الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

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Department of Biotechnology

Practical Works Booklet

Module: Analytical Techniques

Intended for 3rd Year Food Biotechnology Students

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Practical Work 1. Hygiene and safety in laboratories

Hygiene and safety are fundamental pillars in any laboratory environment, especially in fields like food biotechnology where work involves chemical, biological, and physical hazards. This practical work is designed to introduce students to the essential principles and practices that ensure a safe working environment. The primary goal is to minimize the risks of accidents, injuries, and exposure to hazardous substances by fostering a culture of safety awareness and proactive risk management. Through this session, students will become familiar with the standard tools and procedures, including Personal Protective Equipment (PPE) and Safety Data Sheets (SDS), which are critical for preventing incidents and promoting responsible laboratory conduct.

The main objectives of this practical work are:

- ✓ To understand and apply the basic rules of hygiene and safety in a laboratory setting.
- ✓ To learn to identify and manage various types of risks, including chemical, physical, and biological hazards.
- ✓ To handle chemicals safely by understanding and following established protection guidelines and procedures.
- ✓ To manage waste effectively and maintain an organized workspace to prevent contamination and accidents.
- ✓ To become proficient in the correct use of Personal Protective Equipment (PPE) such as lab coats, gloves, and safety glasses.
- ✓ To learn to read and interpret Safety Data Sheets (SDS) and understand globally standardized hazard pictograms for risk identification.

1. Presentation of Personal Protective Equipment (PPE)

• Wearing of the lab coat, gloves, glasses, and other PPE according to the risks.

• Correct and mandatory use of hoods for handling volatile or dangerous products.

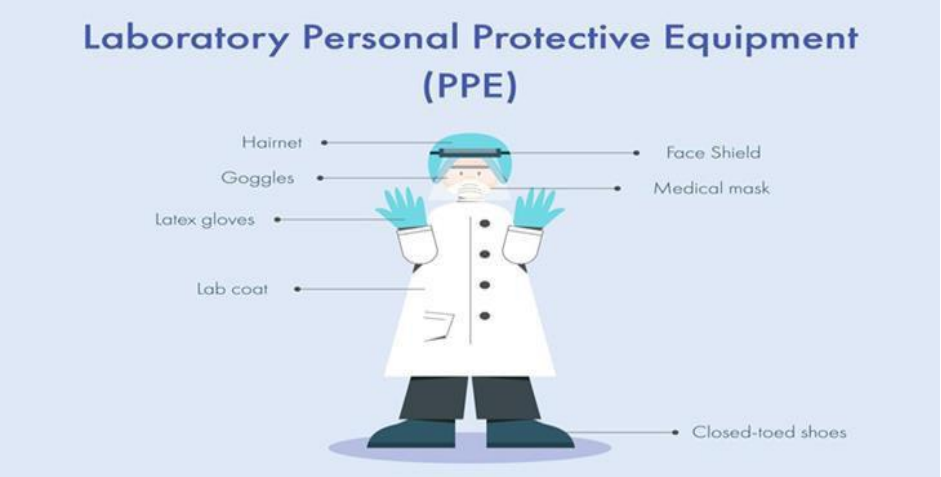


Figure1.Laboratories personal protective equipment

2. Reading and Analysis of Safety Data Sheets (SDS)

• Identification of hazards associated with different chemicals.

• Understanding of hazard pictograms (flammable, corrosive, toxic, etc.).

Chemical pictograms are standardized symbols, primarily defined by the Globally Harmonized System (GHS) of classification and labeling of chemicals. They allow for quick identification of the hazards associated with a chemical substance used in a laboratory. Here is a description of the main pictograms



Figure2. Hazard pictograms

- Substances or mixtures that can explode under the effect of heat, shocks, or friction.
- Products likely to ignite upon contact with air, under the effect of heat or flames. Ex: EtOH
- Substances that promote or intensify the combustion of other materials. Ex: H2O2
- Risk of explosion in case of heating or rupture. Ex: Carbon dioxide, propane gas.
- Products that can cause serious, even fatal effects, even at low doses. Ex: methanol.
- Products that can attack or destroy living tissues, Ex: Sulfuric acid.
- Substances that can cause irritations, skin sensitizations, or narcotic effects. Ex: Acetone, bleach
- Products that can cause cancer, genetic mutations, organ damage. Ex: benzene.
- Substances dangerous to the aquatic environment, capable of causing long-term effects. Ex: Mercury, chloroform.

3. Laboratory equipment and glassware

In a laboratory, equipment and glassware play an essential role in conducting experiments. These tools are designed to measure, contain, heat, mix, or manipulate chemical and biological substances with precision and safety. Here is a list of commonly used laboratory equipment and glassware.



Figure 3. Laboratory equipments



Figure 4. Laboratory glassware

Practical Work 2. Preparation of Solutions and Buffers

The accurate preparation of solutions and buffers is a fundamental and routine technique in all fields of biotechnology. Mastering these skills is essential, as the precision of experiments and the validity of their results are directly dependent on the correct concentration and stability of the solutions used. This practical work is designed to provide hands-on experience in preparing solutions using different concentration units and to explore the critical properties of buffer solutions, which are vital for maintaining stable pH conditions in biological and chemical processes.

This practical session aims to:

- ✓ Master the fundamental techniques for preparing solutions using different concentration units (percentage, molarity, and mass/volume).
- ✓ Understand the concept and importance of a buffer solution.

- ✓ Learn how to prepare a phosphate buffer and experimentally verify its property of resisting pH changes upon the addition of small amounts of acid or base, in contrast to water.

I. Principle

❖ Solution Preparation

A solution is a homogeneous mixture of a solute (the dissolved substance) and a solvent (the dissolving medium, often water). The concentration can be expressed as:

- ✓ Weight/Volume Percent (%): Mass of solute (g) per 100 mL of solution.
- ✓ Molarity (M): Number of moles of solute per liter of solution.
- ✓ Mass Concentration (g/L): Mass of solute (g) per liter of solution.

❖ Buffer Solutions

A buffer solution resists significant changes in pH when small amounts of strong acid or base are added. It typically consists of a weak acid and its conjugate base (or vice versa). The phosphate buffer system ($H_2PO_4^-$ / HPO_4^{2-}) is one of the most important in biological systems, effective in the pH range of approximately 6.2 - 8.2.

II. Experimental Procedure

Part 1: Preparation of a Sodium Chloride (NaCl) Solution

Preparation of three sodium chloride solutions (Molecular Weight, MW = 58.44 g/mol) in a final volume of 7 ml of distilled water with the following concentrations:

- 0.5% (w/v percentage).

- 0.5 M (molar concentration).

- 10 g/L (mass concentration).

a. Calculate the mass of NaCl to be weighed for each concentration

1. For 0.5% (w/v) Solution

- A 0.5% w/v solution means 0.5 g of solute in 100 mL of solution.

- For 7 mL , the mass is calculated as:

Mass=0.5g×(5/100) =0.025g=25mg

2. For 0.5 M Solution

- Molarity (M) = moles of solute / liter of solution.

M=m/MW so m=M×MW=0.5×58.44=29.22g

m=0.146 g

3. For 10 g/L Solution

- A 10 g/L solution means 10 g of solute in 1 L of solution.

- For 5 mL (0.005 L), the mass is: 0.05g

b. Prepare the solutions according to the following operating procedure:

1. Using an electronic balance, weigh the calculated quantity of NaCl for each solution.

2. Transfer each mass of NaCl into separate, labeled containers (e.g., beakers or test tubes).

3. Add a small amount of distilled water (e.g., 2-3 mL) to each container and swirl or stir to dissolve the NaCl completely.

4. Carefully transfer the dissolved solution into a 5 mL volumetric flask (or graduate the solution to 5 mL using a graduated cylinder).

5. Rinse the original container with a little distilled water and add the rinsings to the volumetric flask to ensure all solute is transferred.

6. Add distilled water to bring the final volume to the 5 mL mark.

7. Agitate or invert the flask to ensure complete mixing and a homogeneous solution.

Part 2: Preparation of a Buffer Solution

Preparation of a phosphate buffer solution (0.1 M, pH = 7.2).

1. Calculate the masses of the solutes Na_2HPO_4 and NaH_2PO_4 required to prepare 0.5 L of a 0.1 M solution for each salt.

2. Prepare the two separate solutions, then mix them to obtain a buffer solution with a pH of 7.2.
3. Perform a 1:10 dilution of this buffer solution and measure its pH.
4. Measure the pH of distilled water.
5. Into a 50 ml volumetric flask, add 1 ml of 0.1 M sodium hydroxide (NaOH). Fill to the mark with distilled water, mix well, and measure the pH.
6. Into a 50 ml volumetric flask, add 1 ml of 0.1 M sodium hydroxide (NaOH). Fill to the mark with the buffer solution, mix well, and measure the pH.
7. Into a 50 ml volumetric flask, add 1 ml of 0.1 M hydrochloric acid (HCl). Fill to the mark with distilled water, mix well, and measure the pH.
8. Into a 50 ml volumetric flask, add 1 ml of 0.1 M hydrochloric acid (HCl). Fill to the mark with the buffer solution, mix well, and measure the pH.

Given: MW Na_2HPO_4 = 141.96 g/mol; MW NaH_2PO_4 = 119.98 g/mol; MW HCl = 36.5 g/mol; MW NaOH = 40 g/mol.

- Create a comparison table of the pH values for each preparation. What can you conclude?

$m(Na_2HPO_4)=M \times MW=0.1 \times 141.96= 14.19g$ so m=7.09 g/500ml

$m(NaH_2PO_4)=M \times MW=0.1 \times 119.98= 11.99g$ so m=5.99 g/500ml

$m(HCl)=M \times MW=0.1 \times 36.5= 3.65$ g so m=0.04 g/10 ml

$m(NaOH)=M \times MW=0.1 \times 40= 4g$ so m=0.04g/10 ml

Table1. Comparison Table of pH Values for Each Preparation

Solution	**System Type**	**Composition**	**Expected pH**	**Buffering Behavior**
Distilled Water	Unbuffered	Pure H_2O	~5.5 – 7.0	No buffering; pH may vary slightly due to CO_2
Phosphate Buffer (Stock)	Buffered	Na_2HPO_4 + NaH_2PO_4 (concentrated)	7.2 (target)	Strong buffering capacity
Diluted Phosphate Buffer	Buffered (weaker)	1:10 dilution of phosphate buffer	~7.2 (slightly lower)	Slightly reduced buffering capacity
NaOH in Water	Unbuffered	1 mL 0.1 M NaOH + H_2O to 50 mL	Significantly > 7	No buffering; pH will rise sharply
NaOH in Buffer	Buffered	1 mL 0.1 M NaOH + buffer to 50 mL	~7.2 (very small change)	Buffer neutralizes added base
HCl in Water	Unbuffered	1 mL 0.1 M HCl + H_2O to 50 mL	Significantly < 7	No buffering; pH will drop sharply
HCl in Buffer	Buffered	1 mL 0.1 M HCl + buffer to 50 mL	~7.2 (very small change)	Buffer neutralizes added acid

Conclusion

Based on the expected results in the table, we can conclude the following:

1. Effective Buffer Action: The phosphate buffer solution resists drastic changes in pH when small amounts of a strong acid (HCl) or a strong base (NaOH) are added. The pH remains stable at approximately 7.2.

2. Lack of Buffering in Water: In contrast, distilled water shows very little resistance to pH change. Adding the same small amounts of acid or base causes a dramatic shift in pH, making the solution strongly acidic or basic.

3. Principle Verification: This experiment visually demonstrates the fundamental principle of a buffer: it is a system that uses a weak acid and its conjugate base to neutralize added strong acids or bases, thereby maintaining a stable pH environment.

4. Dilution Effect: Diluting the buffer has a minimal effect on the pH itself but reduces its buffer capacity (its ability to neutralize future additions of acid or base).

Practical Work 3: Extraction of Non-Protein Compounds from a Food Sample

The biochemical composition of food is complex, comprising proteins, carbohydrates, lipids, and a wide range of secondary metabolites. Analytical food science often requires the isolation of specific classes of compounds for identification and quantification. This practical work focuses on the fundamental technique of solid-liquid extraction, which leverages the solubility of target compounds in specific solvents. By selecting an appropriate solvent based on the polarity of the compound of interest, it is possible to efficiently isolate non-protein components such as sugars, lipids, and polyphenols from a food matrix. Subsequent separation by centrifugation yields a clear extract ready for various analytical applications.

The main objectives of this practical work are:

- ✓ To master the technique of solid-liquid extraction for isolating specific compounds from food.
- ✓ To understand the principle of solvent selection based on the polarity of the target compound.
- ✓ To learn how to prepare a food sample efficiently for extraction (homogenization, drying).
- ✓ To perform a separation using a centrifuge and correctly recover the fraction of interest (supernatant).

I. Materials and Reagents

Materials

1. Mortar and pestle or grinder
2. Analytical balance
3. Pipettes + necessary glassware (Erlenmeyer flasks, funnels, etc.)

4. Magnetic stirrer and stir bars
5. Paper filter or gauze
6. Centrifuge
7. 50 mL Centrifuge tubes

Reagents

1. Distilled water
2. 70% Ethanol or Methanol
3. Hexane or Petroleum ether
4. Sample: Ripe banana (without peel)

II. Experimental Procedure

1. Sample Preparation

1. Weigh exactly 2 g to 5 g of the food sample (e.g., a fruit, seed, or vegetable).
2. Grind the sample in a mortar and pestle or a grinder until a homogeneous paste is obtained.

2. Solvent Choice and Extraction

Banana (Carbohydrates)

1. Quantitatively transfer the paste into a 100 mL beaker.
2. Add 50 mL of distilled water.
3. Add a stir bar and place the beaker on a magnetic stirrer.
4. Stir vigorously for 20 minutes at room temperature.
5. Filter the mixture through a Whatman paper filter placed in a funnel.
6. Collect the filtrate in a clean centrifuge tube.

Nuts (Lipids)

1. Weigh precisely 4.00 g of finely ground nuts.
2. Dry the sample in an oven at 105°C for 1 hour to remove water and improve the extraction yield.

3. Transfer the nut powder into a centrifuge tube.
4. Add 50 mL of hexane.
5. Cap the tube and shake vigorously on a vortex for 1 minute, then place on a magnetic stirrer for 30 minutes.

Green Tea (Secondary Metabolites)

1. Weigh precisely 2.00 g of green tea powder.
2. Place the powder in a centrifuge tube.
3. Add 40 mL of 70% ethanol.
4. Stir the mixture on a magnetic stirrer for 30 minutes, protected from light to avoid photo-degradation.

3. Centrifugation

1. Place the tube containing the mixture (or filtrate for banana) in a centrifuge.
2. Set the centrifuge to between 3000 g and 5000 g for 15-20 minutes (at room temperature or at 4°C if the compounds are heat-sensitive).
3. Start the centrifugation.

4. Recovery of the Soluble Fraction

After centrifugation, recover the supernatant (the liquid phase) without collecting the solid deposit (pellet). This supernatant contains the extracted carbohydrates, lipids, or secondary metabolites.

5. Storage and Analysis

Store the supernatant at 4°C for immediate use or at -20°C for longer storage.

Analyze the obtained fraction as needed (spectrophotometric assay, HPLC, GC-MS, etc.).

Synthesis Question

Based on the three extraction methods presented (carbohydrates, lipids, and polyphenols), compare and justify the choices of solvents and operating conditions (sample preparation, time, temperature, centrifugation) in relation to the chemical nature of the target compounds and the properties of the solvents used. Then, discuss the impact of these parameters on the

efficiency and quality of the extracts obtained, illustrating your answer with specific examples taken from the protocols.

Table 2. Extraction Methods, Conditions, and Results

Parameter	**Carbohydrate Extraction (Banana)**	**Lipid Extraction (Nuts)**	**Polyphenol Extraction (Green Tea)**
Method	Aqueous extraction + filtration	Non-polar organic solvent extraction	Polar organic solvent extraction
Solvent	Distilled water	Hexane	70% Ethanol
Sample Condition	Hydrated paste	Dried powder (105°C, 1h)	Fine powder
Time/Temperature	20 min, room temperature	30 min, room temperature	30 min, room temperature
Special Treatment	Filtration before centrifugation	Vortex agitation + magnetic stirring	Light protection
Result Appearance	Clear, colorless solution	Transparent organic phase	Colored yellow-green solution
Advantages	Simple, fast, economical	Good selectivity, easy evaporation	Preservation of sensitive compounds
Limitations	Non-selective extraction	Volatile, flammable solvent	Partial extraction only

Practical Work 4: Spectrophotometric Determination of Vitamin C in a Food Sample

Vitamin C (ascorbic acid) is a crucial water-soluble vitamin and a powerful antioxidant found in many fruits and vegetables. Its quantification is essential in the food industry for quality control, nutritional labeling, and assessing the freshness of products. While several methods exist for its determination, direct spectrophotometry offers a rapid and straightforward technique based on the inherent UV absorption property of ascorbic acid. This practical work introduces students to this direct method, from preparing standard solutions to quantifying the vitamin in real food samples.

The main objectives of this practical work are:

- ✓ To understand the principle of direct spectrophotometric quantification based on UV absorption.
- ✓ To master the technique of preparing a standard stock solution and subsequent serial dilutions.
- ✓ To learn how to construct and use a calibration curve for quantitative analysis.
- ✓ To determine the concentration of vitamin C in various food samples (mandarin, lemon, tomato, parsley).

I. Required Materials

1. Standard solution of Vitamin C at a known concentration.
2. Food sample containing Vitamin C.
3. Cuvettes for the spectrophotometer.
4. UV-Visible spectrophotometer.
5. Pipettes, beakers, and flasks.

II. Experimental Procedure

1. Preparation of the Vitamin C Standard Solution

Prepare a Vitamin C solution of known concentration (1 mg/mL) to be used for the calibration curve.

1. Weigh a precise quantity of pure Vitamin C (e.g., 0.1 g of sodium ascorbate or ascorbic acid).
2. Dissolve the weighed amount in a 100 mL volumetric flask containing distilled water.
3. Add a small amount of distilled water and stir until complete dissolution.
4. Fill to the 100 mL mark with distilled water to obtain a solution with a concentration of 1 mg/mL.
5. Stir or mix the solution well so that it is homogeneous before use.

This solution will serve as the stock solution, and dilutions will be made to prepare various concentrations for the calibration curve.

2. Measurement of Vitamin C Absorbance

1. Measure the absorbance of the standard solution and the samples at the wavelength where Vitamin C has a characteristic absorption (approximately 265 nm).

2. Turn on the spectrophotometer and let it stabilize.
3. Select the wavelength of 265 nm on the spectrophotometer. This corresponds to the maximum absorption of Vitamin C in the UV region.
4. Place distilled water in a clean cuvette and put it in the spectrophotometer compartment to perform a zero calibration. This corrects for any absorption due to water or other external factors.
5. Pipette a volume of the standard Vitamin C solution (e.g., 1 mL) and transfer it into a clean cuvette.
6. Measure the absorbance of this standard solution at 265 nm by inserting the cuvette into the spectrophotometer. Record the absorbance at this wavelength.

3. Establishing the Calibration Curve

1. Obtain a curve that relates the measured absorbance to the concentration of Vitamin C to determine the concentration of Vitamin C in unknown samples.
2. Prepare several dilutions from the Vitamin C stock solution (1 mg/mL) to obtain different concentrations. For example:

- Dilution 1: 1 mL stock + 4 mL H_2O → 0.2 mg/mL
- Dilution 2: 1.5 mL stock + 3.5 mL H_2O → 0.3 mg/mL
- Dilution 3: 2 mL stock + 3 mL H_2O → 0.4 mg/mL
- Dilution 4: 2.5 mL stock + 2.5 mL H_2O → 0.5 mg/mL
- Dilution 5: 3 mL stock + 2 mL H_2O → 0.6 mg/mL
- Dilution 6: 3.5 mL stock + 1.5 mL H_2O → 0.7 mg/mL
- Dilution 7: 4 mL stock + 1 mL H_2O → 0.8 mg/mL
- Dilution 8: 4.5 mL stock + 0.5 mL H_2O → 0.9 mg/mL
- Dilution 9: 5 mL stock + 0 mL H_2O → 1.0 mg/mL

3. Measure the absorbance of each dilution at 265 nm, as described in the previous step. This will give you a series of absorbances corresponding to different Vitamin C concentrations.
4. Plot a graph with the measured absorbances on the y-axis and the corresponding Vitamin C concentrations on the x-axis.

4. Sample Analysis

1. Determine the concentration of Vitamin C in an unknown sample using the calibration curve.
2. Pipette a volume of the sample (mandarin, lemon, tomato, parsley) and transfer it to a clean cuvette.
3. Measure the absorbance of the sample at 265 nm using the spectrophotometer.
4. From the measured absorbance, locate this value on the y-axis of the calibration curve and determine the corresponding Vitamin C concentration.

Note: If necessary, adjust the concentration by taking the dilution factor into account (if you diluted the sample before measurement). For example, if you diluted the sample 1:10, multiply the found concentration by 10 to obtain the actual Vitamin C concentration in the original sample.

Synthesis Question

Based on the protocol and your experimental results for the four samples (mandarin, lemon, tomato, parsley):

1. Rank the analyzed samples from highest to lowest Vitamin C content.

2. Justify the use of a calibration curve in this quantitative analysis instead of a single standard.

3. Discuss one potential limitation of this direct spectrophotometric method for determining Vitamin C in complex food matrices and suggest how it could be mitigated.

1. Ranking of samples by vitamin C content

According to typical experimental data , the ranking would likely be:

Parsley > Lemon > Mandarin > Tomato

- Parsley is known for its very high vitamin C content (approximately 130-190 mg/100g)
- Lemon contains about 50-60 mg/100g
- Mandarin contains 30-40 mg/100g
- Tomato has the lowest content (15-25 mg/100g)

3. Justification for using a calibration curve

The calibration curve is essential for several reasons:

- **Instrument calibration:** It verifies the spectrophotometer's linear response

- **Greater precision:** A multi-point curve helps detect errors and establish a reliable regression line

- **Quantitative determination:** The linear absorbance-concentration relationship enables accurate calculation of unknown concentrations

3. **Method limitation and improvement suggestion**

- **Main limitation**

Lack of specificity - at 265 nm, other UV-absorbing compounds (such as organic acids, polyphenols, pigments) can interfere and distort results.

- **Proposed solution**

- Dual-wavelength method: Measure absorbance at 265 nm (vitamin C maximum) and at a reference wavelength (e.g., 290 nm) where vitamin C doesn't absorb, then subtract non-specific absorbance.

- Selective extraction: Use a specific solvent or preliminary purification step.

- Complementary method validation: Confirm results with a more specific method like HPLC.

Practical Work 5: Thin-Layer Chromatography of Sugars (TLC)

Thin-layer chromatography (TLC) is a widely used qualitative and semi-quantitative analytical technique for separating and identifying the compounds in a mixture. It is based on the difference in affinity of substances between two phases: a stationary phase (fixed) and a mobile phase (eluent). Under the effect of capillary action, the components migrate at different speeds, allowing their separation into distinct spots after revelation.

In this practical work, we will apply TLC to separate and identify the sugars present in common products such as milk and orange juice, by comparing them to standard sugars.

- **Objectives**

- Master the technique of thin-layer chromatography.

- Separate the sugars present in biological samples (milk, orange juice).

- Identify sugars by comparison with standards (glucose, lactose, maltose, sucrose).

- Calculate the retention factors (Rf) to characterize each sugar.

- Interpret the results and draw conclusions about the composition of the analyzed samples.

❖ **Materials and Products**

1. Materials

- TLC plates
- Beaker
- Pasteur pipette
- 100ml and 25ml graduated cylinders
- Hair dryer
- Spatula spoon
- Wash bottle with distilled water (30mL)
- 4 empty glass test tubes with a stopper
- Precision balance (to 0.01g)
- Chromatography tank

2. Products

- Standard sugars (glucose, lactose, maltose, sucrose) at 20 g/L

- Revealing agent ($KMnO_4$)

- Eluent: chloroform / methanol / water = (6/4/1)

- Milk and orange juice

❖ **Procedure**

1. Preparation of the Elution Tank

- Pour the eluent into the tank to a height of 0.5 cm.

- Close the tank with a lid to saturate the atmosphere with solvent vapor.

2. Sample Preparation

- Gently press the fruit (mandarin) over a 25 mL beaker to collect the juice.

- Use the milk as is.

3. Preparation of the TLC Plate

- Draw a line with a pencil 1 cm from the bottom edge of the plate.

- Mark 3 equidistant points for sample application.

- Apply using a capillary tube:

  - 3 drops of each standard solution (glucose, lactose, maltose, sucrose)

  - 3 drops of each sample (milk, orange juice)

- Dry the deposits with a hair dryer.

4. Elution

- In a 100 mL graduated cylinder, measure:

  - 60 mL of chloroform

  - 40 mL of methanol

  - 10 mL of distilled water

- Carefully introduce the plate into the tank without touching the walls.

- Allow the eluent to migrate until it reaches 0.5 cm from the top edge.

- Remove the plate and immediately mark the solvent front.

- Dry the plate with a hair dryer.

5. Revelation

- Weigh precisely 0.5 g of $KMnO_4$ using the precision balance.

- In a 100 mL beaker, dissolve the $KMnO_4$ in 50 mL of distilled water.

- Transfer to a 100 mL volumetric flask and fill to the mark with distilled water.

- Agitate until completely dissolved (homogeneous violet solution).

- Transfer to an amber spray bottle or store protected from light.

- Immerse the plate in a tray containing the revealing agent ($KMnO_4$) for 2 seconds.

- Dry again with a hair dryer: the sugar spots appear light against a violet background.

6. Analysis

- Circle the spots and mark their centers.

- Measure the distances traveled by each spot and by the solvent front.

❖ **Analysis of Results**

a) Calculation of the Retention Factor (Rf)

For each spot, calculate the Rf using the formula:

Rf = Distance traveled by the spot/ Distance traveled by the solvent.

Calculate the average value for each type of sugar.

b) Interpretation

- Compare the Rf values of the samples with those of the standards to identify the sugars present.

- Note the possible presence of multiple spots in the same sample, indicating a mixture of sugars.

- Conclude on the sugar composition of the analyzed milk and orange juice.