Biochemical techniques

Introduction

The extraction and **separation** of biological molecules and/or macromolecules constitute an essential step to undertake a study or to carry out an assay. The analysis techniques used in biochemistry and biology, based on the behavior of macromolecules in solution, are very diverse. In addition to **electrophoresis** and **chromatography**, which are among the most widespread methods, other analytical techniques deserve to be presented. Thus, aqueous phase separation and ultrafiltration methods, for example, constitute other strategies for implementing a separation protocol, or even purification.

Equipment technology and Analysis techniques

I.1. Introduction

A chemical analysis can be defined as a series of elementary operations, statistically independent of each other, which begin at the time of taking the test (taking an analytical sample from the laboratory sample) and culminate in the expression of an analysis result that will have to be validated in order to finally have an analytical data.

It is customary to group these elementary operations into a few main steps, which are inserted in an analytical procedure, this is the method of analysis. The detection of a biomolecule (protein, amino acid, sugar, nucleic acid, fatty acid, vitamin, enzyme, hormone, lipid ...) as well as the evaluation of its concentration or its quantity can be done using several analytical methods such as spectral, electrophoretic, chromatographic methods ... ect. In fact, the choice of a sample processing method constitutes one of the greatest scientific concerns in the analytical field.

I.2. Performance and criteria for choosing an analysis method

Each analysis method has a certain number of characteristic properties, criteria that qualify the performance of the method, the first objective always being to obtain relevant information at the lowest cost.

The methods must be chosen on the basis of their performance taking into account the agreed specifications. The main technical specifications are as follows :

I.2.1. Loyalty/ Fidélité

Represents the narrowness of the agreement between the results of independent tests carried out on different test samples of the same homogeneous sample. More precisely, represents the narrowness of the agreement between the results of independent tests obtained with the same method, on the same homogeneous sample, in the same laboratory, by the same operator using the same equipment and in a short time interval.

I.2.2. Accuracy/ Justesse

Represents the narrowness of the agreement between the average value obtained from a wide series of test results and the conventionally true value of the sample (the accepted reference value).

I.2.3.Reproducibility

Which, unlike repeatability, considers the results obtained with the same method and on the same homogeneous sample, but in different laboratories and by different operators using different equipment. Collaborative studies "also called inter-laboratory analyses or analysis circuits" make it possible to evaluate this reproducibility. We will also sometimes give a restricted meaning to this notion of reproducibility, by considering, for example, in the same laboratory, different operators using the same equipment ... or the same operator who performs the same analysis but at very distant dates from each other, etc

I.2.4.Sensitivity

It is expressed by a concentration, this quality is especially important in the determination of compounds usually present in very small quantities, such as enzymes and especially trace elements or hormones. It represents the slope of the calibration line; if the calibration curve is not a straight line, the sensitivity at a given concentration will be defined as the slope of the tangent to the curve at that concentration. It is clear that the higher the sensitivity, the easier it will be to distinguish 2 samples from neighboring concentration. It also appears that an increase in sensitivitywill make it possible to obtain lower detection or quantification limits.

I.2.5.Robustness

The robustness of the method characterizes the fact that a slight modification of the experimental conditions (one or more parameters) only slightly modifies the measured response. This property is of course very interesting if several operators must intervene to carry out the same series of analyses

I.2.6.Specificity

It deserves a very particular mention, because it informs on the fact that the measured response is not disturbed by physicochemical species other than the analyte considered. The application of a specific analysis method will therefore not require taking special precautions if the starting matrix and consequently the measuring medium have been modified. If the measurement method is even specific, it will result that the preliminary step of processing the sample will be very lightened with, consequently, a considerable saving of time and a sharp reduction in the causes of errors.

I.3. Classification of analysis techniques

Biological analysis methods can be classified according to three modalities :

Depending on the type in qualitative or quantitative methods, quantitative analysis makes it possible to determine the concentration of the various compounds sought. In this it is complementary to the qualitative analysis where it is sought only to determine whether this or that compound is present or not.

According to the automaticity in manual or automatic analysis, automatic analysis is used a lot in laboratories that receive many samples of the same type.

- Depending on the quantity of sample used in macro or microanalysis, depending on the technique used, this quantity may be of the order of a few grams or fractions of a milligram. Microanalysis techniques have mainly been developed in qualitative analysis (reactions on drops of solution).

I.4. Validation of an analysis method

This validation constitutes an essential tool for periodically checking the performance of the reagents supplied by the manufacturers or developed by the laboratory. The validation of a method makes it possible to know its characteristics in order to define and judge the quality of the analytical process and to specify the limits of validities. The analytical performance of the methods is evaluated on several universally recognized criteria. The protocols for evaluating these criteria are standardized and vary little depending on the country. Standardization makes it possible to simplify and optimize the evaluation work, to standardize the presentation of the data and to allow a comparative judgment of the results obtained by different laboratories.

DEFINITION: Separation

Separation techniques are those techniques that can be used to separate two different states of matter such as liquids and solids.

Separation processes or a separation method or simply a separation is methodology to attain any mass transfer phenomenon that convert a mixture of substances into two or more distinct product mixtures.

Separation is an important asset to purify component of interest from a mixtures.

NEED OF SEPARATION TECHNIQUES:

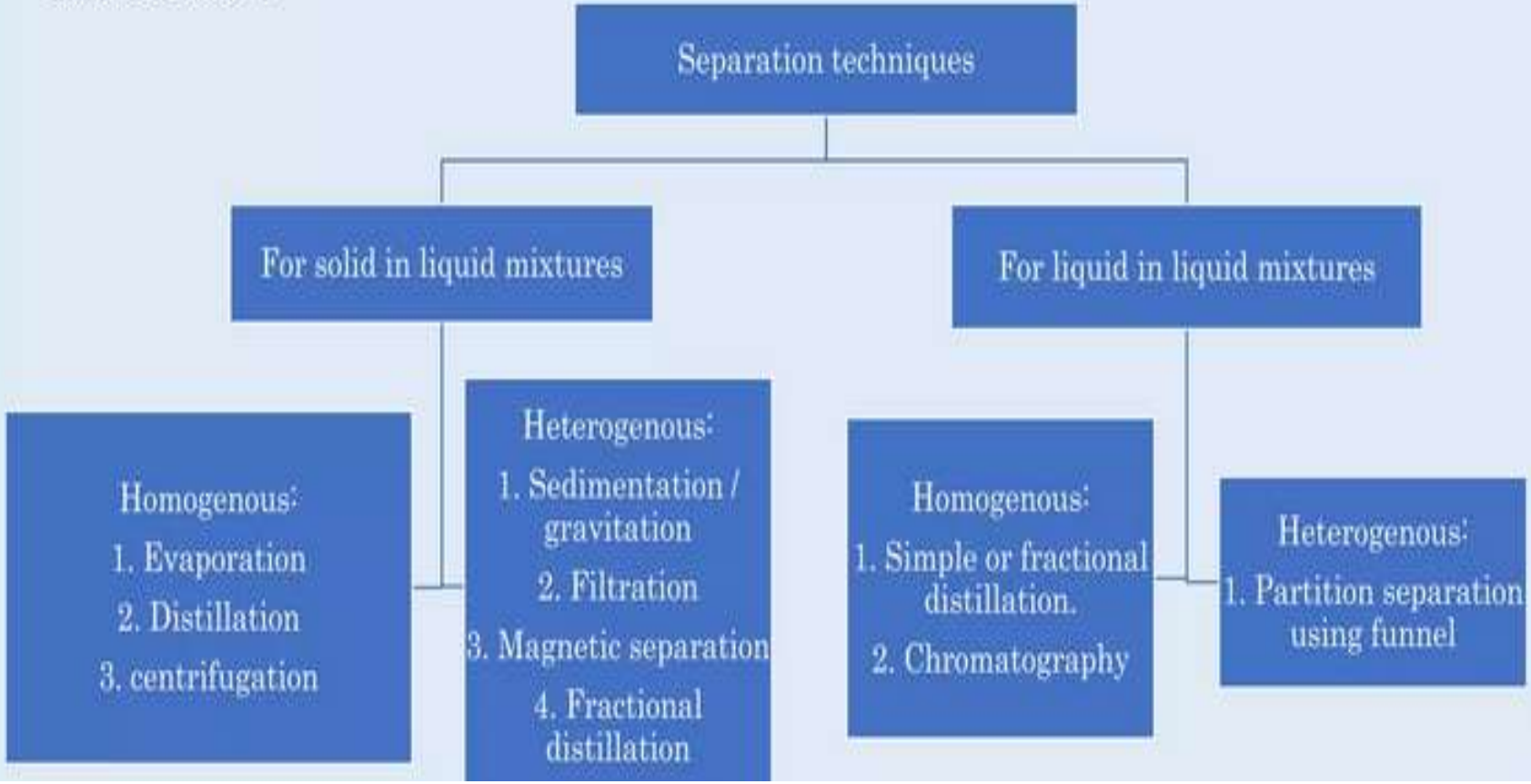
TO IDENTIFY WHAT TO BE SEPARATED FROM MIXTURE.

TO OBTAIN IMPORTANT AND PURE SUBSTANCES.

TO REMOVE UNWANTED PARTICLES.

TYPES OF SEPARATION TECHNIQUES:

- Separation techniques are classified based on type of mixtures:

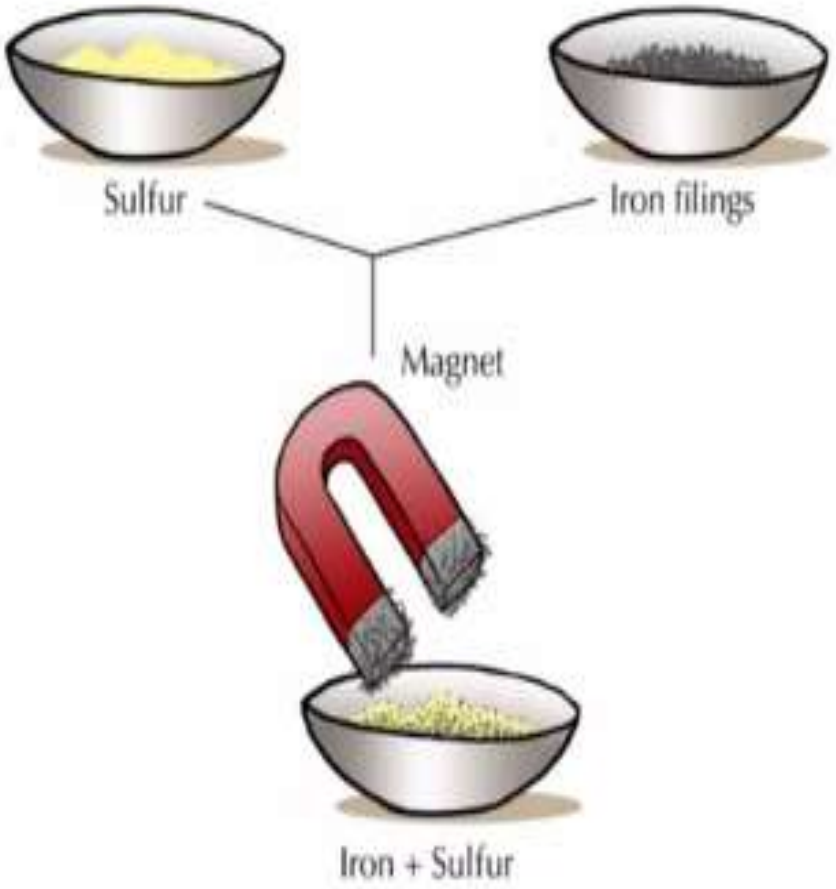


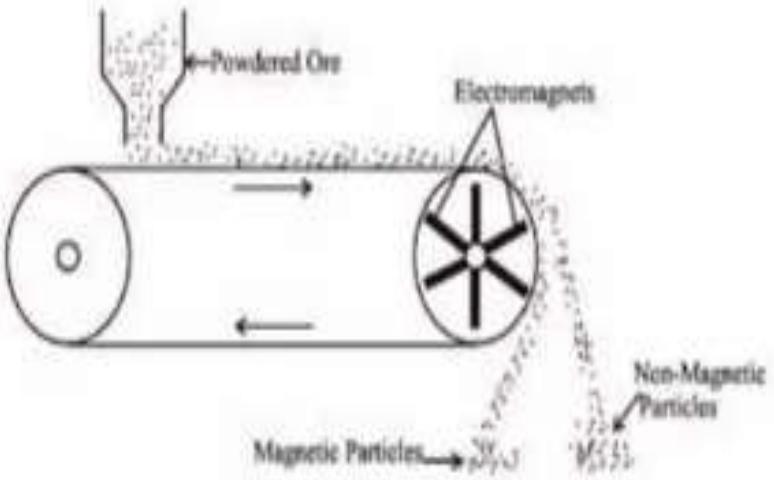
MAGNETIC SEPARATION:

This method involves the separation of magnetic substances from non-magnetic substances by means of magnet.

Takes advantage of physical property of magnetism, so it useful only for certain substances such as ferromagnetic (materials strongly affected by magnetic fields) and paramagnetic (materials that are less affected, but the effect is still noticeable).

This method involves the separation of magnetic substances from non-magnetic substances by means of magnet.


Sulfur
Iron filings
Magnet
Iron + Sulfur


Powdered Ore
Electromagnets
Non-Magnetic Particles
Magnetic Particles

- Applications:
  - Waste management, low-magnetic field separation in water purification and separation of complex mixtures.
  - To remove metal contaminants from pharmaceutical product streams.
  - Magnetic cell separation. It is currently being used in clinical therapies, more specifically in cancer and hereditary diseases researches.
  - These techniques are combined with PCR (polymerase chain reaction), to increase sensitivity and specificity of results.

DECANTATION:

Decantation is a process for the separation of mixtures of immiscible liquids or of a liquid and a solid mixture such as a suspension.

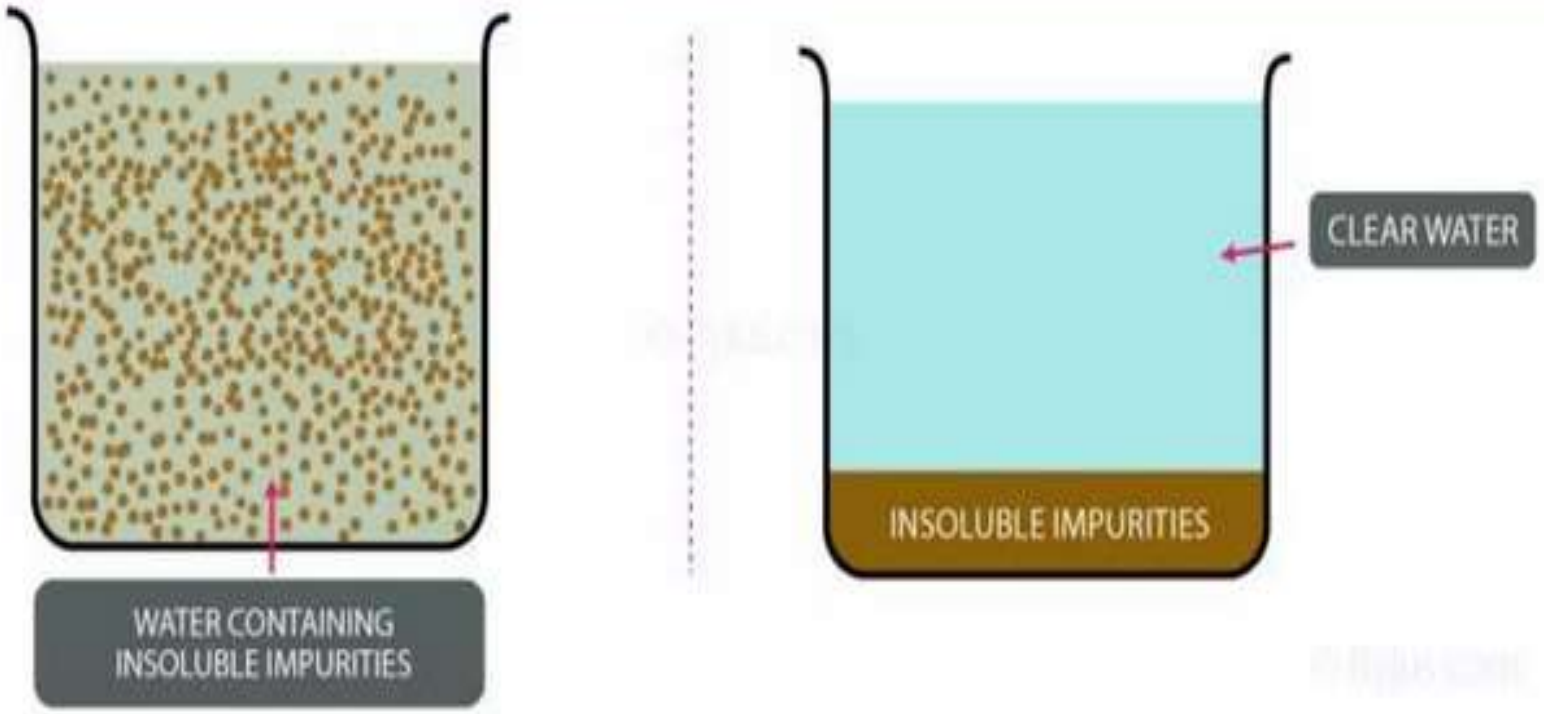
Immiscible liquid separation:

- Takes advantage of differences in density of the liquids.
- A separatory funnel is an alternative apparatus for separating liquid layers. It has a valve at the bottom to allow draining off the bottom layer. It can give a better separation between the two liquids.
- Example: separation of mixture of oil and water.

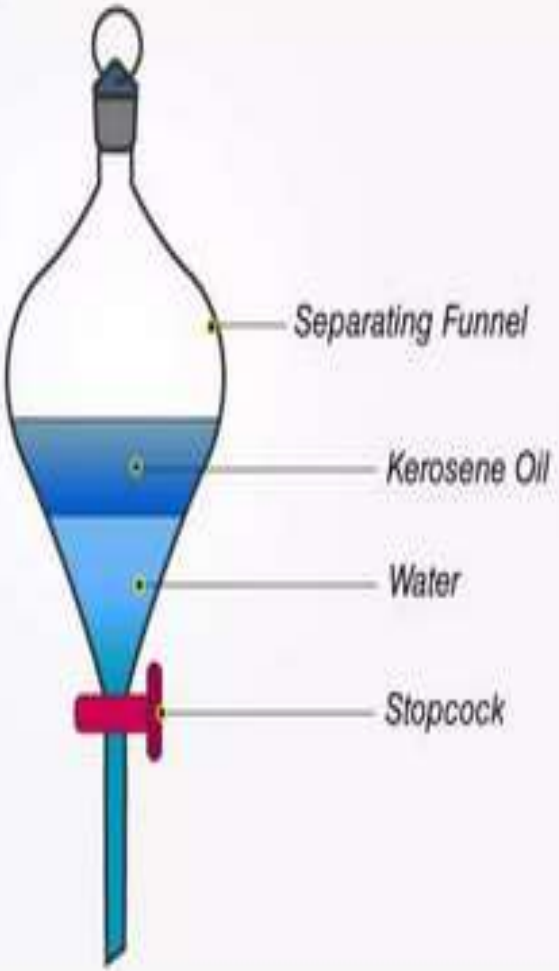
Liquid-solid separation:

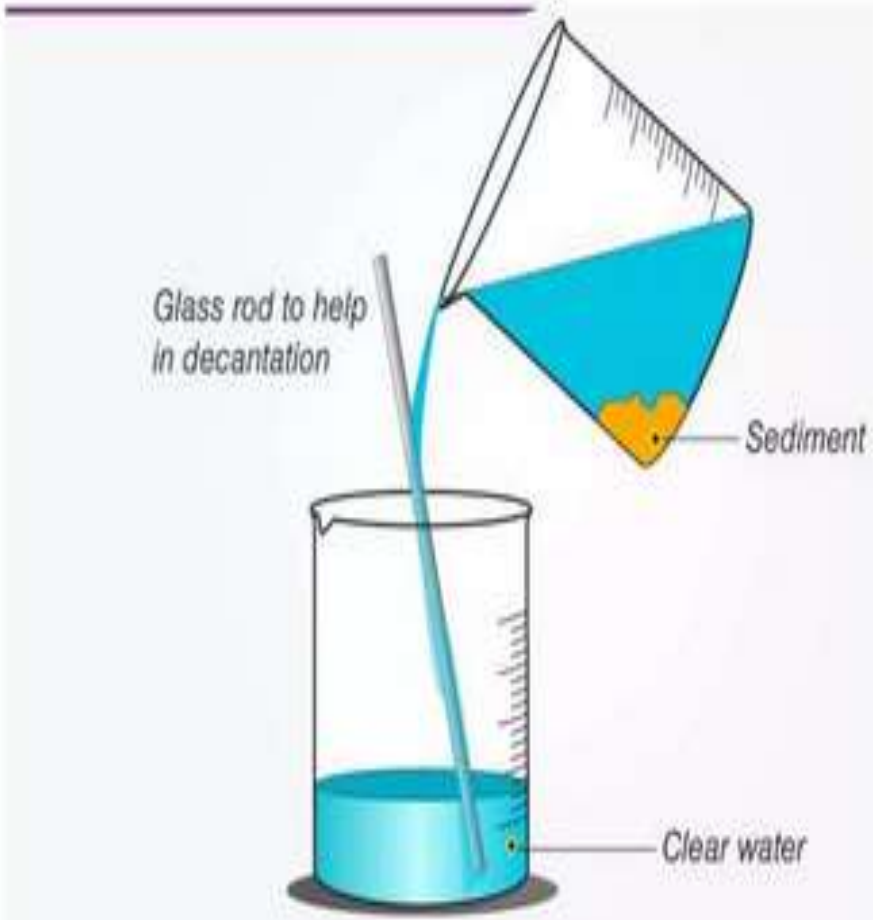
- Takes advantage of gravity/sedimentation of solids in case solid-liquid separation.
- **Sedimentation:** The tendency of particles in suspension to settle down in the fluid due to certain forces like gravity, centrifugal acceleration, or electromagnetism is called as sedimentation.
  - The solid that gets settled down is called as sediment.
- In laboratory it can be done in test tubes. To enhance productivity test tubes should be placed at 45° angle to allow the sediments to settle at the bottom of the apparatus.
- A decanter centrifuge may be used for continuous solid-liquid separation.

SEDIMENTATION



DECANTATION





Examples/applications:

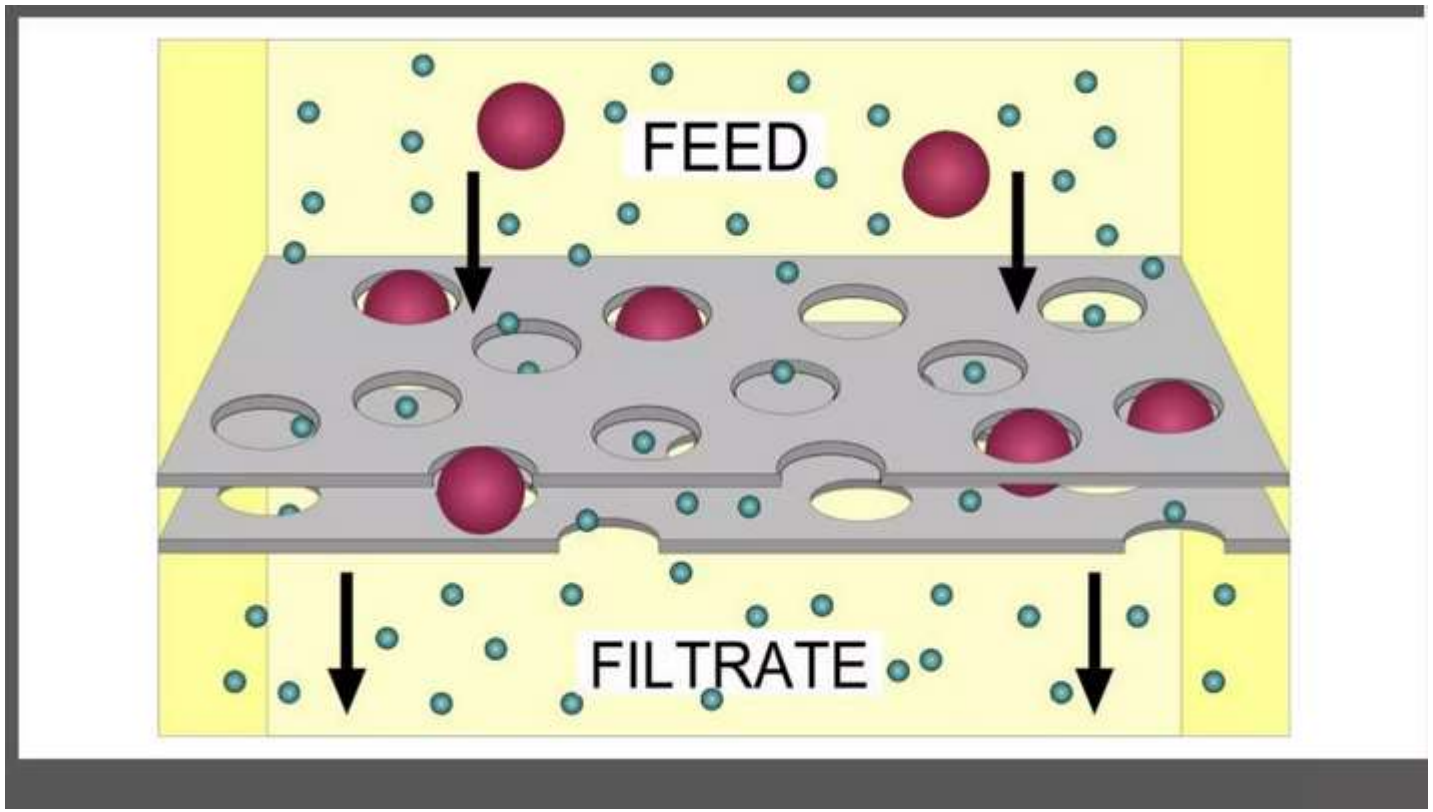
- Decantation is frequently used to purify a liquid by separating it from a suspension of insoluble particles.
- Decantation is also present in nanotechnology. In the synthesis of high-quality silver nanowire (AgNW) solutions and fabrication process of high-performance electrodes, decantation is also being applied which greatly simplifies the purification process.
- Fat is determined in butter by decantation.
- In sugar industry, processing of sugar beets into granular sugar many liquid - solid separations are encountered.

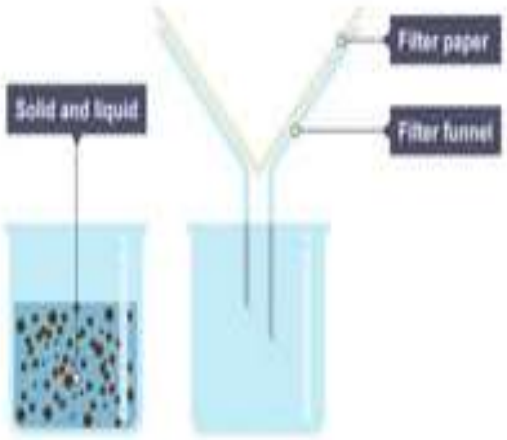
Takes advantage of physical property of state of matter, its size and solubility in liquid.

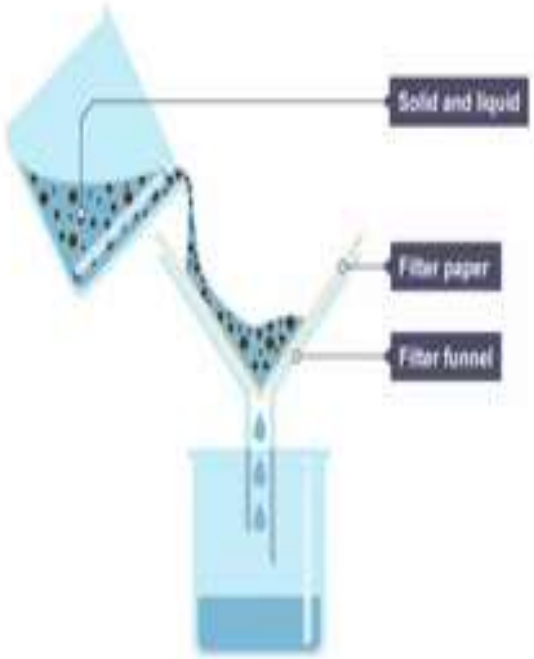
FILTRATION:

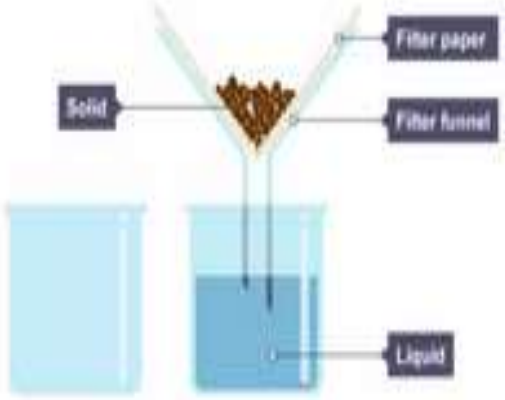
Mechanical, physical or biological operations that separates solids from fluids (liquids or gases) by adding a medium through which only the fluid can pass can be called as filtration.

- The substance which is left behind in the filtration medium is called **residue**.
- The liquid which passes through the filtration medium is called **filtrate**.


FEED
FILTRATE


Solid and liquid
Filter paper
Filter funnel


Solid and liquid
Filter paper
Filter funnel


Solid
Filter paper
Filter funnel
Liquid

Filtration

Filtration is the operation that consists of separating solid particles that are suspended in a liquid. For this purpose, the suspension to be filtered is poured onto a filter which allows the liquid to pass through but retains the solids. The liquid collected after filtration is called filtrate, the solid deposited on the filtering medium is called cake. The filter medium is composed of paper or a granular material that forms narrow channels in which the liquid circulates (for example sintered glass).

I.1.1. Different types of filters

Two types of filters are available: the so-called "in-depth" filters and the so-called "screen" filters, they differ in their principle and their applications

I.1.1.1. The in-depth filters

They consist of fibrous substances (glass fibers, cellulose, cotton, etc.) or agglomerated substances (sand, coal, sintered glass) which create a network of channels inside the filter, a network in which the substances stopped by the filtration are blocked. The efficiency of a filter "in depth" therefore increases with its thickness, but decreases when the pressure exerted on the filter increases. There are different kinds :

Filter papers

The filter, used on a conical or Büchner funnel type support, can be flat or pleated. The type of paper is different depending on whether the desired filtration is fast, for example :

Here are the filters for fast filtrations (Whatman n°= 4 and 9)

All filters for medium speed filtrations (Whatman n°= 1 and 7)

Here are the filters for very slow filtrations (Whatman n°= 5 and 6)

Sintered plates/Plaques frittées

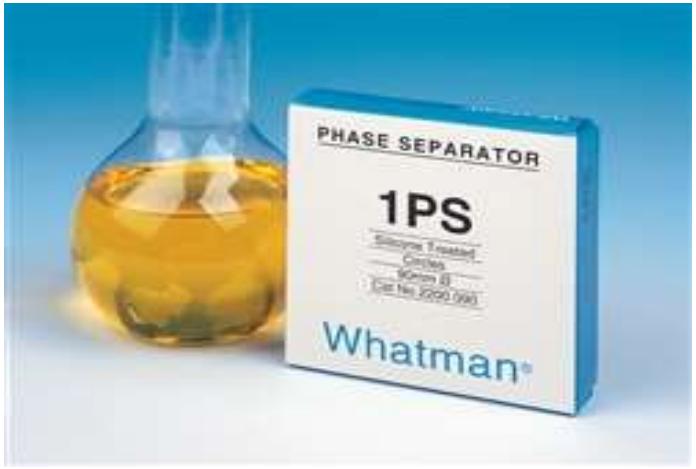
These are porous plates made of agglomerated glass powder presented in a funnel and allowing vacuum filtration. Their cleaning requires washing with sulfochromic acid and abundant rinsing with distilled water.

Filter tubes

They consist of glass microfibers connected together by a resin and are in the form of hollow cylinders that can be mounted on connection tips. They can be used to quickly clarify large volumes of solutions.

Phase separator filter paper

The Whatman IPS phase separator filter paper can replace a settling funnel, it is indeed a hydrophobic paper which, placed on a glass funnel, retains the aqueous phase while the organic phase passes through the filter.



I.1.1.2. Filters "screens"

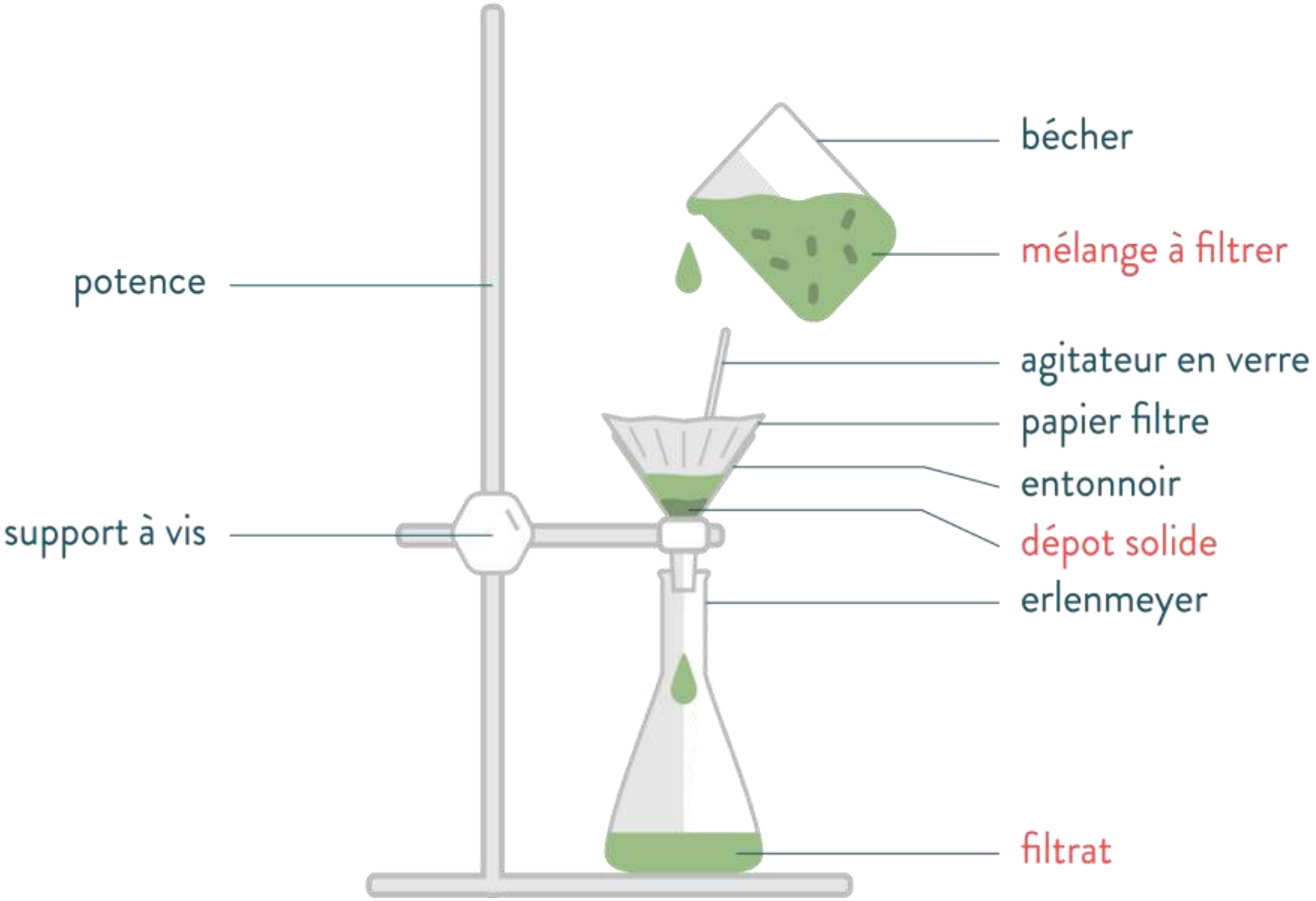
Are milliporous membranes, they are formed of cellulose polymers (cellulose nitrate) comprising a very large number of calibrated pores. The dimensions of these pores vary according to the filters from 0.025 um to 8 um while the thickness of the filter is 0.15 mm. These filters withstand for at least 30 minutes both at temperatures of 125 ° and at high pressures. This allows them to be sterilized by autoclaving. The "screen" filters stop the particles on their surface so that their ability to retain impurities is low. This is why, usually, the filtration on a "screen" filter is preceded by filtration on an asbestos-cellulose or glass fiber in-depth filter; this filter is often then called a prefilter


Millipore Millex-LCR
hydrophilic
PTFE 0.45µm

Filtration processes

I.1.2.1. Gravity filtration

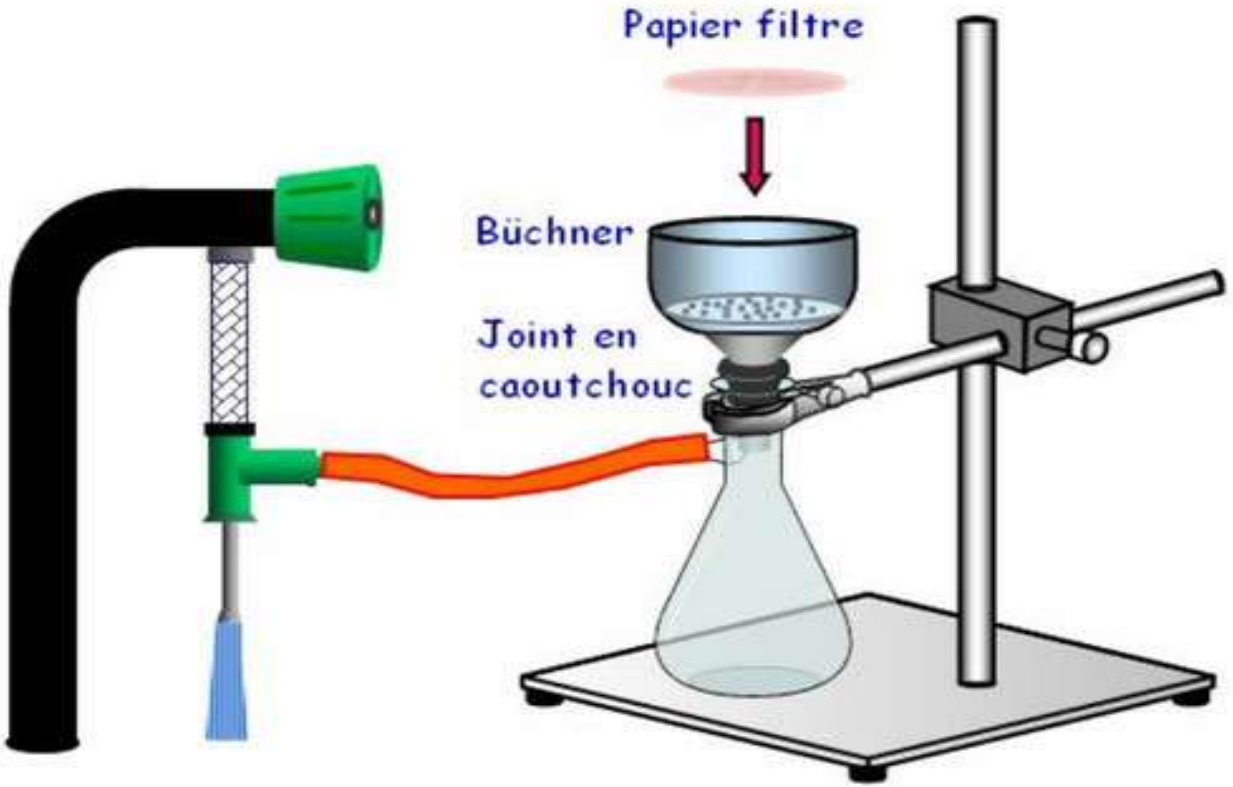
It uses a simpler device which is simply a conical funnel equipped with a membrane filter (pleated paper filter) that is deposited on a container or a volumetric flask. This method is very slow, and often poses the problem of clogging, with the possibility of adsorption of proteins on the surface of the filter.


potence
support à vis
bécher
mélange à filtrer
agitateur en verre
papier filtre
entonnoir
dépot solide
erlenmeyer
filtrat

I.1.2.2. Vacuum or Büchner filtration/ La filtration sous vide ou sous Büchner

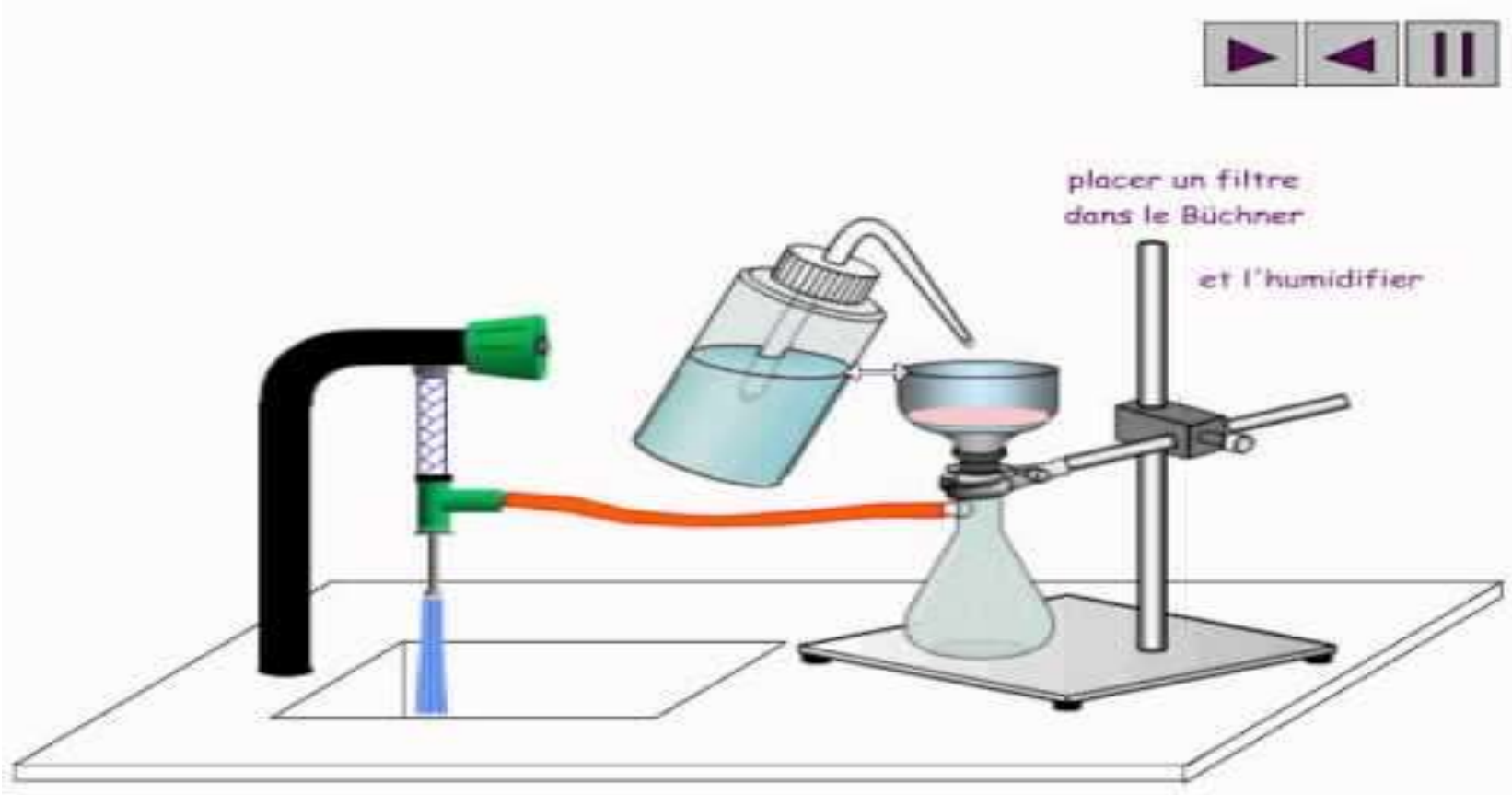
Principle :

The filtration is amplified by the suction of the filtrate using a water pump. As the water circulates, the air is sucked in by the movement of the water, a vacuum is created, the filtrate is thus sucked in.



I.1.2.3.Pressure filtration

It is a question of exerting a pressure on the liquid to be filtered so as to accelerate the flow of the filtrate. This involves the use of synthetic membranes or filters that resist.



I.2. Ultrafiltration

Ultrafiltration is a process for separating dissolved molecules and/or their material suspended in the solvent as a function of their size. This technique uses membranes with selective permeability which allow any substance smaller in size than the pores of the membrane to pass through. The larger molecules are then retained but also concentrated since the membrane is permeable to the solvent. Pressure is exerted on the solution to be ultrafiltrated. The process, which is very simple to implement, does not require any phase change or chemical process. The very mild conditions do not destroy the biological activity of the most fragile molecules.

Applications:

- Most important techniques used by chemists to purify compounds.
- HEPA filters in air conditioning to remove particles from air.
- In the laboratory, a Büchner funnel is often used, with a filter paper serving as the porous barrier.