Separation Chemistry

Subject Area(s): Chemistry

Associated Unit: Unit 6

Lesson Title: Relating Separation Chemistry by Precipitation and Temperature Dependent Phase Transitioning Proteins

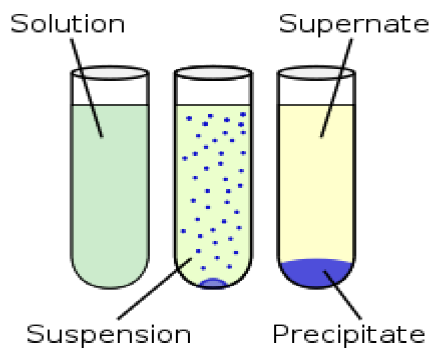


Image 1
ADA Description: Chemical precipitation diagram showing three separate vials displaying solution, suspension and precipitate
Source/Rights: Wikipedia/Public Domain
Caption: Figure 1. Precipitation from Solution

Grade Level: 11-12

Time Required: 50 min

Groups: 2-4 students

Summary

Students will investigate reactions in aqueous solutions by mixing ionic solutions and determine if the reactants formed a precipitate from solution. They will then write complete ionic equations for the precipitation reactions that occurred. Students will also investigate the basics of temperature dependent phase transitioning peptides and how they relate to precipitates and their uses for purifying proteins.

Engineering Connection

Precipitation reactions are something that engineers use quite frequently. Precipitation reactions can be used for making pigments, removing salts from water in water treatment, and in classical qualitative inorganic analysis. Processes of precipitation can form nanoparticles which have a wide variety of engineering applications. Precipitation reactions are useful for finding out whether or not a certain element is present in a solution. If one knows that a precipitate is formed when a chemical reacts to form a precipitate with lead, for example, one could test for lead in water sources by seeing if a precipitate forms. In addition, precipitation reactions can be used to extract elements, such as magnesium from seawater. Biomedical engineers can use genetically designed proteins that can easily be purified using the Inverse Transition Cycle method which takes advantage of the protein's phase transition temperature.

Engineering Category =

Choose the category that best describes this lesson's amount/depth of engineering content:

1. Relating science and/or math concept(s) to engineering

Keywords

Precipitate, ionic (cation and anion), pellet, solute, supernatant, solution, phase transition, hydrophobic, hydrophilic

Educational Standards (List 2-4)

State STEM Standard (required)

Teach Engineering, Florida: Science, 2008, Standard 8: Matter, 9-12, Characterize types of chemical reactions, for example: redox, acid-base, synthesis, and single and double replacement reactions.

Source, year, standard number(s)/letter(s), grade band and text (its unique ID# is optional)

ITEEA Standard (required)

Teach Engineering, International Technology and Engineering Educators Association: Technology [2000] Standard 15. Students will develop an understanding of and be able to select and use agricultural and related biotechnologies

NGSS Standard (strongly recommended)

Teach Engineering, Next Generation Science Standards: Science, 2013, Matter and Its Interactions

Pre-Requisite Knowledge

-Understand definitions of pellet, supernatant, solute, cation, anion and solution

-Complete ionic equation, net ionic equation

-General rules for solubility of ionic compounds

-Basic understanding of hydrophobicity and hydrophilicity

Learning Objectives

After this lesson, students should be able to:

- **Learn how precipitation reactions can be used as separators and purifiers**
- **Predict precipitation reactants products based on rules of solubility**
- **Balance complete and net ionic equations**
- **Make predictions of metal ions present in solution**

Introduction / Motivation (5E – Engage)

Precipitation reactions are fundamental in chemistry and taught in every high school and college level chemistry course. In this lesson you will examine how precipitation reactions and protein phase transitioning are used in separation chemistry. Both precipitation reactions and protein phase transitioning cause the solute to form a solid and "fall out" of solution. The purpose of this lesson is to have you recognize a couple different methods of purifying solutions and explore quantitative analysis of unknown element in solution. You will also be introduced to different ideas of the benefits of precipitation methods.

Lesson Background & Concepts for Teachers (5E – Explain)

See Handout; Separation Reactions (lab)

IMPORTANT: The Elastin-Like Polypeptide can be obtained for the instructors use by contacting Dr. Piyush Koria at the University of South Florida, Chemical and Biomedical Engineering Department. Please allow 2-3 weeks advanced notice if requesting ELP from his lab.

Besides ionic precipitation reactions used for separation and analysis, students will also discover that a non-ionic compound can precipitate or "fall out" of solution at a dependent temperature or ion concentration. At laboratories at the University of South Florida researchers are working with peptides that demonstrate this quality.

The peptide is Elastin-Like Polypeptides (ELP's), they are:

•Have phase transition that is entropically driven

•Soluble below transition temperature

•Insoluble above transition temperature

•ELP's purified rapidly using ITC method

•Increase cellular internalization of protein when the ELP peptide is fused

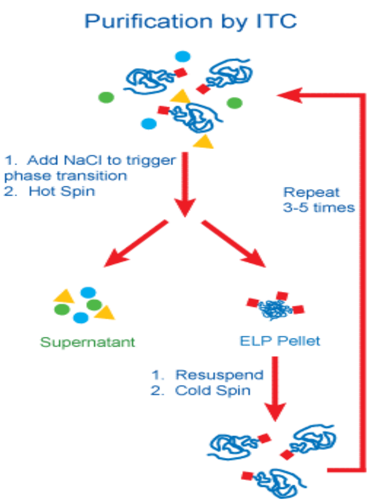


Figure 2

ADA Description: Diagram depicting purification process of ELP by Inverse Transition Cycling

Caption: Figure 2. ITC

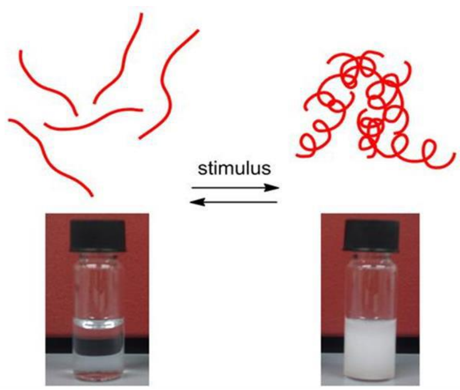


Figure 3

ADA Description: depiction of the phase transition of Elastin-Like Polypeptides

Caption: Figure 3. ELP Phase Transitioning

Vocabulary / Definitions

Word	Definition
Supernatant	denoting the liquid lying above a solid residue after crystallization, precipitation, centrifugation, or other process
Solute	The dissolved substance in a solution
Precipitate	cause (a substance) to be deposited in solid form from a solution
Ion	An ion is an atom or molecule in which the total number of electrons is not equal to the total number of protons, giving the atom a net positive or negative electrical charge.

Hydrophobic	Water repelling, lacking affinity to water
Hydrophilic	Water loving, affinity to water

Associated Activities (5E – Explore)

http://www.teachengineering.org/view_activity.php?url=collection/mis_/activities/mis_pharma/mis_pharma_lesson01_activity1.xml

Hands-on Activity: If You're Not Part of the Solution, You're Part of the Precipitate! Contributed by: Bio-Inspired Technology and Systems (BITS) RET, College of Engineering, Michigan State University

Assessment (5E – Evaluate)

Pre-Lesson Assessment

Descriptive Title: Understanding of precipitation reactions, net ionic equations, complete ionic equations and spectator ions to be determined by the student's teacher. Teachers should give classroom instruction on these concepts prior to exercising the Separations Chemistry lab. For phase transitioning proteins please have students read the Separations Chemistry lab background and read the additional handout, ITC made easy.

Post-Introduction Assessment

Record observations from Separation Chemistry Lab and complete net ionic equations for each reaction, see lab Separation Chemistry, Relating Separation Chemistry by Precipitation and Temperature Dependent Phase Transitioning Proteins

Lesson Summary Assessment

Descriptive Title: Extended learning questions at the end of the Separation Chemistry lab

Homework

None

References

1) Piyush Koria, Hiroshi Yagi, Yuko Kitagawa, Zaki Megeed, Yaakov Nahmias, Robert Sheridan, and Martin L. Yarmush. "Self-assembling elastin-like peptides growth factor chimeric nanoparticles for the treatment of chronic wounds.", PNAS 2011, 108, 1034-1039

Attachments

1) Separations Chemistry Lab: Relating Separation Chemistry by Precipitation and Temperature Dependent Phase Transitioning Proteins
2) ITC Made Easy handout

Contributors

Steven Sanden

Supporting Program

The Research Experience for Teachers (RET) at the Functional Materials Research Institute at USF

Classroom Testing Information

Attachments

Separation Chemistry Lab
Grade 11-12

Relating Separation Chemistry by Precipitation and Temperature Dependent Phase Transitioning Proteins

Purpose: To determine which combinations of ions form water-insoluble precipitates and investigate the temperature dependent phase transitioning protein Elastin-Like Polypeptide (ELP) and relate each method to purifying solute or protein from a solution.

Learning objectives:

1) Classify ions as cations or anions in an ionic solution and determine the precipitate based on the rules of solubility.
2) Understand how precipitates are used in separation chemistry.
3) Become familiarized with protein purification using the temperature dependent behavior of Elastin-Like Polypeptides (ELPs).

Background: In chemistry and chemical engineering the separation process is a method that converts a mixture of substances into two or more distinct product mixtures. In some cases, a separation may fully divide the mixture into its pure constituents. One of many methods used for the separation process is separation by precipitation. Precipitation is the creation of a solid in a solution or inside another solid during a chemical reaction or by diffusion in a solid. When the reaction occurs in a liquid solution, the solid formed is called the precipitate. The precipitate can be then separated from solution by many means. Some examples are by filtration, centrifuge, distillation and evaporation.

A mixture of metal ions in a solution can be separated by precipitation with anions such as Cl^-, Br^-, SO_4^{2-}, CO_3^{2-}, S^{2-}, $Cr_2O_4^{2-}$, PO_4^{2-}, OH^- etc. When a metal ion or a group of metal ions form insoluble salts with a particular anion, they can be separated from others by precipitation. We can also separate the anions by precipitating them with appropriate metal ions.

Elastin-Like Polypeptides (ELP) will undergo a phase transition by varying temperature, ionic strength of the solution, and by varying pH levels. (Important: phase transitioning here refers to the ELP changing its shape (conformation) by becoming mostly linear or tangled). At higher temperatures ELPs will consolidate to form a nanostructure that will form a solid that is visible in solution. ELPs are composed of tandemly repeated blocks of Val-Pro-Gly-X-Gly and are protein-based polymers which are thermoresponsive and whose solubility changes at a particular temperature called transition temperature. Elastin-Like Polypeptides are genetically designed molecules that can be tailored and fused to other proteins using a technique called ligation transformation. (Ligation transformation is a way that bioengineers use bacteria to produce a desired protein amass)

After removing the lysing bacterial cells and removing the large particles from solution the proteins can then be easily be purified using a method called Inverse Transition Cycle (ITC). The ITC method takes advantage of the protein's phase transitioning at temperature by centrifuging the ELP at above or below transition temperature. When centrifuging the ELP and colder temperatures the ELP's will remain in the supernatant but when centrifuging at higher temperatures the ELP will become a solid and form a pellet. By cycling the protein fused ELP between cold and hot centrifuging the protein can be easily purified. (additional handout that depicts the ITC method, *ITC made easy*)

In the first part of the experiment you will mix pairs of different ionic solutions in all combinations to determine which pairs result in precipitate formation. Based on your results you will determine the reactions that have taken place and write net ionic equations for each.

In the second part of the experiment you will dissolve the protein ELP in solution and slowly increase the temperature of the solution until the solution becomes white and cloudy. Once the solution becomes cloudy and the color turns white the ELP has precipitated from the solute. The ELP transition is reversible and can be put back into solution by cooling the temperature solute back down.

Materials per group:

- Safety googles
- Well plate or small test tubes (the ELP will best be visible in test tubes)
- Glass stirring rod
- Plastic wash bottle
- Distilled water
- Pipets
- 1X PBS buffer

SAFETY FIRST!!

In this lab the solutions you use may contain harmful materials. Never touch any of the chemicals. In the event of a spill, inform your teacher immediately. Observe all precautions, especially the ones listed below.

- Wear your safety googles (all steps)
- Return or dispose of all materials according to the instructions of your teacher.
- Wash your hands thoroughly after completing this experiment.
- **Caution:** Aluminum chloride is an irritant. Avoid skin contact.
- **Caution:** Hydroxides are corrosive. Avoid contact with these chemicals and wash your hands thoroughly after use.
- For all other safety concerns, refer to the MSDS for the chemicals used.

Experiment 1
Ionic Solutions

1. 0.1M Aluminum Sulfate, $Al_2(SO_4)_2$ + 0.1M Sodium Hydroxide, NaOH

2. 0.1M Iron(III) Chloride, $FeCl_3$+ 0.1M Potassium Hydroxide, KOH

3. 0.1M Silver Nitrate, $AgNO_3$ + 0.1M Sodium Chloride, NaCl
   -

Procedure: Place metal ionic solutions into individual wells on a well plate or test tube. Drop wise, add salt solutions and mix. Precipitate should form. Create a table in your lab notebook and record your observations. Write complete ionic equation, identify the spectator ions then write a balanced net ionic equation for the reactions in your notebook.

Example table:

Solutions	Did Precipitate Form	Color of Solution after Mixing	Complete ionic Equation	Spectator Ions	Net Ionic Equation
$Al_2(SO_4)_2$ + NaOH					

Experiment 2
Phase Transitioning Protein ELP

In a clear test tube pipet 1mL of 20^{o}C 1X PBS buffer. Add 1mg of ELP protein to PBS buffer and mix until clear. Prepare a water bath in a 100mL beaker by adding 50mL of water to the beaker and placing the beaker on a hot plate. Once the temperature reaches 37^{o}C maintain temperature and place the test tube in the water bath for 1 to 2 minutes or until the water becomes white and cloudy. At this point the protein ELP has undergone a phase transition and has precipitated from solution. Record your observations in your lab notebook. After you record your results place the test tube on ice until the solution becomes clear. Now the protein has reversed its transition and has become solute again. DO NOT DISCARD ELP SOLUTION. THE SOLUTION IS REUSABLE.

Extended learning: Group learning

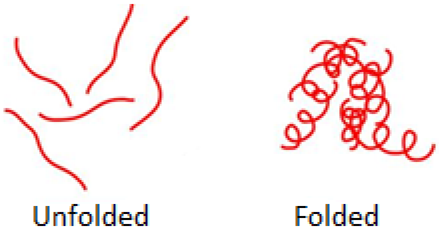
1) If you did not know the initial states of the solutions (what elements where present), based on the reactions that occurred, what can be determined about the initial states of the solutions?

2) Given the observed precipitation reactions, could you theoretically remove all the metals from a solution? And could you also determine how much metal was initially present and how?

3) Based on your knowledge of hydrophobicity and hydrophilicity, what could you predict as to why the ELP is “falling out” of solution at higher temperatures?

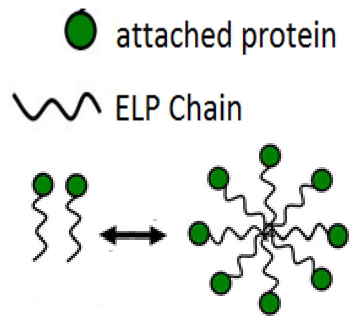
ITC Made Easy

Inverse Phase Transitioning (ITC) takes advantage of protein's ability to be unfolded (linear) or folded (nonlinear) when in a solution. As an example, if you had a string and you stretched the string out straight, this would be linear. Now if you took the string and balled it up with your fingers, this would be nonlinear or in the protein's case, it would be folded.



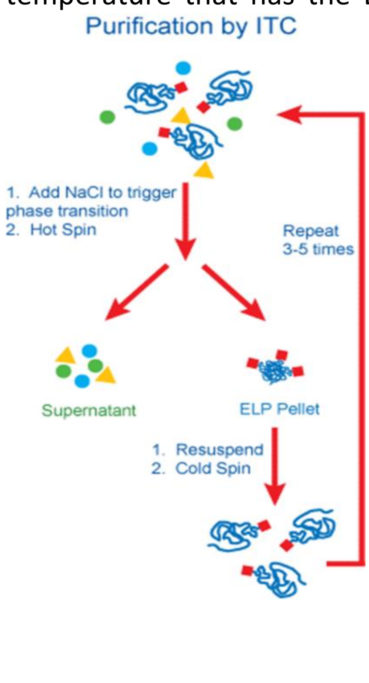
Researchers have taken advantage of this folding and unfolding and designed a protein so that when it is unfolded in a solution, it is solute, meaning that the protein is suspended in solution. But when the protein is folded it drops out of suspension and becomes insoluble.

One protein that researchers have designed is called an Elastin-Like Polypeptide (ELP for short). ELP's can fold and unfold by varying the temperature, pH or ionic strength of the solution that they are suspended in. One of the big advantages of this is that researchers can fuse other proteins to the ELP and use the polypeptides folding and unfolding properties to purify the protein from an impure solution.



Here's how it works:

When the ELP is in an impure solution it can be centrifuged (spun at high RPM) at a temperature that has the ELP unfolded (below the transition temperature). By spinning the solution with the protein unfolded in suspension all of the heavy material that is in the solution with the ELP will fall to the bottom and form a solid pellet. Now you can collect the supernatant and get rid of the pellet.



But we're not done yet, there are still impurities in the supernatant because we got rid of the heavier material but there are lighter materials that still need removed. Now we can heat it up! And by heating the supernatant up the ELP will fold up and become a precipitate. The solution is then centrifuged and now the only material falling to the bottom is the ELP. The pellet can be collected and the supernatant discarded. Do this process a couple more times and as simple as that we have our pure ELP or protein.