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Chromatography 1	عنوان المحاضرة باللغة الانجليزية
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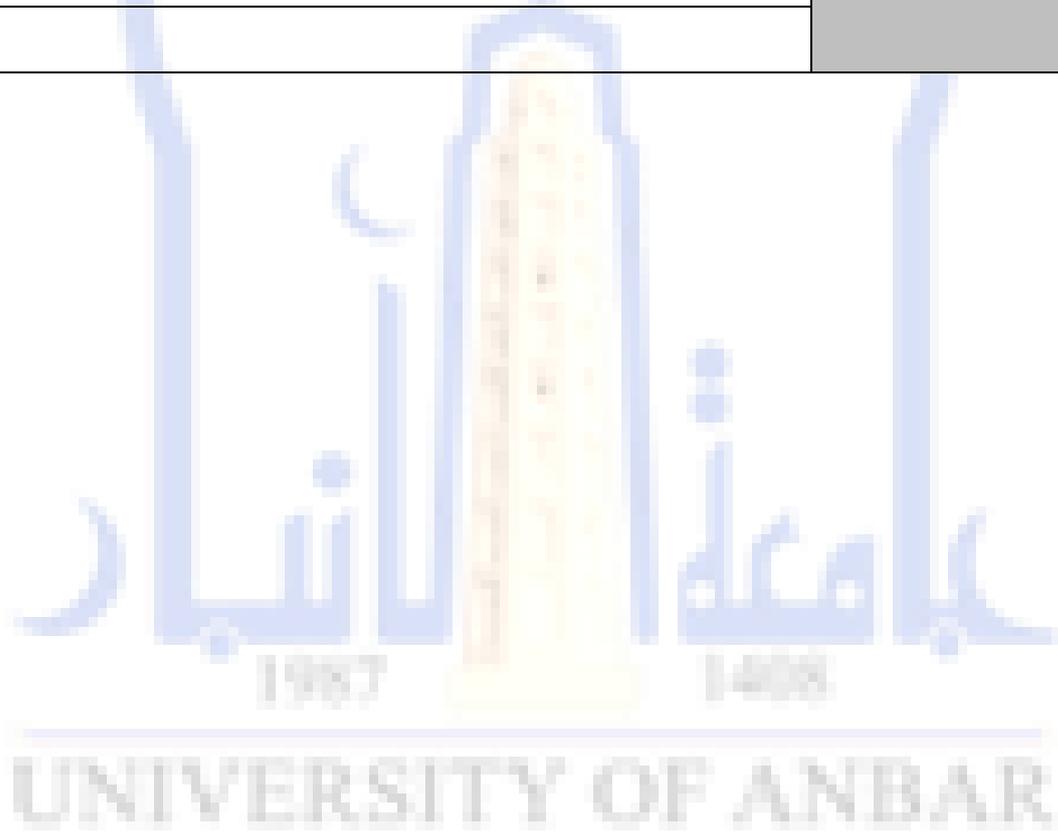
Chromatography

Introduction Chromatography is a physicochemical method for separation of complex mixtures was discovered at the very beginning of the twentieth century by Russian Italian botanist M. S. Tswett. In his paper "On the new form of adsorption phenomena and its application in biochemical analysis" presented on March 21, 1903 at the regular meeting of the biology section of the Warsaw Society of Natural Sciences, Tswett gave a very detailed description of the newly discovered phenomena of adsorption-based separation of complex mixtures, which he later called "chromatography" as a transliteration from Greek "color writing". Serendipitously, the meaning of the Russian word "tswett" means color. Although in all his publications Tswett mentioned that the origin of the name for his new method was based on the colorful picture of his first separation of plant pigments, he involuntarily incorporated his own name in the name of the method he invented. The chromatographic method was not appreciated among the scientists at the time of the discovery, as well as after almost 10 years when L. S. Palmer in the United States and C. Dhere in Europe independently published the description of a similar separation process.

Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography.

However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography.

However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated. High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of online detectors.

In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge.

By the 2000 very fast development was undertaken in the area of column material with small particle size technology and other specialized columns. The dimensions of the typical HPLC column are 100-300 mm in length with an internal diameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns, ranges from 3 μm to 200 μm. In this decade, sub 2-micron particle size technology (column material packed with silica particles of < 2μm size) with modified or improved HPLC instrumentation becomes

popular with different instrument brand name like UPLC (Ultra Performance Liquid Chromatography) of Waters and RRLC (Rapid Resolution Liquid Chromatography) of Agilent.

General Description of Chromatography

The term chromatography is difficult to define rigorously because the name has been applied to several systems and techniques. All these methods, however, have in common the use of a stationary phase and a mobile phase. Components of a mixture are carried through the stationary phase by the flow of a mobile phase, and separations are based on differences in migration rates among the mobile-phase components.

Classification of Chromatographic Methods

Chromatographic methods are of two basic types. In column chromatography, the stationary phase is held in a narrow tube, and the mobile phase is forced through the tube under pressure or by gravity. In planar chromatography, the stationary phase is supported on a flat plate or in the pores of a paper, and the mobile phase moves through the stationary phase by capillary action or under the influence of gravity. We consider here only column chromatography. As shown in the first column of Table 7-1, chromatographic methods fall into three categories based on the nature of the mobile phase: liquid, gas, and supercritical fluid. The second column of the table reveals that there are five types of liquid chromatography and two types of gas chromatography that differ in the nature of the stationary phase and the types of equilibria between phases.

Elution in Column Chromatography

Figure 7-2a shows how two components A and B of a sample are resolved on a packed column by elution. The column consists of narrow-bore tubing that is packed with a finely divided inert solid that holds the stationary phase on its surface.

TABLE 7-1

Classification of Column Chromatographic Methods			
General Classification	**Specific Method**	**Stationary Phase**	**Type of Equilibrium**
1. Gas chromatography (GC)	**a.** Gas-liquid (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	b. Gas-solid		
2. Liquid Chromatography (LC)	**a.** Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	b. Liquid-solid, or adsorption		
	c. Ion exchange	Ion-exchange resin	Ion exchange
	d. Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	e. Affinity	Group specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
3. Supercritical fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

The mobile phase occupies the open space between the particles of the packing. Initially, a solution of the sample containing a mixture of A and B in the mobile phase is introduced at the head of the column as a narrow plug as shown in Figure 7-2a at time t_0 . The two components distribute themselves between the mobile phase and the stationary phase. Elution then occurs by forcing the sample components through the column by continuously adding fresh mobile phase.

With the first introduction of fresh mobile phase, the eluent, the portion of the sample contained in the mobile phase moves down the column, where further partitioning between the mobile phase and the stationary phase occurs (time t1). Partitioning between the fresh mobile phase and the stationary phase takes place simultaneously at the site of the original sample.

Further additions of solvent carry solute molecules down the column in a continuous series of transfers between the two phases.

Because solute movement can occur only in the mobile phase, the average rate at which a solute migrates depends on the fraction of time it spends in that phase. This fraction is small for solutes that are strongly retained by the stationary phase (component B in Figure 7-2, for example) and large where retention in the mobile phase is more likely (component A). Ideally, the resulting differences in rates cause the components in a mixture to separate into bands, or zones, along the length of the column (see Figure 7-3). Isolation of the separated species is then accomplished by passing a sufficient quantity of mobile phase through the column to cause the individual bands to pass out the end (to be eluted from the column), where they can be collected or detected (times t3 and t4 in Figure 7-2a).

Chromatograms

If a detector that responds to solute concentration is placed at the end of the column during elution and its signal is plotted as a function of time (or of volume of added mobile phase), a series of peaks is obtained, as shown in Figure 7-2b. Such a plot, called a chromatogram, is useful for both qualitative and quantitative analysis. positions of the peak maxima on the time axis can be used to identify the components of the sample. The peak areas provide a quantitative measure of the amount of each species.

Methods for Improving Column Performance

Figure 7-3 shows concentration profiles for the bands containing solutes A and B on the column in Figure 7-2a at time t1 and at a later time t2. Because B is more strongly retained by the stationary phase than is A, B lags during the migration. We see that the distance between the two increases as they move down the column. At the same time, however, broadening of both bands takes place, lowering the efficiency of the column as a separating device. While band broadening is inevitable, conditions can often be found where it occurs more slowly than band separation. Thus, as shown in Figure 7-3, a clean separation of species is possible provided the column is sufficiently long.

Several chemical and physical variables influence the rates of band separation and band broadening. As a result, improved separations can often be realized by the control of variables that either (1) increase the rate of band separation or (2) decrease the rate of band spreading.

The variables that influence the relative rates at which solutes migrate through a stationary phase are described in the next section. Following this discussion, we turn to those factors that play a part in broadening zones.

Migration Rates of Solutes

The effectiveness of a chromatographic column in separating two solutes depends in part on the relative rates at which the two species are eluted. These rates in turn are determined by the ratios of the solute concentrations in each of the two phases.

Distribution Constants

All chromatographic separations are based on differences in the extent to which solutes are distributed between the mobile and the stationary phase. For the solute species A, the equilibrium is described by the equation

The equilibrium constant K_d for this reaction is called a distribution constant, which is defined as

$$Kd = \frac{(aA)S}{(aA)M}$$

where (aA)S is the activity of solute A in the stationary phase and (aA)M is the activity in the mobile phase. We often substitute CS, the molar analytical concentrations of the solute

in the stationary phase, for (aA)S and CM, the molar analytical concentration in the mobile phase, for (aA)M. Hence, we often write equation 7-2 as

$$Kd = {^{CS}}/{_{CM}}$$

Ideally, the distribution constant is constant over a wide range of solute concentrations, that is, CS is directly proportional to C_M.

Retention Times

Figure 7-5 is a simple chromatogram of a two-component mixture. The small peak on the left is for a species that is not retained by the stationary phase. The time t_M after sample injection for this peak to appear is sometimes called the dead or void time. The dead time provides a measure of the average rate of migration of the mobile phase and is an important parameter in identifying analyte peaks. All components spend at least time t_M in the mobile phase.

To aid in measuring tM, an unretained species can be added if one is not already present in the sample or the mobile phase. The larger peak on the right in Figure 7-5 is that of an analyte species. The time required for this zone to reach the detector after sample injection is calledthe retention time and is given the symbol t_R. The analyte has been retained because it spends a time tS in the stationary phase. The retention time is then

$$tR = tS + tM$$

The average linear rate of solute migration, $\bar{v}$ (usually cm/s), is

$$v = {^{L}}/{_{tR}}$$

where L is the length of the column packing. Similarly, the average linear velocity, u, of the mobile phase molecules is

$$u = {^{L}}/{_{tM}}$$

Migration Rates and Distribution Constants To relate the rate of migration of a solute to its distribution constant, we express the rate as a fraction of the velocity of the mobile phase:

$\bar{v} = u \times$ fraction of time solute spends in mobile phase

After the derivation of the equation and arrange them become:

$$v = u \times \frac{1}{1 + KdVs/Vm}$$

The two volumes can be estimated from the method by which the column is prepared.