Chromatography

(A Refresher Course CPDHE)

Submitted by

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Chromatography

Chromatography, firstly introduced by the Russian botanist Micharl Iswett is a method for separating the components of a mixture by differential distribution of the components of the mixture between a stationary phase and a mobile (moving) phase. Initially used for the separation of colored substances from the plants (Greek, *Chromos* meaning colored) is now the most extensive technique of separation and purification of colored/colorless organic compounds.

Which means ...
Chromatography is the physical separation of a mixture into its individual components.

But why so special…

- In any chemical or bio-processing industry, the need to separate and purify a product from a complex mixture is a necessary and important step in the production line. Chromatography is a *very* special separation process for a multitude of reasons!
- It can separate complex mixtures with great precision. Even very similar components, such as proteins that may only vary by a single amino acid, can be separated with chromatography,
- Chromatography can purify basically any soluble or volatile substance if the right adsorbent material, carrier fluid, and operating conditions are employed.
- Chromatography can be used to separate delicate products since the conditions under which it is performed are not typically severe. For these reasons, chromatography is quite well suited to a variety of uses in the field of biotechnology.
- Chromatography to separate the components of inks and dyes, such as those found in pens, markers, clothing, and even candy shells.

- Chromatography can also be used to separate the colored pigments in plants

Principle……

Different affinity of the different components to stationary phase causes the separation.

They are then flushed through the system at different rates. These differential rates of migration as the mixture moves over adsorptive materials provide separation. Repeated sorption/ desorption acts that take place during the movement of the sample over the stationary bed determine the rates. The smaller the affinity a molecule has for the stationary phase, the shorter the time spent in a column.

Types of Chromatography…

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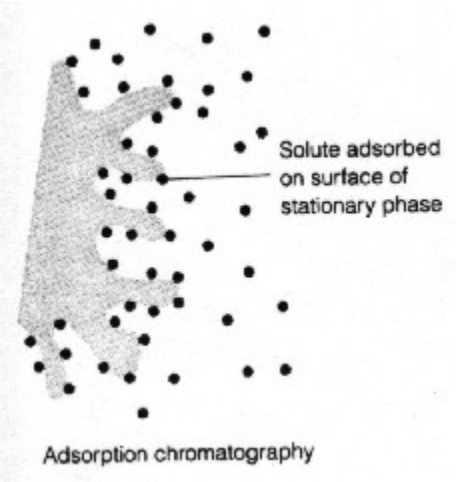
Liquid/Solid Chromatography (adsorption chromatography)

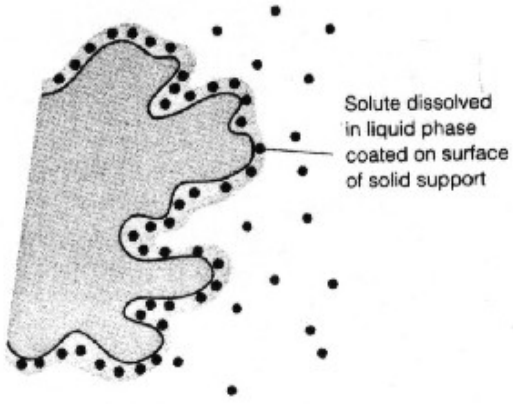
Adsorption Chromatography



Adsorption chromatography is one of the oldest types of chromatography around. It utilizes a mobile liquid or gaseous phase that is adsorbed onto the surface of a stationary solid phase. The equilibration between the mobile and stationary phase accounts for the separation of different solutes.
The separation mechanism in LSC is based on the competition of the components of the mixture sample for the active sites on an absorbent such as Silica Gel. e.g. Thin Layer Chromatography and Column chromatography.

Liquid/Liquid Chromatography (partition chromatography)

Partition Chromatography



Partition chromatography

This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase. Solute equilibrates between the mobile phase and the stationary liquid. Mobile phase may be either a liquid or a gas.

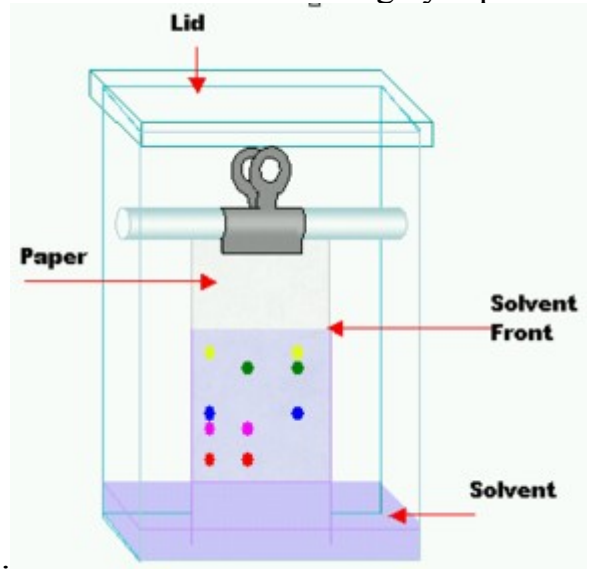
The stationary solid surface is coated with a 2nd liquid (the Stationary Phase) which is immiscible in the solvent (Mobile) phase. Partitioning of the sample between two phases delays or retains some components more than others to effect separation. E.g. Paper Chromatography.

Paper Chromatography is one of the most common types of this chromatography in which filter paper serves as a support for immobile liquid phase. Removing liquid flows between the fibers of the cellulose but these are not the stationary phase. The true stationary phase is the very thin film of liquid usually water adhering o the surface of the fibers. (Water is adsorbed on the fibers/ cellulose by strong hydrogen bonds with – OH of the cellulose). The substrate to be separated is distributed between the two liquids, stationary liquid that is held on the fibers of the paper and moving liquid in developing solvent.

It uses a strip of paper and capillary action is used to pull the solvents up through the paper to separate the solutes. A small concentrated spot of solution that contains the sample is applied to a strip of chromatography paper about 2 cm away from the base of the plate, usually using a capillary tube for maximum precision. This sample is absorbed onto the paper and may form interactions with it. Any substance that reacts or bonds with the paper cannot be measured using this technique. The paper is then dipped in to a suitable solvent, such as ethanol or water, taking care that the spot is above the surface of the solvent, and placed in a sealed container. The solvent moves up the paper by capillary action, which occurs as a result of the attraction of the

solvent molecules to the paper,also this can be explained as differential absorption of the solute components into the solvent. As the solvent rises through the paper it meets and dissolves the sample mixture, which will then travel up the paper with the solvent solute sample. Different compounds in the sample mixture travel at different rates due to differences in solubility in the solvent, and due to differences in their attraction to the fibers in the paper

This method has been largely replaced by thin layer chromatography



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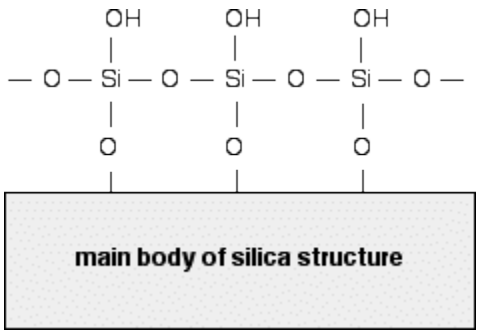
Thin-layer Chromatography

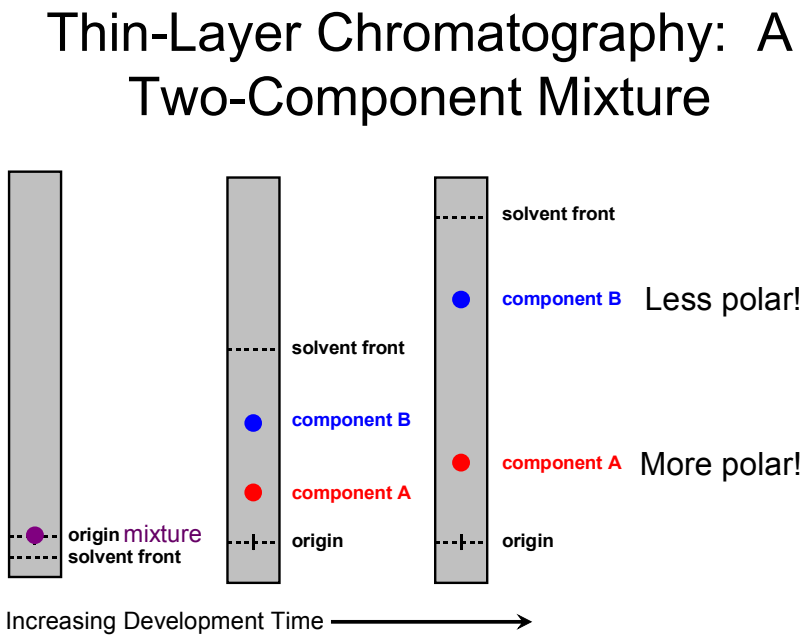
The surface of the plate consists of a very thin layer of silica gel on a plastic or Aluminium backing. Silica gel is a form of silicon dioxide (silica). At the surface of the silica gel, the silicon atoms are attached to -OH groups. The silica is very polar. This is the stationary phase.



The surface of the silica gel is very polar and, because of the -OH groups, can form hydrogen bonds with suitable compounds around it as well as Van der Waals dispersion forces and dipole-dipole attractions. The other commonly used stationary phase is alumina - aluminium oxide. The aluminium atoms on the surface of this also have -OH groups attached.
Spot the material at the origin (bottom) of the TLC plate.
Place the plate into a glass jar with a small amount of a solvent in the glass jar.
This solvent acts as the moving phase.
Remove the plate from the bottle when the solvent is close to the top of the plate.

Visualization of the spots

Non-polar compounds are less strongly attracted to the plate and spend more time in the moving phase. This compound will move faster and will appear closer to the top of the plate. Polar compounds will be more strongly attracted to the plate and will spend less time in the moving phase and appear lower on the plate. It is used to detect pesticide or insecticide residues in food. Thin-layer chromatography is also used in forensics to analyze the dye composition of fibers.



Column Chromatography

Column chromatography is frequently used by organic chemists to purify liquids (and solids.) An impure sample is loaded onto a column of adsorbent, such as silica gel or alumina. An organic solvent or a mixture of solvents (the eluent) flows down through the column. Components of the sample separate from each other by partitioning between the stationary packing material (silica or alumina) and the mobile elutant.

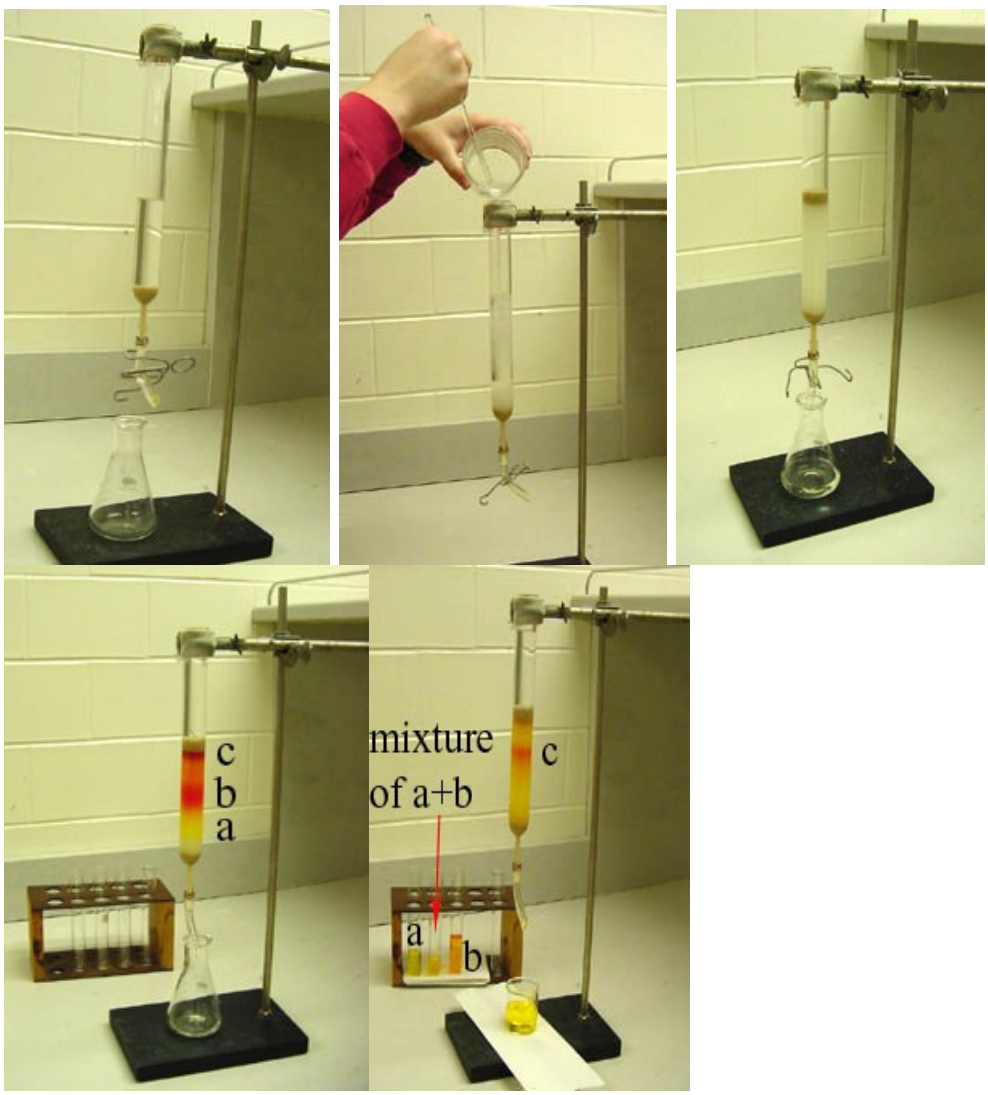
In column chromatography, the stationary phase is packed into a glass tube to form a cylinder or **column** of granules. Solvent or buffer can flow freely between the granules Stationary phase may be silica gel or ion exchange resin or a variety of other substances that may have particular affinity for amino acid molecules.

The sample is applied with care as a layer on top of the stationary phase. Then solvent is added and flows through the column. Samples molecules move while they enter the flowing solvent.

The stationary phase is polar compounds are attracted to the polar column packing by hydrogen bonding or dipole-dipole attractions The more polar component interacts more strongly with the stationary phase. Polar compounds are move slowly.
Non-polar compounds are going to come off the column first, while the polar compounds are going to come off the column last.

Usually, one starts will a less polar solvent to remove the less polar compounds, and then slowly increase the polarity of the solvent to remove the more polar compounds.

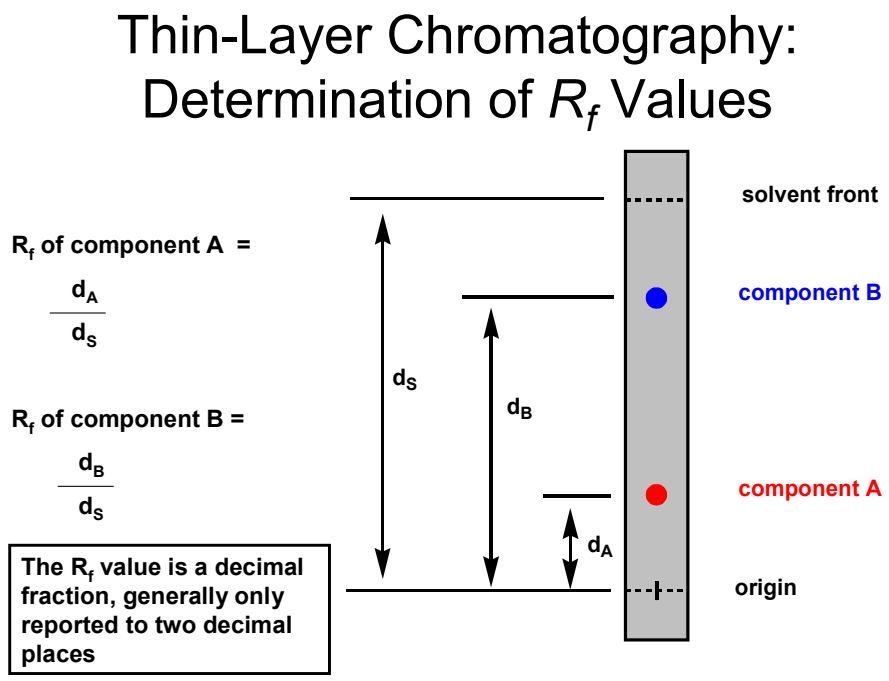
Molecules with different polarity partition to different extents, and therefore move through the column at different rates. The eluent is collected in fractions.



Analysis of the sample

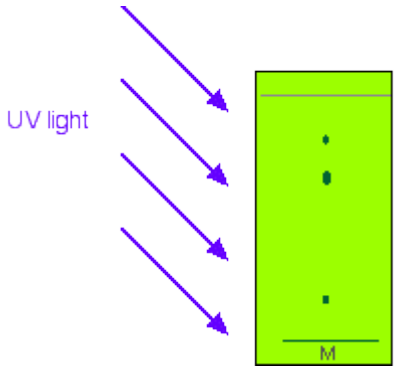
Analyze the fractions by thin-layer chromatography to determine a) if the fraction contains more than one component and b) if fractions can be combined without affecting the purity of those fractions.

Measuring R_f values



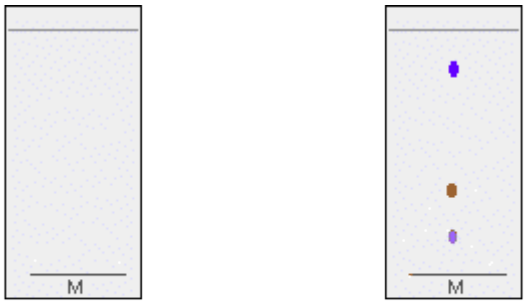
Visualization Methods

Most of the time, the spots don't show unless they are visualized. Visualization is a method that is used to render the TLC spots visible.
A visualization method can be:



- Ultraviolet light
- Iodine vapors to stain spots

Colored reagents to stain spots e.g. The chromatogram is allowed to dry and is then sprayed with a solution of ***ninhydrin***. Ninhydrin reacts with amino acids to give colored compounds, mainly brown or purple. Reagents that *selectively* stain spots while leaving others unaffected.



before spraying with ninhydrin after spraying with ninhydrin