3.4: Chromatography

"Chroma" refers to color and "graphy" refers to writing.

Chromatography is a method by which a mixture is separated by distributing its components between two phases. The stationary phase remains fixed in place while the mobile phase carries the components of the mixture through the medium being used. The stationary phase acts as a constraint on many of the components in a mixture, slowing them down to move slower than the mobile phase. The movement of the components in the mobile phase is controlled by the significance of their interactions with the mobile and/or stationary phases. Because of the differences in factors such as the solubility of certain components in the mobile phase and the strength of their affinities for the stationary phase, some components will move faster than others, thus facilitating the separation of the components within that mixture.

Theory

The distribution of a solute between the mobile and stationary phases in chromatography is described by κ, the partition coefficient, defined by:

$$\kappa = \frac{C_s}{C_m}$$

where Cs is the concentration of solute in the stationary phase and Cm is the concentration of the solute in the mobile phase. The mobile phase serves to carry the sample molecules through the chromatographic column. During the sample molecules transportation through the column, each analyte is retained according to that compound's characteristic affinity for the stationary phase. The time that passes between the sample injection and peak maximum is called the **retention time**. The area underneath each peak is proportional to the amount of co responding analyte in solution.

Retention Time

The retention time, tR, is given in seconds by:

$$t_R = t_S + t_M$$

where tS is the time the analyte spends in the stationary phase and tM is the time spent in the mobile phase. tM is often referred to as the dead, or void time, as all components spend tM in the mobile phase.

An example you can do at home involves a coffee filter (stationary phase) and water (mobile phase) in the separation and analysis of the dye in a water soluble marker pen (the mixture of compounds). If you draw a line with a purple marker on a strip of the filter paper, and place the bottom in a dish of water, the water will wick up the paper, and the differential affinity of the dye inks for the solid support (cellulose) results in differential migration. In the example below, the red ink has some affinity for the paper, but the blue ink does not (and migrates with the front edge of the mobile phase)

Figure 3.4.1: *Chromatography of purple pen*

Chromatography in biochemistry typically utilizes not paper, but beads of polysaccharide (often chemically derivatized) packed into a column, as the solid support. The solid support is often called the chromatography "resin". Some common types of

chromatographic resins include:

- Ion exchange
- Affinity
- Hydrophobic
- Gel filtration
