Video Article

Separation of Mixtures

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Abstract

Most samples of interest are mixtures of many different components. Sample preparation, a key step in the analytical process, removes interferences that may affect the analysis. As such, developing separation techniques is an important endeavor not just in academia, but also in industry.

One way to separate mixtures is to use their solubility properties. In this short paper, we will deal with aqueous solutions. The solubility of a compound of interest depends on (1) ionic strength of solution, (2) pH and (3) temperature. By manipulating with these three factors, a condition in which the compound is insoluble can be used to remove the compound of interest from the rest of the sample.[1]

Introduction

A number of parameters can be used to separate a sample of interest from impurities by reducing its solubility, and removing it from a solution as a solid, as shown in **Figure 1**. First, changing the ionic strength of the solution can change a substances solubility. This often involves the addition of extra salt (also called salting out), or the addition of a counter-ion, which forms a less soluble species with the compound of interest.[2]

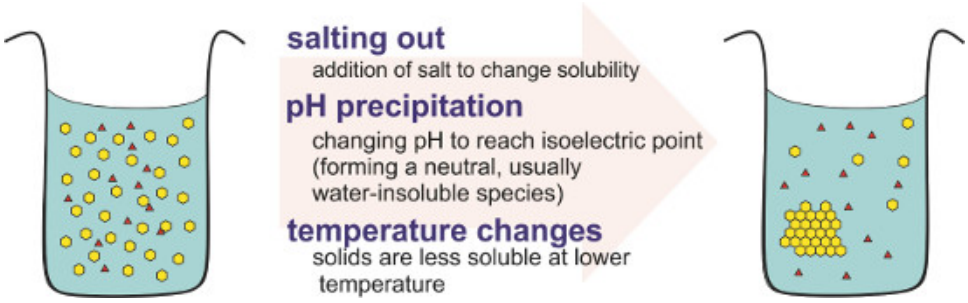

Figure 1. Solubility equilibria are affected by ionic strength, pH, and temperature. A compound of interest (yellow) is separated from impurities (red) by changing its solubility in a given solvent.

Changing the pH of a solution may change the net charge of the compound. At a certain pH, the net charge becomes zero (also called isoelectric point) and the compound becomes less soluble in water, eventually forming a solid. Temperature also affects solubility, as higher temperature increases solubility of solids.

The rate of solid formation determines relative purity (**Figure 2**). In general, the term precipitation refers to the formation of a solid at a rapid pace, thereby producing an amorphous sample with some impurities trapped within. This is common in salting out and pH change-induced processes. When this process is slowed down, the impurities are not trapped within the compound and a relatively pure solid is produced. This technique is employed in recrystallization. In this process, a compound is dissolved in enough solvent to be just at the saturation point at an elevated temperature. This saturated solution is then allowed to cool down slowly. As the solution cools, the solubility of the component decreases, and the compound in excess of the solubility forms a well-ordered solid (otherwise known as crystals) instead of an amorphous solid. Impurities in the solution do not get trapped as the slow process allows the removal of these impurities at the surface of the solid before they are trapped.[1]

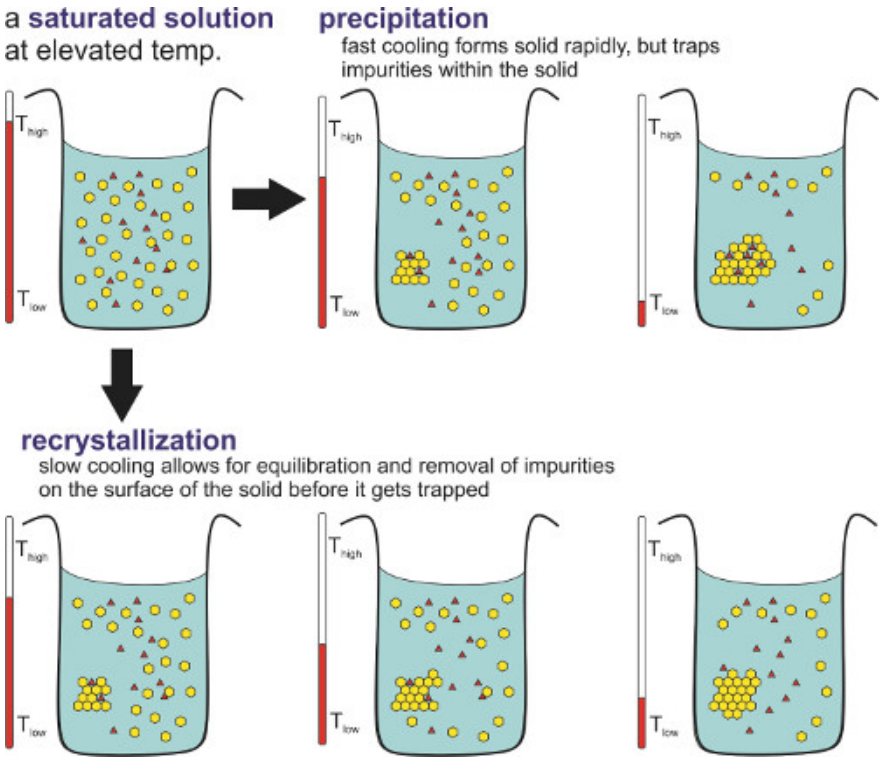

Figure 2. Difference between precipitation and recrystallization.

Once the solid has formed (whether as a crystal or as a precipitate), it should be separated from the rest of the mixture. Filtration is one way to separate them. This employs a porous material which selectively inhibits the passage of the solid material but not the solution.

Centrifugation is another way to separate the precipitate from the rest of the mixture. Centrifugation uses centripetal acceleration to separate mixtures based on their densities. Since solid is denser than the aqueous solution, the solid sediments at the bottom of the container. The solid is also called the pellet and the aqueous solution, the supernatant. The supernatant can then be decanted or extracted using a pipet or syringe. Crystals are fragile and centrifugation is often not employed to separate them from the solution.

This video will cover different methods of separating compounds through solid formation (salting out, pH changes, and recrystallization) and their subsequent removal from the aqueous solution through filtration or centrifugation

Protocol

1. Precipitation of $CaCO_3$

1. Prepare 5 mL of 1M $CaCl_2$.
2. Prepare 5 mL 1M Na_2CO_3.
3. In a small centrifuge tube (1.5 mL), add 750 µL of $CaCl_2$ and 750 µL of Na_2CO_3.
4. Wait 2 min for the reaction to occur. The solution should turn cloudy.
5. Centrifuge the mixture at 10,000 × g for 5 min.
6. Decant the supernatant.
7. Add 1 mL of cold water to the pellet.
8. Resuspend the pellet by mixing in a vortex mixer for 10 s.
9. Centrifuge the mixture at 10,000 × g for 5 min.
10. Decant the supernatant.

2. Precipitation of Milk Proteins

1. Pour the milk in a beaker and add a stir bar.
2. Warm milk gently until 40 °C in a stirring hot plate. Do not heat over 40 °C.
3. Prepare a 15% (v/v) of acetic acid by mixing 7.5 mL of acetic acid and diluting in enough water to reach 50 mL.
4. Immerse the electrode of a pH meter in warm milk, and monitor the pH.
5. Add the acetic acid drop-wise to the milk until a pH of 4.6 is reached.
6. Filtration of Milk
    1. Flute a piece of filter paper and place it in a funnel.

2. Place the funnel in an flask, and pour the acidified milk solution into the funnel.
3. As the solution is poured, the filter paper may get clogged. Using a stirring rod, agitate the solution and filter paper occasionally to unclog. If it does not improve the passage of solution, change the filter paper.
4. Place a new filter paper on the bench top and transfer as much of the wet solid to the new filter paper. This should absorb more water from the solid.
5. If the new filter paper gets too wet, continue to change it until there is minimal amount of wetness on the filter paper. Press it lightly to absorb more water if needed.
6. Take the dried solid and re-suspend in about 70% ethanol. Filter the solid again following steps 2.6.1 to 2.6.5.

7. Centrifugation of Milk (as an alternative to filtration)
   1. Transfer 50-mL portions of the mixture to 50-mL centrifuge tubes.
   2. Centrifuge at 4,500 × g for 10 min, then decant the supernatant.
   3. Add 50 mL 70% ethanol to the pellet.
   4. Using a stirring rod, resuspend the pellet in the ethanol.
   5. Centrifuge this suspension following step 2.7.2.

8. Resuspend the pellet in buffer for further analysis such as SDS-PAGE, otherwise store it at 4°C.

3. Recrystallization of KCl

1. Weigh 50 g of KCl in an Erlenmeyer flask, and add 100 mL of water
2. Heat the mixture until water boils. Make sure all KCl powder is dissolved. Some impurities may not dissolve in water.
3. Heat another (empty) Erlenmeyer flask along with the mixture and keep it very warm.
4. Place a funnel with filter paper in the warm, empty flask.
5. Pour the solution through the filter paper to remove undissolved impurities. The receiving flask is kept warm to make sure no temperature changes occur during the filtration, otherwise a crude precipitate will form. If that happens, re-heat the mixture until all the precipitate dissolves.
6. Remove the flask with the solution from heat.
7. Keep it in a cool place in the room and let it cool down slowly for about 30 min., or until it is no longer warm to touch.
8. Once cooled down to room temperature, place the flask in an ice bath to further lower the temperature. Alternatively, one can leave the flask inside the fridge or a temperature-controlled room at 4 °C.
9. Crystals can be harvested by filtering as in steps 3.4-3.5 (use a flask and funnel at room temperature).

Representative Results

Solubility equilibria is employed in many purification processes. Calcium can be removed from water using sodium carbonate. The solubility product (K_{sp}) of $CaCO_3$ is 4.8×10^{-9}. Mixing 1M of $CaCl_2$ and 1M of Na_2CO_3 produced $CaCO_3$ precipitate. The precipitate was separated from the rest of the solution using centrifugation.

Casein (a key protein in milk) has an isoelectric point at pH 4.6 and formed insoluble curds at this pH. The curds were then separated from the rest of the solution (also called whey) using either filtration or centrifugation (**Figure 3a**). The curd was washed with ethanol to remove phospholipids and other water-soluble compounds that were also trapped in the curd. Centrifugation prevented loss of proteins better than filtration as there were some proteins that stuck to the filter paper. The separated components were analyzed using SDS-PAGE (**Figure 3b**), showing that the precipitation reaction separated most of the casein from the whey. Other milk proteins, such as globulins, precipitate together with casein. Further steps may be applied for isolating casein from the rest of the proteins.

Precipitation removes most impurities from the solid, however it can also trap some impurities within the matrix. Recrystallization is often employed to further purify a solid (**Figure 4**). In this experiment, the solid was mixed with a solvent in which the solid was not very soluble. The temperature of the mixture was then raised to the solvent's boiling point and enough solid is added to saturate the hot solvent. Other insoluble impurities could then be removed via a filtration step. The hot solution was then gradually cooled to room temperature and cooled further in a refrigerator/cold room/ice bath. The slow process resulted in crystals instead of amorphous precipitate. The soluble impurities were not incorporated into the crystal lattice and the resulting crystals were relatively more pure than the crude precipitate. The crystals were then harvested using filtration and left to dry in air (or in vacuum).

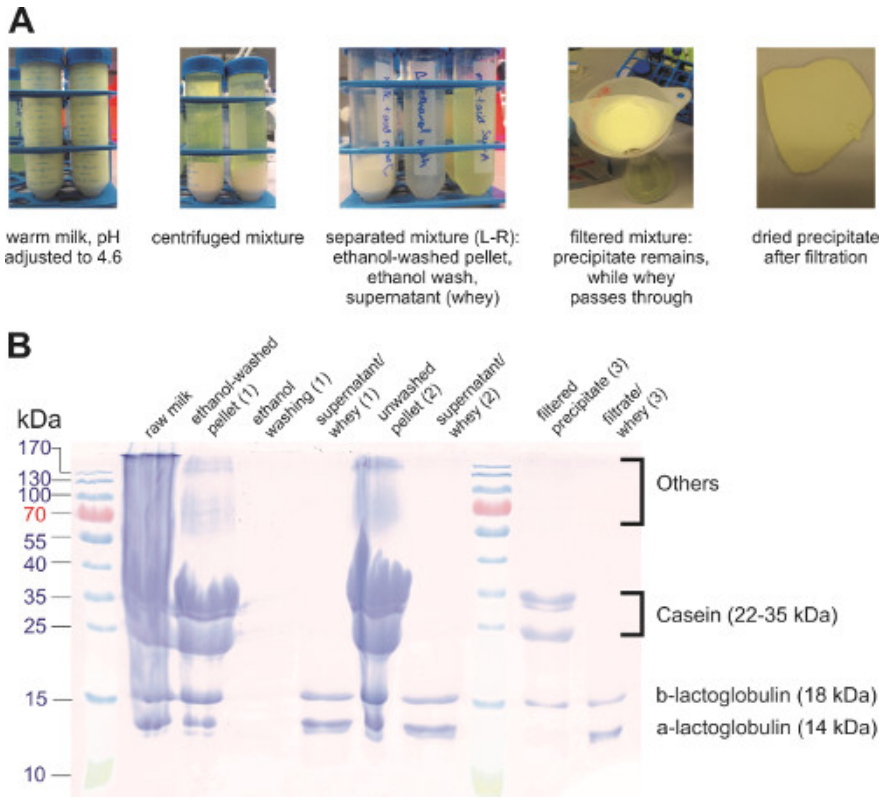

Figure 3. Precipitation of milk proteins. (a) Pictures of different steps in milk protein isolation. (b) SDS-PAGE of the different samples.

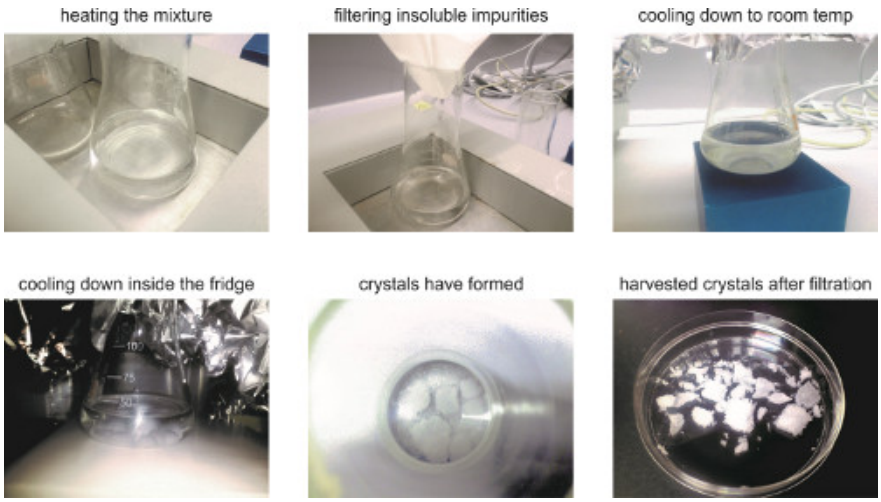

Figure 4. Recrystallization of KCl.

Discussion

Precipitation reactions are applied to many sample preparation processes. As mentioned before, they can be used to remove salts or specific ions depending on their solubility equilibria. They can also be used to remove proteins and other biomolecules from mixtures.

Recrystallization is often employed to further purify solids. This process removes trapped impurities within the solid. Among others, recrystallization can be used to purify salts and organic molecules.

Centrifugation and filtration techniques are applicable to most sample preparation demands to separate insoluble components from the solvent. Filtration is often used in organic chemistry to separate pure crystallized compounds from its solvent. It is also used after solid-liquid extractions in natural products chemistry or analytical chemistry. Centrifugation is often used to separate mixtures of different densities and as shown here applied to separation of milk components and precipitated salt.

In biochemistry, most processes such as protein, lipid, and DNA isolation involves precipitation reactions, centrifugation and filtration methods to purify samples. And while most of these processes have been fully standardized into commercial kits, there is still a lot of room for optimization, as different biological molecules require different conditions.

Disclosures

No conflicts of interest declared.

References

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