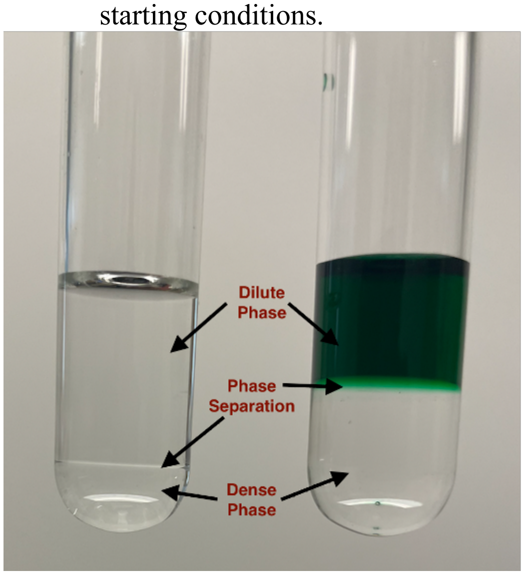
1. Hydrophobic and hydrophilic properties of substances reflect the nature of intermolecular forces of attractions between molecules.
2. Molecular interactions or non-covalent interactions are attractive or repulsive forces between molecules and between non-bonded atoms.
3. In biological systems, proteins fold into globular structures called native states, which are stabilized by molecular interactions.

Teacher Background:

- The three salts ($NaCl$, $NaC_2H_3O_2$ and Na_2SO_4) have the same cation (Na^{1+}) but different anions (Cl^{1-}, $C_2H_3O_2^{1-}$, SO_4^{2-}). In this lab we will keep the Na^{+1} concentrations in solution constant, and hence use 4 M $NaCl$, 4 M $NaC_2H_3O_2$ but 2 M Na_2SO_4.
- We expect the anions to follow the Hofmeister series. The weak charged density anions like Cl^{1-} should have the weakest salting out effect and the densely charged anion SO_4^{-2} should have a strong salting out effect.
- Hofmeister series:



- Liquid-liquid phase separation is the separation of a protein or polymer solution into two aqueous solutions, or phases. Both solutions contain the protein/polymer dissolved in water, but the one on top is more dilute and the one on the bottom is more concentrated (dense) than starting conditions.



Materials: Refer to lesson plan page 4

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Advance Preparation: Refer to page 5

Safety Information

- Students must wear safety glasses, gloves, and closed toed shoes.
- Students should exercise caution when working with glass and heating elements.
- Students should not over heat closed containers.

See SDS (Safety Data Sheet) for full chemical information.

NaCl (sodium chloride) - Skin irritant; Serious eye irritant; May cause respiratory irritation.
https://fscimage.fishersci.com/msds/21105.htm

Na_2SO_4 (sodium sulfate) - May cause skin irritation, eye irritation, respiratory irritation, gastrointestinal irritation.
https://fscimage.fishersci.com/msds/21630.htm

$NaC_2H_3O_2$ (sodium acetate) - May cause skin irritation, eye irritation, respiratory irritation, digestive tract irritation.
https://fscimage.fishersci.com/msds/20860.htm

poly(ethylene glycol) - PEG - This chemical is not considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)
https://www.fishersci.com/store/msds?partNumber=BP233100&productDescription=PEG+8000+100G&vendorId=VN00033897&countryCode=US&language=en

Teaching Strategies:

This lab can be done in groups of 3 or 4.

Before students begin the lab show them some before and after pictures/videos so they are aware of what changes they will be looking for.

Day 1:
Note to teachers: If you just talked about atoms and ions in the previous lesson with your students you can go ahead with "Exploring the Hofmeister Series" part and skip the review of ions part. If not, you must give them a quick review of atoms, ions and how salts are formed.

Objective: Students will be able to identify different salts that can "salt-out" or "salt-in" polymers.

Essential question: What is the Hofmeister Series?

Materials:
- Beaker
- Glass rod
- PEG
- Water
- Worksheet

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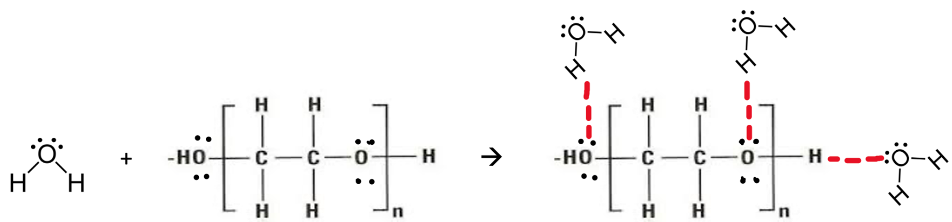
Activation: (15 min.)

- Distribute the "Exploring the Hofmeister Series" Worksheet 1.
- Ask students to complete section A (1) on the worksheet. Give the students a minute to answer and then call on different students to share their answers.
- Ask students whether poly(ethylene glycol) (PEG) will dissolve in water and instruct them to write their prediction in the first column of section A (2). Ask a few students to share their predictions.
- Draw students' attention to your demo as you take about a spoonful (~10g) of PEG in a 250 mL beaker. Add ~100 mL water to it and stir the mixture with a glass rod. (The PEG will dissolve in water, and you will get a clear solution). Ask students to write at least 2 observations in the second column a possible explanation for why this happened in the third column.
- Discuss the answers to the above (Predict, Observe, Explain) as a class.
- Ask students to complete sections B to E. They may work in pairs/groups. Walk around the room, have a casual conversation with students, and ask why they arranged it the way they did.
- Discuss answers as a class and teach/revisit hydrogen bonds if needed.
    - **Teacher Note:** During this activity, student's prior knowledge of intermolecular forces of attraction, especially hydrogen bonding, is determined.
- Show the following structure



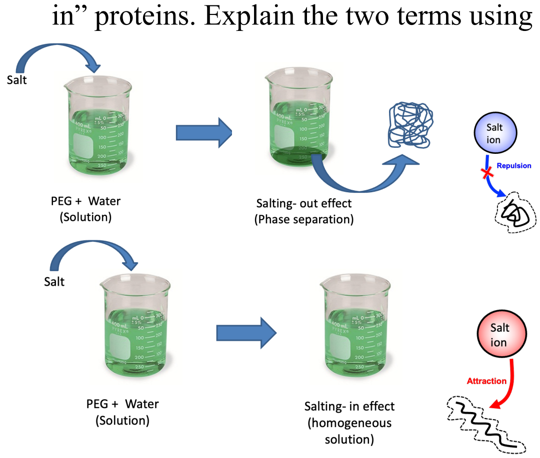
Water + Polyethylene glycol (PEG) → PEG dissolves in water

Hydrogen bonds hold the PEG and water molecules together

- Tell students that they will be able to verify their hypothesis by the end of class today.

Teaching: (25 min)

- Tell students that in 1888 Franz Hofmeister ranked the ions based on their ability to "salt-out" or "salt-in" proteins. Explain the two terms using diagrams.



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- Draw their attention to the **Hofmeister series of anions** in their worksheets. These anions are arranged with respect to their "salting-out" effect.

$$\mathbf{SO_4^{2-} > HPO_4^{2-} > C_2H_3O_2^{1-} > Cl^{1-} > NO_3^{1-} > Br^{1-} > ClO_3^{1-} > I^{1-} > ClO_4^{1-} > SCN^{1-}}$$

- Ask students to arrange the given salts in the order with which they will salt-out or salt-in the polymer in water.
- Call on different students to share their answers.
- Tell students to go back to Section E and check whether their hypothesis holds good with respect to the Hofmeister series.
- **Announcement to students:** After reviewing atoms, cations and anions and exploring about the Hofmeister series you will work with your group on Day 2 to gather data by performing a lab with different salts and evaluate your results.

- Show students the video https://youtu.be/UL96SOGVl6U if time permits.

Day 2: Advanced preparation is needed for Day 2 lab.

Materials per lab group (3-4 students):
- Hotplate with stirrer
- Test tube rack
- 3 – 25mL test tubes*
- Stand
- 2 clamps
- Thermometer
- 400 ml beaker
- 4M NaCl *
- 4M $NaC_2H_3O_2$ *
- 2M Na_2SO_4 *
- 400mg/ml *poly(ethylene glycol)* - PEG*
- Disposable pipets or graduated cylinders
- Beaker tongs
- Food coloring (Optional) (water and propylene glycol-based) (One bottle per group)
- Worksheet per student
- *Total volume of solution in each test tube should not exceed 25%.

Advance Preparation (For six groups of 4):
Prepare the following solutions: Adjust the quantity of solution to be prepared as per your requirement.
- *400mg/ml of polyethylene glycol* (PEG). Polyethylene glycol (Miralax, ClearLax, Glycolax) is an over-the-counter medication that treats constipation. You can purchase 'Polyethylene glycol 3350 powder for solution'. Various brands like ClearLax, MiraLAX and others are available at grocery stores, pharmacies and on Amazon.
  Mass 20.0g PEG. Transfer it to a 50 mL volumetric flask. * Add distilled water up to the calibration mark. Shake to dissolve.
- *4M NaCl*
  Mass 5.844g NaCl. Transfer it to a 25 mL volumetric flask. * Add distilled water up to the calibration mark. Shake to dissolve.

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- *4M $NaC_2H_3O_2$*
  Mass 8.307g $NaC_2H_3O_2$. Transfer it to a 25 mL volumetric flask. Add distilled water up to the calibration mark. Shake to dissolve.
- *2M Na_2SO_4*
  Mass 7.1025g Na_2SO_4. Transfer it to a 25 mL volumetric flask. Add distilled water up to the calibration mark. Shake to dissolve.
  *If the appropriate size volumetric flasks are not available, a graduated cylinder could be used instead while clapped with Parafilm or plastic wrap. Make sure to edit Worksheet 2 to match the glassware available for your lab.

Safety Information for students

- Students must wear safety glasses, gloves, and closed toed shoes.
- Students should exercise caution when working with glass and heating elements.
- Students should not overheat closed containers.

See SDS for full chemical information.

Expected Observations (For Teacher Reference)

- Na_2SO_4 will make the PEG phase separate immediately at room temperature (20-30 °C). Two clear layers form.
- $NaC_2H_3O_2$ w/ PEG will have a cloud point around 52-55 °C. Solution turns cloudy and separated.
- NaCl w/ PEG will have the highest cloud point around 72-75 °C. Solution turns cloudy and separated.

Effect of Hofmeister Salts on PEG

Objective: Students will be able to describe how salts impact the behavior of polymers.

Essential question: How does the identity of an anion affect the temperature at which a polymer separates out in solutions?

Note to teacher: It is also important that the teacher shows before and after pictures of solutions.

Activation: (5 min.)
- Distribute the "Effect of Salts on PEG" Worksheet 2.
- Post key terms (hydrogen bond, salting-out, salting-in, etc.) and the Hofmeister series from the previous day's class on the board/slide. Ask students to define/describe these terms to group members.
- Allow the students to read the background information and Key Terms on the lab worksheet.
- Walk around the room and answer questions if needed. Before starting the lab, supplement students understanding on the structure of polymer and the effect of temperature.

Explore: (20 min.)

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- Using equitable grouping strategy, have the class divided into groups of four.
- Remind students of safety considerations.
- Have them get their lab worksheets, read background information, and formulate hypotheses.
- Have students get their materials and work to gather data by following the procedure in the experiment with different salts on PEG.
- Circulate and provide support to students as they complete the investigation.

Evaluate: (5 min.)

- Have students organize, complete their data table, create a bar graph to show a visual representation of their data, and answer the analysis questions.

- State some applications of Hofmeister series.
  1. Pickling Cucumbers: Putting cucumbers in salty water forces the water in the cucumbers to be drawn out into the brine. This effect is due to an imbalance in osmotic pressure, and the effect that ions have on the osmotic pressure follows the Hofmeister series.
  2. Lightning Storms & Static Electricity: When static is transferred between surfaces, it is not because electrons are being transferred, but usually because anions are transferred. When an anion goes from one surface to another, this causes a difference in electrical potential. That potential difference leads to "zaps" that you can see from lightning clouds or a fleece blanket in the winter.
  3. Pharmaceuticals: Studying phase separation can help in taking care of unwanted behaviors in protein-based drugs. Protein-based drugs often need to be used at very high concentrations where phase separation sometimes occurs. Salts can be added to the solution to reduce the likelihood of phase separation.
  4. Research – study of membrane-less organelles, specifically understanding their use in the cell and how to engineer artificial organelles. Membraneless organelles are similar to organelles because they have a function in the cell and organize proteins, enzymes, and other cellular cargo for specific tasks. It is easier for molecules to enter membraneless organelles due to their lack of a membrane, and they can form or disperse depending on cellular conditions.

Extension:

- Instead of stating some applications, you can ask students to conduct online research on some applications of the Hofmeister series.

  Note: The teacher can provide a rubric for this assignment.

- You can include salts like NaBr and $NaNO_3$ in the experiment.

  Note: NaBr and $NaNO_3$ do not salt out if the water bath temperature is raised to 95ºC. The cloud point temperature with these salts added is close to or above the boiling point of water, and students will need to be advised to stop the experiment once the water bath starts bubbling.

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Resources:
https://pubs.acs.org/doi/10.1021/acs.jpcb.6b10797

https://youtu.be/UL96SOGVl6U

Exploring the Hofmeister Series

Worksheet #1

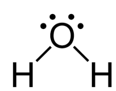
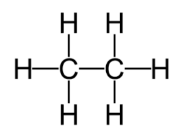
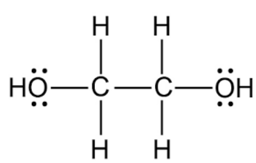
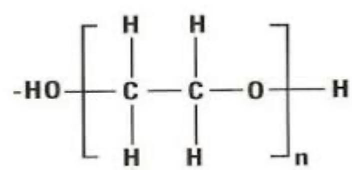
Name____Period___________Date____________

Objective: Identify how different salts "salt-out" or "salt-in" polymers.

Essential question: What is the Hofmeister Series?

A. Directions:

1. State whether the following molecules are polar or nonpolar:

Water: polar or nonpolar? 	Ethane: polar or nonpolar? 
Ethelene glycol: polar or nonpolar? 	Polyethylene glycol (PEG): polar or nonpolar? 

B. Will poly(ethylene glycol) (PEG) dissolve in water?

1. Write your prediction in the first column.
2. Your teacher will mix PEG with water. Write your observations in the second column.
3. Why did this happen? Write a possible explanation in the third column.

Predict	Observe	Explain
What would happen to the polymer when added to water?	What changes did you see on the polymer, and the water in the beaker?	Did you predict this would happen? Why did this happen?

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C. Diagram. With a partner, draw the structure of PEG and place 3 molecules of water around it. Explain why you placed the water molecules in a specific spot and in a specific way.

What is the force of attraction between a water molecule and PEG? Show these in the above diagram.

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D. What If: I have a homogeneous mixture of PEG and water. Can I get a more concentrated solution of PEG from this mixture? If so, how?

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E. Brainstorm: I have a homogeneous mixture of PEG and water. What do you think will happen if I add salt to this mixture and then heat it? Write you hypothesis below using "If... then… statement"

Hypothesis:

If_________________________________then____

____

F. Sequencing anions: "Salt-out > Salt-in"

Hofmeister series of anions:

$SO_4^{2-} > HPO_4^{2-} > C_2H_3O_2^{1-} > Cl^{1-} > NO_3^{1-} > Br^{1-} > ClO_3^{1-} > I^{1-} > ClO_4^{1-} > SCN^{1-}$

Arrange the salts below in the order with which they will salt-out or salt-in the polymer in water from left to right. Use the Hofmeister series to complete the series below:

Fill in the table below:

"Salt-out"						**"Salt-in"**	

1. NaCl
2. NaSCN
3. NaBr
4. $NaC_2H_3O_2$
5. NaI
6. $NaNO_3$
7. Na_2SO_4
8. $NaClO_4$

G. In your own words discuss what you have learned about the "Hofmeister series"? How is it useful in knowing the effects of salts in solutions?

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Exploring the Hofmeister Series

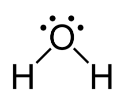
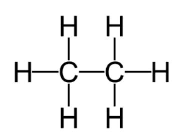
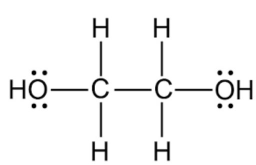
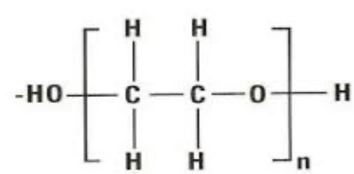
Worksheet #1 (Teacher's guide)
Name______________________________________Period___________Date_______________

Objective: Arrange salts/ions in the order with which they easily "salt-out" or "salt-in" polymers.

Essential question: What is the Hofmeister Series?

A. Directions:

1. State whether the following molecules are polar or nonpolar:

Water: polar or nonpolar?	Ethane: polar or nonpolar?
	
Ethelene glycol: polar or nonpolar?	Polyethylene glycol (PEG): polar or nonpolar?
	

B. Will poly(ethylene glycol) (PEG) dissolve in water?

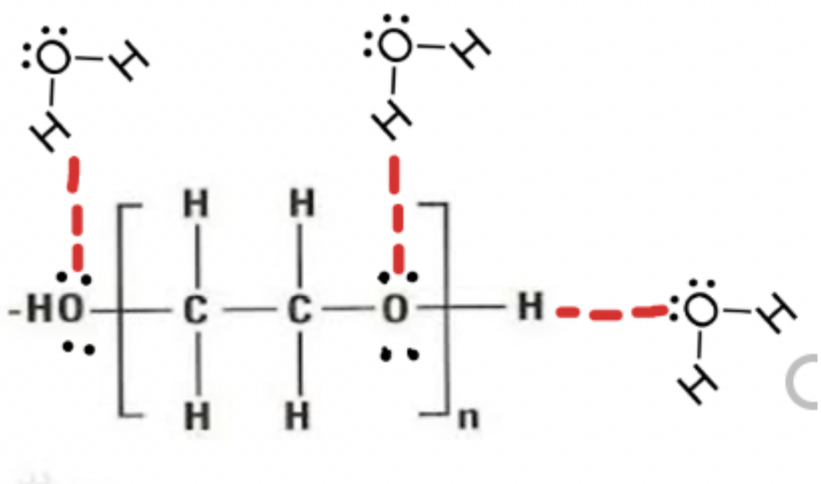
2. Write your prediction in the first column.
3. Your teacher will mix PEG with water. Write your observations in the second column.
4. Why did this happen? Write a possible explanation in the third column.
5.

Predict	Observe	Explain
What would happen to the polymer when added to water?	What changes did you see on the polymer, and the water in the beaker?	Did you predict this would happen? Why did this happen?

PEG will dissolve in water.	PEG dissolves in water.	Yes. “Like dissolves like”

C. Diagram. With a partner draw the structure of PEG and place 3 molecules of water around it. Explain why you placed the water molecules in a specific spot and in a specific way.

(Make sure that all hydrogen-bonding waters only interact with hydrogen bond donors (OH. NH, FH)



C. What is the force of attraction between a water molecule and PEG? Show these in the above diagram.
Hydrogen bond (shown in Green in the above diagram)

D. What If: I have a homogeneous mixture of PEG and water. Can I get a more concentrated solution of PEG from this mixture? If so, how?

Student’s answers may vary: Mostly it would be based on prior knowledge. Common answers will be Boiling or distillation.

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E. Brainstorm: I have a homogeneous mixture of PEG and water. What do you think will happen if I add salt to this mixture and then heat it? Write you hypothesis below using "If... then… statement"

Hypothesis:

If salt is added to the PEG solution, then PEG will separate from water. (Most students will draw this conclusion because of the previous question)

Note to teacher: PEG will still be dissolved in solution, but there will be a much more concentrated solution sitting under a much more dilute solution than starting conditions. Both solutions are PEG dissolved in water but now you have one solution that is much more concentrated.

F. Sequencing anions: "Salt-out > Salt-in"

Hofmeister series of anions:

$\mathbf{SO_4^{2-} > HPO_4^{2-} > C_2H_3O_2^{1-} > Cl^{1-} > NO_3^{1-} > Br^{1-} > ClO_3^{1-} > I^{1-} > ClO_4^{1-} > SCN^{1-}}$

Arrange the salts below in the order with which they will salt-out or salt-in the polymer in water from left to right. Use the Hofmeister series to complete the series below:

Fill in the table below:

"Salt-out"						**"Salt-in"**	
Na_2SO_4	$NaC_2H_3O_2$	NaCl	$NaNO_3$	NaBr	NaI	$NaClO_4$	NaSCN

1. NaCl
2. NaSCN
3. NaBr
4. $NaC_2H_3O_2$
5. NaI
6. $NaNO_3$
7. Na_2SO_4
8. $NaClO_4$

G. In your own words discuss what you have learned about the "Hofmeister series"? How is it useful in knowing the effects of salts in solutions?

The Hofmeister series is a ranking of ion-specific effects originally discovered by Franz Hofmeister in 1888. It is important to know that different salts affect how polymers separate out in solutions.

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Effect of Hofmeister Salts on PEG

Worksheet #2

Name________________________________Period__________Date___________________

Objective: Students will be able to describe how salts impact the behavior of polymers in solution.

Essential question: How does the identity of salts affect the temperature at which polymers separate out of solutions?

Background:

Phase separation is the process by which polymers separate into two aqueous solutions through the application of heat or compounds such as acids, bases, salts, or organic solvents. The two aqueous solutions are called the dilute phase and the dense phase, because one is less concentrated, and one is more concentrated than the original solution.

The temperature at which this change occurs can be strongly influenced by the addition of salts such as $NaC_2H_3O_2$, NaCl and Na_2SO_4. Different anions lower or raise the phase separation temperature according to the Hofmeister series, which is a ranking of ion-specific effects originally discovered by Franz Hofmeister in 1888. Cations and anions can affect surface tension, water activity and even solubility behavior of polymers. Anions appear to have a larger effect than cations, and are usually ordered:

$SO_4^{2-} > HPO_4^{2-} > C_2H_3O_2^{1-} > Cl^{1-} > NO_3^{1-} > Br^{1-} > ClO_3^{1-} > I^{1-} > CO_4^{1-} > SCN^{1-}$

Early members of the series (the ones on the left) have high **charge density** which tends to increase solvent surface tension and cause molecules to collapse on itself ("**salting-out**"), in effect they strengthen the hydrophobic interaction which decreases polymer solubility. By contrast, later salts in the series (the ones on the right) have low charge density which makes the ions bind to the polymer molecules thus increasing their solubility ("**salting-in**") and in effect, they weaken the hydrophobic effect. In this lab, you will learn how salts impact the behavior of the polymer *poly(ethylene glycol)* (PEG).

Key terms:

1. **Cloud-point temperature-** it is the temperature at which the solution turns cloudy (two distinct phases may appear).
2. **Salting-out -** when ions are excluded from the polymer surface resulting to decreased polymer solubility.
3. **Salting- in -** when ions are bound to the polymer surface resulting to increased polymer solubility.
4. **Charge density-** is the amount of charge spread out over a certain area.
5. **High charge density ions-** bind water molecules strongly; strongly hydrated ions.
6. **Low charge density ions-** bind water molecules weakly; weakly hydrated ions.

Materials for each lab group:

- Three 25 mL test tubes
- Thermometer or temperature probe
- Hot plate

- 400 mL beaker for hot water bath
- Magnetic stir bar
- Iron stand and 2 clamps (one for the thermometer and one for the test tube)
- Beaker tongs
- Disposable pipets or graduated cylinders
- Student worksheets
- Prepared solutions (4 M NaCl, 4 M $NaC_2H_3O_2$, 2 M Na_2SO_4, 400 mg/mL PEG)
- (Optional) One bottle of food coloring

Safety Information for students

- Students must wear safety glasses, gloves, and closed-toed shoes.
- Students should exercise caution when working with glass and heating elements.
- Students should not overheat closed containers.

I. Hypothesis

Write a hypothesis about which salt (NaCl, $NaC_2H_3O_2$ or Na_2SO_4) could lower the temperature of PEG in solution the most.

____.

II. Procedure

1. Wear safety glasses. Do not open the solution bottles unless told by your teacher.

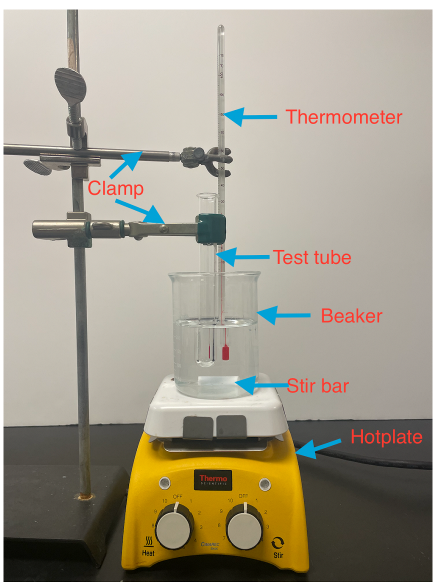

Figure 1: Hotplate setup

2. Fill the 400 mL beaker with approximately 250 mL of tap water. Place a magnetic stir bar in the beaker. Prepare the set-up shown in Figure 1 using two iron clamps. Clamp the

thermometer to one making sure that it is not touching the sides and the bottom of the beaker. Set up the second clamp for the test tube.

3. You will record the **cloud-point temperature** in your data table.
   Note: Cloud-point is the temperature when the solution **starts** to get cloudy (or separate into two phases).
4. Label 3 clean test tubes given to you as 1, 2 and 3. Using a graduated cylinder or pipet, add 2mL of PEG solution to each test tube. If you are using a graduated cylinder, make sure to rinse it with water in between measurements.
5. Add 2 mL of 2 M Na_2SO_4 to test tube labeled 1. Make sure the solution is evenly mixed by carefully swirling the test tube. Write your observation in the data table. (You may add a tiny drop of food color to the solution to see the two separated layers distinctly.)
6. Add 2 mL of 4 M $NaC_2H_3O_2$ to test tube labeled 2. Make sure the solution is evenly mixed by carefully swirling the test tube. Clamp the test tube making sure that it is not touching the sides and the bottom of the beaker and the solution in the test tube is completely below the water line in the bath. Turn the hot plate to medium or high heat setting. Record the **cloud-point temperature** in your data table.
   Note the temperature when the solution **starts** to get cloudy (or separate into two phases). Turn off the hot plate once the cloud point is reached.
7. Add 2 mL of 4 M NaCl to test tube labeled 3. When you are about to test the next test tube, make sure you dump off half of the hot water from your beaker and replace it with cold water to cool down the water temperature. You will then reheat the hot plate to a low setting.
8. After the cloud-point temperatures have been recorded for all your samples, clean up, and return the materials except the hot plate. Allow time for the hot plate to cool before putting it away.

III. Data Collection: Record your data in the table below

Test Tube #	PEG Concentration (mg/ml)	Salt Concentration (M)	Observations	Cloud-point Temperature (^oC)
1	200	2 M Na_2SO_4		
2	200	4M $NaC_2H_3O_2$		
3	200	4M NaCl		

IV. Analyze results: Create a bar graph to visually represent your data above. Use different colored pencils and create a legend.

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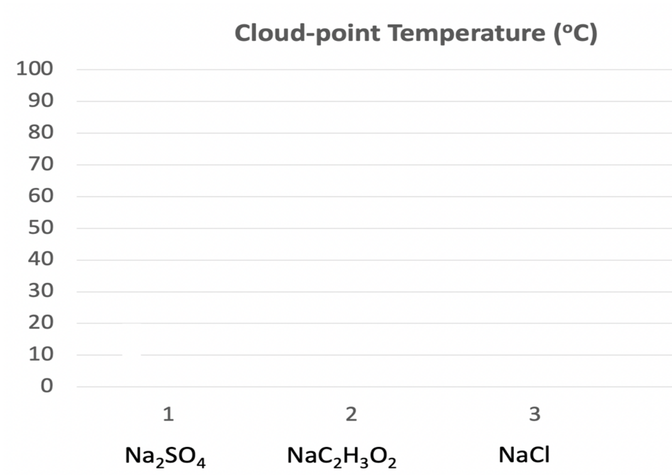

Draw conclusion

From your data above, compare which of the 3 salt solutions lowered the cloud point temperature the most. (Typically, room temperature is between 20-30 degrees Celsius)

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Briefly describe how the chemical identity of the salt impacts the behavior of polymer to separate out in solution. Discuss in terms of the position of the anion in the Hofmeister series and the charge density (low or high) of the ion.

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Confirm or Reject your Hypothesis:

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Effect of Hofmeister Salts on PEG (Teacher's guide)

Worksheet #2

Name________________________________Period__________Date___________________

Objective: Students will be able to describe how salts impact the behavior of polymers in solution.

Essential question: How does the identity of salts affect the temperature at which polymers separate out of solutions?

Background:

Phase separation is the process by which polymers separate into two aqueous solutions through the application of heat or compounds such as acids, bases, salts, or organic solvents. The two aqueous solutions are called the dilute phase and the dense phase, because one is less concentrated, and one is more concentrated than the original solution.

The temperature at which this change occurs can be strongly influenced by the addition of salts such as $NaC_2H_3O_2$, NaCl and Na_2SO_4. Different anions lower or raise the phase separation temperature according to the Hofmeister series, which is a ranking of ion-specific effects originally discovered by Franz Hofmeister in 1888. Cations and anions can affect surface tension, water activity and even solubility behavior of polymers. Anions have a larger effect than cations, and are usually ordered:

$\mathbf{SO_4^{2-} > HPO_4^{2-} > C_2H_3O_2^{1-} > Cl^{1-} > NO_3^{1-} > Br^{1-} > ClO_3^{1-} > I^{1-} > CO_4^{1-} > SCN^{1-}}$

Early members of the series (the ones on the left) have high **charge density** which tends to increase solvent surface tension and cause molecules to collapse on itself ("**salting-out**"), in effect they strengthen the hydrophobic interaction which decreases polymer solubility. By contrast, later salts in the series (the ones on the right) have low charge density which makes the ions bind to the polymer molecules thus increasing their solubility ("**salting-in**") and in effect, they weaken the hydrophobic effect. In this lab, you will learn how salts impact the behavior of the polymer *poly(ethylene glycol)* (PEG).

Key terms:

1. **Cloud-point temperature-** it is the temperature at which the solution turns cloudy (two distinct phases may appear).

2. **Salting-out -** when ions are excluded from the polymer surface resulting to decreased polymer solubility.
3. **Salting- in -** when ions are bound to the polymer surface resulting to increased polymer solubility.
4. **Charge density-** is the amount of charge spread out over a certain area.
5. **High charge density ions-** bind water molecules strongly; strongly hydrated ions.
6. **Low charge density ions-** bind water molecules weakly; weakly hydrated ions.

Materials for each lab group:

- Three 25 mL test tubes
- Thermometer or temperature probe
- Hot plate
- 400 mL beaker for hot water bath
- Magnetic stir bar
- Iron stand and 2 clamps (one for the thermometer and one for the test tube)
- Beaker tongs
- Disposable pipets or graduated cylinders
- Student worksheets
- Prepared solutions (4 M $NaCl$, 4 M $NaC_2H_3O_2$, 2 M Na_2SO_4, 400 mg/mL PEG)
- (Optional) One bottle of food coloring

Safety Information for students

- Students must wear safety glasses, gloves, and closed-toed shoes.
- Students should exercise caution when working with glass and heating elements.
- Students should not overheat closed containers.

I. Hypothesis

Write a hypothesis about which salt ($NaCl$, $NaC_2H_3O_2$ or Na_2SO_4) could lower the temperature of PEG in solution the most.

Na_2SO_4 will lower the temperature the most.

II. Procedure

1. Wear safety glasses. Do not open the solution bottles unless told by your teacher.

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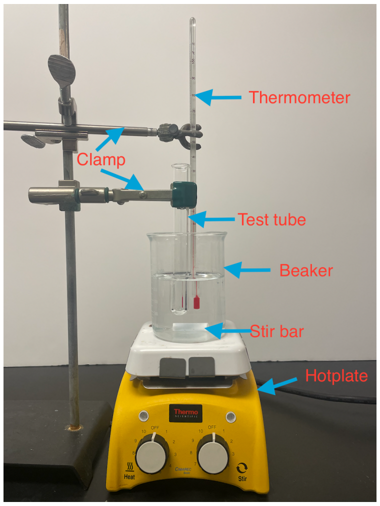

Figure 1: Hotplate setup

2. Fill the 400 mL beaker with approximately 250 mL of tap water. Place a magnetic stir bar in the beaker. Prepare the set-up shown in Figure 1 using two iron clamps. Clamp the thermometer to one making sure that it is not touching the sides and the bottom of the beaker. Set up the second clamp for the test tube.
3. You will record the **cloud-point temperature** in your data table.
   Note: Cloud-point is the temperature when the solution **starts** to get cloudy (or separate into two phases).
4. Label 3 clean test tubes given to you as 1, 2 and 3. Using a graduated cylinder or pipet, add 2mL of PEG solution to each test tube. If you are using a graduated cylinder, make sure to rinse it with water in between measurements.
5. Add 2 mL of 2 M Na_2SO_4 to test tube labeled 1. Make sure the solution is evenly mixed by carefully swirling the test tube. Write your observation in the data table. (You may add a tiny drop of food color to the solution to see the two separated layers distinctly.)
6. Add 2 mL of 4 M $NaC_2H_3O_2$ to test tube labeled 2. Make sure the solution is evenly mixed by carefully swirling the test tube. Clamp the test tube making sure that it is not touching the sides and the bottom of the beaker and the solution in the test tube is completely below the water line in the bath. Turn the hot plate to medium or high heat setting. Record the **cloud-point temperature** in your data table.
   Note the temperature when the solution **starts** to get cloudy (or separate into two phases). Turn off the hot plate once the cloud point is reached.
7. Add 2 mL of 4 M NaCl to test tube labeled 3. When you are about to test the next test tube, make sure you dump off half of the hot water from your beaker and replace it with cold water to cool down the water temperature. You will then reheat the hot plate to a low setting.
8. After the cloud-point temperatures have been recorded for all your samples, clean up, and return the materials except the hot plate. Allow time for the hot plate to cool before putting it away.

III. Data Collection: Record your data in the table below

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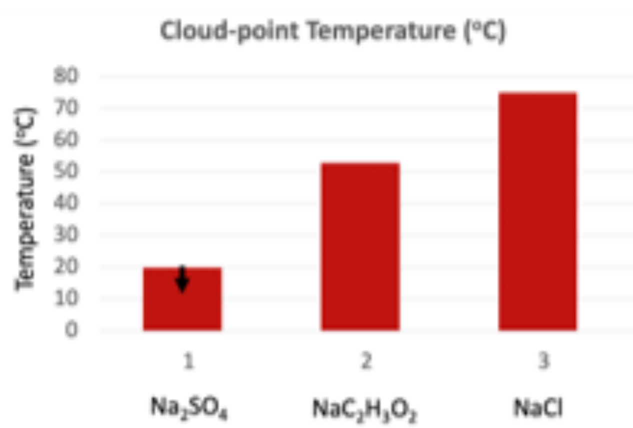
Test Tube #	PEG Concentration (mg/ml)	Salt Concentration (M)	Observations	Cloud-point Temperature (oC)
1	200	2 M Na_2SO_4	The phases separate out as soon as Na_2SO_4 is added.	< 20 (Room temperature)
2	200	4M $NaC_2H_3O_2$	Milky phase at the bottom and clear on top	52-55
3	200	4M NaCl	Milky phase at the bottom and clear on top	72-75

IV. Analyze results: Create a bar graph to visually represent your data above. Use different colored pencils and create a legend.



Draw conclusion

From your data above, compare which of the 3 salt solutions lowered the cloud point temperature the most

Na_2SO_4

Briefly describe how the chemical identity of the salt impacts the behavior of polymer to separate out in solution. (Discuss in terms of the position of the anion in the Hofmeister series and the charge density (low or high) of the ion.

Ions differ in their ability to salt out polymers in solution.
Comparing the 3 salts, SO_4^{2-} (lies to the left on the Hofmeister series) has higher **charge density** which tends to increase solvent surface tension and cause molecules to collapse and decreases polymer solubility. On the other hand, Cl^{1-} in the series has lower charge density which makes the ions bind to the polymer molecules thus increasing their solubility thus requiring higher temperature before reaching a cloud-point.

Confirm or Reject your Hypothesis:

Students either confirm or reject their hypothesis and state the reason.