**Chromatography**

This page covers topics common to the different types of chromatography. Links to the separate pages for the sub-categories of chromatography are below:

* [TLC](http://orgchem.colorado.edu/hndbksupport/TLC/TLC.html)
* [Column Chromatography](http://orgchem.colorado.edu/hndbksupport/colchrom/colchrom.html)
* [GC](http://orgchem.colorado.edu/hndbksupport/GC/GC.html)

**Overview of common undergraduate chromatography techniques.**

Three types of chromatography are routinely used in the organic chemistry teaching labs:

Column Chromatography

Thin Layer Chromatography (TLC)

Gas Chromatography (GC)

In these (and all types of) chromatographies, a mixture is separated by distributing the components between a stationary phase and a mobile phase. The mixture is first placed on the stationary phase (a solid or a liquid) and then the mobile phase (a gas or a liquid) is allowed to pass through the system.

**Column chromatography:** The **stationary phase** is a powdered adsorbent which is placed in a vertical glass column. The mixture to be analysed is loaded on top of this column. The **mobile phase** is a solvent poured on top of the loaded column. The solvent flows down the column, causing the components of the mixture to distribute between the powdered adsorbent and the solvent, thus (hopefully) separating the components of the mixture so that as the solvent flows out of the bottom of the column, some components elute with early collections and other components elute with late fractions.

**Thin Layer Chromatorgraphy:** The **stationary phase** is a powdered adorbent which is fixed to a aluminum, glass, or plastic plate. The mixture to be analyzed is loaded near the bottom of the plate. The plate is placed in a reservoir of solvent so that only the bottom of the plate is submerged. This solvent is the **mobile phase**; it moves up the plate causing the components of the mixture to distribute between the adsorbent on the plate and the moving solvent, thus separating the components of the mixture so that the components are separated into separate "spots" appearing from the bottom to the top of the plate.

**Gas Chromatography:** The **stationary phase** is a high-boiling liquid. (Think of it as a viscous oil, or waxy substance.) This high-boiliing liquid is packed into a long, narrow glass or metal column. The mixture to be analyzed is loaded by syringe into the beginning of this column. The **mobile phase** is an inert gas which continuously flows through the column. The components of the mixture distribute between the stationary high-boiling liquid (these components are either condensed or absorbed on the high-boiling liquid) and mobile gas (vapor) phase moving through the column. The gaseous mixture flows through a detector at the end of the column and if it has been successfully separated, the components show as different 'blips' or peaks on a recorder.

In all three of these chromatographies, separation of chemical components of a mixture is achieved due to the selective interaction of chemicals with both the stationary and mobile phases:

In **Gas Chromatography**, the determining factor in how fast a component travels is usually (but not always) the boiling point of the compound. (If a polar high-boiling liquid adsorbent is used in the GC column, the *polarity* of the components determines the elution order.)

In **Column** and **Thin Layer chromatographies**, the stationary phase (the adsorbent: silica gel or alumina) is polar, and the polarities of both the component of the mixture and the solvent used as the mobile phase are the determining factors in how fast the compound travels.

Column chromatography is used to separate and purify components of a mixture. TLC and GC are usually (but not always!) used only to analyze mixtures: to determine the number of components and to see if a desired component is present. TLC is often used to determine the "ideal system" for a column chromatography procedure (as explained in the following paragraphs).

**Determining solvent systems for TLC and Column Chromatography**

When you need to determine the best system (a "system" means the eluting solvent, itself often a mixture of solvents) to develop a TLC plate or chromatography column loaded with an unknown mixture, vary the polarity of the solvent in several trial runs -- a process of trial and error. Carefully observe and record the results of the chromatography in each solvent system. You will find that as you increase the polarity of the solvent system, all the components of the mixture move faster (and visa versa with lowering the polarity). The ideal solvent system is simply: the system that separates the components.

TLC elution patterns usually extrapolate to column chromatography elution patterns. Since TLC is a much faster procedure than column chromatography, TLC is often used to determine the best solvent system for column chromatography. For instance, in determining the solvent system for a flash chromatography procedure, the ideal system is the one that moves the desired component of the mixture to a TLC Rf of 0.25-0.35 and will separate this component from its nearest neighbor by difference in TLC Rf values of at least 0.20. Therefore a mixture is analyzed by TLC to determine the ideal solvent(s) for a flash chromatography procedure.

Beginners often do not know where to start: What solvents should they pull off the shelf to use to elute a TLC plate? Because of toxicity, cost, and flammability concerns, the common solvents are hexanes (or petroleum ethers, ligroin) and ethyl acetate (an ester). Diethyl ether can be used, but it is very flammable and volatile. Alcohols (methanol, ethanol) can be used. Acetic acid (a carboxylic acid) can be used, usually as a small percentage component of the system, since it is corrosive, non-volatile, very polar, and has irritating vapors. Acetone (a ketone) can be used. Methylene chloride (halogenated hydrocarbon) is a good solvent, but it is toxic and should be avoided whenever possible. If two solvents are equal in performance and toxicity, the more volatile solvent is preferred in column chromatography because it will be easier to remove from the desired compound after isolation from a column chromatography procedure.

Ask the lab instructor what solvents are available and advisable. Then, mix a non-polar solvent (hexanes, a mixture of 6-carbon alkanes) with a polar solvent (ethyl acetate or acetone) in varying percent combinations to make solvent systems of greater and lesser polarity. The charts below should help you in your solvent selection. Download the pdf file (linked below the charts) for a printable version to keep for ready reference.

**Download:** Expected eluting order of organic classes and eluting power of solvents, [PDF file](http://orgchem.colorado.edu/hndbksupport/images/polarity.pdf).

**References**

The above discussion is intended to be informal, brief, and not all-inclusive. Please read the following references for detailed information on chromatography:

*Handbook for Organic Chemistry Laboratory*, CU Chemistry Department (available only at the CU Bookstore)

*Organic Laboratory Techniques*, 3rd Ed., Fessenden, Fessenden, Feist, Brooks/Cole, 2000.

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