CHAPTER II

THEORY

Band Broadening Mechanisms

As a sample traverses a column its distribution about the zone center increases in proportion to its migration distance or time in the column. The extent of zone broadening determines the chromatographic efficiency which can be expressed as either the number of theoretical plates (N) or the height equivalent to a theoretical plate (H or HETP). If the column is assumed to function as a Gaussian operator then the column efficiency is readily expressed in terms of the peak retention time and variance according to equation (2.1)

$$N = (t_R / \sigma_t)^2 \qquad (2.1)$$

where t_R is the retention time.

σ_t is the band variance in time unit.

In practice , the number of theoretical plates (N) was computed from peak profiles by using the formula (2.2)

$$N = 5.54 (t_R / W_{1/2})^2 \qquad (2.2)$$

where $W_{1/2}$ is the full peak width at the half-maximum points.

t_R is the retention time.

The height equivalent to a theretical plate is given by the ratio of the column length (L) to the number of theoretical plates (N)

$$H = L / N \qquad (2.3)$$

The term plate number and plate height have their origins in the plate model of the chromatographic process (27-29) . The plate model assumes that the column can be visualized as being divided into a number of volume elements or imaginary sections called plates. At each plate the partitioning of the solute between the mobile phase and stationary phase is rapid and the equilibrium is reached before the solute moves onto the next plate. The distribution coefficient of the solute is the same in all plates and is independent of the solute concentration. The mobile phase flow is assumed to occur in a discontinuous manner between plates and the diffusion of the solute in the axial direction is negligible (or confined to the volume element of the plate occupied by the solute). Axial diffusion contributes significantly to band broadening. The distribution constant is independent on concentration only over a narrow concentration range ,and , quite obviously , the assumption that flow occurs in a discontinuous manner is false. Perhaps the largest shortcoming of the plate model is that it fails to relate the band broadening process to the experimantal parameters (e.g., partical size , stationary film thickness , mobile phase velocity, etc.) that are open to maniputation by the investigator. The various rate models of the chromatographic process enable a similar expression for the theoretical plate to be derived (29).

When a sample band migrates through a packed bed , the individual flow paths around the packing particles are of different lengths. These variations in the flow direction and the rate lead to band broadening that should depend only on the density and homogeneity of the column packing. Its contribution to the total plate height is proportional to the partical size and can be described by

$$H_E = 2\gamma d_p \qquad (2.4)$$

where H_E is the contribution to the total plate height from eddy diffusion.

γ is the packing factor.

d_p is the average particle diameter.

The contribution to the plate height from molecular diffusion in the mobile phase arises from the nautural tendency of the solute band to diffuse away from the zone center as it moves through the column (30,31). Its value is proportional to the diffusion coefficient and the time the sample spends in the column. Its contribution to the total plate height is given by

$$H_L = 2\varphi D_m / u \qquad (2.5)$$

where H_L is the contribution to the plate height from longitudinal molecular diffusion in the mobile phase.

φ is the obstruction or tortuosity factor.

D_m is the solute diffusion coefficient in the mobile phase.

u is the average mobile phase velocity.

Mass transfer in either the stationary phase or mobile phase is not instantaneous and , consequently , complete equilibrium is not established under normal separation conditions. The result is that the solute concentration profile in the stationary phase is always displaced slightly in advance of the equilibrium position. The combined peak observed at the column outlet is broadened about its band center which is located where it would have been for instantaneous equilibrium , provided the degree of nonequilibrium is small. The stationary phase contribution to mass transfer is given by equation (2.6)

$$H_S = [\, 2kd_f^2 u \,] / [\, 3D_s \,(1+k)^2 \,] \qquad (2.6)$$

where H_s is the contribution to the plate height from the resistance to mass transfer in the stationary phase.

d_f is the stationary phase film thickness.

D_s is the diffusion coefficient in the stationary phase.

When a liquid flows through a packed bed an appreciable fraction of the interstitial fluid is essentially stagnant with respect to the actual stream in the center region of the interparticle channels. The fluid space in the column is depicted as consisting of three domains : the free , streaming fluid space ; the stangnant interstitial fluid space ; and the intraparticle fluid space , which is also assumed to be stagnant. Diffusion is relatively slow in the stagnant mobile phase and its influence on band broadening in liquid chromatography is often significant. Thus equation (2.7) more adequately accounts for the contribution of mass transfer in the stationary phase to the total plate height in liquid chromatography than does equation (2.6)

$$H_S = [\theta(k_0 + k + k_0k)^2 d_p^2 u_e] / [30 D_m k_0 (1 + k_0)^2 (1 + k)^2] \quad (2.7)$$

where θ is the tortuosity factor for the pore structure of the particles.

k_0 is the ratio of the intrapartical void volume to the interstitial void space.

u_e is the interstitial mobile phase velocity.

Mass transfer resistance in the mobile phase is more difficult to calculate because it requires an exact knowledge of the flow profile of the mobile phase. This is only known exactly for open tubular columns for which the contribution of mass transfer resistance in the mobile phase to the total plate height can be described by equation (2.8)

$$H_M = [(1 + 6k + 11k^2) / 96 (1 + k)^2] [(d_c^2 u / D_m)] \quad (2.8)$$

where H_M is the contribution to the plate height from the resistance to mass transfer in the mobile phase.

d_c is the column diameter.

In a packed bed the mobile phase flows through a tortuous channel system and lateral mass transfer can take place by a combination of diffusion and convection. The diffusion contribution can be approxomated by equation (2.9)

$$H_{M,D} = (wud_p^2) / D_M \quad (2.9)$$

where $H_{M,D}$ is the contribution to the plate height from diffusion controlled resistance to mass transfer in the mobile phase .

w is the packing factor function that corrects for radial diffusion (ca. 0.02 to 5).

To account for the influence of convection , that is band broadening resulting from the exchange of solute between flow streams moving at different velocities , the eddy diffusion term must be coupled to the mobile phase mass transfer term , as indicated below

$$H_{M,C} = 1 / (1/H_E + 1/H_{M,D}) \quad (2.10)$$

where $H_{M,C}$ is the contribution to the plate height resulting from the coupling of eddy diffusion and mobile phase mass transfer term.

The above listing of contributions to the plate height , it encompasses the major band broadening factors and the overall plate height can be expressed as their sum , equation (2.11)

$$HETP = H_E + H_L + H_S + H_M \quad (2.11)$$

A plot of HETP as a fuction of mobile phase velocity is a hyperbolic function most generally described by the van Deemter equation (2.12)

$$HETP = A + B/u + (C_S + C_M)u \quad (2.12)$$

The A term represents the contribution from eddy diffusion , the B term the contribution from longitudinal diffusion , and the C terms the contributions from mass transfer in the mobile and stationary phase to the total column plate height.

When the mobile phase is a liquid , a variety of equations can be used in additional to the van Deemter equation (2.12) to describe band broadening as a function of the mobile phase velocity as shown in equations (2.13) to (2.16) (29,30,32-33)

$$HETP = A/[\,1 + (E/u)] + B/u + Cu \qquad (2.13)$$

$$HETP = A/[\,1 + (\,E/u^{1/2})\,] + B/u + Cu + Du^{1/2} \qquad (2.14)$$

$$HETP = Au^{1/3} + B/u + Cu \qquad (2.15)$$

$$HETP = A/[\,(1 + E/u^{1/3})] + B/u + Cu + Du^{2/3} \qquad (2.16)$$

A , B , C , D and E are appropriate constants for a given solute in a given chromatographic system.