INSTITUTE OF MEDICAL BIOCHEMISTRY AND LABORATORY MEDICINE

Introduction to Work in Laboratory

Measuring volumes, filtration, centrifugation, solubility, separation

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Practical in Medical Biochemistry

General Medicine

Martin Vejražka

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Content

The aim of our first practical exercise is to get acquainted with the laboratory, the most common tools and instruments in it. At the same time, you will learn about some separation techniques that are commonly used in processing of biological samples – centrifugation, filtration, and separation of mixtures by shaking or adsorption. The properties of substances and components of mixtures, on the basis of which the mixtures are separated by these techniques, are discussed in more detail.

Task 1 – Separation of dispersion by means of centrifugation

1. Principle

In this practical lesson, diluted milk colored with two dyes will be used as a model dispersion. The first dye is red lipophilic substance, named Sudan III (Fig. 1); the second one is a hydrophilic food dye brilliant blue (also known under the code E-133, Fig. 2). Milk is an emulsion of the type oil in water. The hydrophilic components form the dispersion environment and the dispersed part consists mostly of fat. The emulsion is stable largely due to proteins whose molecules have both a hydrophilic and a lipophilic part. During the practical lesson, we will try to separate the model mixture into its components using various techniques. No technique is able to separate the mixture completely. Different procedures are suitable for isolating different components; each technique has a different efficiency and different demands on time or equipment.

In the first experiment, we will try to separate components of the emulsion by means of simple centrifugation. Two different centrifugation forces will be used: a bench-top microcentrifuge reaches approximately 300×g, whereas a high-speed centrifuge can be set to 30,000×g. The time of centrifugation will be the same in both cases.

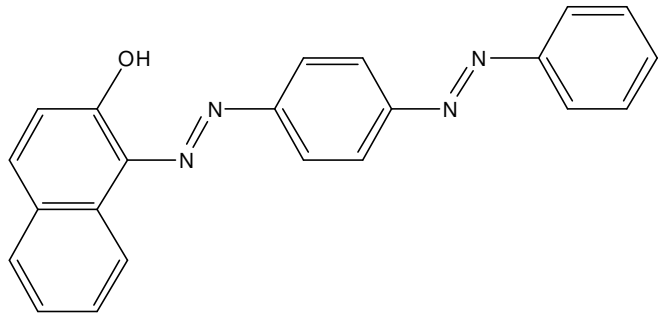

Fig. 1: Sudan III

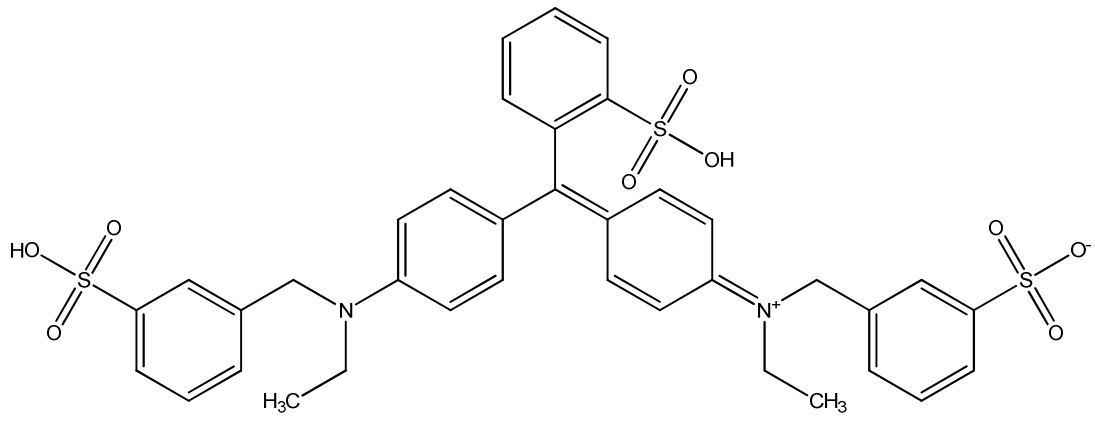

Fig. 2: Brilliant blue (E-133)

2. Procedure

1. Milk
2. Purified water
3. Sudan III 4 g/L in alcohol 
4. Brilliant blue (E-133) 0.25 g/L in purified water

Procedure

Sample preparation

Dilute milk 1:2 with purified water: in a beaker, mix 5 mL of milk with 10 mL of purified water. Use the glass pipettes to measure volumes. Mix gently.

Combine approximately 10 mL of diluted milk, 10 drops of red colorant Sudan III in alcohol, and 10 drops of aqueous solution of the blue colorant brilliant blue (E^-133). Seal the test-tube with laboratory film (Parafilm) and shake with vortex.

Centrifugation

1. Measure 1 mL of the mixture to each of two micro-test tubes. Close the tubes with the attached caps.
2. One test-tube is to be spun with a bench-top microcentrifuge for 10 minutes.
3. The second test-tube is to be centrifuged for 10 minutes at 30,000×g in a high-speed centrifuge.

3. Tasks

Compare separation of the mixture at different centrifugation speeds and explain the differences.

Task 2 – Separation of a dispersion by shaking with chloroform

1. Principle

A mixture of compounds of various polarities can be split between two immiscible liquid phases. Chloroform is a strongly hydrophobic, non-polar organic solvent. It is heavier than water and practically immiscible with hydrophilic substances. When mixtures of hydrophilic and hydrophobic compounds (emulsion of fat in water in our case) are shaken with chloroform, hydrophobic components are dissolved in chloroform, while the hydrophilic ones remain in the aqueous phase.

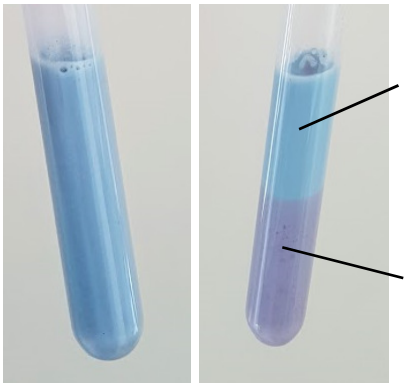

Fig. 3: Separation of emulsion by shaking with chloroform.

Left: emulsion stained with blue hydrophilic and red hydrophobic dye during shaking with chloroform.

Right: after phase separation.

2. Procedure

1. Diluted milk stained with Sudan III and brilliant blue from the previous experiment
2. Chloroform (trichlormethan) 

Procedure

1. Pour approx. 4 mL of stained diluted milk from the previous experiment into an equilibration test-tube. Add approximately the same volume of chloroform.
2. Close the test-tube with the jointed plug and shake with vortex (the highest speed) three times, 30 seconds each time. The content must always be completely shuffled.
3. Put the closed test-tube in a rack. Let it stay until the aqueous (upper) phase gets separated from the organic (bottom) phase.

Caution: Residues that contain chloroform are disposed as hazardous waste.

3. Tasks

1. Compare the appearance of two phases. Compare their colors with the original mixture. Explain the differences.
2. Look at the structural formulas of the two dyes, and explain which parts of their molecules are likely responsible for the observed behavior.

Task 3 – Adsorption of dissolved compound on activated charcoal

1. Principle

Activated charcoal is pure carbon in a form that has an extremely large surface area. It consists of graphite particles, in which micropores (size of the order from several nanometres to tens of nanometres). Porosity of particles greatly increases their adsorptive surface.

Activated charcoal binds, adsorbs, many compounds in a non-specific way. It is generally stated that the adsorption of substances on the coal increases with molecular weight, polarity of molecules, number of multiple bonds and functional groups.

Activated charcoal is often used for purification of solutions from small quantities of contaminants. It is usually added to the solution as a powder, the resulting suspension is stirred over a period of time in order to bind contaminants, and carbon particles are then filtered or centrifuged out.

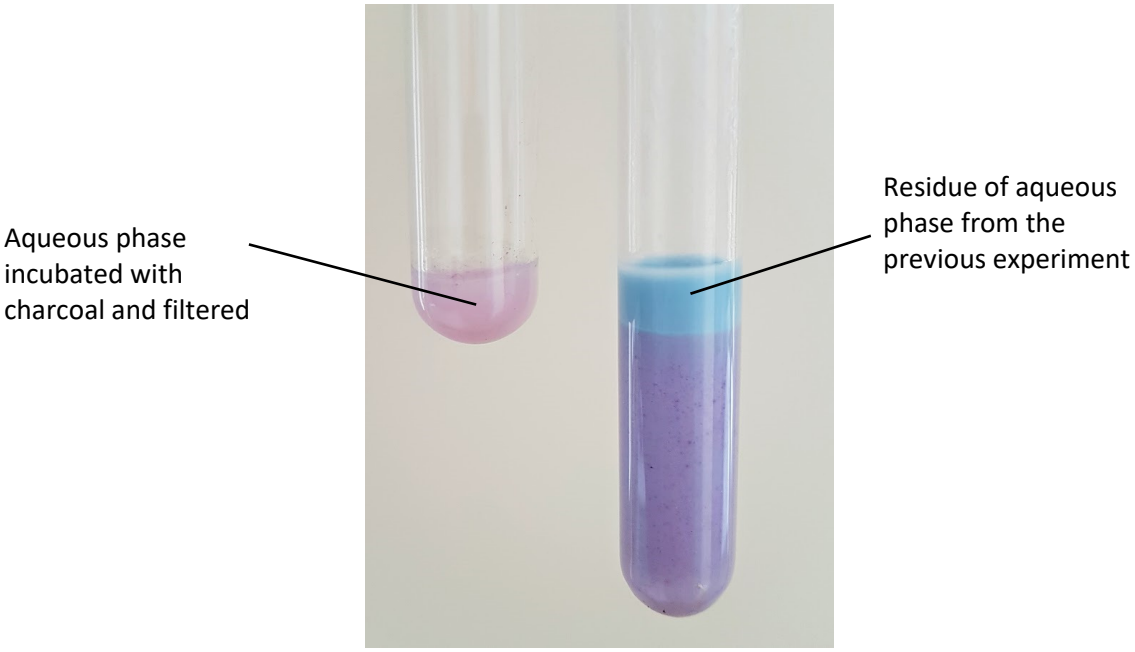

Fig. 4: Adsorption on activated charcoal

Right: a residue of the mixture from the previous experiment after separation with chloroform.

Left: the aquous phase after combining with charcoal and filtration. The blue dye has been removed by adsorption to the activated charcoal. Pink color of the filtrate is caused by traces of the red hydrophobic dye that contaminates the hydrophilic phase and binds much less to activated charcoal.

2. Procedure

1. Separated mixture from the previous experiment
2. Activated charcoal (powder)
3. Folded filter paper Filtrak 3b, equipment for filtration

Procedure

1. Use the plastic dropper to aspirate the aqueous phase from the previous experiment and transfer it to a clean test-tube.
2. Add about 0.2 g of activated charcoal. Mix by vortex about 1 minute at the highest speed.
3. Unfold the filter paper, place it to a funnel, and wet with purified water.
4. Pour the mixture of aqueous phase with charcoal on the filter and allow to pass through. Collect the filtrate to a clean tube.

3. Tasks

Compare the appearance of the filtrate with the original mixture and with the aqueous phase from the previous experiment. Compare it with the diluted milk as well. Explain the differences.

Task 4 – Sublimation of caffeine from coffee

1. Principle

Some compounds can be separated from a mixture by means of distillation or sublimation. In both cases the fact that the compound of interest has the boiling point different from the other compounds in the mixture is utilized. When the mixture is heated, the isolated compound is converted into gaseous state. The gas is removed from the mixture and cooled so that it condensates back to a liquid or solid. In case the compound is isolated from a liquid, the process is described as distillation. In sublimation, the solid state is converted directly to gas when the mixture is heated.

In this experiment we will try to isolate caffeine from roasted and ground coffee. Caffeine is relatively thermostable and its boiling point is 178 °C. Its melting point is higher (about 235 °C), therefore sublimation takes place easily when it is heated. After the vapors have cooled, white needle-like crystals of caffeine are formed.

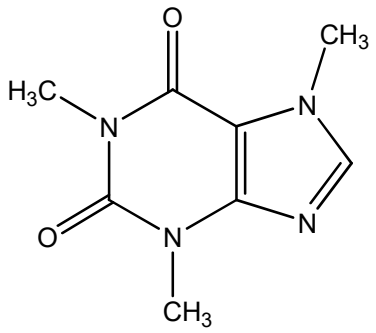

Fig 5: Caffeine

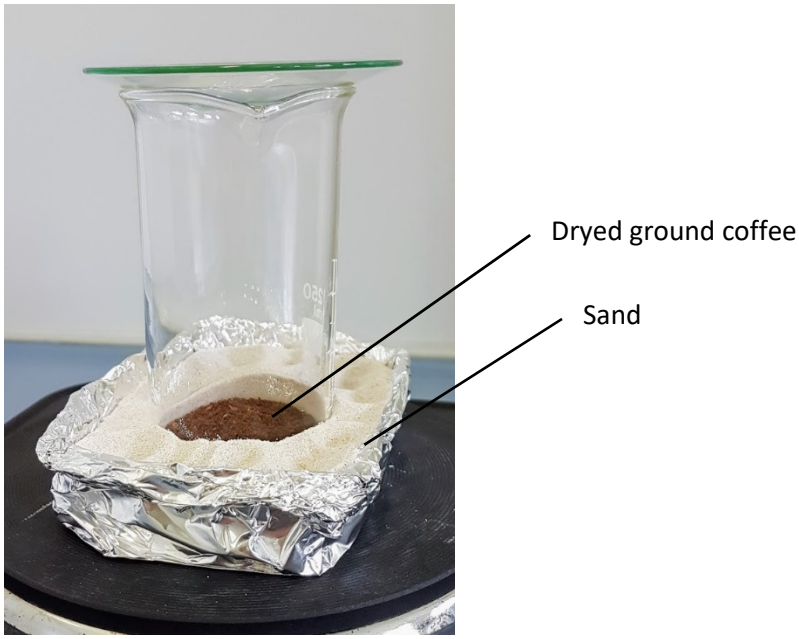

Fig. 6: Sublimation of caffeine from coffee

2. Procedure

1. Roasted ground coffee
2. Sea sand, aluminium foil, beaker 250 mL, watch glass

Procedure

1. Dessicate approx. 7 g of coffee for 5 minutes at 120 °C.
2. Prepare a sand bath: Pour the sea sand into a bowl of thicker aluminum foil. Place on the cooker in the fume hood and heat to about 200 ° C (grade 3).
3. Pour the dessicated ground coffee to a clean and thoroughly polished beaker. Put the beaker into the sand and cover it with a polished watch glass.
4. Let the sublimation run for about 5 minutes. Then take the beaker with the glass from the sand and let it cool down.
   Caution: Both the beaker and the watch glass are hot! Use forceps or an insulating glove.

3. Tasks

Observe crystals of caffeine on the wall of the beaker or on the watch glass.

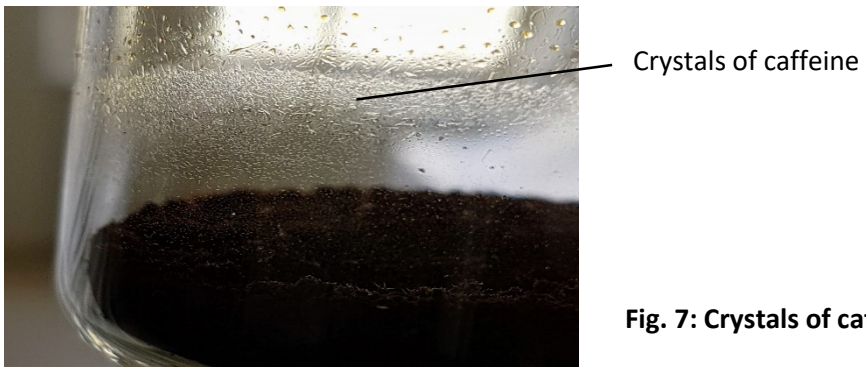

Fig. 7: Crystals of caffeine

Task 5 – Dissolution of a weak acid in an environment of various pH

1. Principle

Polarity of organic acids and bases strongly depends on pH of environment. Carboxylic acids, for example, form carboxylate anions in alkaline solutions and thus turn strongly polar; hence they become easily soluble in aqueous solution. On the other hand, in acidic environment, carboxylic acids get protonated, less polar and their solubility in water decreases. This is important e.g. for absorption of drugs: most drugs behave as weak acids or weak bases, and pH is different in various sections of gastrointestinal tract.

In this experiment solubility of salicylic acid in acidic, basic and approximately neutral environment will be compared.

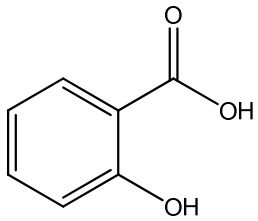

Figure 8: Salicylic acid

2. Procedure

1. Crystalline salicylic acid
2. Hydrochloric acid 2 mol/L 
3. Sodium hydroxide 2 mol/L 

Procedure

1. Pour a small amount (about 0.1 g) of crystalline salicylic acid to each of three test-tubes.
2. Add approx. 2 mL of purified water to the first test-tube, 2 mL of diluted hydrochloric acid to the second one, and 2 mL of diluted sodium hydroxide to the third one.
3. Mix the content of test-tubes and observe how quickly crystals of salicylic acid dissolve.
4. When crystals are completely dissolved in the test-tube with sodium hydroxide, add approx. 3 mL of diluted hydrochloric acid and mix carefully. Watch changes in the mixture.
   Caution: The mixture may get hot during neutralisation. It may start to boil and sprinkle. Take care to keep the mouth of the test-tube is facing away of you and people standing around!

3. Tasks

Compare solubility of salicylic acid in acidic, neutral and basic environment. Explain what happened after neutralisation of the basic solution of the compound. Use chemical equations to describe the observed changes.