CHROMATOGRAPHY

Chromatography is a technique used to separate mixtures based on adsorption differences among a mixture's components. There are several methods of chromatography which range from simple and inexpensive to sophisticated and expensive. The simplest methods include paper chromatography, thin layer chromatography and column chromatography. These methods use relatively inexpensive materials but provide only qualitative results. These methods will separate liquid samples and must have visible components or be able to be detected by staining or viewing with UV radiation. The more sophisticated methods include gas chromatography and high performance liquid chromatography (or high pressure liquid chromatography). These not only provide qualitative results but also allow for quantitative analysis. Using these methods, samples are not limited to visible separations. Gas chromatography (GC) will separate mixtures of gases as well as mixtures of volatile liquids. High performance liquid chromatography (HPLC) will separate liquid samples, including colorless components. These latter two methods use a detection system other than the naked eye and allow for quantitative analysis.

The method of chromatography chosen depends upon the desired results. Regardless of the method of chromatography chosen, the principle remains the same. All methods employ two phases: a stationary phase and a mobile phase. Although the stationary and mobile phases vary from one method to another, their purpose remains the same. The mobile phase carries the sample over the stationary phase, which is where the mixture is separated. Separation occurs due to the interaction of the components of the mixture with the stationary and mobile phases.

If one of the components has a greater affinity for the stationary phase, it will adhere longer to this phase and, hence, will separate from the other components in the mixture. Using paper and thin layer chromatography, this affinity will be detected by the component remaining on the stationary phase near the application of the sample. In column, gas, and high performance liquid chromatography, this component will be removed from the stationary phase last. The reverse is true if the component has a greater affinity for the mobile phase.

If the components have too great of an affinity for the stationary phase or no affinity for the mobile phase, the components will remain at the point of application and will not be separated. In contrast, if the components have too great of an affinity for the mobile phase or no affinity for the stationary phase, they will continue to travel with the mobile phase and will not be separated. The stationary and mobile phases are chosen to be able to separate all of the components. In order to do this, the polarity of the components are considered. One phase is more polar than the other phase, and these are adjusted to give the desired separation. More polar components will adhere more strongly to the polar phase than to the less polar or nonpolar phase. Remember, "like dissolves like".

Paper chromatography use a slightly porous paper (often filter paper) as the stationary phase, which is placed in a liquid mobile phase. The mobile phase is carried over the stationary phase by capillary action. Thin layer chromatography use the same method except the stationary phase is a thin layer of cellulose or silica gel coated onto a plastic or glass plate. In column, gas, and high performance liquid chromatography, the stationary phase is a column packed with a finely divided material, such as alumina, octodecylsilane, etc. In these methods, not only does adsorption play a role in the separation process, but the size of the molecules in the mixture does as well. The smaller molecules will be able to travel through the column quicker than the larger more bulky molecules. The mobile phase for both column and high performance liquid chromatography is a liquid. The liquid mobile phase in column chromatography is carried through the column by the force of gravity, while in high performance liquid chromatography, the mobile phase is pumped through the column using high pressures. Gas chromatography uses gas as the mobile phase. The gas is forced through the column by pressure.

Each component of a mixture can be identified once it is separated by determining the retention time or Rf value of the components. If the sample is removed from the stationary phase as in column, gas, and high performance liquid chromatography, the retention time is used. This is a measure of how long the component was retained on the column before eluting with the mobile phase. In paper and thin layer chromatography, the components are identified by the use of calculated Rf values. The Rf value is calculated using the following equation:

$$Rf = \frac{\text{distance from the point of application of the sample to one of the components}}{\text{distance from the point of application of the sample to the solvent front}}$$

The solvent front is the point where the mobile phase traveled before the stationary phase was removed. Comparing the retention times or Rf values of known compounds analyzed under the same conditions as the mixture will allow for identification of the unknown components in the mixture.

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