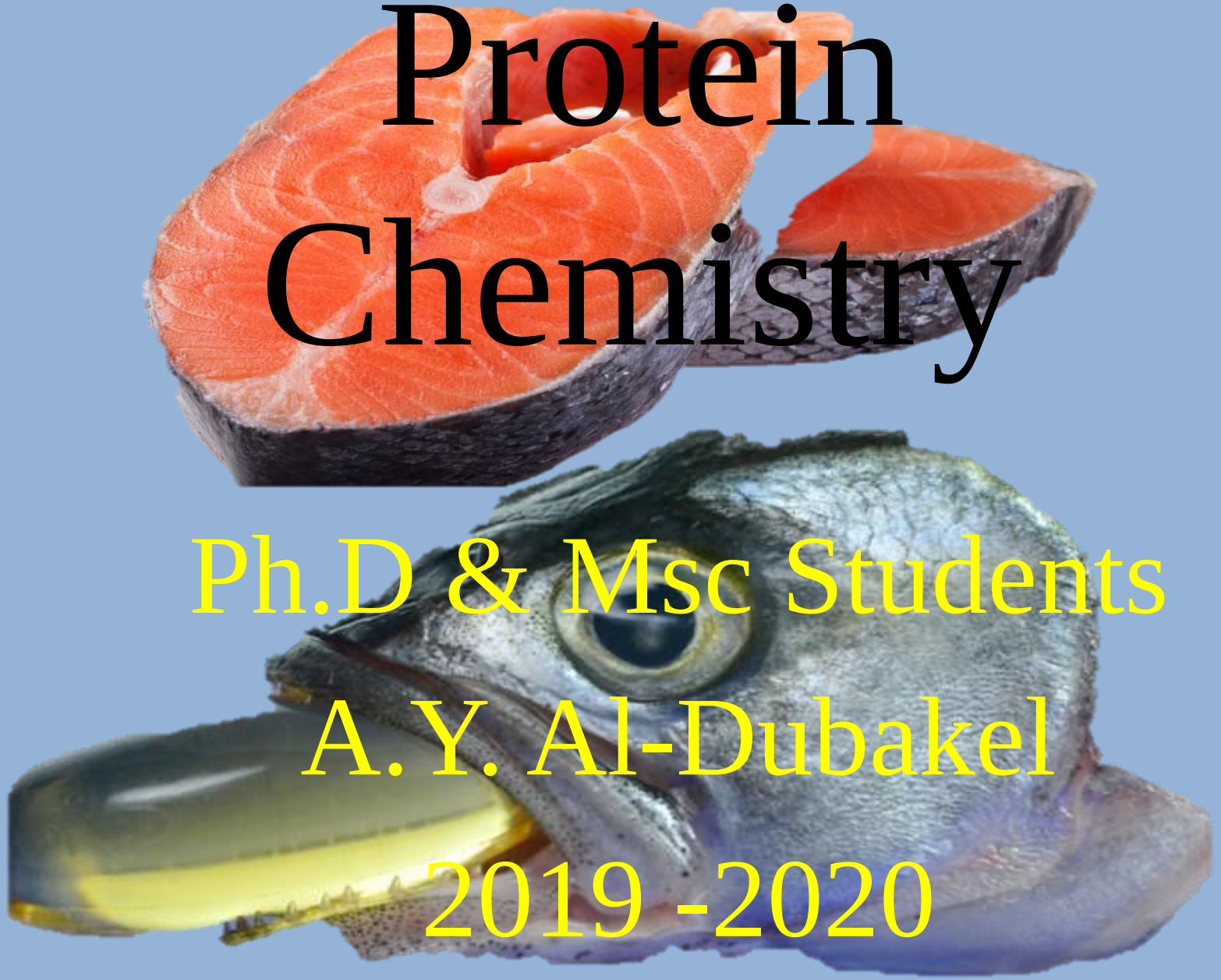
Protein Chemistry



Ph.D & Msc Students

A. Y. Al-Dubakel

2019 -2020

3. PROTEIN IDENTIFICATION

There are two methods that are commonly used to identify proteins: Edman Degradation and Mass Spectrometry.

Edman Degradation is a method of sequencing amino acids in a peptide. The amino-terminal residue is labeled and cleaved from the peptide without disrupting the peptide bonds between other amino acid residues.

Mass Spectrometry is an analytical technique that measures the mass-to-charge ratio of charged particles for determining masses of particles and the elemental composition of a sample of molecules as well as for elucidating the chemical

PROTEIN ANALYSIS TECHNIQUES

1. LIGHT SCATTERING
2. MULTI-DETECTION GPC/SEC
3. CIRCULAR DICHROISM SPECTR
4. ISOTHERMAL TITRATION CALO

https://www.atascientific.com.au/3-protein-analysis-techniques/

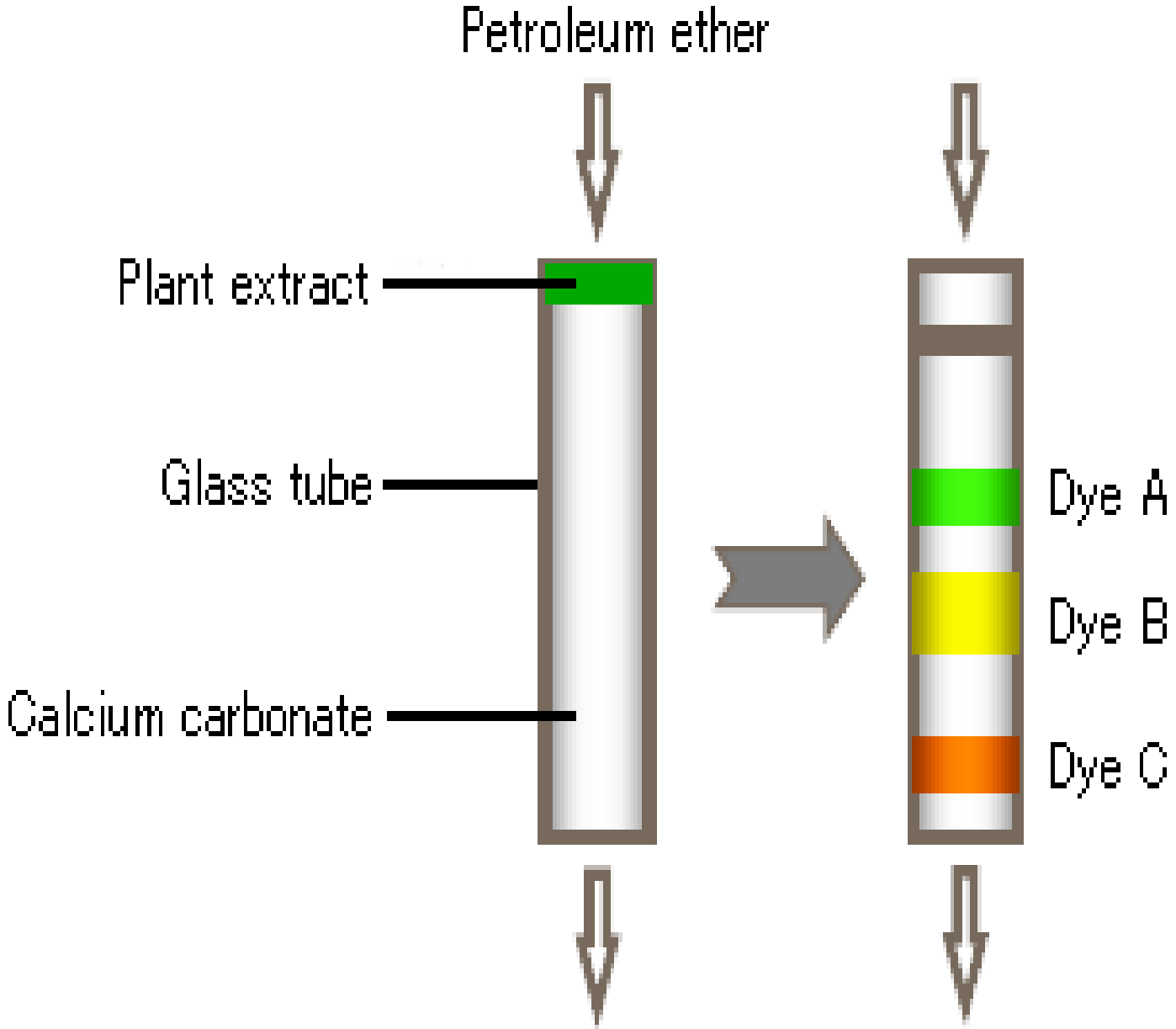
Amino Acid Analysis

Amino acid analysis is used to determine the amino acid content of amino acid-, peptide- and protein-containing samples. With minor exceptions, proteins are long linear polymers of amino acids connected to each other via peptide bonds. The first step of amino acid analysis involves hydrolyzing these peptide bonds. The liberated amino acids are then separated, detected, and quantified. The method was first developed by Moore, Stein and coworkers in the 1950s using HCl acid

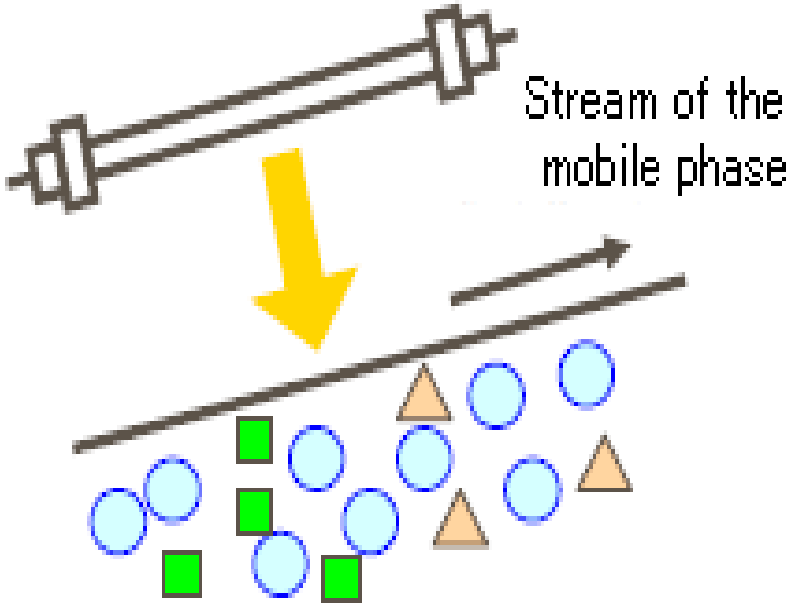
High Performance Liquid Chromatography (HPLC) is the most popular method for analyzing amino acid components

Principle of Chromatography : is a technique by which a mixture sample is separated into components. Although originally intended to separate and recover (isolate and purify) the components of a sample, today, complete chromatography systems are often used to both separate and quantify sample components.

The term, "chromatography" was coined by the Russian botanist, Tswett, who demonstrated that, when a plant extract was carried by petroleum ether through a column consisting of a glass tube packed with calcium carbonate powder, a number of dyes

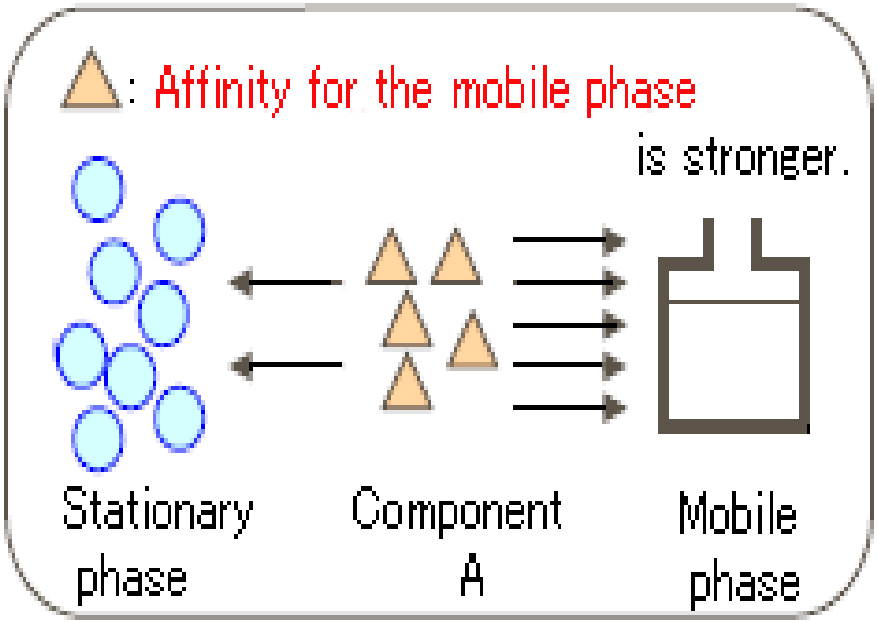

Petroleum ether
Plant extract
Glass tube
Calcium carbonate
Dye A
Dye B
Dye C

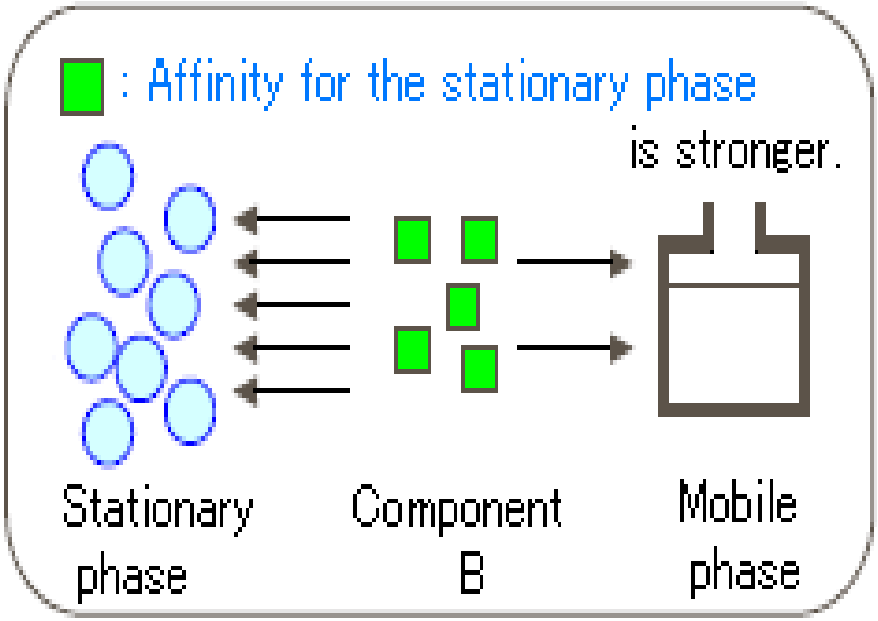
How is a sample separated into its components in the column? The speed of a migrating sample component depends on whether the component has an affinity for the stationary or mobile phase. This affinity appears via various actions: adsorption, partition, ion exchange, etc. As shown in Figure 2, components that have a higher affinity for the mobile phase compared with the stationary phase migrate more rapidly, while components that have a higher affinity for the stationary phase are eluted from the



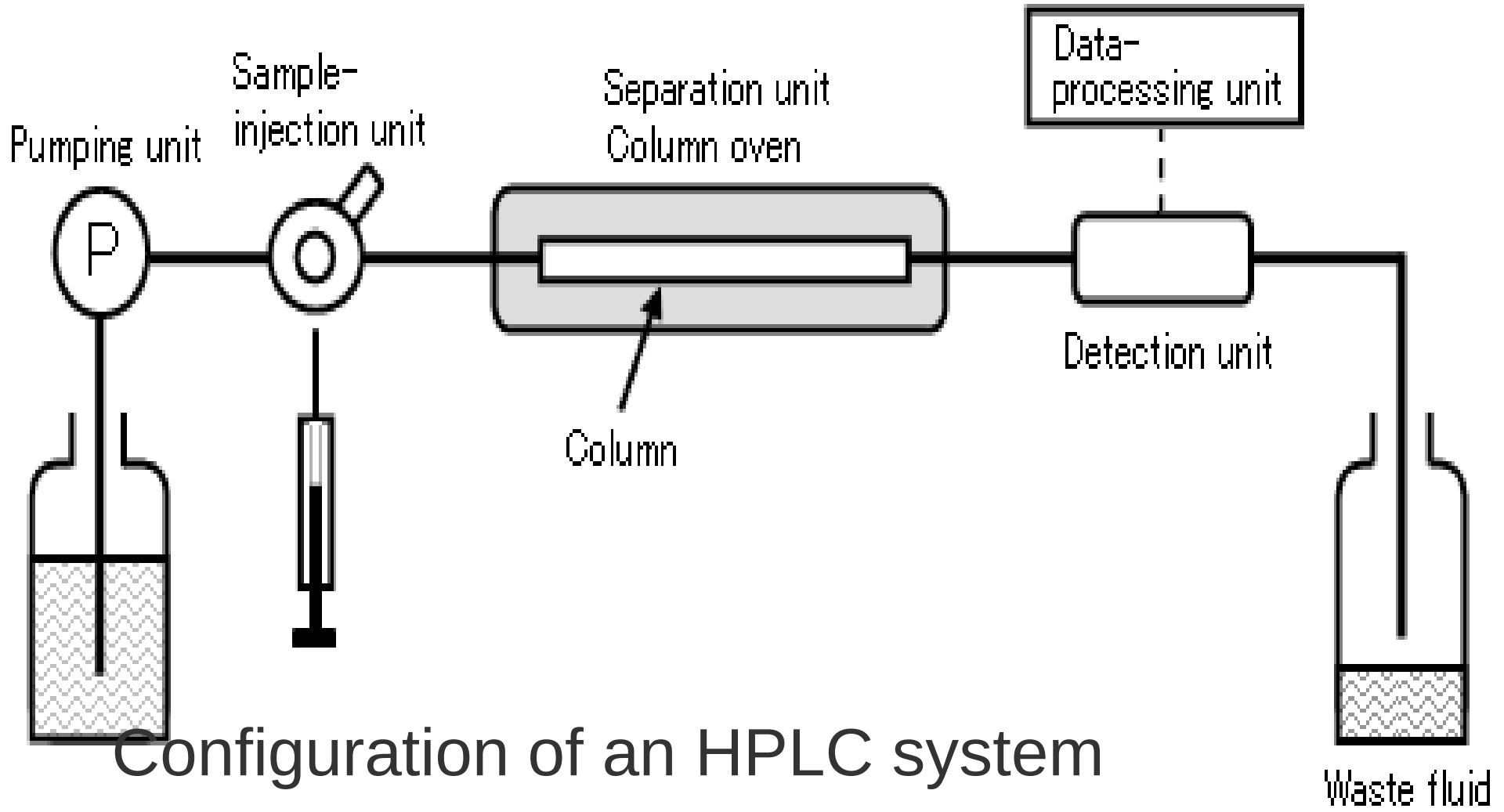
: Filler : Component A : Component B

Component A is eluted earlier into the stream of mobile phase; component B is slower to elute from the column, as it remains in the stationary phase.





An HPLC system consists of a pumping unit, sample-injection unit, separation unit, detection unit, and data-processing unit. Each of these units



Configuration of an HPLC system