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Educational Handout
Subject: Workshop

Pharmaceutical chemistry

Level : 1st year of the second cycle
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Foreword

Pharmaceutical chemistry, also referred to as medicinal chemistry, is a multidisciplinary scientific field at the intersection of chemistry, pharmacology, and medicine. It draws upon concepts from organic chemistry, biochemistry, pharmacology, pharmacognosy, molecular biology, and physical chemistry. This integrated approach enables the design and development of drugs through the definition, preparation, and characterization of chemical compounds, active substances, and excipients **[1].**The main objective of this workshop is to introduce students to the fundamental techniques of pharmaceutical chemistry, such as liquid–liquid extraction, distillation, and recrystallization. Students will not only learn to master these methods, but also to critically analyze experimental protocols, identifying the chemical species involved, their quantities, and the parameters required for successful experiments. This practical work also encourages the application of interdisciplinary knowledge in medicinal chemistry and natural products chemistry, thereby preparing students for the design and development of drug substances in a multidisciplinary context.

This workshop is intended for first-year master's students specializing in **immunotechnology**. It is part of the first-semester program, with a total workload of **45** hours spread over fourteen weeks. The subject carries a coefficient of **2** and is credited with **4**.

Prerequisites include a solid understanding of organic chemistry and analytical chemistry, as well as skills in determining the quantities of chemical species, identifying the limiting reagent, and selecting experimental parameters. Students are also expected to be familiar with purification techniques, product analysis, and yield calculation, while strictly adhering to laboratory safety rules.

At the end of this course, students should be able to:

- Understand the process of drug design, their preparation through organic synthesis, and their nomenclature (particularly that of heterocyclic compounds);
- Establish correlations between the chemical structure of drugs and their biological and therapeutic activities (structure–activity relationships), as well as evaluate their main physicochemical properties (solubility, acid–base character, stability, etc.).

This manual fully covers the program outlined in the training syllabus and includes seven practical workshops**.** It thus provides a comprehensive and structured resource to help students acquire essential skills in pharmaceutical chemistry and prepare for the challenges of drug design and development.

Table of contents

General introduction

Pharmaceutical chemistry is a specialized branch of chemistry that focuses on the design, synthesis, and analysis of chemical compounds intended for therapeutic use. It plays a crucial role in the development of new drugs, ensuring not only their therapeutic efficacy but also their safety and quality **[2].** This discipline bridges fundamental chemical sciences and applied pharmacology, providing the tools necessary to understand how chemical structure influences biological activity.

Pharmaceutical chemistry involves the design, preparation, and mechanistic study of drugs, with particular emphasis on compounds derived from organic synthesis. By establishing structure-activity relationships (SAR), this field enables chemists to optimize molecular structures to enhance pharmacological effects while minimizing undesirable side effects **[3].** It also integrates analytical techniques to characterize compounds, determine purity, and verify dosage forms, making it indispensable in both academic research and industrial drug development.

This laboratory manual is structured around seven hands-on workshops, each targeting essential techniques and concepts in pharmaceutical chemistry:

Workshop 01: Introduction to pharmaceutical chemistry laboratory work, including safety measures, hygiene, and laboratory best practices.

Workshop 02: Study of aqueous solutions and titration of active chlorine, including the preparation of solutions such as sodium hypochlorite (Dakin's solution).

Workshop 03: Extraction and identification of salicylic acid, demonstrating principles of organic extraction and compound characterization.

Workshop 04: Extraction and identification of hyaluronic acid, focusing on biomolecule purification and analytical verification.

Workshop 05: Synthesis of aspirin, illustrating key concepts of chemical reactions, stoichiometry, and pharmaceutical preparation.

Workshop 06: Ointment preparation and quality control, highlighting formulation techniques and evaluation of dermatological products.

Workshop 07: Capsule formulation and preparation, introducing solid dosage forms and ensuring compliance with quality standards.

Through the combination of preparatory exercises, guided questions, and experimental sessions, students are encouraged to develop a deep understanding of the rationale behind each procedure. This approach not only reinforces practical laboratory skills but also fosters critical thinking, enabling students to interpret results, troubleshoot experiments, and relate chemical principles to pharmacological outcomes.

Overall, this manual is designed to provide a comprehensive foundation in pharmaceutical chemistry, equipping students with the knowledge and skills required for both academic progression and professional practice in the pharmaceutical and biomedical fields.

Workshop manual

Workshop N° 01: Introduction to Pharmaceutical Chemistry Laboratory Work *(Safety measures, hygiene, etc.)*

I. Introduction

Practical work in pharmaceutical chemistry presents specific safety challenges due to the complex nature of the chemical substances and processes involved. Handling chemicals—often highly active and sensitive—requires heightened vigilance to prevent accidents and ensure a safe working environment.

II. Objectives

- Apply laboratory safety rules
- Become familiar with laboratory equipment
- Develop documentation and record-keeping skills
- Learn to recognize safety pictograms
- Review some basic calculation concepts

III. Safety Instructions

III.1. Obligations:

- Wear a 100% cotton lab coat of reasonable length with long sleeves
- Wear personal protective equipment such as safety goggles and gloves
- Tie back long hair
- Wear clothing that covers the legs and closed-toe shoes
- Be familiar with emergency procedures, including the location of safety showers and fire extinguishers
- Immediately report any incident or accident in the laboratory to the supervisor
- Read and understand the Safety Data Sheets (SDS) of the substances used

III.2. Recommendations

- Dispose of chemical waste in accordance with current regulations

- Use appropriate containers for hazardous waste
- Maintain a clean and well-organized workspace to avoid accidents
- Immediately clean up any spills
- Students must always handle experiments while standing
- Stools or chairs must be stored under the benches so as not to block the aisles
- At the end of the practical session: empty all containers, rinse and store glassware, fill burettes with distilled water, and clean the workspace
- Wash hands thoroughly after handling chemicals
- Never return a used product to its original container; avoid contaminating a solid by using a soiled spatula
- Use heat-resistant glassware (Pyrex) when heating is required
- Avoid subjecting glassware to thermal shocks (do not cool a hot vessel abruptly)
- Never pour water into a concentrated acid solution (risk of splashing or burns)

III.3. Prohibitions

- Smoking, drinking, or eating in the laboratory
- Mouth pipetting: sucking with the mouth is strictly prohibited; this operation must be carried out using the equipment provided (aspirating bulbs, pipettors, etc.)
- Looking closely into containers holding boiling liquids
- Pouring or transferring liquids while keeping the face close to or above the containers being handled
- Inhaling the contents of a container to identify it by smell
- Handling chemicals directly with fingers or tasting them **[4]**

III.4. Proper Handling of Chemicals

- Read the Safety Data Sheets (SDS)
- Use personal protective equipment (PPE)
- Work in a well-ventilated area
- Handle with care
- Ensure clear labeling
- Store appropriately
- Dispose of waste safely

III.5. Key Practices to Learn

a) For handling a solid:

- Use a metal spatula. Direct contact with fingers is prohibited.

b) For handling a liquid:

- Pour a small quantity into a beaker
- Always close the bottle immediately after use
- Use a pipette with a pipettor to measure precise volumes, or a soft pipette for approximately 1–2 mL

c) Before leaving the laboratory:

- Discard solutions in recovery containers whenever possible
- Wash glassware (use the brush available at the benches)
- Clean and tidy the workbench **[5]**

IV. Safety Pictograms

The vast majority of substances even so-called “natural” ones can be hazardous depending on how they are used. In a laboratory, the potential risks of a substance are indicated on the product’s packaging by a hazard pictogram.

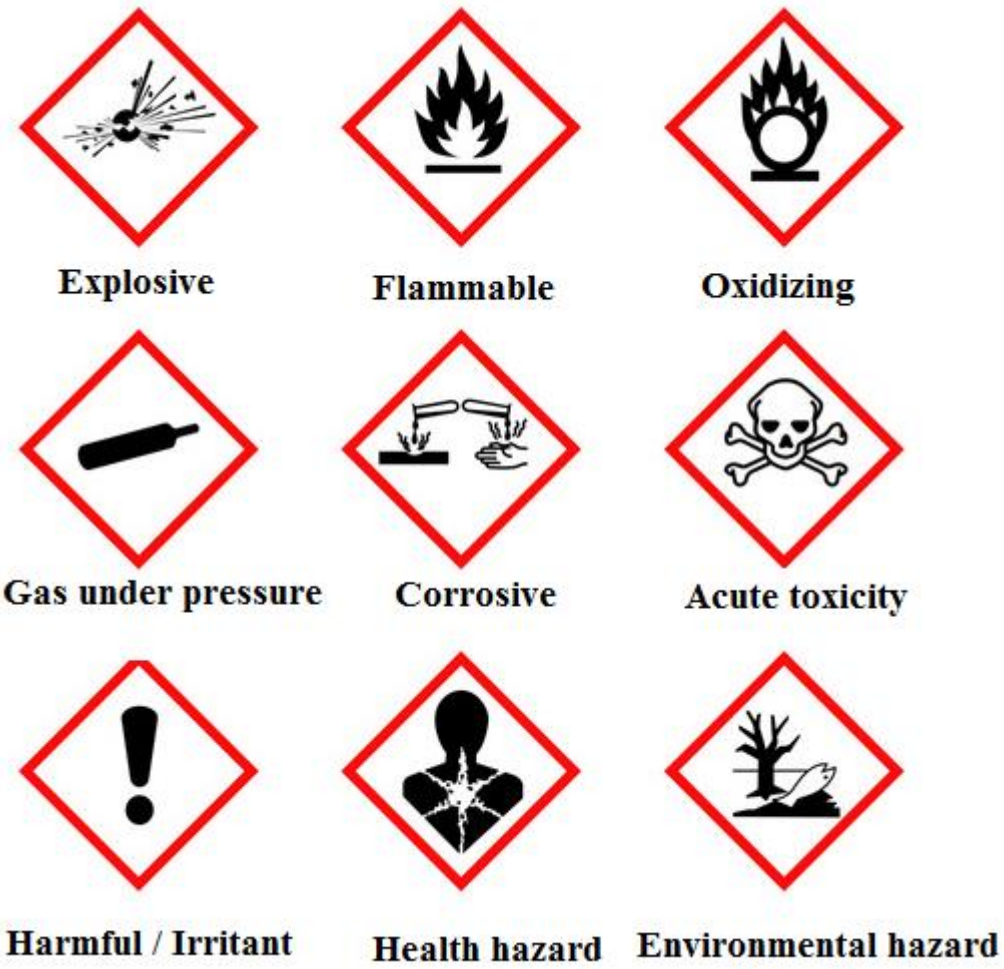

Fig.01. Safety pictograms

V. Glassware

V.1. Volumetric Glassware:

Volumetric glassware is used in laboratories to perform accurate measurements of liquid volumes. Here are some examples of commonly used volumetric glassware:

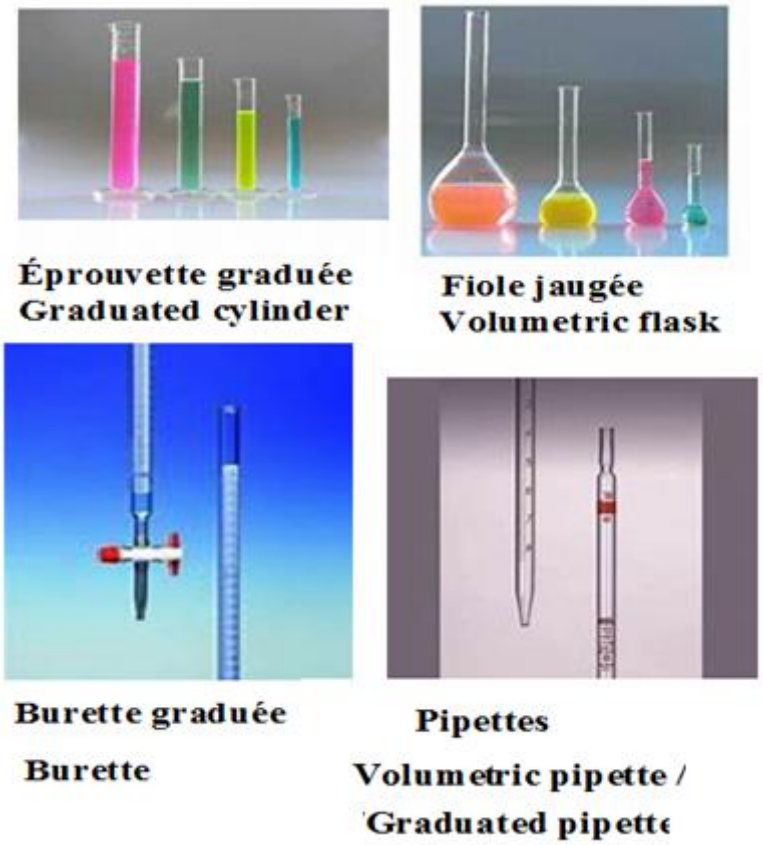

Fig.02. Volumetric Glassware

V.2. General Laboratory Glassware:

General laboratory glassware includes the most commonly used instruments for various operations, whether for heating, mixing, storing, or containing chemical substances. Here is a list of the main types of general glassware:

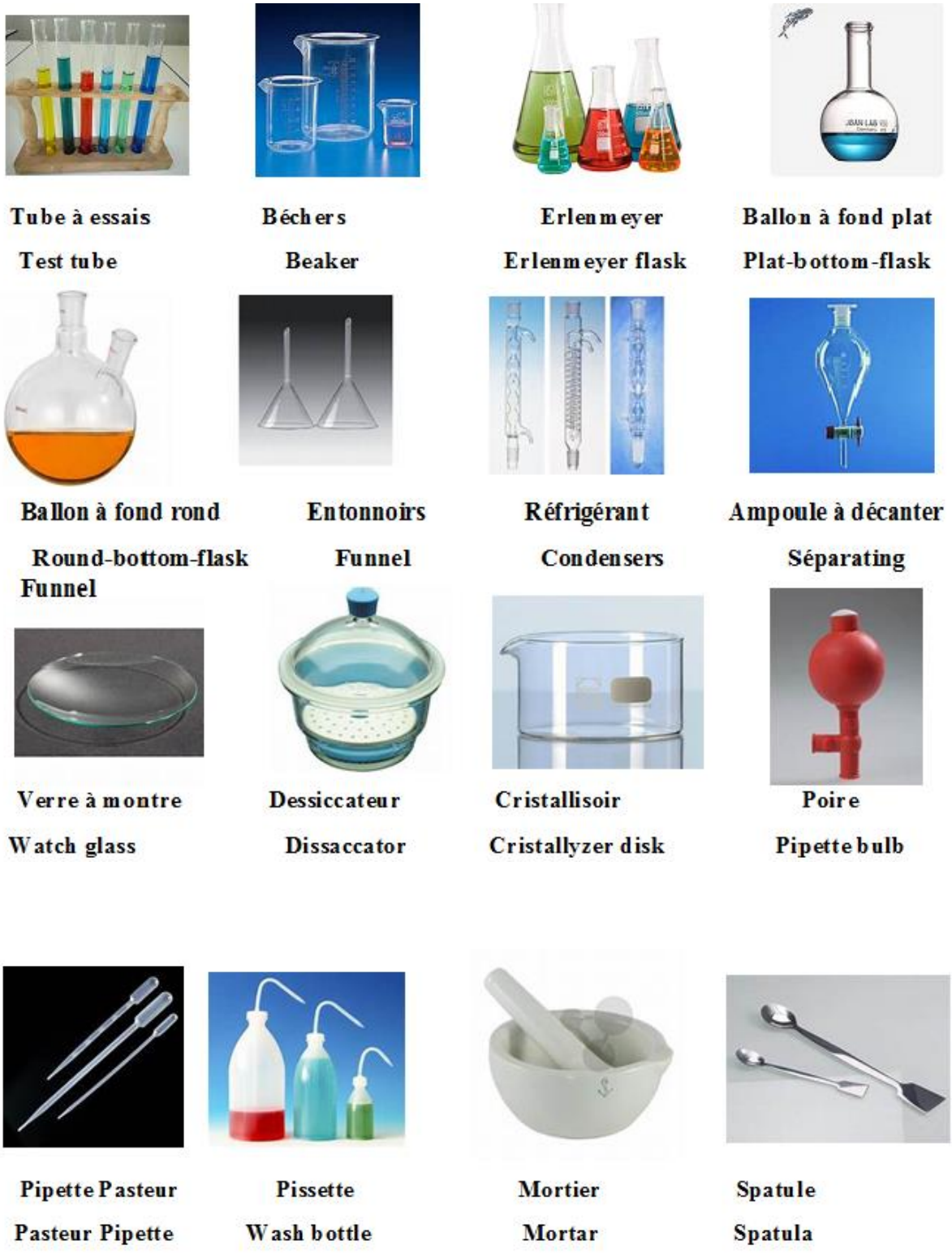

Fig.03. General Laboratory Glassware

V.3. Laboratory equipment

Fig.4. Laboratory equipment

Workshop N° 02 : Aqueous solutions, active chlorine titration – preparation of aqueous solutions, sodium hypochlorite solution (Dakin's solution)

I. Introduction

In the healthcare field, infection prevention is essential, whether in hospitals, laboratories, or daily life. Antiseptics play a crucial role in this prevention: they help eliminate or limit the growth of potentially pathogenic microorganisms on living tissues, such as the skin, thereby reducing the risk of contamination.

II. Objectives

- Learn the basics of antiseptic solution formulation.
- Handle and measure active ingredients to prepare an effective solution.
- Comply with safety and hygiene standards during preparation.
- Understand the antimicrobial properties of antiseptics and their importance in infection prevention.
- Apply dilution and mixing techniques to obtain the desired concentrations.
- Understand the principle of UV-Visible spectrophotometry.
- Acquire practical skills in performing dosage using a UV-Visible spectrophotometer.

III. Definition of an Antiseptic Solution

An antiseptic solution is a liquid preparation containing substances capable of destroying or inhibiting the growth of microorganisms (such as bacteria, viruses, and fungi) on living tissues, particularly the skin and mucous membranes. Its purpose is to prevent infections by reducing the number of microorganisms on treated surfaces, mainly in medical contexts, but also in other areas where hygiene is crucial. **[7]**

III.1. Definition of Dakin's Solution

Dakin's solution is an antiseptic solution made of sodium hypochlorite (NaOCl) diluted in water (hence the bleach-like odor) and potassium permanganate, which gives it a pink coloration.

It was developed during World War I by the British chemist Henry Drysdale Dakin to disinfect wounds and prevent infections.

Dakin's solution works by releasing active chlorine, which eliminates microorganisms present on tissues. Potassium permanganate is used to stabilize the solution; however, it must be stored away from light to slow down its decomposition, as it becomes inactive about 7 days after opening the bottle. It also has a bleaching effect by oxidizing colored pigments. However, it is important to emphasize that Dakin's solution can irritate tissues and should only be applied to healthy, unbroken areas. **[8]**

III.2. Uses of Dakin's Solution

- Cleaning superficial wounds: such as cuts, scrapes, and minor burns.
- Disinfection of chronic wounds: such as skin ulcers and bedsores.
- Postoperative care: as a complement to prevent infection of surgical wounds.

IV. Assay of Antiseptic Solutions

The assay of an antiseptic solution can be performed using different methods depending on the type of active compound present in the antiseptic. In this workshop, we will use two methods:

IV.1. UV-Visible Spectrophotometric Assay

Spectrophotometric assay is a widely used method in pharmacopeia and generally in the pharmaceutical industry. Many active ingredients found in medicines contain chemical groups in their structure that absorb in the ultraviolet region and can thus be quantified. UV absorption mainly depends on electronic energy.

Spectrophotometry is a qualitative and quantitative analytical method:

- *Qualitative*: for identification (by comparison with a reference sample); however, UV/Visible absorption alone is insufficient to identify a substance, but it can usefully complement infrared, mass spectrometry, NMR, and other spectral methods.
- *Quantitative*: useful for dosage; it is a fast and accurate method, based on the Beer-Lambert law, which relates absorption to the concentration of molecules in solution, at a given wavelength (λ). **[9]**

IV.2. Iodometric Assay

Iodometry is a redox titration method that uses iodine (I_2) to determine reducing substances. It is based on a reaction where a reducing agent reduces molecular iodine (I_2) into iodide ions (I^-), while an oxidizing agent, added in excess, generates iodine from iodide ions. **[10]**

V. Protocol for the Preparation of Dakin's Solution

The concentration of permanganates is very low (10 mg/L), which makes volumetric titration difficult. Moreover, the bottle label does not specify this concentration.

In contrast, hypochlorite (ClO^-) is more concentrated, and the label indicates: *"*active chlorine content 0.5 g/100 mL*"*.

Therefore, before preparing Dakin's solution in the laboratory, it is important to determine the active chlorine content (T.a.c, g/L) and the chlorometric degree (°Chl) of the sodium hypochlorite solution used (commercial bleach).

V.1. Determination of Active Chlorine Content by Titration

This is an indirect back titration using the **iodometric method**. It is based on the oxidation of potassium iodide (KI) in an acetic medium and the titration of the released iodine with a 0.1 N sodium thiosulfate ($Na_2S_2O_3$) solution. **[11]**

a- Chemical réactions :

$$\mathbf{ClO^- + 2H^+ + 2é \longrightarrow Cl^- + H_2O}$$
$$\mathbf{2I^- \rightleftharpoons I_2 + 2e^-}$$

$$\mathbf{ClO^- + I^- + 2H^+ \longrightarrow Cl^- + I2 + H2O}$$

In the presence of an oxidizing agent, the colorless iodide ions (I^-) are oxidized to brown iodine (I_2).

Since iodine is only slightly soluble, it is essential to add an excess of iodide ions (I^-), which establishes an equilibrium with iodine (I_2) to form triiodide ions (I_3^-), which are highly soluble:

$$\mathbf{I^- + I_2 \rightleftharpoons I_3^-}$$

The released iodine is then titrated with sodium thiosulfate.

$$2S_2O_3^{2-} \rightleftharpoons S_4O_6^{2-} + 2e^-$$

The overall reaction is:

$$I_2 + 2e^- \rightleftharpoons 2I^-$$
$$2S_2O_3^{2-} \rightleftharpoons S_4O_6^{2-} + 2e^-$$

$$I_2 + 2S_2O_3^{2-} \rightleftharpoons 2I^- + S_4O_6^{2-}$$

b- Titration Protocol:

In an Erlenmeyer flask, introduce:

- 25 mL of distilled water
- 2.5 mL of acetic acid (≈ 5 drops)
- 1 g of potassium iodide (KI)
- 5 mL of bleach diluted to 1/10

Place the Erlenmeyer flask away from light for 10 minutes. Then, using a graduated burette, add a standardized N/10 sodium thiosulfate solution.

Start the titration of starch indicator; the solution will turn blue. And continue the titration until the solution becomes colorless. **[12]**

Record the volume of sodium thiosulfate used, **V ml**.

The amount of chlorine contained in one liter of bleach is: **V mL × 3.546 g/l**

The chlorometric degree is defined as the number of liters of chlorine gas that a chlorinated solution can release (l/l).

Since **1** liter of chlorinated solution releases **3.17 g** of chlorine, the chlorometric degree of the tested bleach is:

$$\mathbf{0° \ chl} = \frac{\textbf{Mass of chlorine contained}}{\mathbf{3.17}}$$

Active chlorine content (g/l) = V × 3.546

V.2. Preparation of Dakin's Solution

For 100 mL of solution :

- In a 250 mL Erlenmeyer flask, dissolve 1.5 g of sodium bicarbonate in 25 ml of distilled water at room temperature.
- Add the calculated amount of bleach (7 ml), and then add potassium permanganate (0.001 g).
- Mix the solution thoroughly, and then transfer it into a 100 ml volumetric flask and make up to the calibration mark with distilled water. **[13]**

Fig.05: Preparation of Dakin's Solution

VI. .Storage

This solution should not be stored for more than one week. It must be kept in a colored glass container, in a cool place, protected from light.

VII. Assay by UV-Visible Spectrophotometry

VII.1. Preparation of Standard Solutions

Dilute the commercial Dakin's solution to obtain a series of solutions with known iodine concentrations.

- *Solution 1*: In a 100 ml volumetric flask, introduce 0.5 ml of Dakin's solution and fill to the mark with distilled water (concentration 0.005 g/l).
- *Solution 2*: In a 100 ml volumetric flask, introduce 1 ml of Dakin's solution and fill to the mark with distilled water (concentration 0.01 g/l).
- Repeat this process to obtain several concentration points within a linear range.

Measure the absorbance of each standard solution at the wavelength of maximum absorption, generally between 500 and 550 nm (to be determined by a wavelength scan).

Plot the calibration curve by placing concentration values on the x-axis and absorbance values on the y-axis.

A straight line should be obtained if the Beer-Lambert law is obeyed in the chosen concentration range.

VII.2. Determination of the Analytical Wavelength

Perform a wavelength scan of Dakin's solution (or one of the standard solutions) in the range of 400 to 600 nm.

Record the wavelength where maximum absorption occurs, e.g., around 500–580 nm, and use this wavelength for all measurements.

VII.3. Measurement of the Concentration of Laboratory-Prepared Dakin's Solution

Once the calibration curve is established, it can be used to determine the concentration of the Dakin's solution prepared in the laboratory by measuring its absorbance and using the calibration curve to calculate the corresponding concentration.

VIII. Matérials and reagents:

Matérials	Reagents
Volumetric flask (10 ml) × 5-(100 ml) ×6 Erlenmeyer flask × 5 Sterile tube (10 ml)	Potassium permanganate ($KMnO_4$) 
Beaker × 5 Burette × 5 Quartz spectrophotometer cuvette Magnetic stir bar × 5	Bleach (Sodium hypochlorite solution)  Sodium thiosulfate ($Na_2S_2O_3$) Sodium bicarbonate ($NaHCO_3$) Starch Commercial Dakin's solution

IX. Questions and Answers

1) Why can the titration be considered an indirect titration?

- Because the target species (hypochlorite ions, ClO^-, responsible for active chlorine) is not titrated directly.
  Instead, it is first reacted with an excess of iodide ions (I^-), which release iodine (I_2).
  Then, this liberated iodine is titrated with a sodium thiosulfate solution.

2) Why should iodide ions (I^-) react with hypochlorite ions (ClO^-) before adding acetic acid?

- Because the oxidation of I^- to I_2 by ClO^- must occur in a mildly acidic medium.
  If acetic acid is added too early, hypochlorite can decompose and release chlorine gas (Cl_2) or oxygen, which would distort the result.

3) What is the role of starch?

- Starch acts as a specific indicator for iodine.

In the presence of small amounts of iodine (I_2 or I_3^-), it forms a dark blue complex.
The disappearance of this blue coloration (turning colorless) marks the equivalence point.

4) How to determine the concentration of the Dakin solution?

- Measure the absorbance of the prepared Dakin solution.
- Use the calibration curve obtained from the standard solutions (Beer–Lambert law).
- The active chlorine concentration (C, in g/l) of the Dakin solution is calculated using the linear equation:

$$\mathbf{A = k \cdot C + b}$$

Where A is the absorbance, C the concentration, and k the slope of the calibration curve.

Workshop N°03: Obtaining and Identification of Salicylic Acid

I. Introduction

Salicylic Acid (2-hydroxybenzoic acid) is an aromatic phenolic compound widely used in the pharmaceutical, cosmetic, and agro-food industries, particularly for its keratolytic, anti-inflammatory, and antiseptic properties **[14].** Historically, it was extracted from natural plant sources such as willow bark (*Salix* spp.), which contains salicin, a natural precursor first isolated in 1828 by Johann Buchner. Over time, the process shifted to industrial chemical synthesis, mainly through the Kolbe-Schmitt reaction — a much more cost-effective and pure method.

II. Objectives:

- To master laboratory techniques related to the preparation and identification of organic compounds.
- To connect theoretical knowledge of organic chemistry with the pharmaceutical and cosmetic applications of salicylic acid.
- To interpret experimental results.

III. Definition of Salicylic Acid

Salicylic acid is an aromatic monocarboxylic acid with a hydroxyl group in the ortho position relative to the carboxyl group on a benzene ring ($C_7H_6O_3$). This arrangement gives it both acidic properties (–COOH group) and phenolic properties (–OH group).

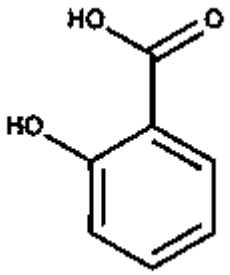

Fig.6. Salicylic Acid structure

IV. Experimental Protocol

IV.1. Extraction of Salicylic Acid:

- Weigh 20 g of plant material (willow bark).
- Grind finely with sand and a small amount of water to facilitate extraction.
- Transfer the plant powder into a round-bottom flask.
- Add 150 ml of ethanol and 10 mL of concentrated hydrochloric acid.
- Heat under reflux for 2 h 30 min at 50 °C.
- During this step, the hydrolysis of salicin is catalyzed by hydrochloric acid to form salicylic acid.
- Filter the reaction mixture to separate the plant residue.
- Rinse the residue with a small amount of ethanol to recover as much active compound as possible.
- Collect the filtrate and gradually add a solution of sodium hydroxide (NaOH) until pH 12–13 is reached. This step converts salicylic acid into sodium salicylate, which is water-soluble.
- Perform an extraction with ethyl acetate (3 × 20 ml). This step removes less polar compounds, particularly saligenin.
- Separate the two phases: the organic phase (containing non-polar compounds) and the aqueous phase (containing sodium salicylate).
- Heat the aqueous phase to concentrate it.
- Acidify the concentrated solution gradually with HCl until pH 2–3. Acidification causes the precipitation of salicylic acid crystals.
- Filter the obtained salicylic acid crystals, wash them with distilled water to remove impurities, and store them protected from light. **[15]**

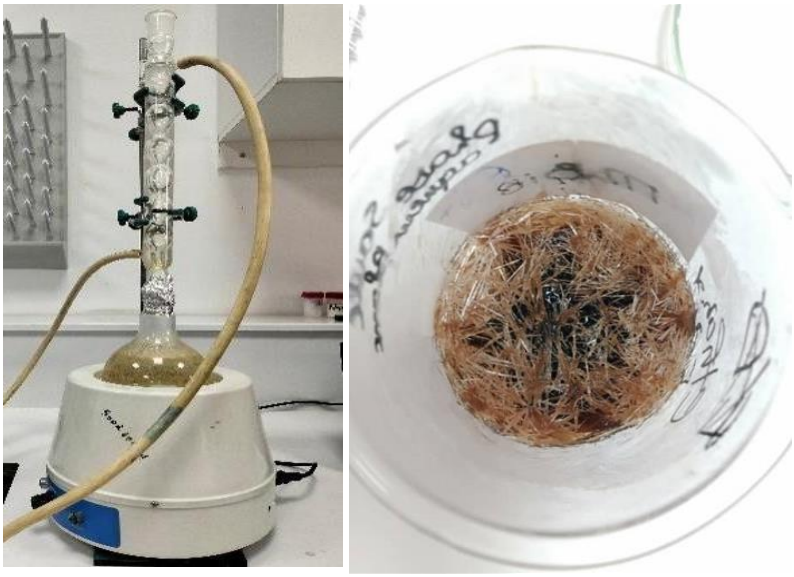

Fig.7. Salicylic Acid extraction

IV.2. Purification by Recrystallization of Salicylic Acid

- Weigh the crude salicylic acid obtained and dissolve it in a minimal volume of hot ethanol to obtain a saturated solution.
- Allow the solution to cool slowly and in a controlled manner.
- The crystallization of pure salicylic acid occurs gradually.
- Impurities, being less soluble in cold ethanol, either remain in solution or are excluded from the crystal lattice.
- Filter the solution to isolate the formed crystals.
- Wash the crystals with a small amount of cold ethanol to remove any residual impurities.
- Allow the salicylic acid crystals to dry in air until the solvent is completely evaporated.

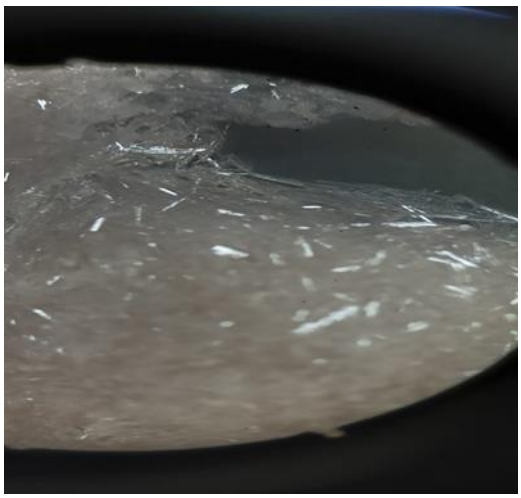

Fig.8. Microscopic image of salicylic acid crystal

V. Matérials and reagents :

Matérials	Reagents
Mortar Single-neck round-bottom flask (250 ml) Water bath Büchner filter Filter paper Erlenmeyer flask Balance Watch glass Spatula Pumice stone Hot plate with magnetic stirrer	Willow bark Concentrated hydrochloric acid  Ethanol  Sodium hydroxide  Concentrated hydrochloric acid  Ethyl acetate 

VI. Questions and Answers

1) What is the role of sodium hydroxide (NaOH) in the protocol?

- It converts salicylic acid into sodium salicylate, which is soluble in water, facilitating separation.

2) Why is extraction with ethyl acetate performed?

- To remove less polar compounds (such as saligenin) and impurities.

3) Which chemical properties of salicylic acid allow its identification?

- Its acidity (–COOH group), phenolic properties (–OH group), and characteristic reactions such as color changes with $FeCl_3$ (purple complex).

4) What is the difference between natural salicylic acid (from willow bark) and the industrially synthesized one (Kolbe-Schmitt)?

- Natural salicylic acid comes from plant extraction, while the industrial method (Kolbe-Schmitt reaction) is more economical and yields purer salicylic acid.

Workshop N°04: Extraction and identification of hyaluronic acid

I. Introduction

Hyaluronic acid is a natural polymer found in numerous animal and microbial species, endowed with remarkable physicochemical properties. For nearly three decades, it has experienced growing success in both therapeutic and cosmetic applications. To meet the increasing demand for this biopolymer, production methods have evolved alongside scientific knowledge. Extraction from animal tissues has been gradually replaced by microbial production. The most commonly used strains for the biosynthesis of this molecule of interest are capsulated bacteria of the genus *Streptococcus*, particularly *Streptococcus equi* subsp. *zooepidemicus*. **[16]**

II. Objectives :

- To extract hyaluronic acid from a biological tissue.
- To purify the obtained extract.
- To identify hyaluronic acid using simple physicochemical and biochemical methods.

III. Definition of hyaluronic acid:

Hyaluronic acid is a natural biopolymer belonging to the family of glycosaminoglycans. It is composed of repeating disaccharide units of glucuronic acid and N-acetyl-glucosamine, alternately linked through β(1-3) and β(1-4) glycosidic bonds (Figure 8).

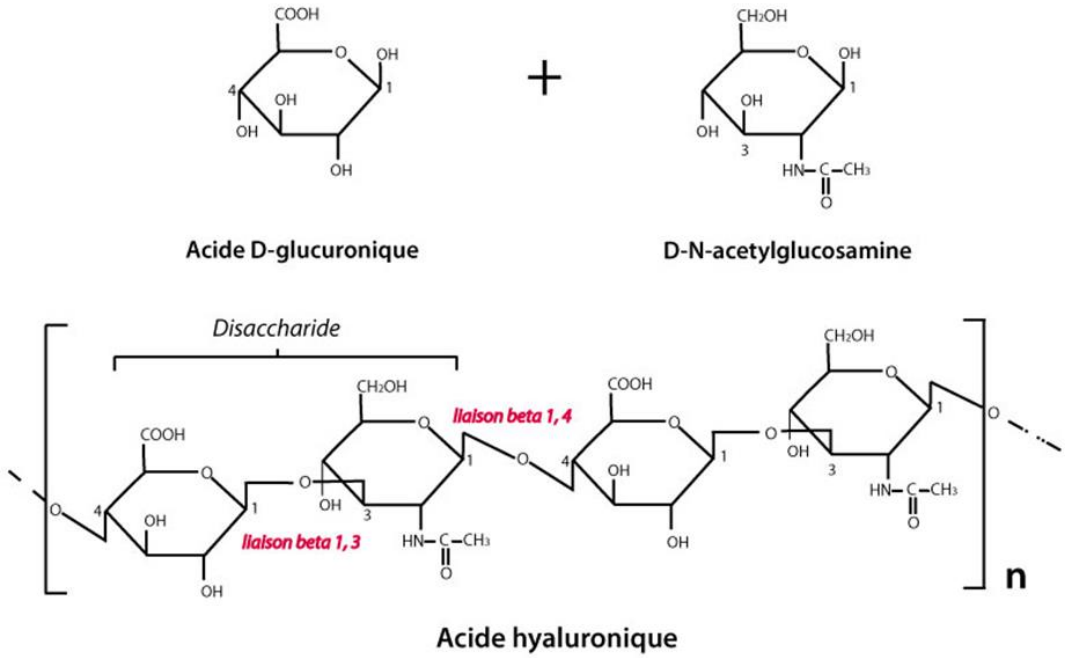

Fig .9. Chemical structure of hyaluronic acid

It can exist in its acidic form, hyaluronic acid, or as a salt, mainly sodium salt, known as sodium hyaluronate. It is never sulfated in its natural state. Hyaluronic acid plays an important role in the human body due to its unique rheological and structural properties. In association with the collagen fiber network, the supramolecular structures of HA determine the shape and architectural organization of tissues. It provides them with mechanical properties (such as cartilage shock absorption and blood vessel elasticity), while also contributing to the maintenance of integrity and hydration in numerous tissues, including the skin and the brain. **[17]**

IV. Extraction protocol of hyaluronic acid:

The source tissue will be fresh rooster combs.

IV.1. Tissue preparation

- Cut 10 g of tissue into small pieces.
- Wash with PBS buffer (0.15 M NaCl) to remove blood impurities.
- Grind finely in a mortar containing cold buffer (~4 °C).

IV.2. Enzymatic digestion

- Transfer the mixture into a flask.
- Add papain at 20 mg/ml in buffer (pH 6.0–7.0).
- Incubate at 55–60 °C for 2–3 hours with gentle stirring. The enzyme degrades proteins and releases HA **[18].**

IV.3. Deproteinization

- Allow to cool at room temperature.
- Add 2 volumes of absolute ethanol.
- Shake, then centrifuge for 10 min at 5,000 rpm.
- Recover the aqueous phase (containing HA).

IV.4. HA precipitation

- Add 2 to 3 volumes of pre-chilled ethanol (–20 °C).
- Leave at 4 °C for 24 h to allow HA to precipitate at low temperature.
- Remove the supernatant and collect the precipitate.

IV.5. Drying and quantification

- Dry in an oven at 40 °C or lyophilize.
- Weigh the solid to calculate the yield.

V. Identification of Hyaluronic Acid.

After drying, a white precipitate appears, confirming the presence of HA. The spectrophotometric method will also confirm this result

V.1. Spectrophotometric analysis

In a test tube, add:

- 0.5 ml of the extract solution
- 3 ml of concentrated sulfuric acid
- Mix gently
- Add 0.1 ml of carbazole solution
- Heat in a water bath (90 °C) for 10 minutes

- Cool to room temperature
- Measure absorbance at 530 nm
- A purple coloration indicates the presence of uronic acid (thus HA)
- Compare with a blank (water) and a standard calibration curve (glucuronic acid)

VII. Matérials and reagents :

Matérials	Reagents
Water bath Büchner filter Filter paper Erlenmeyer flask Balance Watch glass Spatula Graduated cylinder (10 mL, 50 mL) Incubator Magnetic stirrer hot plate Centrifuge	•Fresh rooster combs PBS (phosphate-buffered saline) Sodium chloride NaCl 0.15 M Papain (20 mg/ml) Buffer solution pH 7 Chloroform  Absolute ethanol  Sulfuric acid  Carbazole  Glucuronic acid

VIII. Questions and Answers

1) What is the biological function of hyaluronic acid (HA)?

- Hyaluronic acid plays a key role in maintaining tissue hydration, elasticity, and structural integrity. It acts as a lubricant in joints, contributes to the viscoelastic properties of synovial fluid, and supports tissue regeneration. In the skin, HA helps retain water, ensuring proper hydration and flexibility.

2) Why is an enzyme used to extract HA?

- The enzyme (papain) is used to degrade proteins and break down connective tissue, thereby releasing HA from the extracellular matrix. Enzymatic digestion increases the yield and purity of HA compared to mechanical extraction alone, since it selectively hydrolyzes proteins without degrading polysaccharides.

3) What other tissue could be used for HA extraction?

- Besides rooster combs, HA can be extracted from bovine vitreous humor, umbilical cord tissue, or certain microbial sources (e.g., *Streptococcus zooepidemicus*). However, microbial fermentation is often preferred industrially due to higher scalability and reduced contamination risks.

Workshop N°05: Synthesis of Aspirin (Acetylsalicylic Acid)

I. Introduction

Salicylic acid (o-hydroxybenzoic acid) is the most important of the phenolic acids. It is mainly used for the preparation of aspirin. In 1897, the German chemist Felix Hoffmann successfully synthesized aspirin for the first time. Today, aspirin is one of the most widely prescribed drugs worldwide, and its use continues to be investigated for various health benefits. **[19]**.

II. Objecives

- Prepare a drug, the synthesis of aspirin.
- Purify a substance by recrystallization.
- Find the right recrystallization solvent.
- Identify the product obtained by thin-layer chromatography.
- Carry out a comparative study with commercial aspirin

III. Aspirin Definition

Aspirin, or acetylsalicylic acid, with the chemical formula $COOH–C_6H_4–O–CO–CH_3$, is a widely used non-steroidal anti-inflammatory drug (NSAID) known for its analgesic, antipyretic, and anti-inflammatory properties. Its mechanism of action involves inhibiting the cyclooxygenase (COX) enzyme, thereby reducing the production of prostaglandins, which are chemical mediators involved in pain, fever, and inflammation.

Aspirin is often used to relieve mild to moderate pain, such as headaches, muscle aches, or menstrual cramps. It is also administered at low doses to reduce the risk of blood clot formation, thereby helping prevent strokes and heart attacks. However, its use can be associated with side effects, particularly gastric problems, and it is contraindicated in some patients, such as those suffering from gastriculcers. **[20]**

Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities.

IV. Principle:

Acetylsalicylic acid is synthesized by esterification of salicylic acid and acetic anhydride according to the following reaction mechanism:

a) Oxygen protonation: Salicylic acid is protonated, increasing the reactivity of its hydroxyl group (-OH).

b) Nucleophilicity: Salicylic acid's hydroxyl group acts as a nucleophile, attacking the electrophilic carbon of acetic anhydride.

c) Ester formation: This leads to the formation of an intermediate, followed by the release of acetic acid to produce aspirin.

The reaction also generates acetic acid, which can protonate the intermediate formed and help deactivate unreacted hydroxyl groups.

Acetylsalicylic acid (or aspirin) is formed according to the following reaction scheme: **[21]**

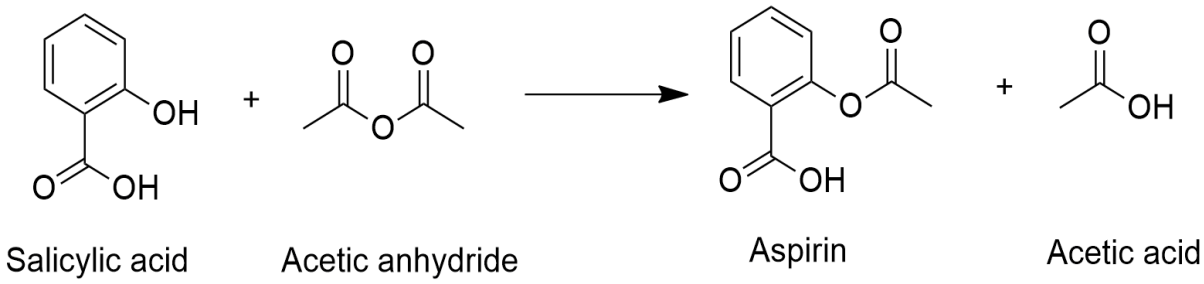

Fig.10. Chemical reaction of acetylsalicylic acid

V. Synthesis procedure:

- Place 5 g of salicylic acid, 7 ml of ethanoic anhydride and approximately 5 drops of concentrated sulfuric acid in a 250-ml flask. And a few pumice stones
- Wear gloves and goggles in a fume hood.
- Heat gently to reflux in a bain-marie with magnetic stirrer for 10 min from boiling point.
- Stop heating. When boiling has subsided, separate the flask from the condenser and add 50 ml distilled water to hydrolyze the excess anhydride.
- Shake until aspirin begins to crystallize, then add 50 ml ice-cold water and shake by placing the flask in an ice bath.

- Filter contents of flask through Büchner or filter paper.
- Rinse the product with cold water and pour this water over the Büchner to remove all the product.
- Wash the crystals with water, spoon the impure aspirin into a watch glass and leave to dry. **[22]**

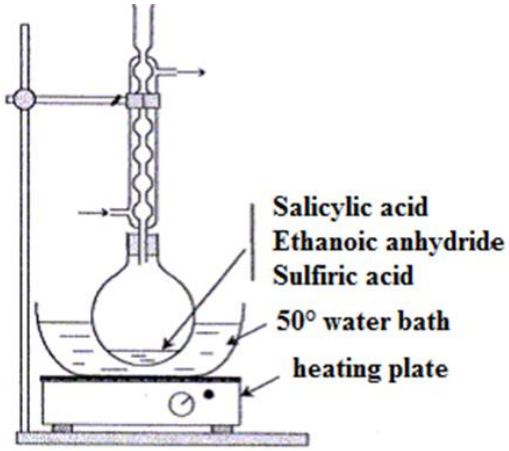

Fig. 11. Aspirin synthesis assembly **[23]**

VI. Recrystallization protocol:

- Perform tests in test tubes with different solvents to identify the correct recrystallization solvent.
- Put an appropriate amount of aspirin in an Erlenmayer
- Add a small quantity of the chosen solvent and heat the mixture to dissolve the solute completely. Adjust the amount of solvent if necessary.
- Allow the solution to cool slowly to room temperature. To promote crystallization, you can also place the beaker in an ice bath.
- Once crystals have formed, use a filter to isolate the crystals from the stock solution.
- Wash the crystals with a little cold solvent to remove any residual impurities.
- Transfer crystals to a watch glass or desiccator to dry completely. **[24]**

VII. Thin layer chromatography:

VII.1 Definition:

Thin layer chromatography (TLC) is an analytical technique commonly used in chemistry and biology to separate and identify components for analysis (analytical TLC) or

purification (preparative TLC). The technique is based primarily on the phenomenon of adsorption.

VII.2 Operating principle

The mixture, in solution, is deposited on the stationary phase, the adsorbent phase, which retains the compounds according to their chemical and physical properties.

During migration (elution), the mobile phase (a solvent or mixture of solvents) entrains the various constituents of the mixture in different ways.

To see the chemical species after elution, it is often necessary to reveal them.

A chemical species can be identified by comparison, on the same chromatogram, with a reference chemical species. **[25]**

VII.3. TLC protocol:

a) Preparation of the elution tank:

- Pour in 50 ml eluent: a mixture of 60% butyl acetate and 40% cyclohexane, with 5 drops of acetic acid.
- Close the tank tightly with the lid for about 10 minutes to saturate the inner atmosphere with the solvent vapors.

b) TLC plate preparation: deposits

- Caution: Never place your fingers on the granular surface of the plate.
- On a chromatographic plate, draw a pencil line 1 cm from the bottom edge. This line is called the deposit line.
- Place three evenly spaced crosses on this line, below which write: A (Aspirin), B (Aspec), C (Aspegic).

c) Sample preparation:

- Dilute each sample in 3 ml ethanol.
- Heat the solution until the solute is completely dissolved (if necessary).

d) Sample deposition:

Apply 3 drops of the corresponding product (A, B, C) to each cross using a capillary: dip the capillary into the bottle, then touch the cross three times with the capillary (a small spot should appear on the plate).

- **Deposit A:** Three drops of synthesized aspirin,
- **Deposit B:** Three drops of Aspec 100 mg (pill),
- **Deposit C:** Three drops of Aspegic cardio 100 mg (capsule).

e) Elution:

- Place the plate in the vat,
- Cover and wait until the solvent front reaches the top line of the plate (solvent front),
- Remove plate and allow to dry for a few seconds.

f) Ultraviolet revelation:

- For colored compounds, the chromatogram can be evaluated directly.
- For colorless compounds, it is necessary to reveal the stains: this is the revelation stage.
- Use an ultraviolet lamp, steam diode or permanganate solution. However, be careful! Do not look directly into the light of an ultraviolet lamp.
- Circle the stains with a pencil (without damaging the plate).

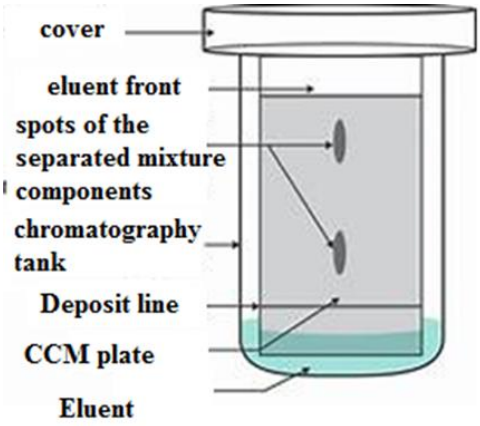

Fig.12: Thin layer chromatography

VII.4 measuring a frontal ratio:

A chemical species can be characterized by a number called the frontal ratio (noted Rf). The frontal ratio of a species is the quotient of the distance h it has covered at the end of the elution by the distance H covered by the eluent during the same time.

$$Rf = \frac{h}{H}$$

Rf has no unit. If several chemical species (several tasks) have the same frontal ratio, then they are the same chemical species. **[26]**

VIII. Matérials and reagents :

Matériels	Produits
Flat bottom flask (250 mL) Water bath Búchner filtre Filtre Paper Erlen mayer flask Scale Watch glass Spatula Graduated cylinder (10 ml, 50ml) Pumice stone Magnétic stirer Magnetic bar Test tubes CCM tank Mortar Beaker (25ml, 100ml) Capillar tubes Pencil Ultraviolet lamp CCM plate	Salycilic acid  Acetic anhydride  Concentrated sulfuric acid  Éthanol  Methanol  Acetonitril  Aspec 100mg (Pill) Aspirin cardio 100mg (capsule) Butyle acétate  Cyclohexane  Acetic acid 

IX. Questions and Answers

1) Give the name of this reaction?

- This is an esterification reaction (specifically, the acetylation of salicylic acid).

2) What are the two drawbacks of this reaction, and what methods can be used to minimize them?

Drawbacks :

- Incomplete conversion
- Side reactions and impurities

Methods to minimize them :

- Use an excess of acetic anhydride to push the reaction equilibrium toward aspirin formation.
- Apply recrystallization as a purification step to eliminate impurities and unreacted salicylic acid.
- Maintain controlled heating (gentle temperature) to avoid decomposition.

3) Why is ice-cold water added?

- Ice-cold water is added at the end of the synthesis in order to:
  a) Precipitate aspirin, since its solubility decreases significantly in cold water.
  b) Keep by-products and impurities dissolved in the aqueous phase, which facilitates their removal during filtration.

4) What is the role of boiling chips (pumice stones)?

- Boiling chips (porous pumice stones) act as boiling regulators. They promote the regular formation of vapor bubbles, which:
- prevents superheating of the liquid,
- reduces the risk of splashing of the reaction mixture,
- ensures safer and more uniform heating.

Workshop N°06: Ointment – Preparation and Quality Control of a Dermatological Formulation

I. Introduction

Ointments occupy an important place in dermatology and pharmaceutical technology, as they allow the direct local administration of treatment. Their preparation requires the careful selection of a suitable base and mastery of formulation techniques in order to obtain a homogeneous, stable, and effective product. Moreover, quality control, through organoleptic and physicochemical tests, is essential to ensure the compliance of the preparation. This practical session aims to introduce students to the preparation of an ointment and the evaluation of its pharmaceutical characteristics before therapeutic use.

II. Objectives

- Understand the fundamental principles of pharmaceutical formulation.
- Master the techniques for ointment preparation.
- Select appropriate excipients.
- Identify and solve formulation-related problems.
- Present the results of a formulation study.

III. Definition of an Ointment

An ointment is a semi-solid pharmaceutical preparation intended for external use, usually applied to the skin or certain mucous membranes. It consists of one or more active ingredients incorporated into a suitable base, which may be greasy, hydrophilic, or absorbent. Due to its consistency, it allows uniform application, promotes local drug penetration, and, depending on its composition, provides protective, emollient, or targeted therapeutic action. **[27]**

IV. Composition:

a) **Hydrophobic base:** Vaseline, lanolin, or a mixture of waxes and oils
b) **Hydrophilic base:** PEG (polyethylene glycol) or other water-miscible bases.
c) **Active ingredients:** such as plant extracts, essential oils, medicinal active ingredient.
d) **Stabilizing agents or preservatives:** such as ascorbic acid, or parabens

e) Optional agents:

- Dyes (natural or synthetic)
- Fragrances (optional, as compatible essential oils) **[27]**

V. Protocol:

In this workshop, we will prepare two different ointments: **[28]**

V.1. Herbal ointment:

To prepare a plant-based ointment, you must first prepare the oily macerate, which will be the mixture between the oil and the plant extract,

Start by selecting a macerate adapted to the desired effect, such as Rosemary macerate for its antioxidant and anti-inflammatory properties, it helps tone the skin and strengthen the hair. Or lavender for its soothing properties. It helps calm skin that is irritated, sensitive or prone to redness. It is also effective against acne thanks to its purifying action.

1) Hot maceration (fast)

- Unlike cold maceration, which lasts weeks, hot maceration can be done in a few hours.
- Weigh 50 gr of the dried and crushed plant and put them in a beaker with 150 ml of olive or sunflower oil.
- Heat in a bain-marie at a temperature between 40 and 60°C for 02 hours then filter the macerate obtained
- In a clean beaker, gently melt (30% of the mass of the oily macerate obtained) a solid fatty base (such as beeswax or shea butter) in a double boiler. Once melted, add the oily macerate, mixing gently to obtain a homogeneous phase. Remove the mixture from the double boiler, let it cool slightly, and then stir in additional active ingredients (such as essential oils or vitamin E) with a spatula if desired. Transfer the still fluid preparation to sterilized jars and let it cool to room temperature before sealing it tightly. **[29]**

Fig. 13. Steps of hot maceration

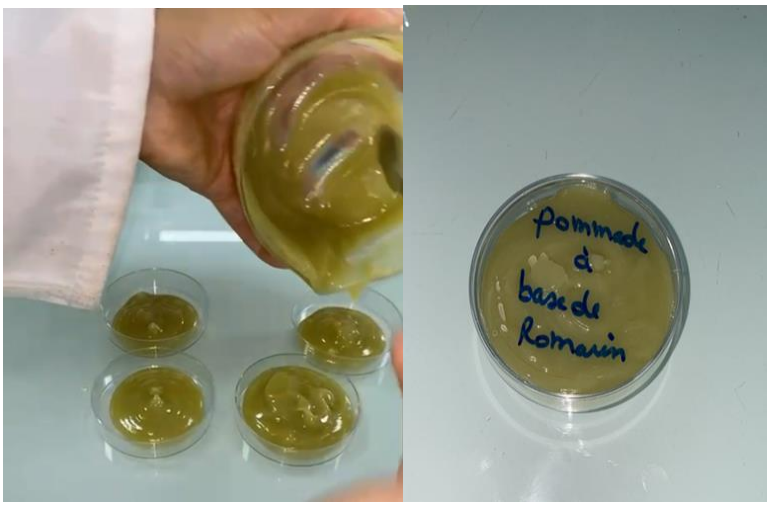

Fig. 14 Ointment Formulation

V.2. Ointment based on a synthetic active agent

Ointments containing a synthetic active ingredient are often used in pharmacies to deliver a specific molecule with therapeutic properties. Such as salicylic acid (treatment for acne, warts, psoriasis, dermatitis and improvement of skin texture), zinc oxide (healing of wounds and skin irritations), or an antifungal molecule (example: ketoconazole).

For 25 gr of 5 % salicylic acid ointment

- Weigh 1.25 g of salicylic acid,
- 21.25 g Vaseline
- 02.5 g petroleum jelly oil
- In a mortar add the salicylic acid and petroleum jelly oil, grind until homogenized

- Add the petroleum jelly and crush again until you get a smooth white ointment without lumps.

Transfer the ointment to sterile jars and allow to cool to room temperature before sealing tightly.

Fig. 15. Ointment **based** on a synthetic active agent Formulation

VI. Matérials and reagents :

Matérials	Reagents
Flat bottom flask (250 mL) Water bath Filtre Paper Erlen mayer flask Scale Watch glass Spatula Graduated cylinder (10 ml, 50ml) Mortar Beaker (500ml)	Rosemary Olive or sunflower oil. Beeswax Essential oils Vitamin E Salicylic acid  Vaseline Petroleum jelly oil

VII. Questions and Answers

1) What is the main advantage of using ointments in dermatology?

- Ointments allow local delivery of drugs directly to the skin or mucosa, ensuring targeted action, reduced systemic side effects, and improved patient compliance.

2) Why is homogenization important in ointment formulation?

- Homogenization ensures that the active ingredient is uniformly distributed throughout the base, improving the efficacy, stability, and safety of the preparation.

3) What quality control tests can be performed on ointments?

- Organoleptic tests: color, odor, consistency.
- Physico-chemical tests: pH, melting point, spreadability, stability.
- Microbiological tests: sterility and absence of contamination.

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Workshop N°07: Capsule – formulation and preparation

I. Introduction

Capsules represent one of the most widely used dosage forms in therapeutics due to their ease of use, good patient acceptability, and accuracy in dosing. Their formulation and manufacture require knowledge of excipients, filling techniques, and storage conditions to ensure product stability and effectiveness. This practical session aims to familiarize students with the different stages of capsule preparation, from formulation to manufacturing, while emphasizing the importance of quality control to guarantee the pharmaceutical safety and compliance of the product.

II. Objectives

- Understand the basic principles of capsule formulation (selection of active ingredient and excipients).
- Learn the different stages of capsule manufacturing: filling, closing, and packaging.
- Become familiar with quality control tests applied to capsules (mass uniformity, appearance, shell integrity).
- Raise awareness of Good Manufacturing Practices (GMP) to ensure product safety, efficacy, and pharmaceutical compliance.

III. Capsule definition

A capsule is a solid pharmaceutical dosage form consisting of a shell, usually made of gelatin, containing a unit dose of active ingredient in the form of powder, granules, or liquid. Its role is to mask the taste and odor of active substances, facilitate oral administration, and allow either rapid or controlled release of the drug in the body, depending on the nature of the shell and the chosen formulation. **[30]**

IV. Capsule composition

a) Active ingredient (API): paracetamol, ibuprofen, plant extracts.

b) Excipients: for example:

- Lactose monohydrate (hydrophilic diluent).
- Starch or microcrystalline cellulose (Avicel) to improve flowability.

c) Empty capsules: Generally made of gelatin or HPMC *(hydroxypropylmethylcellulose)* for a vegetarian alternative, with size adapted to the intended dose. **[31]**

V. Choice of capsule size

The size of the capsules depends on the maximum capacity of the envelope to introduce the total volume of the powder**: [32]**

Size (number)	Size (mm)	Volume (ml)	Capacity (mg)
000	26.1	1.37	822
00	23.3	0.93	558
0	21.7	0.68	408
1	19.4	0.50	300
2	18	0.37	222

Table.01 Capsule capacity and size table according to (ANSM)

VI. Protocol:

VI.1. Preparation of the powder for 10 capsules:

In this workshop, we will prepare capsules based on rosemary and black lentil.

a) First capsule:

- Weigh 05 gr of black lentil and grind it into a fine powder
- Weigh 01 gr of licorice and grind it into a fine powder
- Mix 1500 mg of lentil powder with 400 mg of cellulose and 600 mg of licorice powder.
- Sift the mixture

b) Second capsules:

- Weigh 05 gr of dried rosemary and grind them finely
- Mix 02 gr of rosemary powder with 500 mg of corn starch.
- Sift the mixture

VI.2. Capsule filling

- Separate the two parts of the capsules (body and cap).
- Place the bodies in the holes of the machine.
- Pour the powder onto the machine and use a spatula to fill the capsule bodies.
- Lightly tamp down the powder using the compression tool that came with the machine (or a spatula).
- Place the capsule caps in the second orifice
- Place the hole in the caps on the filled bodies.
- Squeeze to close capsules.

Fig.16. Capsule filling

Fig.17. Final product

VII. Matérials and reagents :

Matérials	Reagents
Scale	Black lentil
Watch glass	Licorice
Spatula	Cellulose
Mortar	Rosemary
capsular	Corn starch

VIII. Questions and Answers

1) What are the main advantages of using capsules as a dosage form?

- Capsules mask the unpleasant taste and odor of active substances, ensure accurate dosing, are easy to swallow, and allow flexibility in formulation for either rapid or controlled release.

2) Why is quality control essential in capsule production?

Quality control ensures the uniformity of mass, integrity of the shell, and stability of the product, which are necessary to guarantee therapeutic efficacy, patient safety, and regulatory compliance.

3) How does capsule size relate to the amount of drug that can be filled?

Capsule sizes range from 000 (largest) to 5 (smallest). Larger capsules can hold more powder or granules, while smaller sizes are used for lower doses or for patients who have difficulty swallowing.

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