SOLID LIQUID CHROMATOGRAPHY

Chromatography is a widely used technique to separate analyte mixtures of two or more compounds based on differences in polarity. The word “chromatography” literally means “color writing” and refers to the first time this technique was used: to separate brightly colored pigments of leaves. This separation occurs because of an intermolecular competition between the stationary phase and the mobile phase for the components of the analyte mixture.

The stationary phase, as suggested by its name, does not move; rather, chemical compounds travel through it at different rates depending on their polarity. The most common stationary phase is extremely fine particles (3.5 x 10^{-5} m to 7.5 x 10^{-5} m) of silica (SiO_{2}). Silica (SiO_{2}) is a solid with an extended structure of tetrahedral silica atoms bridged together by bent oxygen atoms (Figure 1). On the surface of the silica particles, the solid terminates in very polar silanol (Si-O-H) groups. The small particle size produces a large surface area that strongly adsorbs other molecules via intermolecular forces such as hydrogen-bonding and dipole-dipole interactions. (Adsorption is an attraction to a surface, whereas absorption means to take in a fluid internally.) The more polar an analyte compound is, the more strongly it adheres to the silica stationary phase and the more slowly it moves through the stationary phase.

Figure 1. Silica extended structure and surface.

The mobile phase consists of liquid solvents (eluents) of different polarities that move and separate analyte compounds through the stationary phase. The more polar the mobile phase, the faster all compounds run through the stationary phase. Eluents are typically used in order of polarity: nonpolar eluents are used first, to move the least polar analyte compounds, then eluents
of increasing polarity are used until the most polar analyte compound has moved through the stationary phase. Here are some solvents commonly used in chromatography:

![Diagram of solvents](image)

Very polar liquids capable of hydrogen bonding (such as water and methanol) are not shown. These liquids are rarely used as eluents, their polarity is similar enough to the stationary phase to dissolve it.

The separation of the different analyte compounds results from the competing intermolecular forces of the stationary and mobile phases for the analyte compounds. (The following concepts are extension of the “like dissolves like” adage.) The silica stationary phase is very polar, so the more polar analyte compounds will adsorb more strongly to the silica and are more likely to be retained by stationary phase and not move. In fact, if the analyte is too polar it will not move from its the origin (its initial spot). The mobile eluent phase can vary from moderately polar to nonpolar depending on the identity of the organic solvent or mixture of organic solvents. Nonpolar eluents tend to move only nonpolar compounds of the analyte mixture a significant distance. Choosing a more polar eluent results in the movement of more of the compounds of the analyte mixture. The reason: a more polar eluent is a better competitor for the analyte molecules versus the polar silica stationary phase.

The two main types of liquid-solid chromatography are thin layer chromatography (TLC) and column chromatography. TLC is used to determine what stationary and mobile phases should be used in column chromatography, to check reaction progress, and to determine product purity. Column chromatography is used to separate reaction mixtures to obtain pure products.
Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is used frequently to visualize components of a mixture. The most common TLC plate is typically a rectangular piece of glass or plastic (2.5 cm x 7.5 cm) coated with silica powder. The silica is the stationary phase because it remains adhered to the glass plate and does not move during the chromatography process.

The analyte mixture is typically delivered by capillary (a small glass tube with a very small inner diameter (0.5 mm)) to the bottom of the silica plate in a very concentrated (if not saturated) solution.

![Figure 2. TLC plate.](image)

The analyte solution is added in one spot, dropwise to a pencil line drawn about 1 cm from the bottom of the TLC plate. (This spotting procedure is similar to putting a straw into soda, placing a fingertip snuggly over the top end of the straw, and then pulling the straw out of the soda. The soda is then released by removing the finger.) Add drops gently, do not to disturb the silica when spotting the plate.
Once all solvent has evaporated from the analyte “spot”, the TLC plate is placed into a developing chamber (Figure 3). To create a TLC developing chamber a small glass jar with a lid just large enough to fit the TLC plate is needed. An eluent (an organic solvent or mixture of organic solvents) is added to a height of ~0.5 cm. The level must be below the origin line created on the TLC plate. A piece of paper towel (or filter paper) is then fitted around approximately half the vertical walls of the jar with the bottom edges immersed in the eluent. *Capillary action* will draw the eluent throughout the paper. This wetted paper insures the vapor of the eluent is uniform throughout the developing jar once its lid is secured. When the TLC plate is placed in the jar (without touching the filter paper), the eluent serves as the mobile phase – moving up the silica on the plate (also by capillary action). The different compounds in the analyte mixture will be carried in the eluent (up the plate) at different rates depending on polarity. The TLC plate must be removed before the eluent moves beyond the top of the silica powder on the plate. The line which the eluent moves to is called the *solvent front*. The solvent front must be marked immediately after removing the plate from the developing jar.

TLC results can be quantified by calculating the retardation factor (R_f). An R_f value is the ratio of the distance the analyte traveled versus distance the eluent travelled.

$$R_f = \frac{\text{distance from origin to analyte}}{\text{distance from origin to solvent front}} = \frac{x_a}{x_t}$$

The labeling of x_a and x_t are shown in Figure 4. The distance an analyte travels is dependent on its structure as well as the identity of the stationary and mobile phases. Furthermore, as long as
the stationary and mobile phases are the same, the $R_f$ ratio for an analyte travels remains the same.

![Diagram](image)

**Figure 4.** Developed TLC plate.

**Column Chromatography**

Column chromatography is often employed to separate relatively large quantities of an analyte mixture. This technique is utilized many times at the end of a synthesis to separate a desired product from an unwanted byproduct.

To create a column a narrow glass cylinder is needed. Special cylinders are made with a stopcock to control flow at the bottom (similar to a buret), but a simple glass pipet (flow will be controlled by using a finger to seal the top of the pipet) can also be used. A cotton plug is stuffed in the bottom of the cylinder to keep the next components inside the cylinder. A thin layer of sand is added to create a flat surface horizontal with the bench top. Then silica gel is added as dry powder or as a slurry (silica and a small amount of eluent are poured into the column while being stirred). Once the silica settles, a thin layer of sand is then added to the top of the silica. The sand is heavier than the silica so it protects the top of the silica column from disturbances that occur when analyte or eluent is added. Analyte is then added – as a solid if the silica was added dry, or, as a very concentrated solution if the silica was added as a slurry.
Figure 5. Column in a Disposable Glass Pipet

The first (least polar) eluent is then added gently to the column. From this point on, the eluent level should never go below the top sand layer. If it does, the “bands” containing the separated analyte compounds will streak, leading to incomplete separation. As the first purified analyte compound begins to come off the column place a clean beaker beneath the tip of the column to collect the band. Then, if needed, change to a more polar eluent to get the next analyte compound off of the column and repeat the collection procedure. The solid analyte compounds can be collected from solution by evaporating the solvent or adding the analyte solution to another liquid to initiate precipitation.