

Department of Biochemistry & Microbiology
Chemistry of Biomolecules Lab (CHE 203 L)

Experiment 1 A & B.

Separation mixtures by using simple distillation & Preparation of Buffer.

EXP 1 A

Separation mixtures by using simple distillation

Background:

Distillation is one of the most common methods of purifying a liquid. It is a very simple method: a liquid is brought to a boil, the liquid becomes a gas, the gas condenses and returns to the liquid state, and the liquid is collected. As heat is applied, water molecules increase their kinetic energy. Some molecules acquire sufficient energy to escape from the liquid phase and enter into the vapor phase. The vapor above the liquid exerts a pressure, called the vapor pressure. As more and more molecules obtain enough energy to escape into the vapor phase, the vapor pressure of these molecules increases.

Eventually the vapor pressure equals the pressure exerted externally on the liquid (this external pressure usually is caused by the atmosphere). Boiling occurs when this condition is met, and the temperature where this occurs is called the boiling point. The liquid in the boiling flask is heated to a boil, and the vapor rises through tubing. The vapor then travels into a tube cooled by water, which serves as a condenser, where the vapor returns to the liquid state. If the mixture has a low-boiling component (a volatile substance with a high vapor pressure), it will distill over first and can be collected. Higher-boiling and nonvolatile components (substances with low vapor pressure) remain in the boiling flask. Only by applying more heat will the higher-boiling component be distilled.

Normal distillations, procedures carried out at atmospheric pressure, require “normal” boiling points. However, when boiling takes place in a closed system, it is possible to change the boiling point of the liquid by changing the pressure in the closed system. If the external pressure is reduced, usually by using a vacuum pump or a water aspirator, the boiling point of the liquid is reduced. Thus, heat-sensitive liquids, some of which decompose when boiled at atmospheric pressure, distill with minimum decomposition at reduced pressure and temperature.

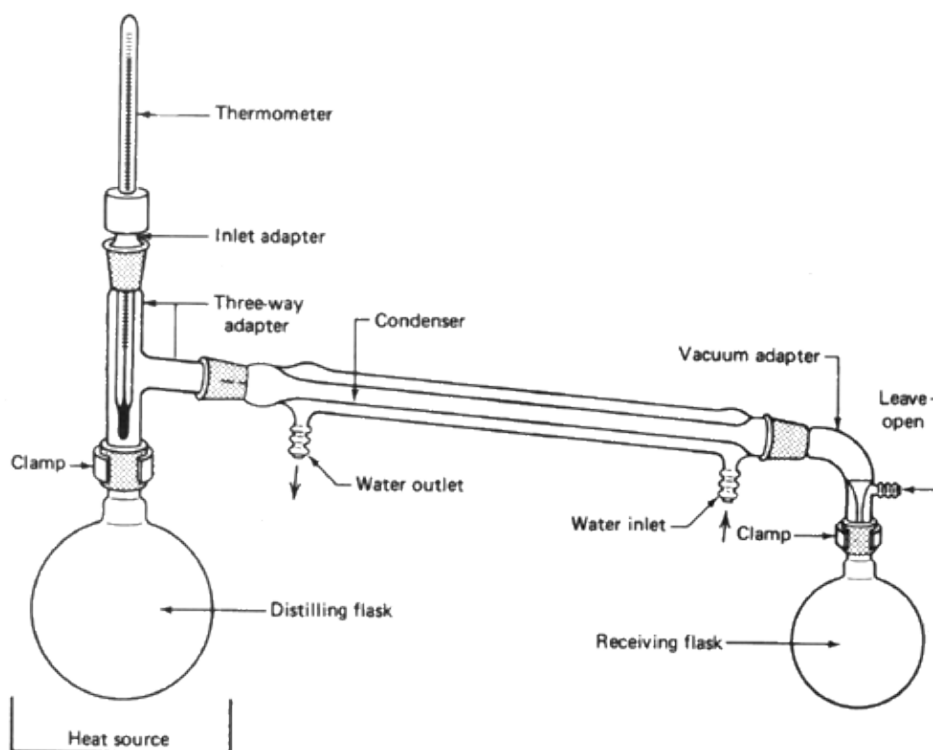


Fig 1.1: A distillation Kit

Objectives

1. To use distillation to separate a mixture of water, acetone and alcohol.
2. Identify the distillates by chemical test.

Procedure

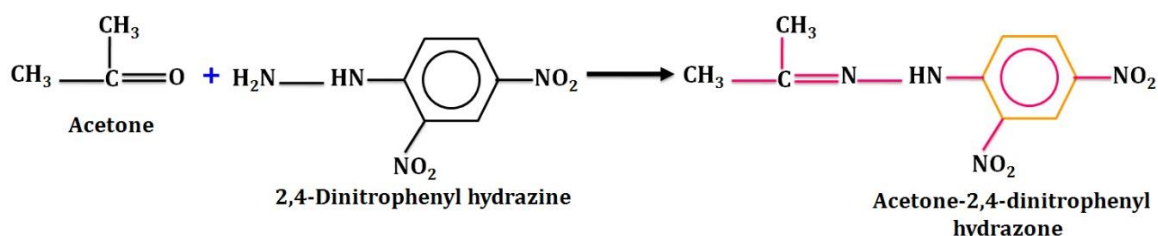
1. Assemble an apparatus as illustrated in Fig 1.1. A kit containing the necessary glassware can be obtained from your instructor. The glassware contains standard taper joints, which allow for quick assembly and disassembly. Before fitting the pieces together, apply a light coating of **grease** to each joint to prevent the joints from sticking.
2. Use 250ml/500-ml (as instructed by lab instructor) round-bottom flasks for the boiling flask and the receiving flask. Fill the boiling flask with 100 mL of the prepared mixture. Add two boiling chips/pebbles to the boiling flask to ensure smooth boiling of the mixture and to prevent bumping. Be sure that the rubber tubing to the condenser enters the lower opening and empties out of the upper opening. Turn on the water faucet and allow the water to fill the jacket of the condenser slowly, so as not to trap air. Adjust the bulb of the thermometer to below the junction of the condenser and the distillation column.

3. Gently heat the boiling flask with a heater. Eventually the liquid will boil, vapors will rise and enter the condenser, and liquid will re-condense and be collected in the receiving flask. The liquid with lower boiling point would come out first followed by liquid with higher boiling point.
4. Record the temperature of the vapors as soon as the 1 mL of eluent has been collected.
5. Keep an eye on the thermometer, the temperature would rise once the liquid with lower b.p is fully distilled. Record the elevated temperature.
6. Collect the second eluent. Stop once the temperature rises again.
7. Test the first two liquids for acetone and alcohol
8. Take four test tubes and place 4 ml of each distillate in each test tube name them as 1A,1B for first distillate and 2A & 2B for second distillate.

a) Test for acetone : 2,4-dinitrophenyl hydrazine test (2,4-DNP test)

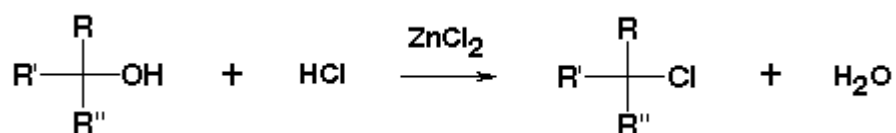
Add 2,4-dinitrophenyl hydrazine to 1A and 1 B.

A positive result is indicated by the formation of an yellow or orange-red precipitate of 2,4-dinitrophenyl hydrazone.



b) Test for alcohol:

To ___ mL of the unknown in a test tube add 2 mL of the Lucas reagent at room temperature. Stopper the tube and shake vigorously, then allow the mixture to stand. Note the time required for the formation of the alkyl chloride, which appears as an insoluble layer or emulsion.



Positive test

Appearance of a cloudy second layer or emulsion

- 3° alcohols: immediate to 2-3 minutes
- 2° alcohols: 5 -10 minutes
- 1° alcohols: no reaction

9. Record your observations in Table 1.1.

Table 1.1: Data table

Distillate	Boiling Point	Test Result Observation	Functional Group Confirmed
1			
2			

Lab Report

Answer the following questions

1. Taking your results and recordings into consideration, explain what is distillate 1 and 2?
2. Describe why the rubber tubing to the condenser enters the lower opening and empties out of the upper opening? What would have happened if it was other way round?
3. The distillation that was used in this lab was a form of simple distillation. There are other forms of distillation used in organic chemistry. Find out at least two similar processes and write a few lines on their uses.

EXP 1 B

Preparation of TE Buffer & PBS Buffer

1. Preparation of 10X Tris EDTA Buffer (TE) Stock (10 mM Tris; 1 mM EDTA), pH 8.0

Background

Tris [tris-(hydroxymethyl)-aminomethane] is a frequently used buffer in biological experiments. The reasons for this are that Tris is comparatively inexpensive, very freely soluble in water, is inert in many enzymatic systems (no interactions with other components) and has a high buffer capacity.

TE buffer is prepared at pH 7.4 - 8.0. **This buffer has become the standard buffer for the storage of nucleic acids.** It is generally prepared by mixing Tris buffer stock solutions with an EDTA stock solution (0.5 M; pH 8.0). The prepared buffer can be stored at room temperature. TE stock solutions are prepared in concentrations of 100X to 1X.

Tris can maintain a stable pH. It is an effective buffer between pH 7 and 9. Because of its neutral pH range, Tris is a commonly used buffer in biological labs.

DNAses are inactivated due to chelation of Mg^{2+} and Ca^{2+} by EDTA.

EDTA, a chelating agent, chelates ions preventing degradation by nucleases. Nucleases require bi-valent cations for their activity.

Method:

● Preparation of 1 M Tris-Cl, pH 8.0 (Stock)

Required reagents: Tris Base (MW 121), Distilled Water, 1N HCl

For 1 liter

- Dissolve 121 g **Tris base** [tris(hydroxymethyl)aminomethane] in 800 ml dH_2O
- Adjust to desired pH with concentrated HCl
- Mix and add H_2O to 1 liter
- Store up to 6 months at $4^{\circ}C$ or room temperature
(Approximately 42 ml of 1N HCl was required for a solution that is pH 8.0)

Prepare 10 ml 1M Tris-Cl, pH 8.0:

- Weigh **Tris base** _____ g using electronic balance and put in a small (25ml) Beaker
- Dissolve in _____ ml dH_2O
- Adjust pH to 8.0 by adding _____ ml concentrated HCl; Mix and Transfer into Falcon measuring tube
- Adjust final volume by adding _____ ml dH_2O to final volume of 10 ml
- Label the tube (1 M Tris-HCl)

● **Preparation of 0.5 M EDTA, pH 8.0 (Stock)**

Required reagents: EDTA (MW 186.1), Distilled Water, 1N NaOH

For 1 liter

- Add 186.1 g EDTA (disodium, dihydrate) to 800 ml of dH₂O.
- Adjust the pH to 8 with NaOH pellets (you will need about 20 g) or NaOH solution.
- Add the last few grams slowly to avoid overshooting the pH.
- Note that the EDTA won't completely dissolve until the pH is around 8.
- Bring volume up to 1 L with distilled water (dH₂O), put in vessel or container, and autoclave.

Prepare 10 ml 0.5 M EDTA, pH 8.0:

- Weigh **EDTA** _____g using electronic balance and put in a small (25ml) Beaker
- Dissolve in _____ ml dH₂O
- Adjust pH by adding _____ ml NaOH; Mix and Transfer into Falcon measuring tube
- Adjust final volume by adding _____ ml dH₂O to final volume of 10 ml
- Label the tube (0.5 M EDTA)

● **Preparation of 10XTE buffer (10 mM Tris-HCl, 1 mM EDTA) (Stock)- pH is determined by the pH of the Tris-Cl**

Required reagents: 1M Tris-HCl, pH 8.0 (Stock); 0.5 M EDTA (Stock); Distilled Water

Preparation for 50 ml 10 X TE

Calculation: $C_1 V_1 = C_2 V_2$ $1M \times V_1 = 10mM \times 50$ or $V_1 = 10 \times 50/1000 = 0.5$
ml of 1M Tris-HCl for 1X TE
for **10 XTE = 0.5 x 10 = 5.0 ml 1M Tris-HCl is required**

For EDTA $C_1 V_1 = C_2 V_2$ $0.5M \times V_1 = 1mM \times 50$ or $V_1 = 1 \times 50/500 = 0.1$ ml
of 0.5 M EDTA for 1X TE
for **10 XTE = 0.1 x 10 = 1.0 ml 0.5M EDTA is required**

Procedure

- Measure out **5 ml of 1M Tris-HCl** and **1 ml of 0.5M EDTA** at pH 8.0.
- Add together in a 50 ml measuring cylinder.
- Add distilled water to make the volume up to a 50ml.
- Sterilize by autoclaving and store the buffer at 4°C or room temperature.

Calculate for the preparation of 10 ml of 10X TE:

- Measure out -----ml of **1M Tris-HCl** and ----- ml of **0.5M EDTA** at pH 8.0.
- Add then together in a 10 ml measuring cylinder.
- Add distilled water to make the volume up to a 10 ml.
- Sterilize by autoclaving and store the buffer at 4°C or room temperature.
- Label the reagent bottle (10XTE, 10mM Tris-HCl, 1 mM EDTA, pH 8.0)

2. Preparation of 10X Phosphate-buffered Saline (PBS) buffer Stock, pH 7.4

Background

PBS has many uses because it is isotonic and non-toxic to cells. PBS can be used as a diluent in methods to dry biomolecules, as water molecules within it will be structured around the substance (protein, for example) to be 'dried' and immobilized to a solid surface. PBS can be used to take a reference spectrum when measuring the protein adsorption in ellipsometry. PBS with EDTA is also used to disengage attached and clumped cells.

Materials Required:

- KH_2PO_4 ; $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$; NaCl crystals; KCl; Distilled Water

Method:

1X PBS (Phosphate Buffered Saline) Buffer consists of:

- 137 mM NaCl
- 2.7 mM KCl
- 100 mM Na_2HPO_4
- 2 mM KH_2PO_4

To prepare **1 liter of 10X** concentration of stock solution, materials required are as follows:

NaCl	= 80 g
KCl	= 2 g
$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$	= 14.4 g
KH_2PO_4	= 2.4 g

To prepare 10 ml of 10X concentration of stock solution:

Calculate

Materials required: NaCl	=	-----	g
KCl	=	-----	g
Na ₂ HPO ₄ .7H ₂ O	=	-----	g
KH ₂ PO ₄	=	-----	g

Procedure:

1. Weigh and add required amount of NaCl crystals in a 25 ml beaker
2. Weigh and add required amount of KCl
3. Add required amount of Na₂HPO₄.7H₂O
4. Finally add required amount of KH₂PO₄
5. Add 6 ml of distilled water and dissolve.
6. Adjust the pH to 7.4 using 1M HCl and 1M NaOH.
7. Adjust the volume to 10 ml and transfer into reagent bottle or 15 ml Falcon tube
8. Label the bottle or tube (10XPBS, pH 7.4)
9. Autoclave and store at room temperature.