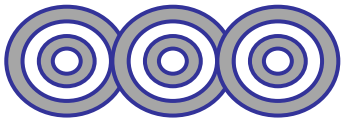
Chemical Separation and Chromatographic Methods



Separations Based on Size

Ahmad Aqel Ifseisi
Assistant Professor of Analytical Chemistry
College of Science, Department of Chemistry
King Saud University
P.O. Box 2455 Riyadh 11451 Saudi Arabia
Building: 05, Office: 1A7 & AA53
Tel. 014674198, Fax: 014675992
Web site: http://fac.ksu.edu.sa/aifseisi
E-mail: ahmad3qel@yahoo.com
aifseisi@ksu.edu.sa

Size is the simplest physical property that can be exploited in a separation.

The separation is accomplished using a **porous medium** through which only the analyte or interferent can pass.

Filtration

Filtration

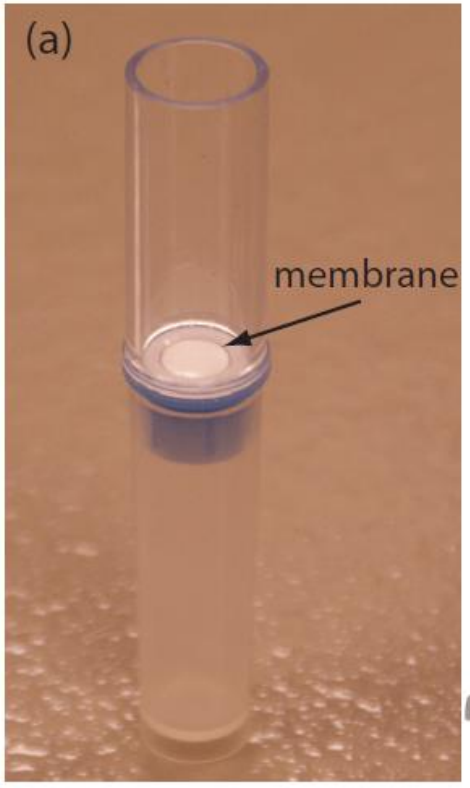
Filtration, in which **gravity**, **suction**, or **pressure** is used to pass a sample through a porous filter is the most commonly encountered separation technique based on size.

In a filtration we separate a particulate interferent from dissolved analytes using a filter whose pore size retains the interferent. The solution passing through the filter is called the **filtrate**, and the material retained by the filter is the **retentate**.

Filter paper (membrane filters) available in a variety of **micrometer pores sizes**, are the method of choice for particulates that are too small to be retained by filter paper.

A filter paper's size is just its **diameter**. Filter paper comes in many sizes, including 4.25, 7.0, 11.0, 12.5, 15.0, and 27.0 cm. Choose a size that fits comfortably into your funnel.

(a) centrifugal filter for concentrating and desalting macromolecular solutions.



(b) A 0.45µm membrane syringe filter (disc filter)

(c) A 0.22µm cellulose acetate membrane filter.

Three types of membrane filters for separating analytes from interferents.

Choosing the membrane filter and its kit depends on:

- Molecular weight cut-off.
- Membrane size (diameter).
- Membrane pore size.
- Sample size (capacity of the filter unit).
- Membrane material (cellulose acetate, graphite, nylon).

The most common filtration method uses filter paper, which is classified according to its **speed**, its **size**, and **its ash content** on ignition.

Speed, or how quickly the supernatant passes through the filter paper, is a function of the paper's pore size. A larger pore allows the supernatant to pass more quickly through the filter paper, but does not retain small particles of precipitate. The proper choice of filtering speed is important. If the filtering speed is too fast, we may fail to retain some of the precipitate, causing a negative determinate error. On the other hand, the precipitate may clog the pores if we use a filter paper that is too slow.

Filter paper is rated as fast (retains particles larger than 20–25 μm), medium–fast (retains particles larger than 16 μm), medium (retains particles larger than 8 μm), and slow (retains particles larger than 2–3 μm).

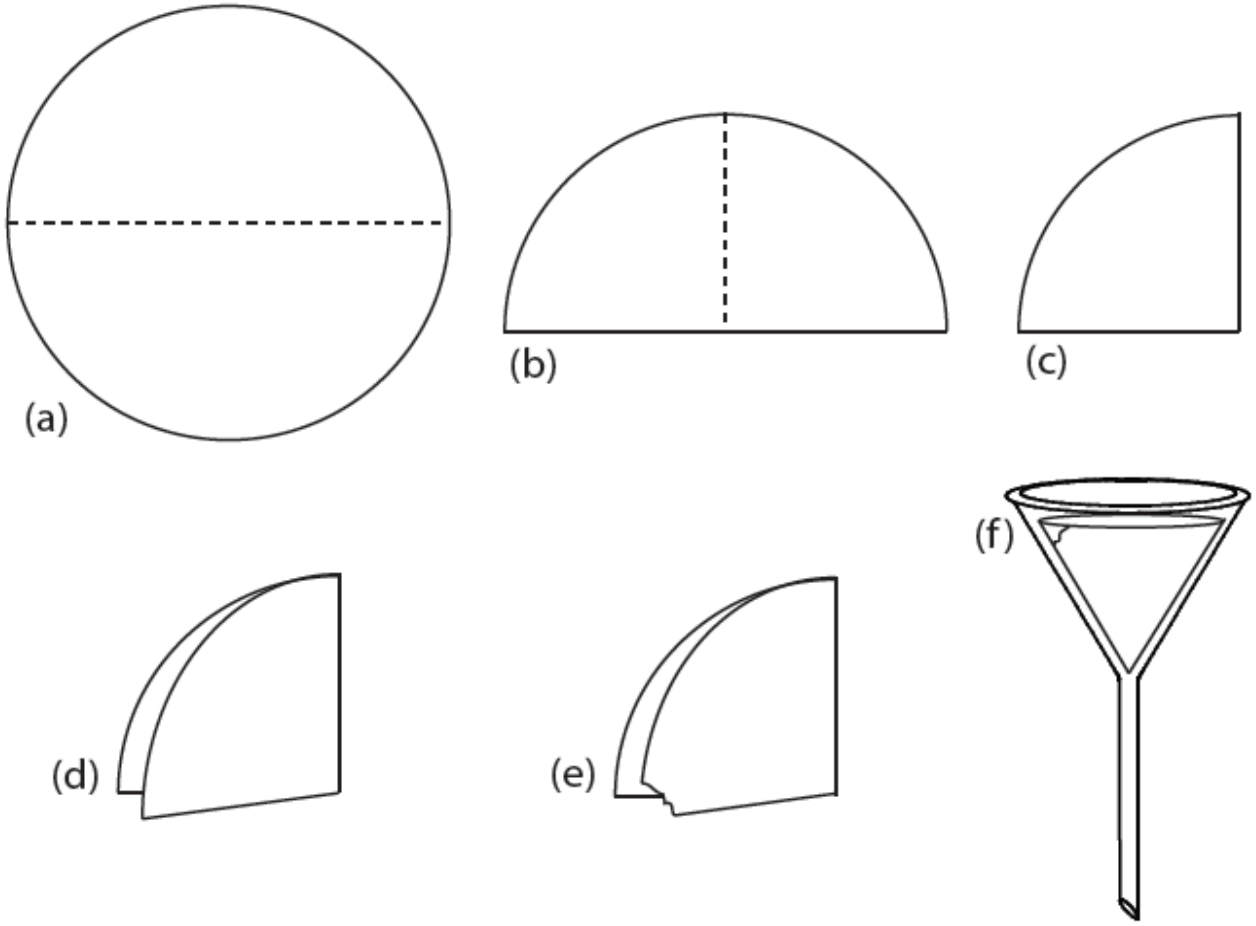
Because filter paper is **hygroscopic**, it is not easy to dry it to a constant weight. When accuracy is important, the filter paper is removed before determining the precipitate's mass. After transferring the precipitate and filter paper to a covered crucible, we heat the crucible to a temperature that converts the paper to **CO_2**(g) and **H_2O**(g), a process called **ignition**.

Igniting a poor quality filter paper leaves behind a residue of inorganic ash. For quantitative work, use a low-ash filter paper. This grade of filter paper is pretreated with a mixture of **HCl** and **HF** to remove inorganic materials. Quantitative filter paper typically has an ash content of less than 0.010% w/w.

Ash is the material remaining after manufacturing (minerals; salts, inorganic ash, Na, Mg, Si, Fe, etc.).

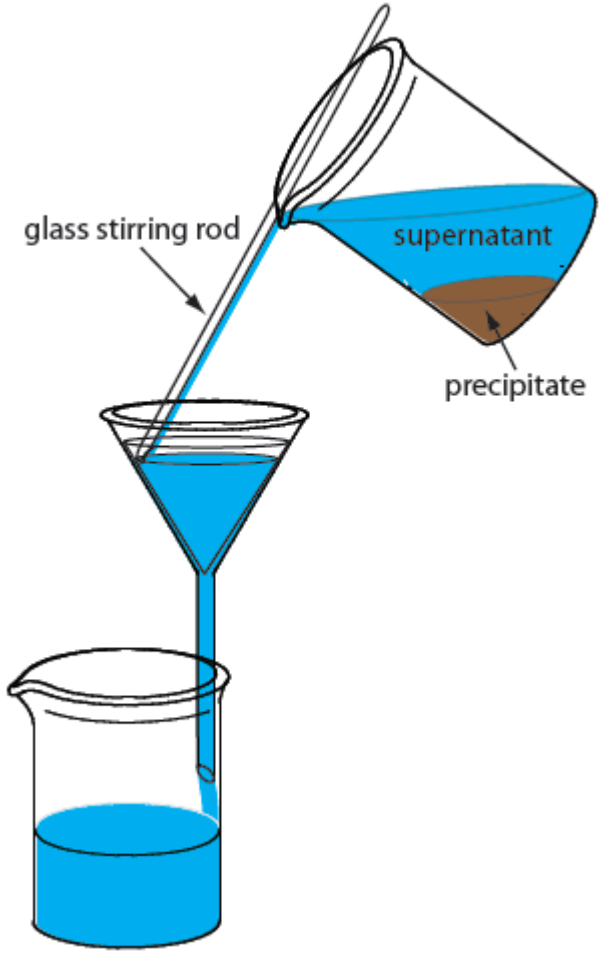
Gravity filtering is accomplished by folding the filter paper into a cone and placing it in a long-stem funnel. A seal between the filter cone and the funnel is formed by dampening the paper with water or supernatant, and pressing the paper to the wall of the funnel. When properly prepared, the funnel's stem fills with the supernatant, increasing the rate of filtration.

Preparing a filter paper cone. The filter paper circle in (a) is folded in half (b), and folded in half again (c). The folded filter paper is parted (d) and a small corner is torn off (e). The filter paper is opened up into a cone and placed in the funnel (f). The small tear at the corner creates a slightly broader cone (lock the air) which will make better contact with the walls of the funnel thus creating a better suction.



The precipitate is transferred to the filter in several steps. The first step is to decant the majority of the supernatant through the filter paper without transferring the precipitate. This prevents the filter paper from clogging at the beginning of the filtration process.

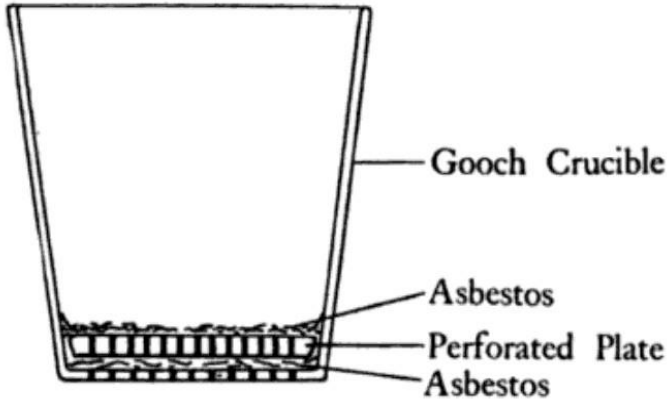
The precipitate is rinsed while it remains in its beaker, with the rinsings decanted through the filter paper. Finally, the precipitate is transferred onto the filter paper using a stream of rinse solution. Any precipitate clinging to the walls of the beaker is transferred using a rubber policeman (a flexible rubber spatula attached to the end of a glass stirring rod).

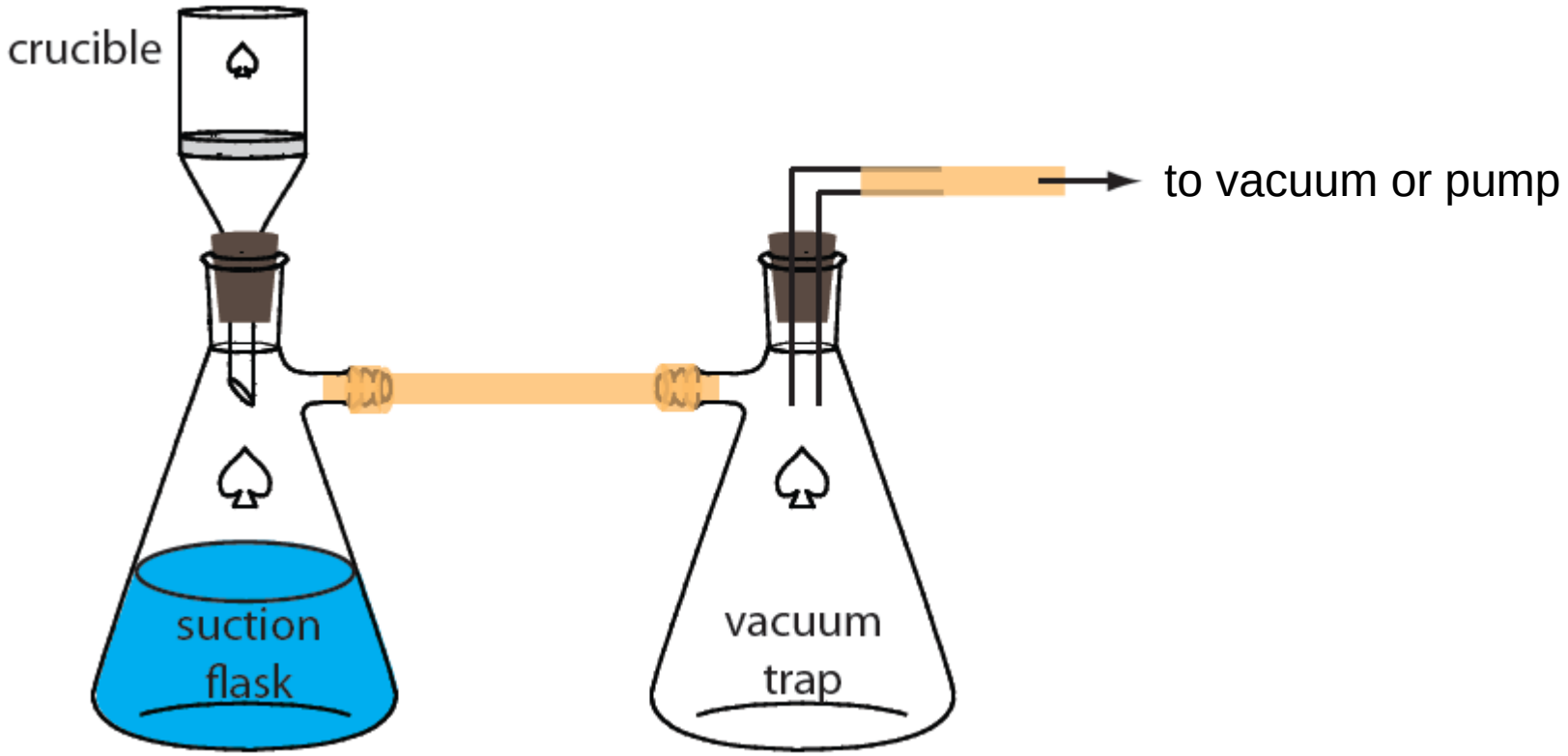


Proper procedure for transferring the **supernatant** to the filter paper cone.

An alternative method for filtering a precipitate is a **filtering crucible**. The most common is a fritted-glass crucible containing a porous glass disk filter. Fritted-glass crucibles are classified by their porosity: coarse (retaining particles larger than 40–60 μm), medium (retaining particles greater than 10–15 μm), and fine (retaining particles greater than 4–5.5 μm).

Another type of filtering crucible is the **Gooch crucible**, which is a porcelain crucible with a perforated bottom. A glass fiber mat is placed in the crucible to retain the precipitate. For both types of crucibles, the precipitate is transferred in the same manner described earlier for filter paper. Instead of using gravity, the supernatant is drawn through the crucible with the assistance of suction from a vacuum aspirator or pump.





Procedure for filtering a precipitate through a filtering crucible. The trap prevents water from an aspirator from back-washing into the suction flask.

Applications

This separation technique is important in the analysis of many natural waters, for which the presence of suspended solids may interfere in the analysis.

Filtration also can be used to isolate analytes present as solid particulates from dissolved ions in the sample matrix. For example, this is a necessary step in gravimetry, in which the analyte is isolated as a precipitate.

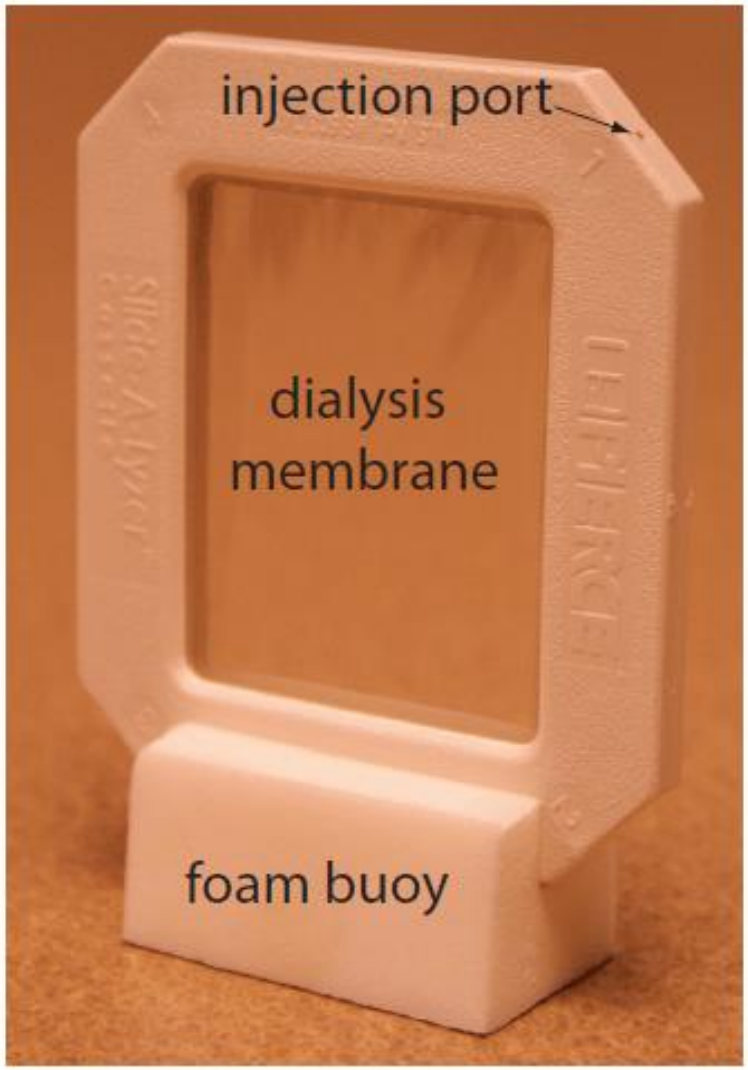
Dialysis

Dialysis

Dialysis is another example of a separation technique that uses size to separate the analyte and the interferent. In this technique, **semipermeable membrane** is used to separate the analyte and interferent.

A dialysis membrane is usually constructed from cellulose with pore sizes of 1–5 nm, and fashioned into tubing, bags, or cassettes. The sample is injected into the dialysis membrane, which is sealed tightly by a gasket, and the unit is placed in a container filled with a solution whose composition is different from the sample.

If the concentration of a particular species is different on the membrane's two sides, the resulting concentration gradient provides a driving force for its diffusion across the membrane. While small species freely pass through the membrane, larger species are unable to pass.



Example of a dialysis cassette. The dialysis membrane in this unit has a molecular weight cut-off of 10 000 g/mol. Two sheets of the membrane are separated by a gasket and held in place by the plastic frame. Four ports, one of which is labeled, provide a means for injecting the sample between the dialysis membranes. The cassette is inverted and submerged in a beaker containing the external solution, which is stirred using a stir bar. A foam buoy, used as a stand in the photo, serves as a float so that the unit remains suspended above the stir bar. The external solution is usually replaced several time during dialysis. When dialysis is complete, the solution remaining in the cassette is removed through an injection port.

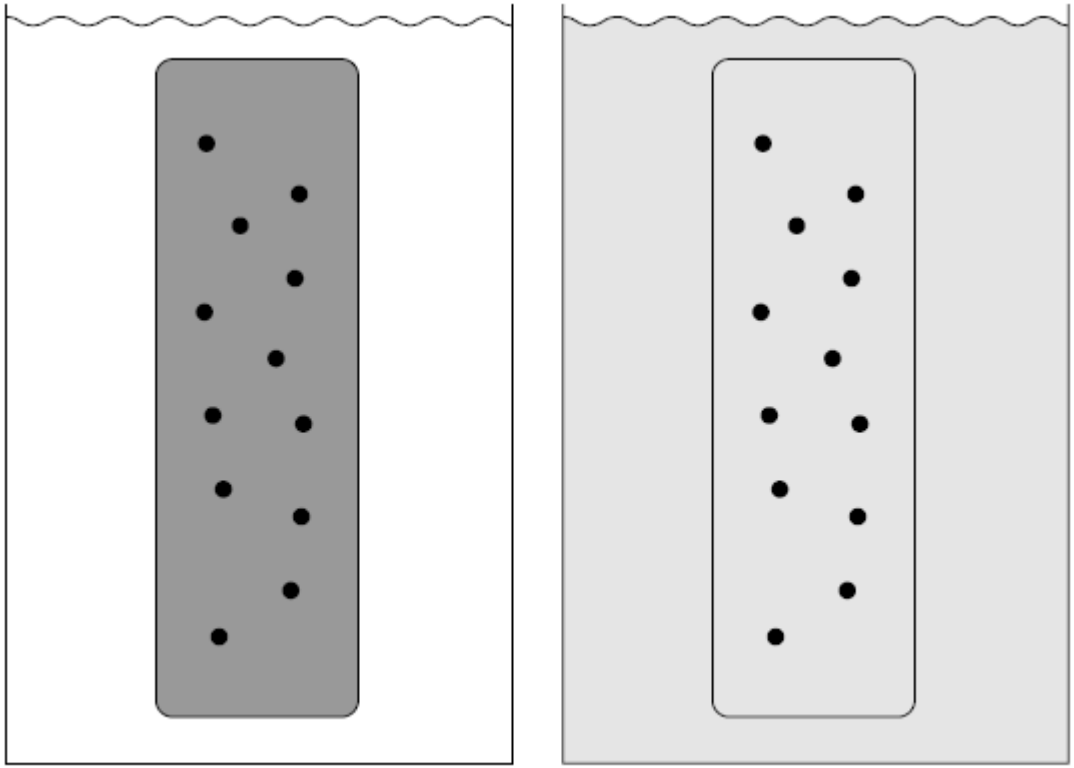


Illustration of a dialysis membrane in action. In (a) the sample solution is placed in the dialysis tube and submerged in the solvent. (b) Smaller particles pass through the membrane, but larger particles remain within the dialysis tube.

Applications

Dialysis is frequently used to purify proteins, hormones, and enzymes.

During kidney dialysis, metabolic waste products, such as urea, uric acid, and creatinine, are removed from blood by passing it over a dialysis membrane.

Size-exclusion chromatography

Size-exclusion chromatography

Size-exclusion chromatography (SEC), which also is called gel permeation, gel filtration or molecular exclusion chromatography.

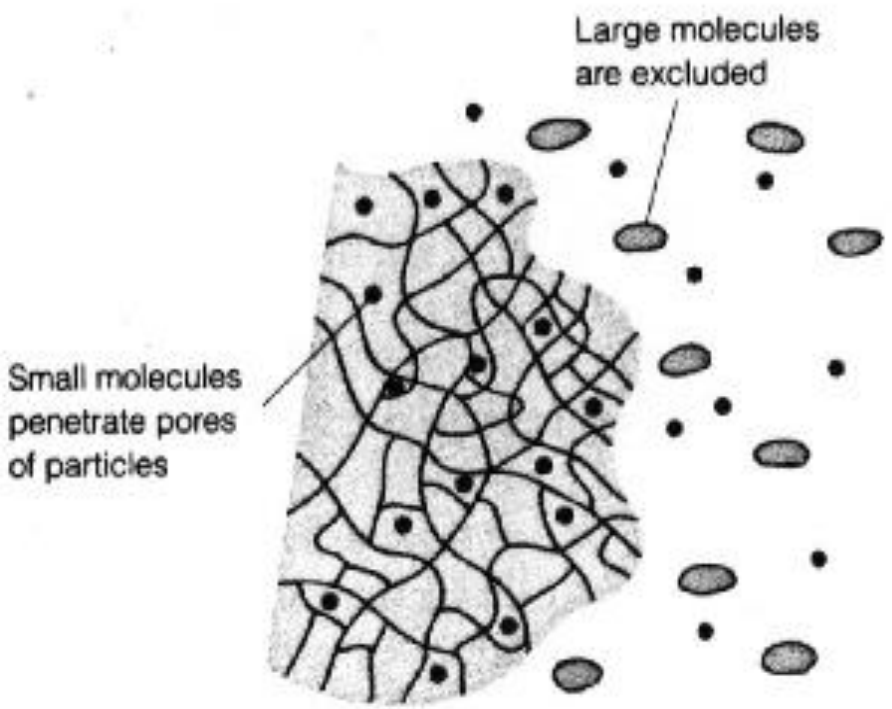
SEC is a chromatographic method in which particles are separated by differences in their molecular size (on the absence of any specific analyte interactions with the stationary phase).

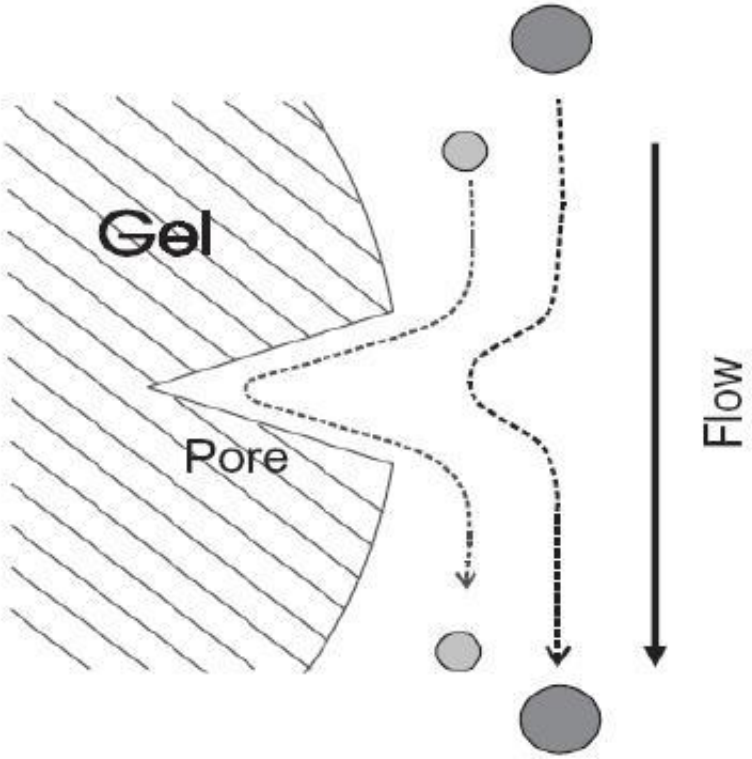
In **SE**, the columns are filled with material having precisely controlled pore sizes (e.g., cross-linked dextrin or polyacrylamide), and the sample is screened or filtered by the stationary phase according to its solvated molecular size.

The sample to be separated is placed into a stream of solvent that is pumped through the column at a fixed flow rate. The separation process in simplified form is based on the ability of sample molecules to penetrate inside the pores of packing material and is dependent on the relative size and shape of analyte molecules and the respective pore size of the absorbent. Therefore, different molecules have different total residence times in the column. The process also relies on the absence of any interactions with the packing material surface.

The molecules are separated in order of decreasing molecular weight, particles too large to enter the pores are not retained and pass through the column first. Smaller particles are capable of entering into the pore structure, those take longer to pass (longer path) through the column, take longer time to pass through the column.

Figures show that a large molecule is excluded from the pores and migrates quickly, whereas a small molecule can penetrate the pores and migrates more slowly down the column.





Because the solutes are all eluted within a small retention volume, peaks in **SEC** are generally narrow, thereby enhancing sensitivity and allowing the use of relatively insensitive detection methods such as refractive index (**RI**) detection.

On the other hand, **SEC** is generally a low resolution chromatography, for small molecules, a size difference of more than about 10% is required for acceptable resolution; for macromolecules a twofold difference in molecular weight is necessary. And thus it is often reserved for the final, polishing step of purification.

Two types of **SEC** are usually distinguished:

(1) Gel filtration chromatography (**GFC**): use aqueous mobile phases and hydrophilic packings to separate and identify aqueous soluble biological macromolecules. Typical stationary phases for **GFC** include polydextrans, polyvinyl alcohol gel and silica gel.

(2) Gel permeation chromatography (**GPC**): usually performed using hydrophobic stationary phases and organic mobile phases to obtain molecular weight distribution information and characterization on organic soluble polymers. Stationary phases for **GPC** are typically crosslinked, rigid polystyrene divinylbenzene gels.

Historically, Per Flodin and Jerker Porath introduced a hydrophilic gel consisting of dextran crosslinked with epichlorohydrin as a cooperative venture under the trade name Sephadex in 1959, this was called **GFC**. On the other hand, the use of hydrophobic polystyrene gels by John Moore of the Dow Chemical Co. led to the development of **GPC**, enabling the determination of the molecular weight distribution of high molecular weight synthetic polymers. In the mid 1960's special instruments were introduced for such measurements, and these may be considered as early dedicated liquid chromatographs.

Generally, columns of 15 to 50 cm length are used, packed with 7 to 10 μm particles and with an i.d. between 0.6 and 0.8 cm. In **SEC**, unlike in other chromatographic modes, the stationary phase is the primary factor controlling retention.

Mobile phases for **SEC** fall into two broad categories: **aqueous buffers** for **GFC** and **organic solvents** for **GPC**. In **SEC**, the mobile phase is selected not to control selectivity but for its ability to dissolve the sample. In addition, the mobile phase should have a low viscosity and be compatible with the detector and column packing.

Applications

SEC is a widely used technique for the purification and analysis of synthetic and biological polymers, such as proteins, polypeptides, macromolecular complexes, polysaccharides, nucleic acids, hydrocarbon polymers, polyamides and polyesters.

It is also useful for determining the tertiary and quaternary structures of purified proteins, and is the primary technique for determining the average molecular weight of natural and synthetic polymers.

One of the major applications of **SEC** is polymer characterization. As many of the properties that characterize a polymer, including hardness, brittleness and tensile strength, are related to the molecular weight distribution, **GPC** can be used to identify subtle differences between polymer materials.


Thank You!