CHAPTER II

THEORY

A : Elution Modelling of Reversed-Phase Chromatography

The chromatographic process is viewed as a reversible association of the solute, S, with the hydrocarboneceous ligand, L, covalently bound to the surface of stationary phase [21]

$$S + L \overset{K_{assoc}}{\rightleftharpoons} SL \qquad (2.1)$$

Where L is the ligand which is the hydrophobic group of phenylpropanolamine bound to the silica support. K_{assoc} is the association constant which is defined

$$K_{assoc} = [SL] / [S][L] \qquad (2.2)$$

It is assumed that the equilibrium constant of the process with both neutral and ionized solutes.

Equilibria

The dissociation of a monoprotic acid, HA, in the mobile phase is governed by the following equilibrium.

$$HA \overset{K_{a\,m}}{\rightleftharpoons} A^- + H^+ \qquad (2.3)$$

where A^- is the dissociated acid and H^+ is the solvated proton. The equilibrium constant is the acid dissociation constant in the eluent proper, $K_{a\,m}$, is given by

$$K_{a\,m} = [H^+]_m [A^-]_m / [HA]_m \qquad (2.4)$$

where $[H^+]_m$, $[A^-]_m$ and $[HA]_m$ are the concentrations of the solvated proton, the dissociated, and undissociated acid in the mobile phase, respectively.

In the chromatographic process under consideration, solute retention is assumed to occur because of a reversible association between the dissociated and / or undissociated acid and hydrocarboneceous ligand, L, of the stationary phase. The binding of the acid is determined by the equilibrium.

$$HA + L \overset{K_{LHA}}{\rightleftharpoons} LHA \qquad (2.5)$$

with the equilibrium constant K_{LHA} which given by

$$K_{LHA} = [LHA]_s / [HA]_m [L]_s \qquad (2.6)$$

where $[LHA]_s$ and $[L]_s$ are the concentrations of the complex, LHA, and the ligand of the stationary phase, respectively.

The interaction between the anion and the ligands results in the formation of the complex LA^- according to the following equilibrium

$$A^- + L \overset{K_{LA^-}}{\rightleftharpoons} LA^- \qquad (2.7)$$

and the corresponding equilibrium constant K_{LA^-} is given by

$$K_{LA^-} = [LA^-]_s / [A^-]_m [L]_s \qquad (2.8)$$

where $[LA^-]_s$ is the concentration of the complex in the stationary phase.

The magnitude of solute retention is expressed by the capacity factor, k', which is the measure of the stoichiometric mass distribution of HA between the stationary and mobile phases. In a given column the volume ratio of the stationary and mobile phases, ϕ, is fixed so that the mass distribution ratio is simply given by

$$k' = \phi \frac{[LHA]_s + [LA^-]_s}{[HA]_m + [A^-]_m} \qquad (2.9)$$

Expressing the species concentrations from Equations (2.4), (2.6) and (2.8) and substituting into Equations (2.9), we obtained the capacity factor as

$$k' = \phi K_{LHA} [L]_s + K_{LA^-} [L]_s \{ Ka_m / [H^+]_m \} \qquad (2.10)$$

It is convenient to define the capacity factor of undissociated acid, k_0, as

$$k_0 = \phi K_{LHA} [L]_s \qquad (2.11)$$

and the capacity factor of the conjugate base k_{-1}, as

$$k_{-1} = \phi K_{LA^-} [L]_s \qquad (2.12)$$

Substituting k_0 and k_{-1} from equation (2.10), we obtain for the capacity factor of a monoprotic acid and the following expression

$$k' = \frac{k_0 + k_{-1}\{ Ka_m / [H^+]_m \}}{\{ 1 + Ka_m / [H^+]_m \}} \qquad (2.13)$$

Equation (2.13) represents a phenomenological relationship between the capacity factor of a partially dissociated weak acid and the hydrogen ion concentration in the eluent, with the approprite acid dissociation constant and the two limiting capacity factors of the undissociated and fully dissociated acid as the parameters. Although it is not shown explicitly, the model also accounts for the acid dissociation equilibrium of the bound species with a dissociation constant $K_{a\,m}$.

B : Elution Modelling of Ion-Exchange Chromatography

The retention characteristics of analytes in ion exchange chromatography which were in some way or another related to the ionic nature of the mobile phase employed were shared by many researchers during the early development of ion chromatography ; however, few attempts have been made to quantitatively model separation behavior based on a theoretical evaluation of the ion exchange process. Two such models have been proposed. Both treat the separation process in terms of classic thermodynamics, that is, that the separation of analytes by an ion exchange resin can be considered to be controlled mechanistically by the thermodynamic equilibrium that exists at the individual resin sites between the analyte species and any ionic component of similar charge type in the mobile phase (eluent ions). These models differ slightly in derivation, but most prominently with

respect to the number of mobile-phase eluent ions with which each is equipped to deal. Gjerde and associates have developed a model that is applicable to a situation in which the mobile phase contains a single active component [32]. Jenke and Pakenkopf have modified and applied a more generalized approach. The details of mobile phase containing single eluent ion were described in the following discussion [3].

One can consider the equilibrium that exists between two species of similar charge type on a strong ion-exchange resin to have the form

$$xA^{y} + y\,[B - R_x] \rightleftharpoons yB^{x} + x[A - R_y] \qquad (2.14)$$

where R represents the ion exchanger, A and B are the competing ions, and x and y are the absolute values of their associated charges. In this derivation, species A represents the analyte ion and species B is the mobile-phase eluent. The equilibrium constant for this expression (K_B^A) becomes

$$K_B^A = \frac{[A - R_y]^x\,[B]^y}{[A]^x\,[B - R_x]^y} \qquad (2.15)$$

where the brackets indicate the activities of the various species. It is noted that although both [A] and [B] are solution-phase activities, $[A - R_y]$ and $[B - R_x]$ both refer to the activity of a resin-bound ion. If the sample size is small, the quantity of eluent ion on the equilibrated resin, $[B - R_x]$, is essentially constant and equal to the column capacity Q. The ratio of the amount of A on the resin to that in the solution phase in contact with the resin ($[A - R_y]$ / [A]) is a distribution ratio D, which classically can be related to a reduced retention volume V_R for analyte A by the equation

$$D = V_R / w \qquad (2.16)$$

where the reduced retention volume is the difference between the measured retention volume for analyte A and the column void volume, and w is the weight of the resin in the column.
Substituting D and Q into equation (2.15) yields

$$K_B^A = D^x ([B] / Q)^y \qquad (2.17)$$

which, when combined with equation (6), produces

$$K_B^A = (V_R / w)^x ([B] / Q)^y \qquad (2.18)$$

Rearranging and taking the logarithm of both sides :

$$\log K_B^A w^x = x \log V_R + y \log [B] - y \log Q \qquad (2.19)$$

Now if the weight of the resin in the column and the capacity of the column are constant, and if the selectivity coefficient is truly independent of the mobile phase composition, then equation (2.19) simplifies to

$$\log V_R = (-y / x) \log [B] - \text{constant} \qquad (2.20)$$

which is consistant with the Gjjerde derivation [24].

C : Peak Width and Column Efficientcy

Peak width (W) in HPLC can be related to the number of theoretical plates (N) as follows [2] :

$$N = (t_R / \sigma_t)^2 \qquad (2.21)$$

where

t_R is the retention time.

σ_t is the standard deviation or band variance in time unit.

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

$$N = 5.54 (t_R / W_{1/2})^2 \qquad (2.22)$$

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

The column efficiency of a given column could be measured by the height equivalent to a theoretical plate (H)

$$H = L / N \qquad (2.23)$$

where L is the column length since N defined as equation (2.21) and if t_R and σ are in the same time units, the substitution of equation (2.21) into equation (2.23) gives

$$H = L (\sigma / t_R)^2 \qquad (2.24)$$