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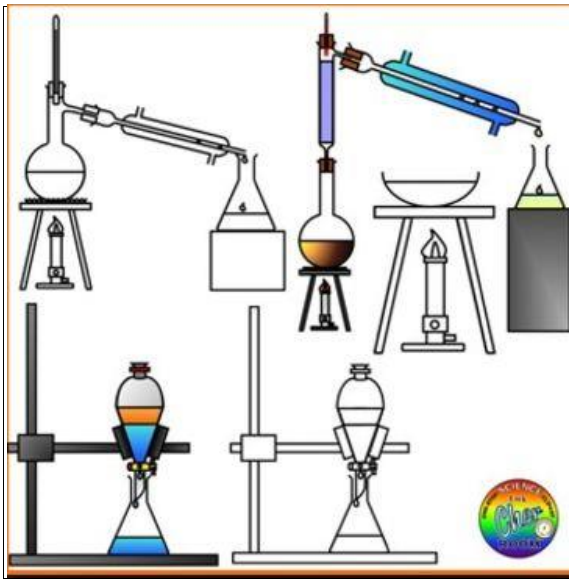
وزارة التعليم العالي والبحث العلمي

المدرسة العليا في العلوم التطبيقية
-تلمسان

Handout

On

Separation techniques in organic chemistry. Support for chemical engineer.



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Preamble

the content of this handout which describes the Techniques for transforming a mixture of substances into two or more distinct components is intended for engineering students, these techniques are intended for purification (by extraction of impurities from the compound of interest), concentration (by eliminating part of the solvent) or fractionation (by separating a complex mixture into several different mixtures).

A separation technique is always based on the use of a difference in properties between the compound of interest (the one we are trying to isolate) and the rest of the mixture. A good knowledge of the properties of the different components is therefore essential in order to retain the method and the parameters that will guarantee a successful separation.

The main objective of this handout is to train students in a wide range of skills that will allow them to mix according to their sectors of activity, how to extract the desired product by these separation methods in heavy chemistry, in fine chemistry, in parachemicals, petrochemicals, etc

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Introduction

The needs of the industry for a product that meets given specifications require prior separation into different constituents or different fractions. For this purpose, there are separation methods, described as a set of mechanical and physical-chemical procedures that allow the separation of the various pure bodies forming a mixture, i.e. they allow the transfer of a solute initially contained in a liquid, solid or gas phase to a phase that is immiscible with the first medium.

The most common substances in nature are mixtures. Salt water, for example, is a mixture of water and salt, while air is a mixture of various gases. Very often a substance has to be purified before it can be used. Seawater, for example, is not drinkable, but distilled water is the separation of various mixtures calls for a variety of purification/separation techniques. This gives us the opportunity to study small-scale techniques.

Mixture separation techniques are used to isolate or separate certain constituents from the mixtures in which they are found. To obtain a pure substance, it is often necessary to separate it from all the other substances that accompany it. The choice of technique depends on the mixture, the substance that needs to be separated from the rest of the mixture and the phases that make up the mixture.

A mixture can take two forms: heterogeneous when it forms two or more phases, and homogeneous when it forms a single phase. The first, heterogeneous mixture, is separated by decantation equipment, while the second, homogeneous mixture requires the use of sometimes complex processes.

The main mixture separation techniques most commonly used are:

- ✓ **Distillation**
- ✓ **Precipitation**
- ✓ **Chromatographic techniques (TLC, GPC, HPLC)**
- ✓ **Membrane separation**
- ✓ **…**

I. Distillation

Distillation is a material transfer operation designed to separate the constituents of a homogeneous or heterogeneous liquid mixture. It consists of boiling a liquid mixture followed by condensation of the vapours obtained, into a "pure" liquid or into liquid fractions that are more or less rich in the constituents of the vaporised mixture. It is based on the difference in volatility between these components. It is one of the most widely used separation operations in the food, chemical and petrochemical industries. Distillation is used to separate the constituents of a solid-liquid (S-L) or liquid-liquid (L-L) mixture.

There are two types of distillation:

- Simple distillation
- Fractional distillation

I.1. Simple Distillation

I.1.1. Principle

The principle of distillation is very simple: a mixture of liquids is heated to the boiling point of one of the components: the most volatile will evaporate first and the vapors are collected and condensed in another container. While t h e first liquid evaporates (distillate), the second does not reach its evaporation temperature and remains in liquid form in the initial container (residue).

It can be summed up in two actions:

➢ **Heating** an impure liquid or a mixture of liquids to transform them into vapors by **boiling**.

➢ Then **condense** the vapors by **cooling** and isolate the pure liquids.

It all depends on the boiling temperatures of the products. If the temperatures are not too high (T < 120°C), distillation under atmospheric pressure is sufficient. On the other hand, if the temperature of the compounds becomes too high, we have to resort to a trick: reducing the pressure. If the pressure is reduced, the boiling temperature (T_{eb}) of a liquid is also reduced.

I.1.2. Assembly

The set-up starts with the flask heater, then the reaction flask, then the distillation head, the condenser, the extension tube and the receiver.

Disassembly is in reverse order.

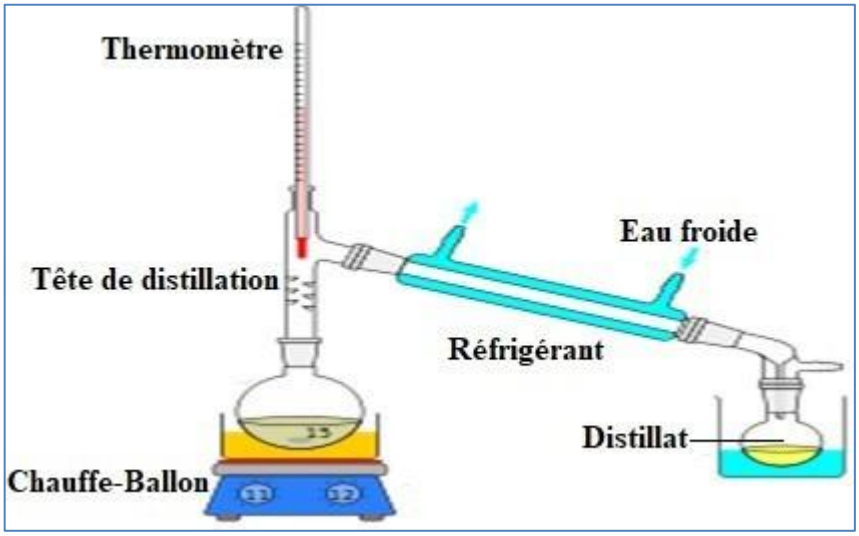


Figure 1: Diagram of a simple distillation process

I.1.3. Example of the treatment of a mixture

The alcohol has a lower boiling point than the water, so it evaporates first. The alcohol vapors are collected and cooled. This condensation allows the alcohol (distillate) to be recovered in another container. The water (residue) will remain in the original container.

I.2. fractional Distillation

It concerns a mixture of several miscible liquids, and uses a separation column (or **Vigorous** column) to separate the different constituents of the mixture according to theirboiling temperatures at each plate of this column.

I.2.1. Principle

Fractional distillation, also known as rectification, is a process for separating liquids by fractionation, based on the difference in their boiling temperatures. The more volatile component has a lower boiling point and therefore evaporates first. The mixture is brought to a slow boil and remains at the same temperature until the more volatile component has evaporated.

The most volatile is completely vaporized, so each liquid can be distilled according to itsboiling point.

I.2.2. Assembly

The mixture to be separated is placed in a flask (boiler) topped by a distillation column. At the head of the column, a straight condenser is placed at an angle to allow the condensing liquids to flow towards a receiver. A thermometer is placed at the head of the column so that its reservoir is level with the junction with the condenser (the temperature of the liquid-vapor equilibrium of the compound recovered in the distillate is thus measured).

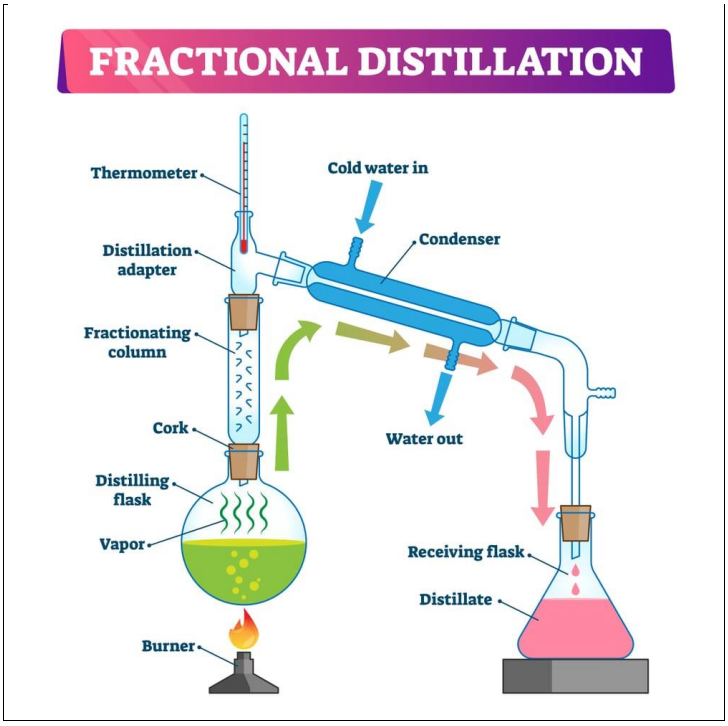


Figure 2: Diagram of fractional distillation

I.3. Example of application

Orange juice without pulp:

Step 1: The heater heats the mixture in the flask, which then boils.

Stage 2: the water vapor thus formed gradually rises in the Vigorous column.

Stage 3: the thermometer is used to read the temperature of the steam entering the cooler: it is close to 100°C.

Stage 4: the (very hot) water vapor comes into contact with the walls of the refrigerant. As the refrigerant is cold, this leads to the circulation of cold water around it, the water vapor cools and turns into liquid water.

Step 5: The drops of liquid water thus formed roll towards the outlet of the condenser and fall into the beaker. The resulting liquid, which contains only water, is called the **distillate**.

II. Filtration

After decantation or centrifugation, the solids and liquids are separated, but in the same container. Filtration physically separates a solid from a liquid by passing the mixture through a filter of varying size. The solids are trapped in the filter and the liquid is collected in a container.

II.1. Definition

Filtration is an operation whose aim is to separate a containing phase (liquid or gaseous) from the solid or liquid matter (dispersed phase) present in suspension, by means of a funnel. It is carried out by passing the suspension through a suitable filter medium capable of retaining the solid particles by physical, and more rarely chemical, action.

The filtering medium is made up of solid particles, which are deposited on a support that may be sheets of special paper, cloth, wire mesh, sand or gravel. To facilitate the operation and increase the speed at which the liquid passes through, which depends on the pressure drop in the channels of the filtering medium, suction is exerted on the filter, or the pressure on the liquid to be filtered is increased.

Continuous or discontinuous filtration is used when it is desired to treat liquids or gases with a very low solids content and in particular when the solids particles have a low sedimentation rate.

Microfiltration is the separation of particles in the micrometer range.

Sterilizing filtration is a special case, as the particles are micro-organisms.

Current filtration applications result from the separation of a solid dispersed in a liquid to obtain :

- A clarified liquid, free of solid particles.

- A solid wrung out of excess liquid.

This technique is widely used in the food and pharmaceutical industries, as well as by many animal species, mainly aquatic.

II.2. Principle of filtration

Filtration is a technique used to separate the constituents of a mixture when one of the constituents is in the liquid phase and the other is in the solid phase. A filter is used to do this. The filter retains solid particles that are larger than the pores (holes) in the filter. The liquid that passes through the filter is called the **filtrate** and the solid collected in the filter is called the **residue**.

There are three types of filtration:

- ✓ Gravimetric filtration
- ✓ Vacuum filtration
- ✓ Pressure filtration

II.3. Gravimetric filtration (filtration by gravity)

Filters are used for this, usually made of paper, either conical or pleated, through which the liquid flows under its own weight. This method uses a laboratory funnel fitted with filter paper. The pressure difference is created by the height of the liquid on the filter.

II.3.1. Principle

It is based on the use of a filter made up of meshes that allow the water to pass through but retain the particles it contains. Filtration produces a homogenous liquid**.** The holes in the filter paper are so small that no particles larger than bacteria can pass through. The large particles retained on the filter paper constitute the residue, while what passes through the filter is called the filtrate.

II.3.2. Assembly

To carry out filtration, you need a filter and a device to support it: the filter holder. Most of the time, a funnel is used.

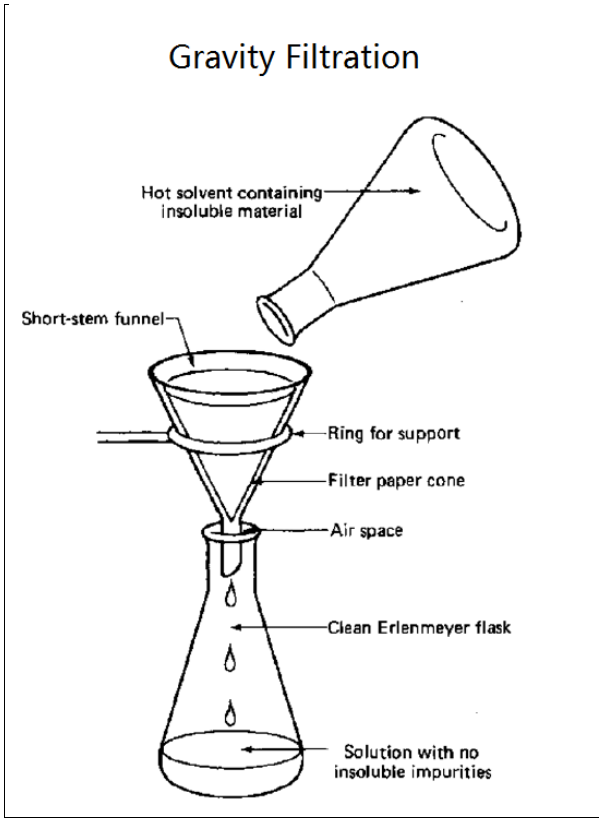


Figure 3: Gravity filtration assembly.

Gravimetric filtration has the following disadvantages:

- Filtration is slow.
- The difficulty of recovering the solid phase is isolated, especially when it is scarce.
- Separation is incomplete: the solid retains a significant quantity of liquid.

This method is generally slow and does not allow optimal separation of solid and liquid.
To overcome these drawbacks, vacuum filtration is often used.

II.4. Vacuum Filtration

Filtration speed is increased by creating a vacuum downstream of the filter material **(Fig. 4)**. This is the filtration method commonly used for glass frits and membrane filters. Special Büchner funnels adapted to a suction flask, in which a vacuum is created, are used. The funnel is fitted to the flask by means of a rubber cone. This is a porcelain or plastic funnel that will stick to the flask and funnel when the vacuum is established.

Sometimes the solid is made up of particles that are too fine to pass black through the filter. A sintered glass funnel, onto which the mixture is poured directly, can then be used. Different porosities of sintered glass exist, and it is important to choose the one that is best suited to the size of the solid particles to be filtered.

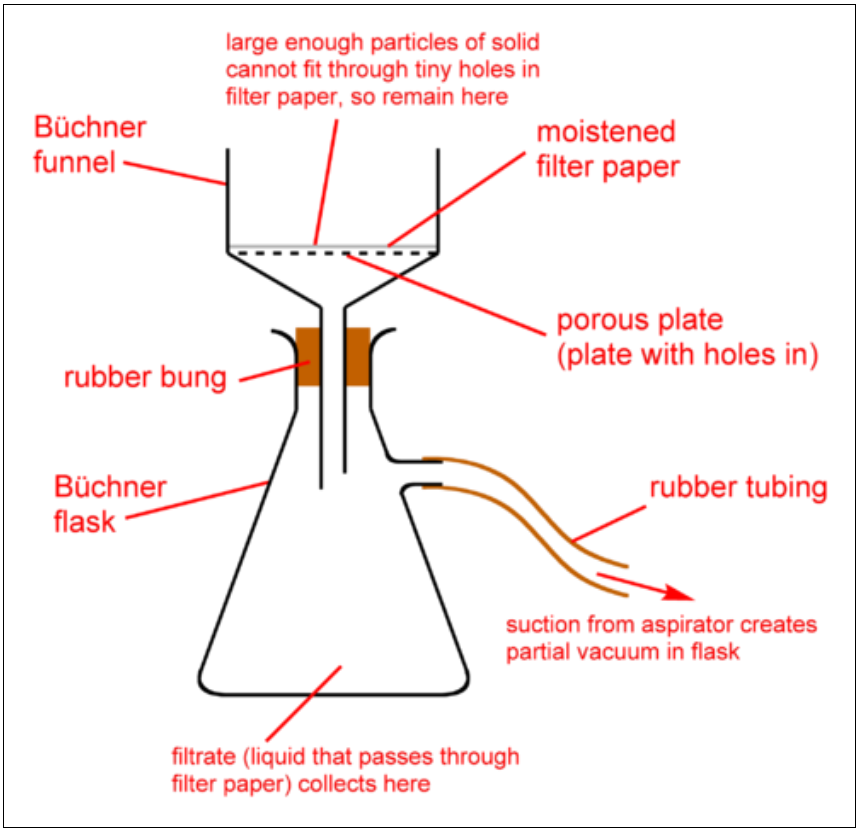


Figure 4: Diagram of vacuum filtration.

- ***the 'Büchner' type filter***

Made of porcelain, cylindrical in shape with a large-hole sieve, a circular paper filter is placed on top, large enough to cover the entire sieve.

- ***sintered glass filter***

This is a glass funnel containing a sintered glass disc with fixed porosity. This type of filter is used in extreme pH conditions where paper would not withstand it. However, this type of filter cannot be used with hydrofluoric acid solutions, which react with the silica in the glass.

II.5. Pressure Filtration

Filtration speed is increased by exerting pressure on the liquid to be filtered in front of the filtering material represented by a membrane filter **(Fig. 5)**. Pressure filtration prevents foaming and solvent evaporation, and is frequently used in industry. This pressure filtration system with filter membranes is also available in the form of filter cartridges (Millipore) that can be fitted to a syringe for filtering small volumes of solution. In the laboratory, sterilizing microfiltration using the Swinnex Millipore device is filtration under pressure. This device consists of two plastic parts, screwed together, enclosing a filter membrane.

Figure 5: Filtration under pressure.

II.6. Performing filtration

- The first step is to choose a suitable filter with meshes tight enough to retain solid particles without reducing the flow rate too much.

- The filter can be placed in a funnel to facilitate recovery of the filtrate.

- The heterogeneous mixture to be filtered is poured slowly and in stages to avoid damaging or submerging the filter. For example, the mixture can be poured by letting it flow along a glass rod.

- The solid residue is recovered in the filter, and if this is the objective of the filtration process, it can be dried in an oven.

- The filtrate can be recovered in an Erlenmeyer flask.

II.7. Examples of application

- *Manufacture of coffee-based food products:*

In the production process, the coffee is transferred from one tank to another during **freeze-drying**. During this stage, it is necessary to remove any agglomerated coffee from the system to maintain product quality and to protect the pump. The scale and design of t h e operation necessitated the use of ten transfer filters for this process application . The filters

had to be of hygienic/sanitary design and withstand aggressivetreatment (chemical cleaning).

III. Precipitation

There are more complex techniques for separating mixtures, which require the addition of reagents to initiate a chemical reaction (precipitation). Precipitation can be used to extract a particular chemical species from a mixture, the precipitated species then being filtered. A number of parameters can be used to separate a sample of interest from impurities by reducing its solubility and disassociating it from the solution in solid form. Firstly, the ionic strength of the solution can change a substances solubility. This often involves the addition of extra salt (also known as salting-out), or the addition of a counter-ion, which forms a less soluble species with the compound of interest. Precipitation is the creation of a solid from a solution. When the reaction occurs in a liquid solution, the solid formed is called a "**precipitate**". The product that causes the solid to form is called the "**precipitant**".

III.1. Principle

Precipitation consists of forming a heterogeneous phase within another phase. If we suspect the presence of certain ions in a solution, we can add another ion which will form a solid substance with them. If the desired ion is indeed present, a solid substance will appear, which can then be filtered and recovered. Precipitation is a means of separating mixtures.

III.2. Diagram of precipitation

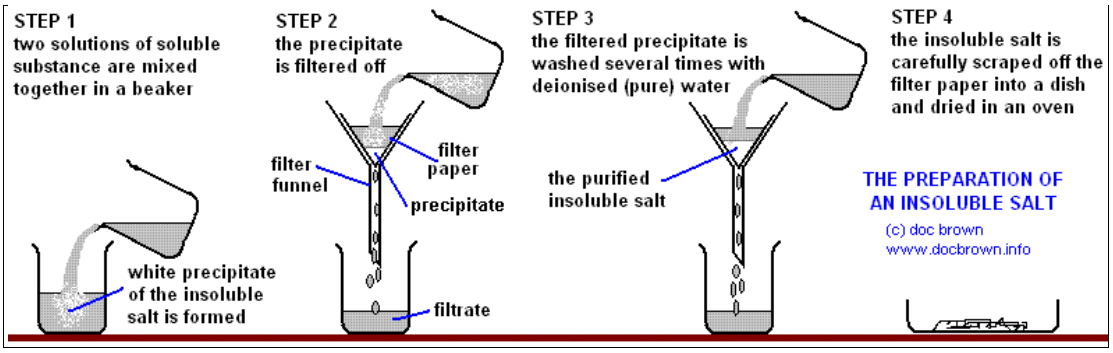


Figure 6: Precipitation protocol

III.3. Precipitation in biochemistry

Precipitation of biomolecules, particles or cells, their aggregates or their complexes with other compounds results f r o m a loss of solubility and an increase in molecular mass or weight (e.g. formation of antigen/antibody complexes; aggregation of cells; association of particles and cells). It can also result from a change in the composition of the medium, or in the characteristics of the biomolecules.

- the protein: by modifying the pH of the solvent; as a general rule, proteins precipitate when the pH of the solution is equal to the pI (izoelectric point) of the protein: the proteins will form agglomerates which will precipitate.

- The DNA solution, brought to high ionic strength by the addition of NaCl, is added to a large excess of absolute ethanol at -20°C. After a maximum of a few minutes, a whitish, translucent, filamentous precipitate (the "jellyfish") appears, made up of long filaments of precipitated DNA.

III.4. Example of application

Precipitation of milk proteins:

1. Put the milk into a bowl.
2. Heat the milk gently to 40°C on a hob, stirring constantly. Do not heat above 40°C.
3. Prepare 15 % (v/v) acetic acid by mixing 7.5 mL acetic acid and diluting with water to 50 mL.
4. Add the acetic acid drop by drop to the milk until you reach a pH of 4.6.
5. Milk filtration or centrifugation (as an alternative to filtration)
6. Resuspend the pellet in buffer for further analysis, otherwise store at 4°C.

IV. Extraction

Extractions are one of the most widely used methods in analysis for separating mixtures. This technique uses a medium to selectively separate one or more compounds from a mixture on the basis of chemical or physical properties. The extraction medium is immiscible or only slightly miscible with the main components of the mixture, while the compound to be extracted has more affinity with the extraction medium than with the main components of the mixture. The extraction operation therefore consists that the compound to be extracted is transferred between the initial mixture and the extraction medium.

The development of techniques was motivated by the diversity of raw materials and the optimization of exchange conditions between phases, while seeking to minimize solvent consumption. It was during the 18ème century that the use of organic solvents for extracting natural materials began.

IV.1. Definition

Extraction is an ancient process used to extract food, pharmaceutical or odoriferous products from plants and certain animal organs, in the form of beverages, drugs or perfumes. The solvents used in these plant product separation processes are generally water, alcohols, organic and/or chlorinated solvents, etc**.** Extraction involves transferring a compound from one phase to another:

• From one liquid phase to another.

• From a solid phase to a liquid phase.

This involves separating certain compounds from an organism (animal or plant) using various techniques.

IV.3. Principle

Extraction involves treating a homogeneous or non-homogeneous mixture of liquids or solids with a pure solvent in order to extract a solid or liquid component.

The extraction operation takes place in two stages:

- a first part for transferring the compound to be extracted between the initial mixture and the extraction means
- a second part for separating the extraction means from the main mixture.

There are several extraction methods:

- Liquid - liquid extraction (**Liq - Liq**)
- Solid - liquid extraction (**Sol - Liq**)

IV.4. Liquid extraction- liquid

Liquid-liquid extraction is one of the oldest sample preparation techniques. It is a fundamental operation involving the transfer of matter between two immiscible liquid phases, without the transfer of heat. This technique allows a substance dissolved in one solvent to be extracted using another solvent, called the **extraction solvent**, in which it is more soluble. The initial solvent and the extraction solvent must not be miscible.

IV.4.1. Principle

Because water does not evaporate easily, the chemical species is difficult to recover if it is in solution in water. In this case, an organic solvent must be used in which the substance is highly soluble (much more so than in water), so that it can be transferred from the water to the organic solvent. The water and the organic solvent must not be miscible.

- There are two types of liquid-liquid extraction:

• **Extraction discontinuous**:Liquid-liquid liquid-liquid discontinuousis carried outby vigorous agitation of the solvent and the solution to be extracted in a separating funnel.

• **Continuous extraction**: For continuous extraction, the solution to be extracted is fed with a pure solvent that is continuously recycled by distillation.

IV.4.2. Extraction protocol

1- The solution to be extracted and the extraction solvent are introduced into the ampoule.

2- After closing the ampoule, hold it upside down with both hands and shake vigorously.To extract the solute optimally, the previous partition equilibrium must be reached.

3- The ampoule should be unplugged when it is placed back on its support, again to avoid excess pressure. The phases should then be allowed to settle.

4- The two phases are then recovered separately: the aqueous phase is generally denser than the organic phases, with the exception of chlorinated solvents.

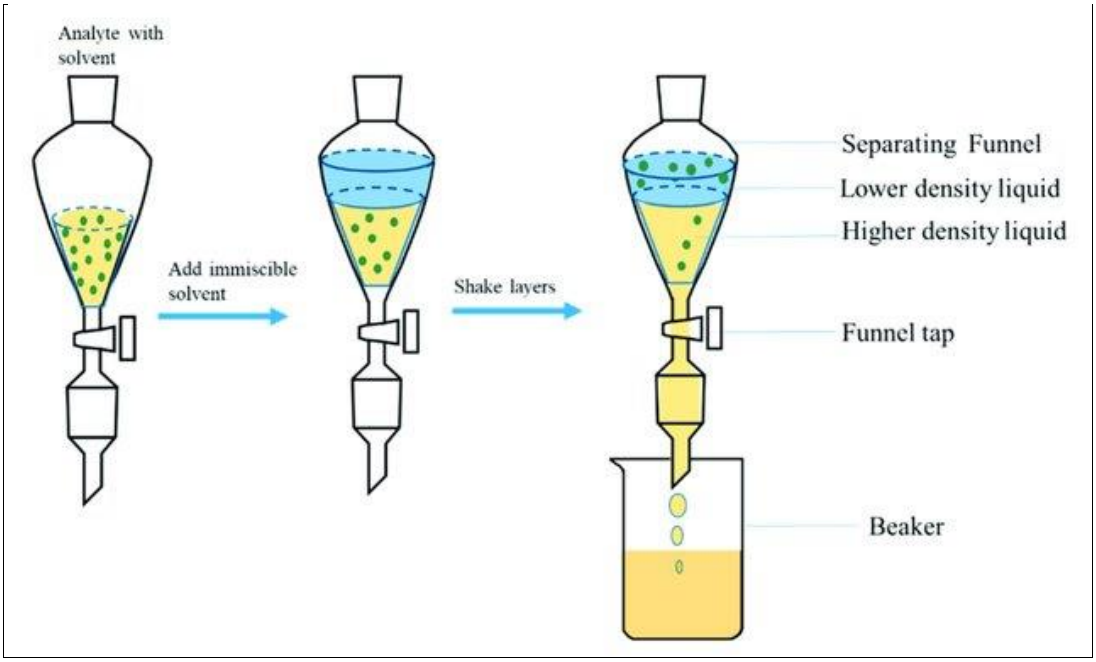


Figure 7: Liquid-liquid extraction diagram

IV.5. Solid-liquid extraction

Historically, solid-liquid extraction, also known as solvent extraction, is one of the oldest unit operations. Commonly performed in the home, where it is directly related to the making of daily coffee, it is also widely used in industry, particularly in hydrometallurgy (selective dissolution of ores or leaching) and in the food and cosmetics industries (beet sugar, oils, natural essences.).

Solid-liquid extraction is a solvent extraction technique which consists of extracting a chemical species from a solid and transferring it to a carefully chosen solvent. This type of extraction is carried out using a reflux heating system.

Solid-liquid extraction poses a particular problem: as a general rule, a solid will not allow a liquid to pass through it. It is therefore necessary to carry out a large number of successive extractions. This is done using a Soxhlet extractor, or its cheaper variant.

The Soxhlet extractor is a piece of glassware used to extract solid liquid with great efficiency. The device is named after its inventor: Franzvon Soxhlet. The Soxhlet is a classic method for solid-liquid extraction. The advantages of Soxhlet are as follows: the sample rapidly comes into contact with a fresh portion of solvent, which helps to shift the transfer equilibrium towards the solvent. This method does not require filtration after extraction. The Soxhlet is independent of the plant matrix.

IV.5.1. Principle

The device consists of:

- A flask containing a supply of solvent.

- A device (the extractor itself) allowing contact between the solvent and the solid in a porous cartridge and evacuation of the solution to the flask via a siphon.

- A water cooler to condense solvent vapours in the porous cartridge.

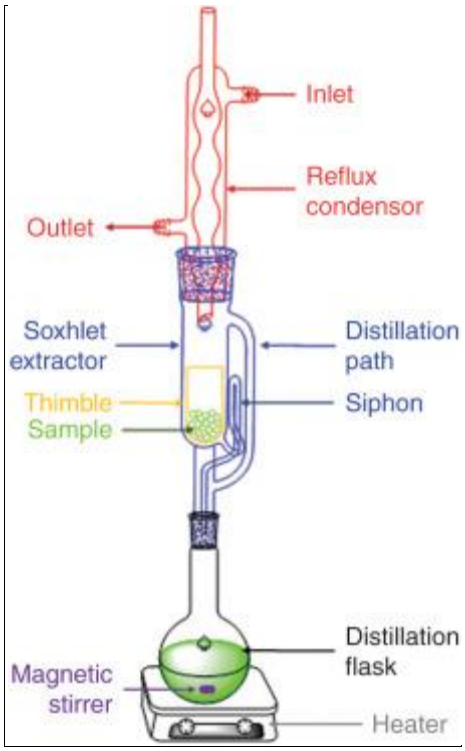


Figure 8: Diagram of a Soxhlet device.

Solid-liquid extraction operations encompass several methods, all of which involve the solvent interacting with the solid material in order to dissolve its soluble components:

IV.5.2. Extraction discontinuous

There are many different methods for batch extraction:

- **Decoction:** is an operation in which the solid is immersed in a boiling liquid solvent. This is a brutal operation which should be reserved for the extraction of non-thermolabile active ingredients. However, it is very rapid and sometimes essential.
- **Infusion:** is a decoction during which the solvent is heated without boiling, followed by cooling of the mixture. The preparation of tea is a typical example of this operation.
- **Maceration:** is an infusion in a cold solvent. Although this operation is generally long and

often yields mediocre results, it is the only method that can be used to extract a group of fragile molecules. Maceration can be carried out in a covered container, protected from light and in some cases kept in a refrigerator.

- **Digestion:** is a hot maceration. This operation and maceration are used particularly in pharmacy and perfumery. It is a faster process than the previous one.

IV.5.3. Extraction continuous

Continuous extraction takes much longer than discontinuous extraction, but is more effective.

- **Percolation:** this consists of slowly passing a solvent through a layer of finely pulverised substance, usually contained in a thick porous paper cartridge or filter paper bag.

IV.5.4. Extraction protocol

1- The material to be extracted is weighed and then placed in the Soxhlet cartridge.

2- The solvent is introduced into the flask and heated to start the extraction.

3- Extraction is stopped when the liquid surrounding the cartridge becomes clear, this colour indicating that the solvent is no longer extracting anything from the solid.

IV.6. Extraction by hydrodistillation

Hydrodistillation consists of distilling a compound by steam distillation. Hydrodistillation is the most widely used method for **extracting** essential oils. However, it has its limitations when the molecules to be extracted are fragile and cannot withstand heating.

IV.6.1. Principle

The natural aromatic raw material is placed in a flask filled with water, which is then brought to the boil. The heat causes the plant cells to burst, releasing the fragrant molecules which are carried away by the water vapour formed. They pass through a cooler where they are condensed by cooling. The water and essential oil are separated by density difference in a separating funnel. The decanted water, known as the distillate, remains highly fragrant. The distillate obtained from the flowers is called floral water.

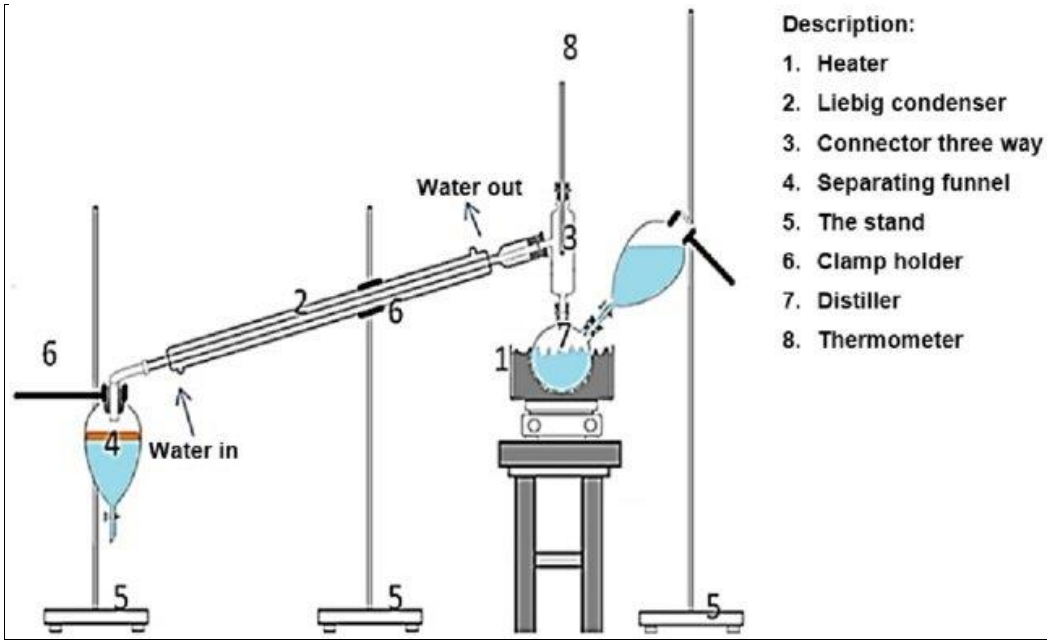


Figure 9: Diagram of a hydrodistillation set-up

IV.6.2. Example of application

Extraction of essential oil from orange peel by hydrodistillation

✓ An orange should be peeled (only the peel should be taken without the white part) and cut into small pieces.

✓ Next, place the pieces of orange peel in a flask with a little water. and a few grains of pumice stone. Start the circulation of cold water in the cooler, then turn on the heating (thermostat at maximum at first, then adjust).

✓ Leave the mixture to heat for around 30 minutes, keeping an eye on the boiling point of around 100ºC with a thermometer. The heat causes the orange peel cells to burst, releasing volatile organic compounds.

✓ The water vapour formed carries the gaseous organic compounds to thecooler.

✓ Condensation of this gaseous mixture causes it to separate into two liquid phases:

- The upper, oily and highly fragrant organic phase, known as essential oil.This is the part of the plant that contains the limonene we need.
- The lower aqueous phase, which contains only a minority of odorous components.

To obtain only the oily part, the two immiscible phases can be decanted using a separating funnel.

V. Techniques chromatography

Chromatography is a separative method used to identify and measure the different compounds in a mixture. The principle is based on the differences in affinity of the compounds in the mixture with the stationary phase and the mobile phase. The chromatogram shows the variation of the solute in the eluent as a function of time. The term chromatography therefore covers several technologies based on a common principle: identification by separation.

Above all, we must remember that: **Chromatography = Separation**.

There are different types of chromatography depending on the separation method used:

➢ Thin layer chromatography (**TLC**).

➢ Gas chromatography (**GC**).

➢ High performance liquid chromatography (**HPLC**).

The various techniques described above are technologically very different, but have many points in common in terms of their operating principle.

They differ in terms of the equipment used. TLC requires a sheet of paper, a solvent and a glass tank. GC and HPLC require high-tech, high-cost equipment.

V.1. Thin Layer Chromatography (TLC)

Thin layer chromatography is the simplest of the chromatographic methods. It involves placing a stain on a sheet (paper, silica or other….) and allowing it to elute by soaking it in a solvent or solvent mixture.

(called the **eluent**), the eluent diffuses along the support. The stain migrates across the sheet more or less quickly depending on the nature of the interactions it undergoes with the support and the eluent.

V.1.1. Principle

When the plate on which the sample has been deposited is placed in the tank, the eluent rises through the stationary phase, essentially by capillary action. In addition, each component of the sample moves at its own speed behind the solvent front. This speed depends, on the one hand, on the electrostatic forces holding the component to the stationary plate and, on the other hand, on its solubility in the mobile phase.

The compounds therefore move alternately from the stationary phase to the mobile phase, the retention action of the stationary phase being mainly controlled by absorption

phenomena.

The main elements of a thin layer chromatographic separation are:

– **the chromatographic cell:** a container, usually made of glass, of variable shape, closed by a tight-fitting lid.

– **the stationary phase:** a layer of approximately 0.25 mm of silica gel or another adsorbent is fixed to a glass plate using a binder such as starch.

– **the sample:** approximately one microlitre (ml) of dilute solution (2 to 5%) of the mixture to be analysed, placed at a reference point above the surface of the eluent.

– **the eluent (mobile phase):** a pure solvent or a mixture: it migrates slowly along the plate, carrying the sample components with it.

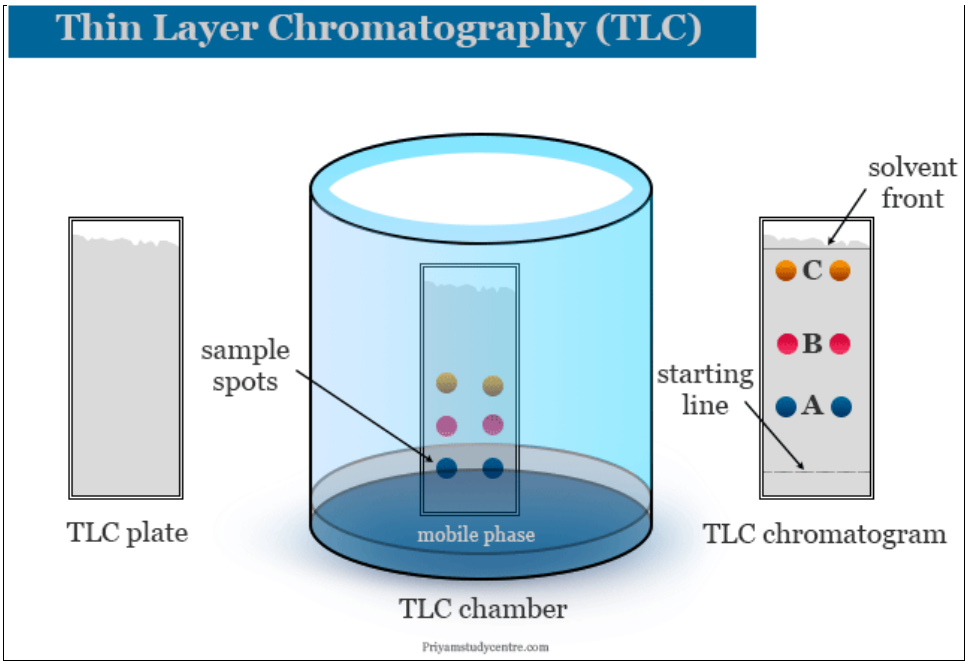
After migration, the stains must be revealed; this can be done either by detection:

➢ Spraying a characteristic reagent

➢ By immersion in a potassium permanganate bath

➢ By spraying diiodine vapor

➢ By observation under Ultra-Violet (UV) light if the silica plate has a fluorescenceindicator.



Colored dots represent sample deposits

Figure 10: TLC diagram

V.1.2. Applications of CCM

When the operating conditions are known, it enables the purity of an organic compound to be checked quickly and easily. If the analysis, carried out with different solvents and different adsorbents, reveals the presence of a single substance, then the sample can probably be considered pure.

In addition, because thin layer chromatography indicates the number of components in a mixture, it can be used to monitor the progress of a reaction.

V.1.3. Example of application

Chromatography of sugars

Check for the presence of 3 carbohydrates (glucose, maltose and sucrose) in a fruit juice.

- Glucose, maltose and sucrose solutions
- Fruit juice (orange juice)

- Using a capillary tube, place a very small drop of each prepared solution and orange juice on the chromatography plate.
- Insert the plate into the chromatography cell containing 0.5 cm of eluent.
- Remove the plate when the eluent is approximately 1cm from the top edge of the plate.

We observed spots during the migration of glucose and sucrose deposits similar to the orange spots, and the absence of the maltose spot compared to orange juice. We can therefore deduce that orange juice contains sucrose and glucose.

V.2. Gas chromatography (GPC)

This is a method of separating compounds that are gaseous or liable to be vaporized by heating without decomposition. It is a partition chromatography in which the mobile phase is a gas. It is the most powerful and accurate method for separating, identifying and quantifying gaseous or volatile substances. It allows the analysis of potentially very complex mixtures whose constituents may differ considerably in nature and volatility.

V.2.1. Principle

GC is based on the partition equilibrium analyses between a stationary phase and a gaseous mobile phase. The separation of analyses is based on the difference in affinity of thesecompounds for the mobile phase and the stationary phase.

The mixture to be analyzed is vaporized and then transported through a column containing a liquid or solid substance, which forms the stationary phase. Transport is carried out using an inert gas, known as a carrier gas (usually He or N_2), which forms the mobile phase.

The result is a chromatogram showing peaks whose integration is proportional to the quantity of product injected. The peak is characterised by its retention time, shown on the abscissa (fig.11).

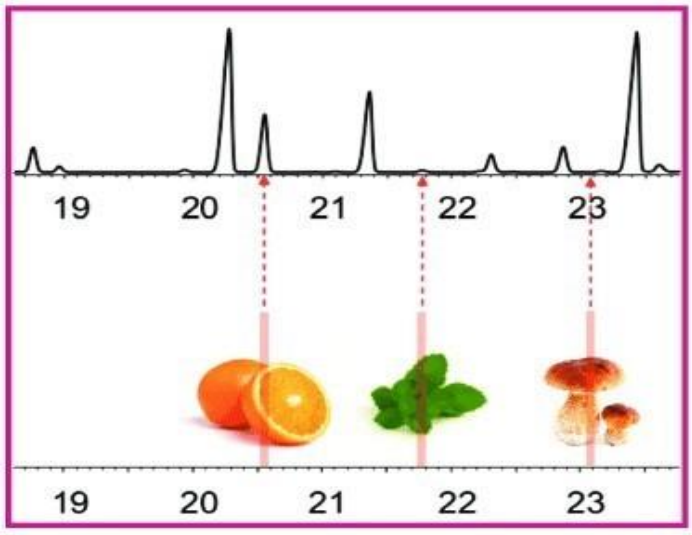


Figure 11: Example of a food mixture chromatogram

V.2.2. Equipment

The apparatus used to carry out a vapor chromatography analysis is called a chromatograph. A gas chromatograph consists of three parts: an injector, a column and a detector, as shown in the diagram below:

- **Injector:** This is used to introduce a liquid which must be vaporized instantaneously before being transferred to the column.
- **Column:** This is the main component. It consists of a tube, generally made of metal internal diameter of around one millimeter.
- **Detector:** Used to detect the passage of the different gases separated by the column. Detectioncan be based on different measurement techniques. The detector most commonly used in GC is the thermal conductivity detector known as a catharometer.

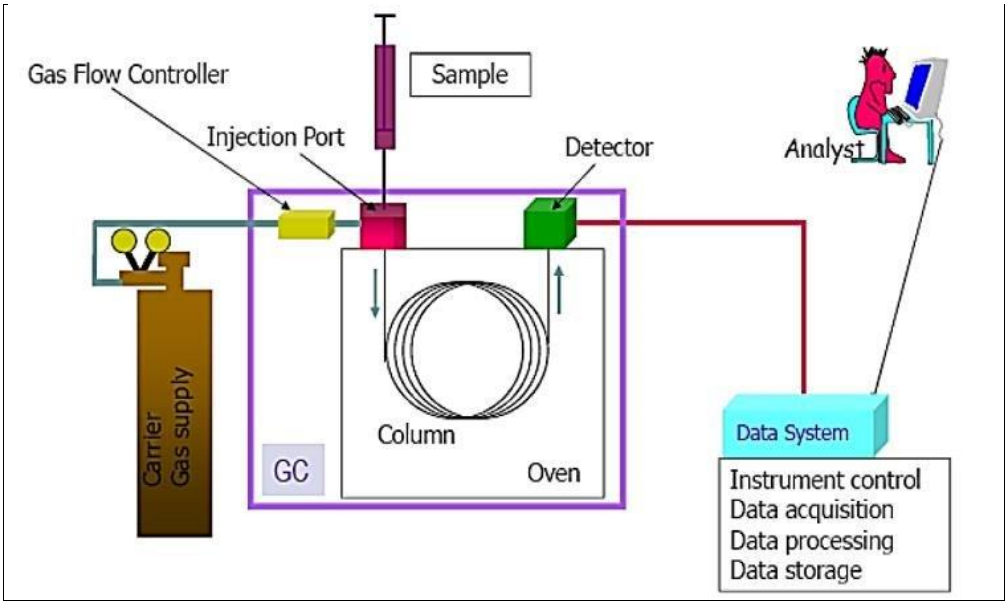


Figure 12: Simplified diagram of the gas chromatograph

V.2.3. Use of CPG

➢ *Toxicology assay:*

- Search for toxic substances in acute intoxication (methanol, EG...)

- Dosage of drugs: amphetamines, opiates, cannabinoids

- Doping control

- Drug screening

➢ *Agri-food:*

- Pesticide research and dosage

- Identification and measurement of nitrosamines (nitrites)

V.2.4. Example of application

Detection of fatty acid methyl esters (FAME) in fats of animal and plant origin. The method is based on the fact that methyl esters are formed by transmethylation with a methanolic solution of potassium hydroxide. Using gas chromatography, the fatty acid methyl esters (FAMEs) are separated on a highly polar stationary phase according to their chain length, degrees of saturation/establishment and the configuration and position of the double bonds.

V.3. High Performance Liquid Chromatography (HPLC)

This is a technique for the analytical separation of molecules present in a mixture. Its high level of precision means that it can be used to detect traces. Liquid chromatography has

made it possible to carry out analyses that were previously impossible using thin layer or gas phase techniques. This form of chromatography is frequently used in biochemistry and toxicology.

V.3.1. Principle

The compounds to be separated (solutes) are dissolved in a solvent. This mixture is introduced into the liquid mobile phase (eluent). Depending on the nature of the molecules, they interact to a greater or lesser extent with the stationary phase in a tube called a chromatographic column. The mobile phase is pushed through the chromatographic system by a high-pressure pump. This is known as high-pressure liquid chromatography (HPLC). The mixture to be analyzed is injected and then transported through the chromatographic system. The compounds in solution are then distributed according to their affinity between the mobile phase and the stationary phase. At the end of the column, an appropriate detector is used to identify the different solutes by means of a peak. The set of peaks recorded is called a chromatogram. The detector signal is amplified and recorded, and the oven temperature is kept constant.

Four types are commonly used, depending on the nature of the stationary phase.

- ➢ Adsorption chromatography
- ➢ Partition chromatography: this is the most widely used technique with an apolar stationary phase.
- ➢ Ion exchange chromatography
- ➢ Exclusion chromatography: also known as gel permeation chromatography.

V.3.2. Equipment

Schematic diagram of how a high-performance liquid chromatograph (HPLC) works.

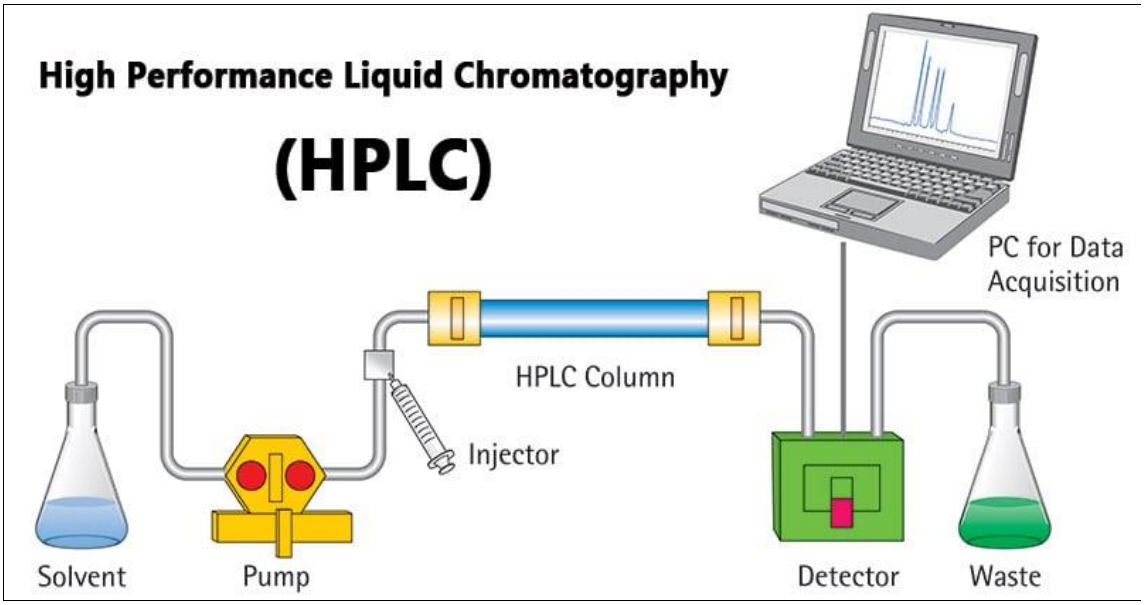


Figure 13: Diagram of an HPLC system

V.3.3. Application area

HPLC is used to detect multiple antibiotic residues of quinolones, sulphonamides, β- lactam antibiotics, macrolides and tetracyclines in a wide range of samples such as milk and muscle tissue.

VI. Electrophoretic techniques

Electrophoresis is used to check the purity of a chromatographic fraction and to determine the molecular mass of an unknown protein using standard proteins.

VI.1. Principle

Electrophoresis is a technique for moving ions (molecules that have lost their electrical neutrality) under the effect of an electric field. These migrate towards their respective electrodes: anions migrate towards the anode and cations migrate towards the cathode. For uncharged molecules, there is no migration. Because of their specific characteristics and the conditions of electrophoresis, the speed of migration and the distance travelled in the matrix by these different ions allow them to be separated.

VI.2. Types of electrophoresis

Electrophoresis can be carried out under non-denaturing or denaturing conditions.

VI.2.1. Electrophoresis under non-denaturing conditions

The molecules are separated in a state as close as possible to their native state. The

speed of migration depends on the molecule's native charge and its three-dimensional structure.

VI.2.2. Electrophoresis under denaturing conditions

The molecules are subjected to a denaturing treatment before electrophoretic separation, destroying the native three-dimensional structure. Separation is therefore a function of molecular weight. The denaturing agents are SDS (Sodium Dodecyl Sulfate) and β-mercaptoethanol, which reduces the disulphide bridges in proteins. SDS is a mild denaturant and surfactant, and acts on proteins in several ways:

- If the protein is oligomeric, its sub-units are separated from each other.

- It binds to proteins, coating them with a negative charge. The proteins transformed into manopolyanions all have the same electrophoretic mobility. The overall negative charge allows migration towards the anode, but the molecules are separated solely on the basis of their molecular weight. Depending on the electrophoretic support, there are two types of electrophoresis:

VI.2.2.1. Liquid vein electrophoresis (free electrophoresis)

This was the first electrophoretic method described. The sample solution is placed in a **U-tube** and covered with a buffer solution of lower density than that of the sample to avoid convection currents. The electrodes are placed in the tube to which the electric field is applied. In this case, migration takes place in a liquid consisting of a buffer solution of suitable pH and concentration, the ions of which conduct the current from one pole to the other. This type of electrophoresis has a number of disadvantages(expensive equipment, long and delicate set-up, incomplete particle separation).

VI.2.2.2. Zone electrophoresis on support

The mobilities obtained in this type of electrophoresis are lower than those obtained in liquid vein electrophoresis. This type uses a porous support to stabilise the liquid phase. The mixture to be separated is deposited on a suitable, homogeneous, porous, inert support impregnated with buffer. This support can be paper, a cellulose derivative, starch, agarose, polyacrylamide (PAGE), etc. The different types of zone electrophoresis are often named according to the type of support (paper, cellulose ester, gel, etc.).

Electrophoresis on paper, cellulose acetate, gel (starch, agar, agarose, polyacrylamide). Fractions separated by zone electrophoresis migrate as individual zones. The size of the pores in the gel support can influence the speed of migration (slower movement of large molecules).

VI.3. Electrophoresis equipment

The usual equipment consists of:

- A stabilised direct current generator connected to the electrodes of the tank. This current creates an electric field that causes the molecules to migrate.

- A closed (horizontal or vertical) electrophoresis tank, which may be thermostated, with two compartments. Each compartment is filled with migration buffer.

- Accessories such as the electrophoresis stand, glass plates for casting the gel and combs for making wells in the gel, in which the compounds to be analyzed will be deposited.

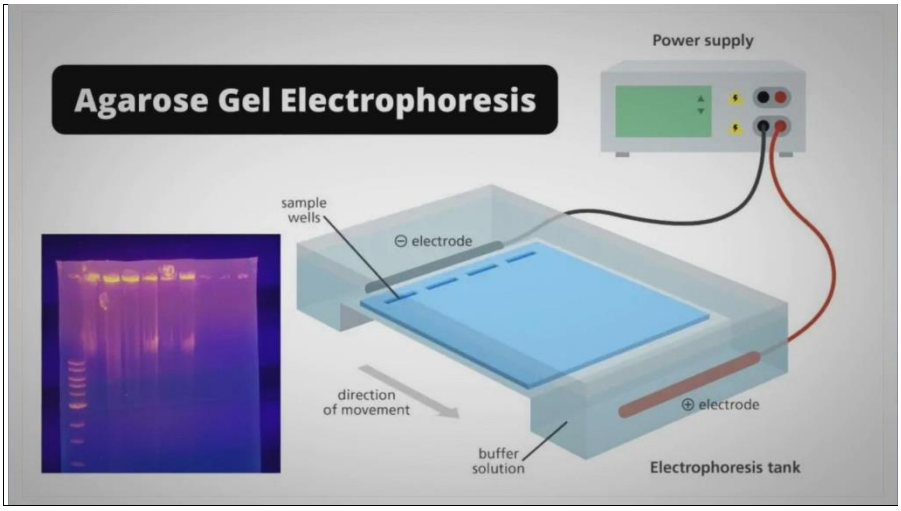


Figure 14: Electrophoresis apparatus.

VI.4. Example of application

Serum protein electrophoresis separates proteins in the blood under the influence of an electric field. It is used to highlight abnormal proteins and to detect an abnormal increase or decrease in proteins in the blood. An increase in certain proteins may indicate an inflammatory syndrome. The test is performed by taking a blood sample, usually from the elbow. The serum is then deposited on cellulose acetate or agarose gel. The proteins migrate under the influence of an electric field from the cathode to the anode.

VII. membrane Separation

Membrane separation operations form a fairly broad class of techniques applying to liquid/liquid, gas/liquid, solid/liquid or gas/gas separations. Membrane processes have undergone rapid development in a few specific sectors. Today, new applications are emerging thanks to progress in developing more suitable membranes and improving equipment design.

VII.1. Principle

A **membrane** is a material barrier (polymeric, ceramic or, rarely, metallic film) which allows the selective passage of certain compounds from the fluid to be treated, under the action of an acting force: pressure gradient, electrical potential or chemical potential.

Membrane processes are used to separate and concentrate molecules or ionic species in solution and/or to separate particles or micro-organisms suspended in a liquid according to their size and charge.

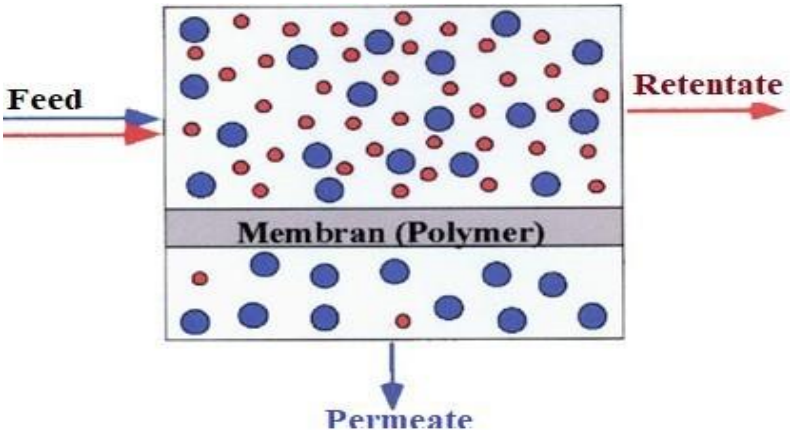


Figure 15: Membrane separation operating principle

VII.2. Microfiltration

Crossflow microfiltration can be defined as a solid-liquid separation process using membranes with pore diameters of between 0.1 and 10 µm. This process allows suspended particles, bacteria, indirectly colloids and certain ions to be retained after they have been attached to larger particles obtained by complexation, precipitation or flocculation.

The average operating parameters are a filtration pressure of between 0.5 and 3 bar, a flow of around 200 to 450 $m^3 .h^{-1} .m^{-2}$ and a water velocity of around 3 m/s. Particles larger than the pore diameter are screened out. Progressive clogging develops. Unclogging is very difficult. In general, pre-filtration to a threshold of around 500 µm is necessary to avoid premature deterioration of the membranes. Microfiltration is suitable for treating groundwater with occasional high turbidity, such as karstic water.

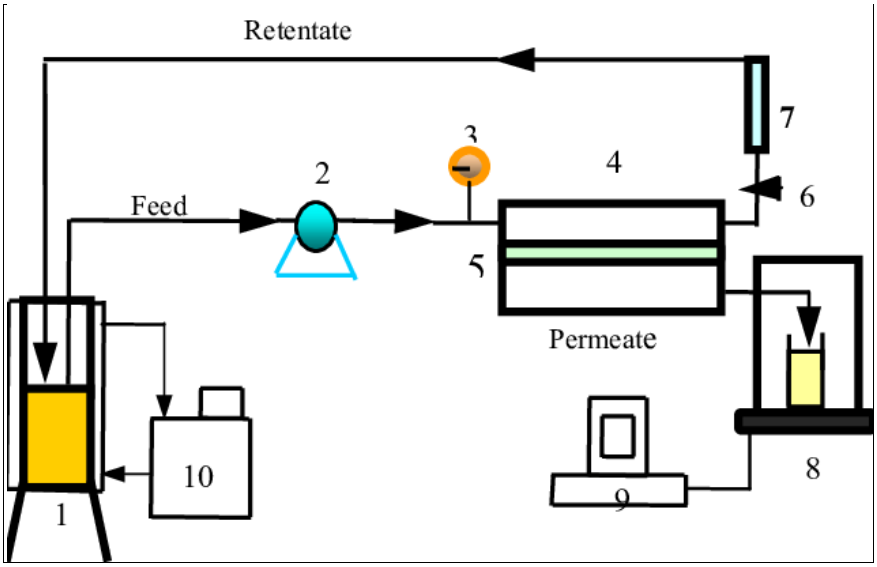


Figure 16: Microfiltration diagram

Microfiltration effectively replaces the clarification stage. Turbidity reduction, mineral and germ microfiltration are remarkable. However, microfiltration does not eliminate viruses, pesticides or oxidation by-products.

VII.2.1. Example of application

A major public application of this microfiltration function is milk.

*"**Marguerite***", distributed by certain supermarkets, has a long shelf life achieved by bacterial reduction through microfiltration, while the nutritional and organoleptic properties of the milk are preserved by reducing the heating phases to a minimum.

VII.3. Ultrafiltration

This technique uses microporous membranes. The membrane porosity is between 0.5 and 0.002 µm. The average operating pressure is 0.5 to 10 bar and the tangential velocity of the water is 1 m/s. Ions are partly retained upstream of the membrane, causing concentration polarisation and clogging.

Ultrafiltration retains all suspended particles, colloids, proteins, polymers and micro-organisms, including viruses. Ultrafiltration does not eliminate organic micropollutants unless powdered activated carbon (PAC) is added. CAP is injected into the circulation loop. Treatment rates are between 10 and 20 g. m^{-3} . The membranes used are generally made of polysulphone, a material that can be used at temperatures of 80 to 121°C. The membrane can be tubular or spiral wound like osmosis membranes. Hourly backwashing and chemical de-clogging every three months are recommended. There are many applications:

- Concentration of macromolecular solutions (proteins, polysaccharides, various polymers)

- Removal of macro-solutes from effluent or domestic water.

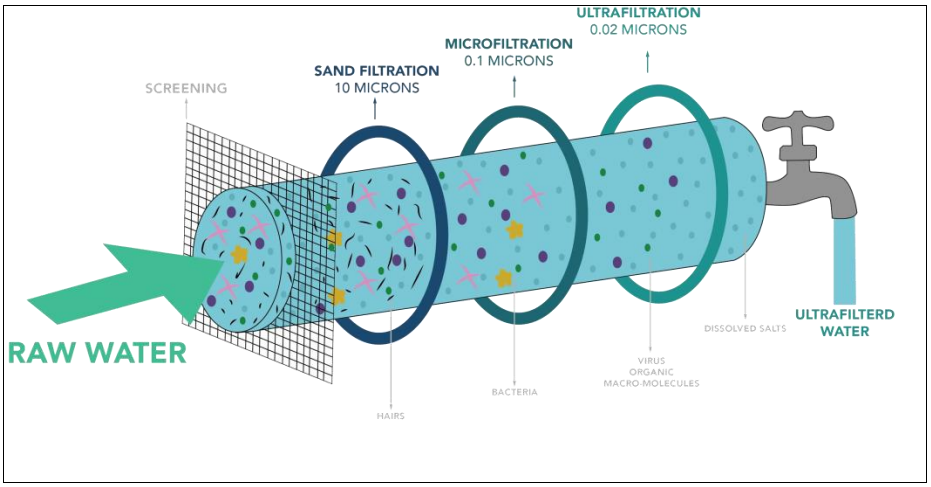


Figure 17: Principle of ultrafiltration

VII.4. Nanofiltration

Nanofiltration (NF) is the term used to describe a new membrane-based separation technique between reverse osmosis and ultrafiltration. It enables the separation of components with a size in solution close to that of the nanometre. Monovalent ionised salts and non-ionised organic compounds with a molar mass of less than approx. 300 g/mol are not retained by this type of membrane. Multivalent ionised salts (Calcium, Magnesium, Aluminium, Sulphates....) and non-ionised organic compounds with a molar mass greater than approximately 300 g/mol are, on the other hand, strongly retained. This type of membrane is very effective at eliminating small dissolved molecules suchas pesticides, humic and fulvic acids that are precursors of organochlorines and biodegradabledissolved organic carbon. The overall reduction in salts is 30-60% for monovalent ions and 5- 15% for bivalent ions. This process is mainly used in drinking water treatment or in wastewater treatment lines. To date, it is rarely used to produce ultra-pure water.

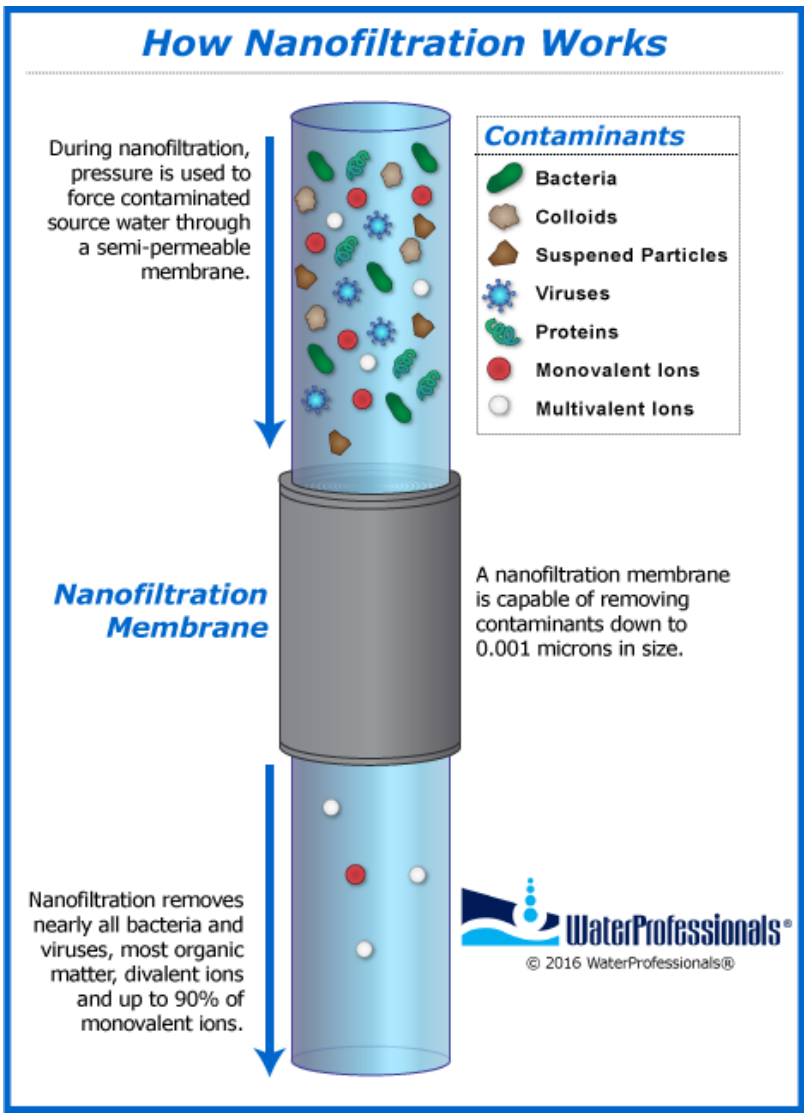


Figure 18: Principle of nanofiltration

VII.4.1. Application area

Generally speaking, the separation by nanofiltration of simple sugars (fructose, glucose and galactose), oligo-polyfructoses, amino acids, short peptides and antibiotics means that they can be used in specialised foods. Nanofiltration has mainly been used for water softening.

VII.5. Reverse Osmosis

Reverse osmosis **(RO) is** a long-established process that uses dense membranes that allow only the solvent to pass through and block all salts. Solvent-solute separation occurs by a solubilisation-diffusion mechanism: the solvent adsorbs to the membrane phase and then diffuses through the material. The pressure applied must be greater than the osmotic pressure exerted upstream of the membrane by the filtered solution in order to observe a flow of permeate through the membrane. The pressures applied vary from 20 to 80 bar.

Reverse osmosis membranes (dense membranes) can retain monovalent ions by a different mechanism involving solubilisation and diffusion. This considers that the rate of transmission of a solute through a reverse osmosis membrane results from a process in which the solute solubilises in the membrane phase and then diffuses through it into the permeate compartment. The main industrial applications of reverse osmosis are as follows:

- Desalination of seawater and brackish water
- Elimination of pesticides and herbicides
- Production of ultra-pure water (electronics, pharmaceuticals, etc.)
- Antibiotic concentration

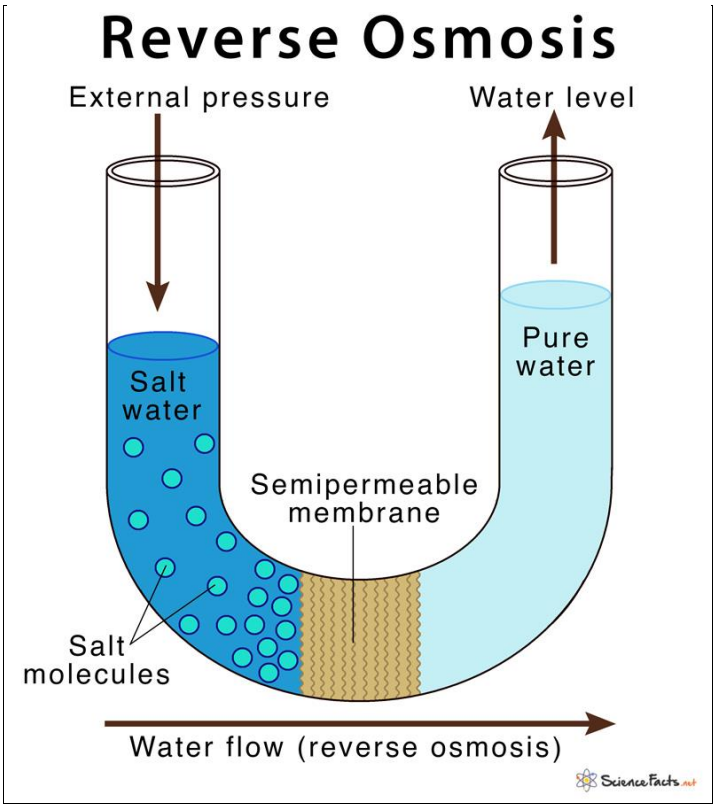


Figure 19: Reverse osmosis operating principle

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