Thin-layer Chromatography

Chromatography represents the most versatile separation technique readily available to the chemist. Conceptually, the technique is very simple—there are only two components: a stationary phase (usually silica or cellulose) and a mobile phase (usually a solvent system). Any two compounds usually have different partitioning characteristics between the stationary and mobile phases. Since the mobile phase is moving (thus the name), then the more time a compound spends in that phase, the farther it will travel.

Chromatographic techniques fall into one of two categories: analytical and preparative. Analytical techniques are often used to follow the course of reactions and determine purity of products. These methods include gas chromatography (GC), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC). Sample sizes for these procedures are usually quite small, from microgram to milligram quantities.

Preparative methods are used to purify and isolate compounds for characterization or further use. The most common techniques in this category are preparative HPLC, preparative TLC, and column chromatography. Collectively, these methods fall under the category of LC (liquid chromatography), meaning the mobile phase is some liquid solvent system. Occasionally, this is a single solvent, but more often than not it is a binary mixture of solvents with different polarities. The advantage of the latter is that the bulk polarity can be modulated by varying the ratio of the two solvents.

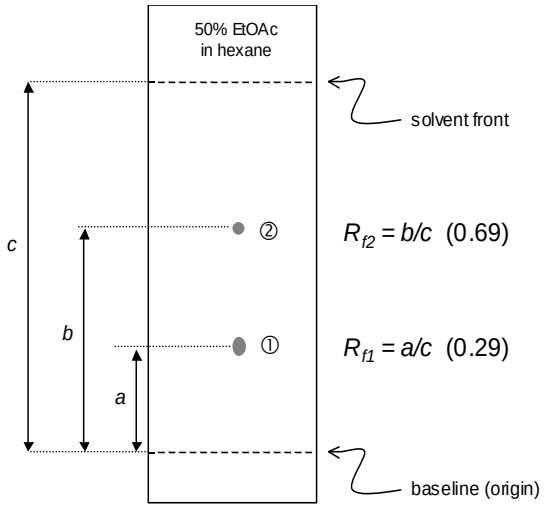

Figure 1. A typical TLC plate.

For example, consider a typical TLC plate (Figure 1) developed in a 1:1 mixture of ethyl acetate and hexane, which exhibits two well-separated components. The spots can be characterized by their R_f value, which is defined as the distance traveled from the origin divided by the distance traveled by the mobile phase. Generally speaking, the slower moving component (R_f 0.29) is either larger, more polar, or both. If we wanted a larger R_f value, we could boost the solvent polarity by increasing the proportion of ethyl acetate in the mobile phase. Conversely, more hexane would result in lower-running spots.

Sometimes one or more of the components interact so strongly with the silica gel that departure from the origin comes only with great difficulty. One answer to this problem is to derivatize the silica with non-polar substituents, such as long-chain aliphatic residues. This creates a situation known as a reverse phase system. In normal phase chromatography, the stationary phase (i.e., silica) is more polar than the mobile phase; in reverse phase, the stationary phase is less polar than the mobile phase. The derivatized silica is not only much less polar itself, but it also allows for the use of very polar solvents, such as water and methanol. The reversed phase results in some counterintuitive outcomes. For example, more polar components actually elute faster, and a more polar solvent system results in an increased retention time (i.e., lower R_f value). These parameters are summarized in Table 1. In this laboratory, you will be using both types: the TLC plates are normal phase and the HPLC column is reverse-phase.

parameter	normal phase	reverse phase
typical stationary phase	underivatized silica gel	C8-hydrocarbon derivatized silica
representative mobile phase	ethyl acetate/hexane mixture	acetonitrile / water mixture
more polar components	have lower R_f values	have hire R_f values
increasing solvent polarity	increases R_f values	decreases R_f values

Table 1. Normal vs. reverse-phase chromatography.

Check out this video about how to set up a TLC plate:

http://www.youtube.com/watch?v=EUn2skAAjHk