Penn State RET in Interdisciplinary Materials

Teacher's Preparatory Guide

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Why Salts Matter?
Three 45-minute class periods

Lesson Overview

Students will be able to identify and explain how the chemical identity of salts and their concentration affects the temperature at which polymers, particularly protein molecules would precipitate out of solutions. Students will refer to the Hofmeister series which shows the ions in order of their ability to "salt- out" or "salt- in" proteins. In this lab, students will record the cloud-point temperature for filtered chicken egg white mixed with various concentrations of three salts within the series namely Na_2S0_4, NaCl and NaSCN. The change in temperatures will be compared with the cloud-point of filtered egg white alone, and the pattern will be analyzed to determine the effect of the identity of the particular anions present as well as the concentration of salts to the cloud-point temperature of egg white.

Content Framework Summary

- In 1888 Franz Hofmeister ranked the ions based on their ability to salt -out or salt- in proteins. The Hofmeister series named after him has become the subject of many researches in recent years. Salts in solutions play a significant role in biology. Cations and anions can affect surface tension, water activity and even solubility behavior of macromolecules such as polypeptides or proteins. Anions appear to have a larger effect than cations, and are usually ordered F^- $S0_4^{2-}$ >$HP0_4^{2-}$ > acetate> Cl^->$N0_3^-$ >Br^->$Cl0_3^-$ >I^->$Cl0_4^-$>SCN^- while the order of cations is usually given as NH_4^+ >K^+>Na^+>Li^+>Mg^{2+}>Ca^{2+} >guanidinium.[1]

- Early members of the series have high charge density which tends to increase solvent surface tension and cause nonpolar molecules or proteins to collapse in itself ("salting-out")in effect they *strengthen* the hydrophobic interaction which decreases protein solubility. By contrast, later salts in the series have low charge density which makes the ions bind closely to the protein molecules thus increasing their solubility ("salting-in") and in effect, they *weaken* the hydrophobic effect.

- Denaturation is the process by which protein molecules lose their native state (quarternary, tertiary or secondary structure), by changing its viscosity or color through the application of heat or compounds such as acids, bases, salts or organic solvents.

Enduring Understandings

- Proteins are made of long chains of amino acids, which are the building blocks of proteins. An egg white, which is composed of about 40 different proteins contain both hydrophilic and hydrophobic amino acids. There are about 40 proteins present in egg whites, ovalbumin is the most abundant with 54%. Lysozyme is also present at 3.5%.

- At room temperature proteins are folded, and at high temperatures they unfold and denature. Then these unfolded proteins become stable when they aggregate.

- The solubility of proteins are influenced by the identity and concentration of salts present. In 1888 Franz Hofmeister showed this in the order called Hofmeister or lyotropic series [2].

- The structure and interactions of matter at the bulk scale are determined by intermolecular forces within and between atoms.

Essential Questions

1. What is the Hofmeister series?
2. How does the identity and concentration of salts affect the temperature at which proteins precipitate out in solutions?
3. What is the significance of the Hofmeister series in biological or chemical processes?

National Science Education Standards Grades 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. 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.

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 reacting particles on the rate at which a reaction occurs.

Chemical Reactions

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

Day 1 - Exploring the Hofmeister Series

Materials:

- Teacher demonstration materials (egg white, hotplate, beaker with water)
- Exploring the Hofmeister series worksheet

Video clips:

1. " Science reveals what happens when you boil an egg" (stop at 0.48 secs)
   https://www.youtube.com/watch?v=3QHOnLh3ziQ
2. Hofmeister series and Thermoresponsive polymers ((start at 4:07-4:56)
   https://www.youtube.com/watch?v=AjYeJKVbnBc&feature=youtu.be

Day 1: Exploring the Hofmeister Series

Objective: Students will be able to arrange salts/ions in the order as to which would easily salt-out or salt-in with proteins.

Essential question: What is the Hofmeister Series?

Engage: (15 mins)

1. Provide a predict, observe and explain (POE worksheet) to students and let them pay attention. With everyone's eyes on you ask the question " Predict what would happen to this egg white if I add this to this water in the beaker, and I heat it up?" Give the students a minute to write their prediction.

2. Ask a few students about their prediction, and then let them watch and observe as you mix the egg white in the beaker (hot plate, on low heat). Write down at least 2 observations and write a possible explanation on how or why this happened. Have students address the following prompts.
    - **Observation**: What changes did you see on the egg white, and the water on the beaker?
    - **Explanation:** How was the egg white affected by the temperature of the water in the beaker?
3. Have students list down a concept web with their elbow partner about what they know an egg white is made up of, and why or how it changed its appearance with temperature.
    - Ask the partners to come to a consensus on the different aspects and clarify through whole group discussion, as needed.

      Teacher Note: During this activity, student's prior knowledge of Biology concepts on macromolecules, such as proteins (polypeptides) and their structures, building blocks (amino acids), and denaturation is determined.

After the partners have shared to the whole class, the teacher shows the video clip

" Science reveals what happens when you boil an egg" (stop at 0.48 secs)
https://www.youtube.com/watch?v=3QHOnLh3ziQ

From the video clip they will be able to gather information, recall and write down important terms such as: denaturation, coiling, or uncoiling of proteins.

Explore:

1. Facilitate a discussion around the following prompt:

   What do you think would happen to the egg white if we heat it up in a beaker with water but this time there is salt in it?

   Write your hypothesis on the worksheet. Use the If…then….format.

2. Have students watch the video (start at 4:07- 4:56)
https://www.youtube.com/watch?v=AjYeJKVbnBc&feature=youtu.be on
" **The Hofmeister series and Thermoresponsive polymers** "

This video would give them a background on the principle behind the Hofmeister series and their ion- specific effects on proteins.

3. Check for understanding and explain further if students needed clarification.

Evaluate:

a. Let your students do the : **Sequencing anions : "Salt-out> Salt-in"** of the worksheet. Have them use the Hofmeister series to predict which ions will raise the " Cloud-point temperature or "Salt- in", which ions will lower the Cloud-point temperature or " Salt out".
b. Wrap up the lesson by letting students write in their own words, " How important is the Hofmeister series in solutions?
c. Check for understanding.

Announce to students: After exploring a little bit about the Hofmeister series you will work with your groups in Day 2 to gather data by performing a lab with different salts on egg whites and evaluate your results.

<u>Day 2-3:</u> Effect of Hofmeister Anions on Egg Whites (lab)

Advanced preparation is needed for Day 2 lab

Materials •

Per class

- 30 ml filtered egg white (prepared from 3-4 large fresh eggs)
- Refrigerator (to keep the egg white)
- Sodium chloride solution (5 M)
- Sodium Iodide solution (5 M) (prepare this if NaSCN is not available in your school)
- Sodium thiocyanate solution (5 M)

Prepare the following stock solutions using an analytical balance:

- **30 ml of filtered egg white:** Get 3-4 large fresh eggs, separate the egg whites from egg yolk, whisk the egg whites into a fine foam, filter, keep in a sealed container, and store in a refrigerator ready for the lab
- **20 ml of 5 M NaCl:** dissolve 5.844 g in 20 ml DI water
- **20 ml of 5 M NaI:** dissolve 14.9894 g in 20 ml DI water
- **20 ml of 5 M NaSCN:** dissolve 8.1072 g. **(it should be oven dried first for 13 hrs at 120^{o}C due to its hygroscopic property)** in 20 ml DI water.

Materials per lab group

- Three, 2 ml glass vials
- Deionized water
- Ice (crushed preferred)
- Thermometers or temperature probes
- Hot plate
- 100 ml beaker
- A piece of paper with black and white lines taped at the back of the beaker (optional)
- Iron stand and clamps to set-up and hold the thermometer as it is placed inside the vial
- Tongs

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 over heat closed containers.

 See SDS for full chemical information.

NaCl; Sodium Chloride - Skin irritant; Serious eye irritant; May cause respiratory irritation.[3]

NaI, Sodium Iodide-Very hazardous in case of ingestion. Hazardous in case of eye contact (irritant), of inhalation. Slightly hazardous in case of skin contact (irritant, permeator).[4]

NaSCN; Sodium Thiocyanate- Very hazardous in case of ingestion. Hazardous in case of skin contact (irritant), of eye contact (irritant), of inhalation. Slightly hazardous in case of skin contact.[5]

Effect of Hofmeister Anions on Egg Whites (lab)

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

Essential question: How does the identity and concentration of salts affect the temperature at which proteins precipitate out in solutions?

Engage: (5 min.)

1. Teacher will pass out cards with pictures of folded proteins, unfolded proteins and aggregated proteins, and will ask them to sequence the card which would be the protein structure at room

temperature, when heated and the final stabilized structure.
2. Teacher will walk around the room and have a casual conversation with students and ask why they arranged it the way they did. Allow the students to read the background information on the lab worksheet to obtain supporting information.
3. Before starting the lab, supplement students understanding on the structure of proteins and the effect of temperature.

Explore: (30 min.)

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

Evaluate: (5 min.)

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

Elaborate/ Explain: (10 min.)

1. Students refer to key terms and definitions to help them formulate their reasoning.
2. The teacher can make clarifying statements if students need some feedback.

Extend:

Salting-out has found widespread applications in different industries. Conduct an online research on an application of Hofmeister series and how it makes an impact in chemical and biological processes. Use the format below and submit in google classroom. It is upon the teacher's discretion to set the due date for this research assignment.

1. The name of application: ex Textile dyeing
2. The process.
3. The benefits or advantages of this process in the field of science.
4. Cite your sources.

Examples: Manufacturing process for dyes, textile dyeing, in the pharmaceutical industry, etc.

Evaluate;Assess

Teacher can use a quick informal assessment on the fourth day before proceeding to the next lesson to check for student's understanding of the Hofmeister series.

1. Facilitate a post- lab discussion regarding the data and results from the lab in the previous day.
   Ask the following questions:

- Which salt lowered the cloud-point temperature & why?
- Which salt raised the cloud-point temperature & why?
- Which salt had the ability to induce the denaturation of protein (from folded to unfolded state)?
- Which salt had the ability to stabilize the folded state the protein in egg white?
- Who confirmed or rejected his/her hypothesis? Why?

Teacher Resources:

The following related literatures on the Hofmeister series were useful resources in developing the lesson. It will be beneficial if the teacher read through them to get a substantial background information.

Jungwirth, Pavel, and Paul S. Cremer. **"Beyond Hofmeister."** Nature Chemistry 6.April 2014 (2014): 26163. Cremer Research Group Publications. Web. 9 July 2015. <http://sites.psu.edu/cremer/?page_id=26>.

Zhang, Y., & Cremer, P. S. (2009). **The inverse and direct Hofmeister series for lysozyme.** *Proceedings of the National Academy of Sciences of the United States of America*, *106*(36), 15249–15253. http://doi.org/10.1073/pnas.0907616106

Note to teacher: If NaSCN is not available in your school and you are using NaI or a substitute salt, please change the corresponding salt in your worksheet.

Student Resources:

- Hofmeister series
- Student worksheet # 1 -POE worksheet
- Stduent worksheet # 2 - Effect of Hofmeister Anions on Egg Whites (lab worksheet)

Key terms:

1. **Cloud-point temperature-** onset of light scattering which makes the solution turn cloudy or milky white.
2. **Salting-out -** when ions are excluded from the polymer surface resulting to decreased protein solubility.
3. **Salting- in -** when ions are bound tightly to the polymer surface resulting to increased protein 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].
6. **Low charge density ions-** bind water molecules weakly ; weakly hydrated ions[6].

Developed by Teacher's Name and Faculty's Name
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References:

1. Hofmeister, F Arch. Exp. Pathol. Pharmakol (Leipzig) 24, 247-260 (1888)

2. Hofmeister, F Arch. Exp. Pathol. Pharmakol (Leipzig) 25, 1-30, (1888)

3. http://www.sciencelab.com/msds.php?msdsId=9927593

4. http://www.sciencelab.com/msds.php?msdsId=9927270

5. http://www.sciencelab.com/msds.php?msdsId=9927605

6. Charge density-dependent strength of hydration and biological structure. Collins, K. D. (1997). Charge density-dependent strength of hydration and biological structure. Biophysical Journal, 72(1), 65–76.

7. Jungwirth, Pavel, and Paul S. Cremer. **"Beyond Hofmeister."** Nature Chemistry 6.April 2014 (2014): 26163. Cremer Research Group Publications. Web. 9 July 2015. <http://sites.psu.edu/cremer/?page_id=26>.

8**.** Zhang, Y., & Cremer, P. S. (2009). **The inverse and direct Hofmeister series for lysozyme.** *Proceedings of the National Academy of Sciences of the United States of America*, *106*(36), 15249–15253.

Student Worksheet #1

Chemistry
Name_____Period_____Date_____

Exploring the Hofmeister Series

Objective: Students will be able to arrange salts/ions in the order as to which would easily salt-out or salt-in with proteins.

Essential question: What is the Hofmeister Series?

A. Directions: 1.Write your prediction first. Your teacher will show a demo with egg whites placed on a beaker with water and heated up on a hot plate.

2. Observe what happens and write at least 2 things in the second column.

3. Write a possible explanation in the third column.

Predict	Observe	Explain
What would happen to the egg white when added to water and heated?	What changes did you see on the egg white, and the water on the beaker?	How was the egg white affected by the temperature of the water in the beaker?

B. Concept map. With a partner draw a concept map about: What makes up egg whites & why or how it changed its appearance with temperature.(**Hint**:Recall your knowledge on Macromolecules/Biology)

C. Science reveals what happens when you boil an egg :
https://www.youtube.com/watch?v=3QHOnLh3ziQ

In your own words, write what happens when you cook an egg_____

_____

D. **Brainstorm with your partner:** What would happen to egg white if you put it in a beaker with water , you heat it up but this time there is salt in it? Write you hypothesis below using "If….then…statement"

Hypothesis:

If_____then_____

_____

E. **Sequencing anions : "Salt-out> Salt-in"**

Have the students watch a video clip on: The Hofmeister series and Thermoresponsive polymers

https://www.youtube.com/watch?v=AjYeJKVbnBc&feature=youtu.be (start at 4:07-4:56)

Hofmeister series of anions: SO_4^{2-} >HPO_4^{2-} > acetate> Cl^- >NO_3^- > Br^- >ClO_3^- >I^- >ClO_4^- >SCN^-

Arrange the salts below in the order of which will easily salt-out or salt-in with polymers 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. $NaClO_4$

F. 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?

_____

_____

_____

_____

_____

Student Worksheet #1 (Teacher's guide)

Chemistry
Name_____Period_____Date_____

Exploring the Hofmeister Series

Objective: Students will be able to arrange salts/ions in the order as to which would easily salt-out or salt-in with proteins.

Essential question: What is the Hofmeister Series?

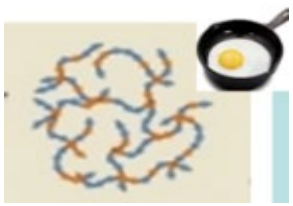
A. Directions: 1.Write your prediction first. Your teacher will show a demo with egg whites placed on a beaker with water and heated up on a hot plate.

2. Observe what happens and write at least 2 things in the second column.

3. Write a possible explanation in the third column.

Predict	Observe	Explain
What would happen to the egg white when added to water and heated ? Student's answers may vary: Mostly it would be based on prior knowledge of cooking eggs.	What changes did you see on the egg white, and the water on the beaker? The water turned cloudy; The egg whites come together as white solid (they clumped to each other.)	How was the liquid egg white affected by the temperature of the water in the beaker? At room temperature proteins are curled/folded at higher temperature they denature or uncurl/unfold.

B. Concept map. With a partner draw a concept map about: What makes up egg whites & why or how it changed its appearance with temperature.(**Hint**:Recall your knowledge on Macromolecules/Biology)

Egg-white proteins are long molecules, made up of chains of amino acids linked together. Students can draw a chain of polypeptide to represent proteins	In a raw egg, these proteins are curled and folded to form a compact ball. 	When you cook an egg, these proteins uncurl and form new bonds with one another. 

C. Science reveals what happens when you boil an egg :
https://www.youtube.com/watch?v=3QHOnLh3ziQ

In your own words, write what happens when you cook an egg: When you cook an egg, the protein structure changes from curled or folded to uncurl or unfolded which is called denaturation.

D. **Brainstorm with your partner:** What would happen to egg white if you put it in a beaker with water , you heat it up but this time there is salt in it? Write you hypothesis below using " If….then…statement"

Hypothesis:

If salt is added to egg white in water, mixed and heated up, t**hen** it would raise or lower the temperature at which the solution would turn cloudy.

E. **Sequencing anions : "Salt-out> Salt-in"**

Have the students watch a video clip on: The Hofmeister series and Thermoresponsive polymers

https://www.youtube.com/watch?v=AjYeJKVbnBc&feature=youtu.be (start at 4:07-4:56)

Hofmeister series of anions: **SO_4^{2-} >HPO_4^{2-} > acetate> Cl^- >NO_3^- > Br^- >ClO_3^- >I^- >ClO_4^- >SCN^-**

Arrange the salts below in the order of which will easily salt-out or salt-in with polymers 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. $NaClO_4$

F. 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 very important in knowing that different salts affect how proteins precipitate out in solutions. Macromolecule phase behavior are strongly influenced on the identity and concentration of the salts added in solution.

Student Worksheet #2

Chemistry
Name_____Period_____Date_____

Effect of Hofmeister Anions on Egg Whites (lab)

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

Essential question: How does the identity and concentration of salts affect the temperature at which proteins precipitate out in solutions?

Background:

Denaturation is the process by which protein molecules lose their native state by changing its viscosity or color through the application of heat or compounds such as acids, bases, salts or organic solvents. At room and physiological temperatures proteins are folded, and at high temperatures they denature. The temperature at which this change occurs can be strongly influenced by the addition of salts such as Na_2SO_4, NaCl, and NaSCN. Different anions lower or raise the precipitation 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 macromolecules such as polypeptides or proteins. Anions appear to have a larger effect than cations,[1] and are usually ordered : F- S04 ^{2}- > HP042- > acetate> Cl-> N03- >Br- > Cl03- > I->Cl04-> SCN-
Early members of the series have high charge density which tends to increase solvent surface tension and cause nonpolar molecules to collapse in itself ("salting-out"), in effect they strengthen the hydrophobic interaction which decreases protein solubility. By contrast, later salts in the series have low charge density which makes the ions bind closely to the protein 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 macromolecules, particularly proteins in egg whites.

Key terms:

1. **Cloud-point temperature-** onset of light scattering which makes the solution turn cloudy or milky white.
2. **Salting-out -** when ions are excluded from the polymer surface resulting to decreased protein solubility.
3. **Salting- in -** when ions are bound tightly to the polymer surface resulting to increased protein 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[2].

6. **Low charge density ions-** bind water molecules weakly; weakly hydrated ions [2].

Materials for each lab group:

- Three 2 ml glass vials
  Vial #1 (1 ml DI water + 1 ml filtered egg white)
  Vial #2 (1 ml 5 M NaCl + 1 ml filtered egg white)
  Vial #3 (1 ml 5 M NaSCN + 1 ml filtered egg white)

- Deionized water
- Ice (crushed preferred)
- Thermometers or temperature probes
- Hot plate
- 100 ml beaker
- A piece of paper with black and white lines taped at the back of the beaker (optional)
- Iron stand and clamps to set-up and hold the thermometer as it is placed inside the vial
- Tongs

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 over heat closed containers.

I. Hypothesis

Write a hypothesis about which salt (NaCl, or NaSCN) could raise or lower the temperature of egg whites in solution the most. Use the " if….then…format.

If _____ then_____
_____.

II. Procedure

1. Wear safety glasses and gloves, and get the lab materials for your group. Do not open the vials unless told by your teacher.

2. Fill the 100 ml beaker with approximately 75 ml of tap water. Prepare the set- up shown in Fig 1 using iron clamps to place the vial inside the beaker with water and a thermometer placed inside the vial.

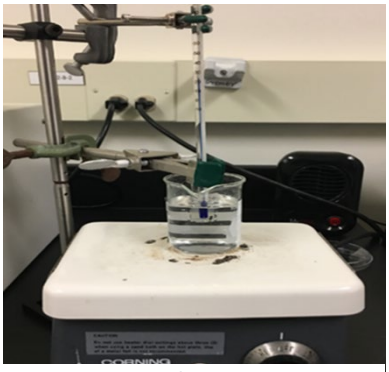


Figure 1 Hot plate set-up

3. Make sure the solution is evenly mixed by carefully swirling the vial before you clamp it inside the water bath. Place the thermometer inside the vial making sure the probe is not touching the bottom. Turn the hot plate into a low heat setting.

4. You will record the **cloud-point temperature**, in your data table. Note the temperature when the solution in the vial **starts** to get cloudy (not just portion of it but the whole solution in the vial). This is the onset of scattering of light which is indicated by cloudy or milky white solution. Turn off the hot plate.

5. You will repeat this procedure for all 3 vials:

   - Vial #1 (1 ml DI water + 1 ml filtered egg white),
   - Vial #2 (1 ml 5M NaCl solution + 1 ml filtered egg white),
   - Vial #3 (1 ml 5 M NaSCN solution + 1 ml filtered egg white).

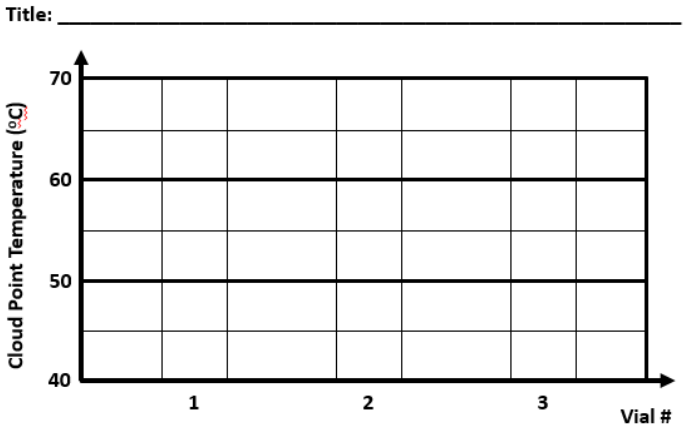
6. When you are about to do the next vial, make sure you dump off half of the hot water from your beaker an replace it with cold water or ice to cool down the water temperature. You will then re-heat the hot plate to a low setting.

7. After the cloud-point temperatures have been recorded for all 3 vials, clean up, return the materials, except the hot plate. Allow time for the hot plate to cool before putting away.

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

Vial #	Species	Cloud-point Temperature (°C)	ΔT
1	1 ml DI water + 1 ml egg white		
2	1 ml 5 M NaCl + 1 ml egg white		
3	1 ml 5 M NaSCN + 1 ml egg white		

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



V. Draw conclusion

1. From your data and graph above, compare which of the solutions from the three vials raised or lowered the temperature to cause proteins in egg white to precipitate out in solution, resulting to a cloud point._____
   _____

2. How do your results for Vial # 2 and # 3 compare with vial #1 without salt in it? Refer to the change in temperature (ΔT) from the data table to support your answer.

   _____
   _____
   _____
   _____

3. How does the effect caused by thiocyanate ion compare to the effect caused by chloride ion?
   _____
   _____
   _____

4. Briefly describe how the chemical identity of the salt impacts the behavior of proteins to precipitate 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.
   _____
   _____
   _____
   _____
   _____

5. **Confirm or Reject your Hypothesis:** _____
   _____

Checking for Understanding:

a. **Circle one**: Which salt had the ability to induce the denaturation of the protein in egg white that lowered the cloud-point temperature?(NaCl, or NaSCN)

b. **Circle one**: Which salt had the ability to stabilize the folded state of the protein in egg white that caused to raise the cloud-point temperature?
(NaCl, or NaSCN)

Extension: Salting-out has found widespread applications in different industries. Conduct an online research on an application of Hofmeister series and how it makes an impact in chemical and biological processes. Use the format below and submit in google classroom**.**

1. The name of application; ex Textile dyeing
2. The process.
3. The benefits/advantages of this process in human life in general.
4. Cite your references

References:

1. Hofmeister, F Arch. Exp. Pathol. Pharmakol (Leipzig) 24, 25; 1-30; 247-260 (1888)

2. Charge density-dependent strength of hydration and biological structure. Collins, K. D. (1997). Charge density-dependent strength of hydration and biological structure. Biophysical Journal, 72(1), 65–76.

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Student Worksheet #2 (Teacher's guide)

Chemistry
Name_____Period_____Date_____

Effect of Hofmeister Anions on Egg Whites (lab)

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

Essential question: How does the identity and concentration of salts affect the temperature at which proteins precipitate out in solutions?

Background:

Denaturation is the process by which protein molecules lose their native state by changing its viscosity or color through the application of heat or compounds such as acids, bases, salts or organic solvents. At room and physiological temperatures proteins are folded, and at high temperatures they denature. The temperature at which this change occurs can be strongly influenced by the addition of salts such as Na_2SO_4, NaCl, and NaSCN. Different anions lower or raise the precipitation 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 macromolecules such as polypeptides or proteins. Anions appear to have a larger effect than cations,[3] and are usually ordered : F- S04 ^{2}- > HP042- > acetate> Cl-> N03- >Br- > Cl03- > I->Cl04-> SCN-
Early members of the series have high charge density which tends to increase solvent surface tension and cause nonpolar molecules to collapse in itself ("salting-out"), in effect they strengthen the hydrophobic interaction which decreases protein solubility. By contrast, later salts in the series have low charge density which makes the ions bind closely to the protein 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 macromolecules, particularly proteins in egg whites.

Key terms:

Cloud-point temperature- onset of light scattering which makes the solution turn cloudy or milky white.

2. **Salting-out -** when ions are excluded from the polymer surface resulting to decreased protein solubility.
3. **Salting- in -** when ions are bound tightly to the polymer surface resulting to increased protein 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 [2].

6. **Low charge density ions-** bind water molecules weakly; weakly hydrated ions [2].

Materials for each lab group:

- Three, 2 ml glass vials
  Vial #1 (1 ml DI water + 1 ml filtered egg white)
  Vial #2 (1 ml 5 M NaCl + 1 ml filtered egg white)
  Vial #3 (1 ml 5 M NaSCN + 1 ml filtered egg white)

- Deionized water
- Ice (crushed preferred)
- Thermometers or temperature probes
- Hot plate
- 100 ml beaker
- A piece of paper with black and white lines taped at the back of the beaker (optional)
- Iron stand and clamps to set-up and hold the thermometer as it is placed inside the vial
- Tongs

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 over heat closed containers.

I. Hypothesis

Write a hypothesis about which salt (NaCl, or NaSCN) could raise or lower the temperature of egg whites in solution the most. Use the " if….then…format.

Students write a hypothesis and choose one from the salts above as to which they think would raise or lower the temperature of egg whites in solution.

II. Procedure

1. Wear safety glasses and gloves, and get the lab materials for your group. Do not open the vials unless told by your teacher.

2. Fill the 100 ml beaker with approximately 75 ml of tap water. Prepare the set- up shown in Fig 1 using iron clamps to place the vial inside the beaker with water and a thermometer placed inside the vial.

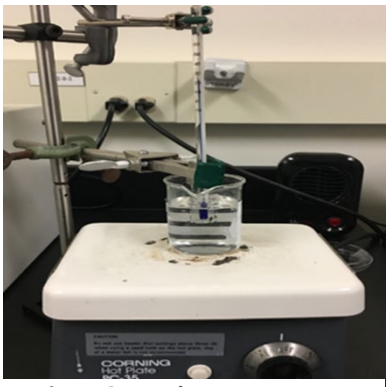


Figure 2 Hot plate set-up

3. Make sure the solution is evenly mixed by carefully swirling the vial before you clamp it inside the water bath. Place the thermometer inside the vial making sure the probe is not touching the bottom. Turn the hot plate into a low heat setting.

4. You will record the **cloud-point temperature**, in your data table. Note the temperature when the solution in the vial **starts** to get cloudy (not just portion of it but the whole solution in the vial). This is the onset of scattering of light which is indicated by cloudy or milky white solution. Turn off the hot plate.

5. You will repeat this procedure for all 3 vials:

- Vial #1 (1 ml DI water + 1 ml filtered egg white),
- Vial #2 (1 ml 5M NaCl solution + 1 ml filtered egg white),
- Vial #3 (1 ml 5 M NaSCN solution + 1 ml filtered egg white).

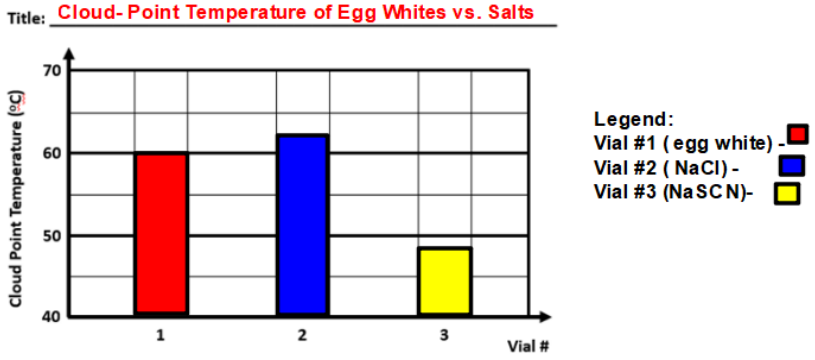
6. When you are about to do the next vial, make sure you dump off half of the hot water from your beaker an replace it with cold water or ice to cool down the water temperature. You will then re-heat the hot plate to a low setting.

7. After the cloud-point temperatures have been recorded for all 3 vials, clean up, return the materials, except the hot plate. Allow time for the hot plate to cool before putting away.

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

Vial #	Species	Cloud-point Temperature (°C)	ΔT
1	1 ml DI water + 1 ml egg white	Temp ranges from 56.5 to 60	
2	1 ml 5 M NaCl + 1 ml egg white	62 to 63.5	Vial 2 temp- Vial 1 temp
3	1 ml 5 M NaSCN + 1 ml egg white	46 to 48	Vial 3 temp- Vial 1 temp

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



7. Draw conclusion

1. From your data and graph above, compare which of the solutions from the three vials raised or lowered the temperature to cause proteins in egg white to precipitate out in solution, resulting to a cloud point. NaCl solution raised the temperature for the solution to get cloudy , while NaSCN lowered the temperature for the solution to get cloudy.

2. How do your results for Vial # 2, & #3 compare with vial #1 without salt in it? Refer to the change in temperature (ΔT) from the data table to support your answer.

   The egg white with DI water (which is the control vial) reached around 60 degrees (or whatever temp your students got from the experiment) before the solution turned cloudy, but the one with NaCl was way higher than the temperature obtained in the control vial , while the one with NaSCN went lower in temperature compared to the control vial.

3. How does the effect caused by thiocyanate ion compare to the effect caused by chloride ion?
   The SCN^- being a weakly hydrated ion binds with the protein molecule of the egg white which induces denaturation, while the Cl^- which is a strongly hydrated ion remains hydrated and stabilizes the proteins folded state.

4. Briefly describe how the chemical identity of the salt impacts the behavior of proteins to precipitate 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 proteins in solution. SCN^- ions have low charge density, which makes them bind directly with the polymer which induces its

unfolding thereby reaching its cloud-point. On the other hand, high- charge density ions, like Cl^- remain hydrated and are excluded from the polymer which results in stabilizing the folded state thus requiring higher temperature (to unfold the proteins) before reaching a cloud-point.

5. **Confirm or Reject your Hypothesis:**
   Students either confirm or reject their hypothesis and state the reason why.

Checking for Understanding:

c. **Circle one**: Which salt had the ability to induce the denaturation of the protein in egg white that it lowered the cloud-point temperature?(NaCl, or **NaSCN)**

d. **Circle one**: Which salt had the ability to stabilize the folded state of the protein in egg white that caused to raise the temperature for the solution to turn cloudy?
(**NaCl**, or NaSCN)

Extension: Salting-out has found widespread applications in different industries. Conduct an online research on an application of Hofmeister series and how it makes an impact in chemical and biological processes. Use the format below and submit in google classroom**.**

1. The name of application; ex Textile dyeing
2. The process.
3. The benefits/advantages of this process in human life in general.
4. Cite your references

Students could come up with different applications of " Salting-out" process based on their online research. The teacher can come up with a rubric to set clear grading expectations to students.