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The Retention Factor, k

The retention factor is an important experimental parameter that is widely used to compare the migration rates of solutes on columns. For solute A, the retention factor kA is defined as

$$kA = \frac{tR - tM}{tM} = \frac{tS}{tM}$$

As shown in Figure 7-5, tR and tM are easily obtained from a chromatogram. A retention factor much less than unity means that the solute emerges from the column at a time near that of the void time. When retention factors are larger than perhaps 20 to 30, elution times become inordinately long. Ideally, separations are performed under conditions in which the retention factors for the solutes of interest in a mixture lie in the range between 1 and 5.

In gas chromatography, retention factors can be varied by changing the temperature and the column packing.

Band Broadening and Column Efficiency

The amount of band broadening that occurs as a solute pass through a chromatographic column strongly affects the column efficiency. Before defining column efficiency in more quantitative terms, let us examine the reasons that bands become broader as they move down a column.

Rate Theory of Chromatography

The rate theory of chromatography describes the shapes and breadths of elution bands in quantitative terms based on a random-walk mechanism for the migration of molecules through a column. We can give a qualitative picture of why bands broaden and what variables improve column efficiency.

Consider a single solute molecule as it undergoes many thousands of transfers between the stationary and the mobile phases during elution. Residence time in either phase is highly irregular. Transfer from one phase to the other requires energy, and the molecule must acquire this energy from its surroundings. Therefore, the residence time in a given phase may be very short after some transfers and relatively long after others. Recall that movement through the column can occur only while the molecule is in the mobile phase. As a result, certain particles travel rapidly by virtue of their accidental inclusion in the mobile phase for a majority of the time while others lag because they happen to be incorporated in the stationary phase for a greater-than-average length of time. The result of these random individual processes is a symmetric spread of velocities around the mean value, which represents the behavior of the average analyte molecule.

As shown in Figure 7-6, some chromatographic peaks are non-ideal and exhibit tailing or fronting. In the former case, the tail of the peak, appearing to the right on the chromatogram, is drawn out while the front is steepened. With fronting, the reverse is the case. A common cause of tailing and fronting is a distribution constant that varies with concentration. Fronting also arises when the amount of sample introduced onto a column is

too large. Distortions of this kind are undesirable because they lead to poorer separations and less reproducible elution times. In the discussion that follows, tailing and fronting are assumed to be minimal.

A Quantitative Description of Column Efficiency

Two related terms are widely used as quantitative measures of chromatographic column efficiency: (1) plate height, H, and (2) plate count or number of theoretical plates, N. The two are related by the equation

$$N = {}^{L}/_{H}$$

where L is the length (usually in centimeters) of the column packing. The efficiency of chromatographic columns increases as the plate count N becomes greater and as the plate height H becomes smaller. Enormous differences in efficiencies are encountered in columns as a result of differences in column type and in mobile and stationary phases. Efficiencies in terms of plate numbers can vary from a few hundred to several hundred thousand, while plate heights ranging from a few tenths to one thousandth of a centimeter or smaller are not uncommon.

Determining the Number of Plates in a Column

The number of theoretical plates, N, and the plate height, H, are widely used in the literature and by instrument manufacturers as measures of column performance. Figure 7-7 shows how N can be determined from a chromatogram. In the figure, the retention time of a peak t_R and the width of the peak at its base W (in units of time) are measured. Where the number of plates can be calculated in relationship .

$$N = 16\left(\frac{tR}{W}\right)^2$$

Many chromatographic data systems report the width at half-height, W1/2, in which case

N = 5.54(tR/W1/2)2.

$$N = 5.54(tR/W_{1/2})^2$$

Variables Affecting column Efficiency

Band broadening reflects a loss of column efficiency. The slower the rate of mass transfer processes occurring while a solute migrates through a column, the broader the band at the column exit. Some of the variables that affect mass-transfer rates are controllable and can be exploited to improve separations.

Effect of Mobile-Phase Flow Rate The extent of band broadening depends on the length of time the mobile phase is in contact with the stationary phase, which in turn depends on the flow rate of the mobile phase. For this reason, efficiency studies generally have been carried out by determining H as a function of mobile phase velocity. The plots for liquid chromatography and for gas chromatography are typical of the data obtained from such studies. While both show a minimum in H (or a maximum in efficiency) at low linear flow

rates, the minimum for liquid chromatography usually occurs at flow rates that are well below those for gas chromatography. Often these flow rates are so low that the minimum H is not observed for liquid chromatography under normal operating conditions.

Generally, liquid chromatograms are obtained at lower linear flow rates than gas chromatograms. Also, plate heights for liquid chromatographic columns are an order of magnitude or more smaller than those encountered with gas chromatographic columns. Offsetting this advantage is the fact that it is impractical to use liquid chromatographic columns that are longer than about 25 to 50 cm due to the high viscosity and high fluid density - compared to gaseous materials. In contrast, gas chromatographic columns may be 50 m or more in length. As a result, the total number of plates, and thus overall column efficiency, are usually superior with gas chromatographic columns.

Theory of Band Broadening Researchers have devoted an enormous amount of theoretical and experimental effort to develop quantitative relationships describing the effects of the experimental variables on plate heights for various types of columns. Perhaps a dozen or more expressions for calculating plate height have been put forward and applied with various degrees of success. None of these models is entirely adequate to explain the complex physical interactions and effects that lead to zone broadening and thus lower column efficiencies. Some of the equations, though imperfect, have been very useful, however, in pointing the way toward improved column performance. One of these is presented here.

The efficiency of capillary chromatographic columns and packed chromatographic columns at low flow velocities can be approximated by the expression

$$H = \frac{B}{u} + C_S u + C_M u$$

where H is the plate height in centimeters and u is the linear velocity of the mobile phase in centimeters per second. The quantity B is the longitudinal diffusion coefficient, while CS and CM are mass-transfer coefficients for the stationary and mobile phases, respectively. At high flow velocities in packed columns where flow effects dominate diffusion, the efficiency can be approximated by Van Deemter equation.

$$H = A + \frac{B}{u} + C_S u$$

They quantity A they express eddy diffusion , where it includes the contribution to unequal pathways or irregularities in the paths that the mobile phase passes, as well as expressing the randomness of the molecules in the column that can indicate that the molecules are in a straight line or go in a polygonal line, that is, there is a random movement, as shown in the figure(7-8).

The Longitudinal Diffusion Term, B/u.

Diffusion is a process in which species migrate from a more concentrated part of a medium to a more dilute region. The rate of migration is proportional to the concentration difference between the regions and to the diffusion coefficient DM of the species. The latter, which is

a measure of the mobility of a substance in a given medium, is a constant for a given species equal to the velocity of migration under a unit concentration gradient.

In chromatography, longitudinal diffusion results in the migration of a solute from the concentrated center of a band to the more dilute regions on either side (that is, toward and opposed to the direction of flow). Longitudinal diffusion is a common source of band broadening in gas chromatography where the rate at which molecules diffuse is high. The phenomenon is of little significance in liquid chromatography where diffusion rates are much smaller. The magnitude of the B term in Equation 7-8 is largely determined by the diffusion coefficient DM of the analyte in the mobile phase and is directly proportional to this constant.

As shown by Equation 7-8, the contribution of longitudinal diffusion to plate height is inversely proportional to the linear velocity of the eluent. Such a relationship is not surprising inasmuch as the analyte is in the column for a briefer period when the flow rate is high. Thus, diffusion from the center of the band to the two edges has less time to occur. The diffusion coefficients in gaseous media are orders of magnitude larger than in liquids. Therefore, band broadening occurs to a much greater extent in gas chromatography than in liquid chromatography.

The Stationary Phase Mass-Transfer Term, CSu.

When the stationary phase is an immobilized liquid, the mass-transfer coefficient is directly proportional to the square of the thickness of the film on the support particles, d^2_f, and inversely proportional to the diffusion coefficient, D_S, of the solute in the film. That is, with thick films, molecules must on the average travel farther to reach the surface, and with smaller diffusion coefficients, they travel slower. The result is a slower rate of mass transfer and an increase in plate height. When the stationary phase is a solid surface, the mass-transfer coefficient C_S is directly proportional to the time required for a species to be adsorbed or desorbed.

The Mobile Phase Mass-Transfer Term, C_Mu.

The mobile-phase mass-transfer coefficient CM is known to be inversely proportional to the diffusion coefficient of the analyte in the mobile phase DM. For packed columns, CM is proportional to the square of the particle diameter of the packing material, d2p. For capillary columns, CM is proportional to the square of the column diameter, d2C, and a function of the flow rate. The contribution of mobile-phase mass transfer to plate height is the product of the mass-transfer coefficient CM (which is a function of solvent velocity) as well as the velocity of the solvent itself.

Zone broadening in the mobile phase is due in part to the multitude of pathways by which a molecule (or ion) makes its way through a packed column. As shown in Figure 7-8, the lengths of these pathways can differ significantly. This difference means that the residence times in the column for molecules of the same species vary. Solute molecules then reach the end of the column over a range of times, leading to a broadened band.

Summary of Methods for Reducing Band Broadening.

For packed columns, one variable that affects column efficiency is the diameter of the particles making up the packing. For capillary columns, the diameter of the column itself is an important variable. To take advantage of the effect of column diameter, narrower and narrower columns have been used in recent years. With gaseous mobile phases, the rate of longitudinal diffusion can be reduced appreciably by lowering the temperature and thus the diffusion coefficient. The result is significantly smaller plate heights at lower temperatures. This effect is usually not noticeable in liquid chromatography because diffusion is slow enough that the longitudinal diffusion term has little effect on overall plate height. With liquid stationary phases, the thickness of the layer of adsorbed liquid should be minimized since CS in Equation 7-8 is proportional to the square of this variable, the table (7-2) shows all the variables that influence column efficiency.

TABLE 7-2

Variables That Influence Column Efficiency

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	cm s^{-1}
Diffusion coefficient in mobile phase*	D_M	cm^2 s^{-1}
Diffusion coefficient in stationary phase*	D_S	cm^2 s^{-1}
Retention factor	k	unitless
Diameter of packing particles	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

*Increases as temperature increases and viscosity decreases

Column Resolution

The resolution, Rs, of a column tells us how far apart two bands are relative to their widths. The resolution provides a quantitative measure of the ability of the column to separate two analytes. The significance of this term is illustrated in Figure 7-9, which consists of chromatograms for species A and B on three columns with different resolving powers. The resolution of each column is defined as

$$R_s = \frac{\Delta Z}{\frac{W_A}{2} + \frac{W_B}{2}} = \frac{2\Delta Z}{W_A + W_B} = \frac{2\left((t_R)B - (t_R)A\right)}{W_A + W_B}$$

where all of the terms on the right side are as defined in the figure. It is evident from Figure 7-9 that a resolution of 1.5 gives an essentially complete separation of A and B, but a resolution of 0.75 does not. At a resolution of 1.0, zone A contains about 4% B, and zone B contains about 4% A. At a resolution of 1.5, the overlap is about 0.3%. The resolution for a given stationary phase can be improved by lengthening the column, thus increasing the number of plates. The added plates, however, result in an increase in the time required for separating the components.

Effect of Retention Factor and Selectivity Factor on Resolution We can derive a very useful equation that relates the resolution of a column to the number of plates it contains as well as to the retention and selectivity factors of a pair of solutes on the column. Thus, it can be shown that for the two solutes A and B in Figure 7-9, the resolution is given by the equation

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{a-1}{a}\right)\left(\frac{k_B}{1+k_B}\right)$$

where kB is the retention factor of the slower-moving species and α is the selectivity factor. This equation can be rearranged to give the number of plates needed to realize a given resolution:

$$N = 16R_s^2 \left(\frac{a}{a-1}\right)^2 \left(\frac{1+k_B}{k_B}\right)^2$$

Effect of Resolution on Retention Time As mentioned previously, the goal in chromatography is the highest possible resolution in the shortest possible elapsed time. Unfortunately, these goals tend to be incompatible, and a compromise between them is usually necessary. The time (tR)B required to elute the two species in Figure 7-9 with a resolution of Rs is given by

$$(R_s)_B = \frac{16R_s^2 H}{u}\left(\frac{a}{a-1}\right)^2 \frac{(1+k_B)^3}{{k_B}^2}$$

where *u* is the linear velocity of the mobile phase.