CHAPTER-2

Fundamentals of chromatography

2.1 Adsorption Phenomena and Molecular Interactions in Chromatography

Chromatographic separations have a common principle, the distribution of analytes *to* be separated between stationary and mobile phases. The mobile phase may be a liquid or a mixture of liquids, a gas, or a supercritical fluid with the characteristics of gas and liquid depending on the chromatographic conditions. A huge variety of stationary phases such as inert or adsorptive solids, liquids bonded by adsorptive forces to solids or to the wall of columns, chemically modified solids, etc. can be used for the separation of analytes The differences in the capacities of analytes to bind to the stationary phase result in different migration velocities and in the separation of the analytes. Many excellent papers [3-9] and books [10-11] have been published on the various aspects of the theoretical principles governing chromatographic separation.

2.2. Adsorption Phenomena in Liquid Chromatography

Due to the presence of multi component eluents, the adsorption processes are more complicated in liquid than in gas chromatography. The various components of the eluent mixture are competitively adsorbed on the surface of the stationary phase. Similar competition can occur between the analytes and the impurities of the sample.

2.2 1 Competitive Adsorption of the Eluent Components

In the case of a two-component eluent system the chemical potential (μ) of components 1 and 2 can be described by

$$\mu_{1l} = \mu_{1l} + RT. \ (1 - r_{12}).\Phi_{1l} + RT.\ln(\Phi_{1l}\tau_{1l}) \qquad (1)$$

$$\mu_{2l} = \mu_{2l} + RT. \ (r_{2l} - 1).\Phi_{2l} + RT.\ln(\Phi_{2l}\tau_{2l}) \qquad (2)$$

where μ_{1l} and μ_{2l} are the standard chemical potentials in the surface phase; Φ_{1l} and Φ_{2l} are the volume fractions of eluent components 1 and 2 in the l-th surface layer; τ_{1l} and τ_{2l} are the corresponding activity coefficients; $r_{12} = r_1/r_2$; $r_{2l} = r_2/r_1$; and r_1 and r_2 are the number of adsorption sites occupied by eluent components 1 and 2, respectively.

The interaction energy (U_{in}) in the adsorbed phase is composed of three components:

$$U_{in} = U_{in}^{(b)} + U_{in}^{(id)} \; U_{in}^{(mix)} \tag{3}$$

$$U_{in}^{(b)} = \Phi_1.\varepsilon_{11}^{(p)} + (\Phi_2 \, \varepsilon_{22}^{(p)} + 2\alpha_{12} \, \Phi_1. \, \Phi_2)N_o/2 \tag{4}$$

Where No is the number of adsorption sites in a surface layer; $\varepsilon_{11}^{(p)}$ and $\varepsilon_{22}^{(p)}$ are the interaction energies of the molecular pairs 1-1 and 2-2, α_{12} is the interchange energy:

$$\alpha_{12} = \varepsilon_{12} - (\varepsilon_{11}^{(p)} + \varepsilon_{22}^{(p)}) / 2 \tag{5}$$

$$U_{in}^{(id)} = (a_p + a_v). \, (\Phi_{11} \, \varepsilon_{11}^{(p)} + \Phi_{21}.\varepsilon_{22}^{(p)}) \, N_o/2 \; \sum_{I=2}^{L} \Phi_{11} \, \varepsilon_{11}^{(p)} + \Phi_{21} \, \varepsilon_{22}^{(p)}).z.N_o/2 \tag{6}$$

where a_p and a_v are the numbers of the nearest-neighbors adsorption sites in the same surface layer (p) and above (v), and z is the total number of nearest-neighbors adsorption sites

$$U_{in}^{(mix)} = \sum N_o \; \alpha_{12} \, [a_p \; \Phi_{11} \; \Phi_{21} + av .(\Phi_{11} \; \Phi_{2(1+l)} + \Phi_{1(1+l)} + \Phi_{2l}]$$
$$+ N_o. \, \alpha_{12}.[a_p. \Phi_{11} \; \Phi_{21} + av \; (\Phi_{11} \; \Phi_2 + \Phi_1 \; \Phi_{21} - \Phi_1. \Phi_2] \tag{7}$$

The activity coefficients can be calculated from the interaction energies.

$$RT.\ln \tau_{1l} = (\delta \, U_{in}^{(mix)} / \delta \, N_{1l})_{N_{2l}, N_{1m}, N_{2m}} \tag{8}$$

$$RT.\ln \tau_{2l} = (\delta \, U_{in}^{(mix)} / \delta \, N_{2l})_{N_{2l}, N_{1m}, N_{2m}} \tag{9}$$

where τ_{1l} and τ_{2l} are the activity coefficients of components 1 and 2 in the surface layer.

$$\ln \tau_{1l} = A_{12} \, [f_y. \Phi_{21}. \Phi_{2(1-l)} + \Phi_{1(1-l)} + f_p \, (\Phi_{21})^2 + f_y \, \Phi_{2(1+l)}] \tag{10}$$

$$\ln \tau_{2l} = r_{21}A_{12} \, [f_y \; \Phi_{11}. \Phi_{1(1-l)} + \Phi_{2(1-l)} + f_p.(\Phi_{11})^2 + f_y \, \Phi_{1(1+l)}] \tag{11}$$

where $f_p = a_p/ z$ and $f_v = a_v/z$.

The adsorption equation in liquid chromatography is

$$(\Phi_{11} \; \tau_{1l}/ \Phi_1. \tau_1). (\Phi_2. \tau_2/ \Phi_{21}. \tau_{21})^{r_{12}} = K_{12}^{(l)} \tag{12}$$

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where $K_{12}^{(1)}$ is the adsorption equilibrium constant in the surface layer:

$$K_{12}^{(1)} = e[(\mu_1^0 - \mu_{11}^0) - r12.(\mu_2^0 - \mu_{21}^0)] \tag{13}$$

The difference between the standard chemical potentials in the bulk phase (μ^0) and in the surface layer (μ_1^0) is equal

$$\mu_1^0 - \mu_{11}^0 = \varepsilon_{11} - RT.\ln(q_{11}/q_1) \tag{14}$$

$$\mu_2^0 - \mu_{21}^0 = \varepsilon_{21} - RT.\ln(q_{21}/q_2) \tag{15}$$

where ε_{11} and ε_{21} are the adsorption energies of components 1 and 2 and q_i is their molecular partition functions in the adsorbed layer and in the bulk phase.

Many efforts have been devoted to the practical application of the theoretical considerations discussed previously in modem high-performance liquid chromatography.[12-17]

The equations discussed previously are the results of theoretical considerations. However, in the practice of chromatographic separations the adsorption centers on the support surface are not homologous. The distribution of the energy of adsorption of the adsorption centers have been modeled many times either by the Gaussian or Poisson equations, or by the assumption of the random distribution of adsorption centers.[18]

It has been proved many times that the prediction power of the theoretical distribution functions describing the real situation on the support surface are sometimes unsatisfactory. Many excellent studies deal with the effect of surface in homogeneity on the retention characteristics of supports both in gas [19] and. in liquid chromatography.[20-22]

2.3. Competitive Adsorption of analytes

Competition occurs not only among the components of the eluent mixture, but also among the analytes present in the sample to be analyzed. In the case of monolayer adsorption and binary eluent mixture, the partition coefficient K can be expressed by

$$K = X_{Sl} / X_s = r_s / K_{1s}^{(1)}.(\Phi_1 + K_{21}^{(1)}. \Phi_2)^{-1} \qquad (16)$$

where Xsl and X_s are the molar concentrations of s in the surface layer and in the bulk eluent, respectively; r_s is the number of sites occupied by one molecule of s; $K_{1s}^{(1)}$ and $K_{21}^{(1)}$ are the equilibrium constants for the adsorption in the surface layer, and Φ values are the volume fractions.

Capacity factor k is related to the partition coefficient:

$$k = K.V_s/V_m \qquad (17)$$

where V_s is the molecular volume of component s in the bulk phase and V_m is the molar volume of the eluent mixture.

When K1 is the partition coefficient in pure component 1:

$$\log (K_1/K) = \log (\Phi_1 + K_{21}^{(1)} \Phi_2) \qquad (18)$$

$$\log (K1/K) = a' \, \alpha_2 \, (\varepsilon_{12}^0 - \varepsilon_1^0) \qquad (19)$$

where a' is the adsorbent activity according to Snyder, α_2 is the surface area occupied by one molecule of component 2, ε_{12}^0 is the solvent strength of a two-component eluent system, and ε_1^0 is the eluent strength of component 1

Under ideal conditions the retention can be described by

$$\varepsilon_{12}^0 = \varepsilon_1^{0 \; a' \; \alpha 2 \, (\varepsilon_2^0 - \varepsilon_1^0)} + \log (\Phi_1 + \Phi_2.10) \qquad (20)$$

The no ideality of the chromatographic system has been discussed in more detail in References [23-25]

When the surface areas occupied by the analyte and by the components of the eluent the equations describing the retention become very complicated.[26-27]

The partition coefficient K in reversed-phase HPLC can be described by

$$\ln K = \ln K^{(in)} + \ln K^{(comp)} \qquad (21)$$

where In $K^{(in)}$ is the contribution of the analyte-solvent interaction to the partition, and In $K^{(comp)}$ is related to the competitive adsorption of solvents on the support surface.

In the case of ternary mobile phases the [28] activity coefficient of the analyte $\tau_{s(f)}$ in a given phase f is described by

$$RT \ln \tau_{s(f)} = v_{s,}(\delta_s - \delta_{(f)})^2 \tag{22}$$

where δ_s and $\delta_{(f)}$ are the solubility parameters for the analyte and for the phase, respectively.

$$\ln K = [(\delta_s - \delta_f)^2 - (\delta_s - \delta_{(s)})^2], v_s / RT \tag{23}$$

where s and m refer to the surface and the mobile phase, respectively When

$$\delta_{(m)} = \overset{3}{\underset{i=1}{\Sigma}} \Phi_i . \delta_i \quad \text{and} \quad \overset{3}{\underset{i=1}{\Sigma}} \Phi_i = 1 \tag{24}$$

the partition coefficient becomes

$$\ln K = \{[\delta_s - \Phi_1 . \delta_1 - \Phi_2 \quad \delta_2 - (1- \Phi_1- \Phi_2) \quad \Delta_3]^2 - (\delta_s - \delta_{(s)})^2\}. v_s / RT \tag{25}$$

In the case of constant δ_s value the partition coefficient is

$$\ln K = A_1. \Phi_1 + B1. \Phi_2 + A_2 . \Phi_1^2 + B_2 \Phi_2^2 + Bz'<p\sim + \Phi_1. \Phi_2 A_2 B_2 + C \tag{26}$$

where

$$A_1 = (\delta_s - \delta_3)'(\delta_3 - \delta_1).2v_s / RT \tag{27}$$

$$B_1 = (\delta_s - \delta_3)'(\delta_3 - \delta_2) 2v_s / RT \tag{28}$$

$$A_2 = (\delta_1 - \delta_3)^2 v_s / RT \tag{29}$$

$$B_2 = (\delta_2 - \delta_3)^2. v_s / RT \tag{30}$$

$$C = [(\delta_3 - \delta_s)^2 - (\delta_{(s)} - \delta_s)^2 v_s / RT \tag{31}$$

The change of the standard free energy of the transfer of an analyte molecule from the eluent to the stationary phase (ΔF_s) is given by [29]

$$- \Delta F_s = \Delta F_{c,s} + \Delta F_{m,s} \tag{32}$$

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where $\Delta F_{c,s}$ is the free energy change of the creation of a cavity in the eluent to accommodate the analyte molecule, and $\Delta F_{m,s}$ is the energy change of the interaction between the analyte molecule and the eluent.

$$- \Delta F_s = C_m^2.I_m^2 + C_m.C_s.I_m.I_s \qquad (33)$$

where s and m refer to the molecules of the analyte and the eluent, I_i are the interaction indexes; and C_i are correcting coefficients for I_i

In the case of binary eluent mixture

$$I_m = \Phi_1 I_1 + \Phi_2.I_2 \qquad (34)$$

$$\ln K = - F_s /RT = c + b_1. \Phi_2 + b_2 \ \Phi_2^2 \qquad (35)$$

the constants being equal

$$c = [C_m, I_1(C_m. I_1 - C_s I_s)] / RT \qquad (36)$$

$$b_1 = [C_m (I_2 - I_1).(C_sI_s + 2C_m.I_1)]/RT \qquad (37)$$

$$b_2 = C_m^2 (I_2 - I_1)^2 / RT \qquad (38)$$

The theory of Horvath et al.[30] supposes the reversible association of analyte S with the a polar ligand L on the support surface

$$S + L \ \rightarrow \ SL \qquad (39)$$

and the equilibrium constant K is

$$K = [SL]/[S] \ [L] \qquad (40)$$

The free energy of association $\Delta F^0_{assoc.}$ Can be expressed by

$$\Delta F^0_{assoc.} = \Delta F_{vdw,assoc} + (\Delta F_{c,SL} + \Delta F_{m,SL}) - (\Delta F_{c,s} + \Delta F_{m,s}) - (\Delta F_{c,L} + \Delta F_{m,L}) - RT. \ \ln RT / P_o, v_m \qquad (41)$$

The free energy of cavity formation $\Delta F_{c,j}$ of the analyte j can be expressed by

$$\Delta F_{c,j} = k_j^e .\alpha^{(v)}_j .\sigma_m.N.[1 - (1 - K_j^s / K_j^e) \ (\delta \ln \sigma_m / \delta \ln T + 2 \ \alpha^{(v)}_j \ T / 3 \qquad (42)$$

where $\alpha^{(v)}{}_j$ is the specific surface area of analyte j; σ_m is the surface tension of the eluent mixture; $K^e{}_j$ is a correction factor expressing the ratio between the energy required for the cavity formation and the energy for expanding the surface of the eluent by the same area; and $K^s{}_j$ is the corresponding entropy.

The energy ratio can be calculated by

$$K^e{}_j = 1 + (k^e - 1) \cdot (v_m/V_j)^{2/3} \tag{43}$$

where k^e is an energy term related to the heat of vaporization.

The term $\Delta F_{m,j}$ is composed of van der Waals ($\Delta F_{m,j,vdw}$) and electrostatic ($\Delta F_{m,j\,es}$) components:

$$\Delta F_{m,j} = (\Delta F_{m\,j,vdw}) + (\Delta F_{m,j\,es}) \quad j = S, L, SL \tag{44}$$

The term ($\Delta F_{m,j,vdw}$) can be calculated from the ionization potentials, refractive indexes, and molecular volumes of the analyte and the eluent, and ($\Delta F_{m,j\,es}$) can be calculated from the corresponding values of the dielectric constants, dipole moment, and polarizability. Assuming that

$$k^s{}_j = k^e{}_j = 1 \quad j = S, L, SL \tag{45}$$

$$\Delta F_{m,SL,vdw} = \Delta F_{m,L,vdw} \tag{46}$$

$$\mu_{SL} = \mu_S \text{ and } \mu_L = 0 \tag{47}$$

the partition coefficient can be expressed by

$$\ln K = - \Delta F^0{}_{assoc.} / RT \tag{48}$$

2.4 Molecular Forces in Chromatography

The molecular forces observed between various molecules are also involved in the process of chromatographic separation. The forces of interest include columbic interac-

tions between oppositely charged ions, ion-dipole interactions, hydrogen bonds, and hydrophobic interactions such as van der Waals and London dispersion forces.

2.4.1. Ion-Ion Interactions

Two oppositely charged particles (q^+ and q^-) separated by a distance (r) exert an interactive force on each other. The potential energy of interaction (U_{i-i}) is given by

$$U_{i-i} = q^+ . q^- / 4\pi.E_o.E.r \tag{49}$$

where q^+ and q^- are charges of the positive and negative ions, respectively; E_o is the permittivity of the free space between the charge ions; E is the dielectric constant. These types of interactions (frequently called salt-bridge or ion-pair formation) can occur between the positively and negatively charged substructures of the analytes and the stationary phase or eluent additives.

2.4.2. Ion-Permanent Dipole Interactions

Ion-permanent dipole interactions occur when one of the molecules is an ion and the other a dipole. (Dipoles are molecules that carry no net charge yet have permanent charge separation due to the nature of the electronic distribution within the molecule itself. Vector addition of the electrical forces derived from its partial charge separation leads to the creation of an electric dipole.) The charge asymmetry in the molecule is given a magnitude and direction by calculating the dipole moment (μ). The dipole moment is calculated by multiplying the separate charge with the distance of separation. The existence of a dipole moment requires an asymmetric molecule The electric dipole is treated like any other charge, although the orientation of the dipoles ($\cos\Theta$) must be taken into consideration in calculating the ion-permanent dipole interactive force (U_{i-pd}):

$$U_{i-pd} = q.\mu \ L \ \cos\Theta \ / \ 4\pi.E_o.E.r^2 \tag{50}$$

where q is the charge of the ion species (positive or negative); μ is the permanent dipole moment of the interacting neutral molecule; $\cos\Theta$ is the orientation of the permanent

dipole. These types of interactions can easily occur in adsorption liquid chromatography between the ionizable analyte and the asymmetric molecules of the mobile phase.

2.4.3 Ion-Induced Dipole Interaction

Even molecules that have no net dipole moment can have a transient dipole induced in them when they are brought into an electric field. Electronic interactions of this type are called ion-induced dipole interactions and the potential of the interaction (U_{i-id}) depends on the ability of the neutral molecule to be induced into a dipole, that is, on its polarizability (α):

$$U_{i-id} = q^2 . \alpha /8\pi.Eo.E^2.r^4$$ (51)

where a is the polarizability of the neutral molecule.

This interaction requires a molecule without permanent dipole moment. As the majority of analytes and eluent components have a considerable permanent dipole moment the importance of this type of interaction in chromatography is negligible.

2.4.4 Hydrogen bonds

Hydrogen bonds are formed by hydrogen atoms located between two electronegative atoms When a hydrogen atom is covalently attached to a highly electronegative atom such as an oxygen or a nitrogen, it takes a partial positive charge due to the strong electronegativity of the oxygen or nitrogen atoms. This interaction is very common in chromatography. Hydrogen bonds have been many times established between the polar analytes and the hydrophilic adsorption centers of various supports.

2.4.5 Van der Waals Interactions

When two molecules are in very close proximity, attractive forces called van der Waals interactions occur, which are cohesive forces that vary with respect to distance as $1/r^6$. The van der Waals interactions are generally subdivided into three types, all derivable from electrostatic considerations. They include permanent dipole-permanent dipole, permanent dipole-induced dipole, and induced dipole-induced dipole interactions.

2.4.5.1 Permanent Dipole-Permanent Dipole Interactions

These interactions occur when molecules with permanent dipole interact. In these cases the orienting forces acting to align the dipoles will be countered by randomizing forces of thermal origin. The calculation of the energy of interaction (U_{pd-pd}) includes the terms temperature and distance, indicating that permanent dipole-permanent dipole interactions are sensitive to both temperature (T) and the distance (r) between the inter- acting molecules:

$$U_{pd-pd} = - 2 . \mu^2_1 \mu^2_2 . / 3 K.T .(4 \pi E_o)^2 . r6 \tag{52}$$

where μ_1 and μ_2 are permanent dipole moments of the interacting neutral molecules 1 and 2

2.4.5.2 Permanent Dipole-Induced Dipole Interactions

Molecules with permanent dipole moment can induce dipole moment in a neutral molecule in a fashion similar to that discussed above for ion-induced dipole interaction. This interaction depends on the polarizability of the neutral molecule, but it is not sensitive to the thermal randomizing forces. The interaction energy (U_{pd-id}) can be calculated by

$$(U_{pd-id}) = - 2 . \mu^2 .\alpha/16\pi^2 E_o \ r6 \tag{53}$$

2.4.5.3 Induced Dipole-Induced Dipole Interactions

Induced dipole moment is a time-averaged value. If a snapshot were taken of any neutral molecule at an instant of time, there would be a variation in the distribution of the electrons in the molecule. At this instant, the "neutral" molecule would have a dipole moment. This instantaneous dipole is capable of inducing an instantaneous dipole in other neutral molecule: thus are born induced dipole-induced dipole forces. These forces are also called London or dispersion forces Dispersion forces fall off very rapidly with distance but can be quite significant for molecules in close proximity. The deformability of the electron clouds, as reflected by α, is obviously important in these interactions. The

rigorous calculation for dispersion forces (U_{id-id}) is quite complicated, but an adequate approximation can be used for the calculation:

$$U_{id-id} = -f(I).\ \alpha_1\ .\ \alpha_2\ /\ 16\pi^2.\ E_o.\ r^6 \qquad (54)$$

where (α_1 and α_2 are polarizabilities of the neutral molecules 1 and 2; f(I) is a function of the ionization energies (I) of the two molecules:

$$f(I) = 3I_1.\ I_2\ /2(I_1 + I_2) \qquad (55)$$

where I_1 and I_2 are ionization energies of the interacting neutral molecules 1 and 2.

When the interaction takes place between two identical molecular species having the same polarizability, Eq. 54 can be written as:

$$U_{id-id} = -f(I).\ a^2\ /\ 16\pi^2.\ E_o.\ r^6 \qquad (56)$$

The overall interactive energy for vander Waals forces (U_{vdW}) can be expressed in terms of electro statically derived interactions defined by the equations listed above:

$$U_{vdW} = .-2\mu^2_1\ .\ \mu^2_2/3K.T.(4\ \pi\ E_0\ )^2\ .\ r^6 -2\ \mu^2\alpha/16\pi^2E_o.\ r6 \qquad (57)$$
$$-\quad f(I).\ \alpha_1.\ \alpha_2\ /\ 16\pi^2.\ E_o.\ r^6$$

Because the above equation is fairly complicated for practical purposes and sometimes the parameters cannot be defined accurately, the attractive interactional energy (U_{vdW}) represented by the van der Waals forces is simplified in the form:

$$U_{vdW} = -A/r^6 \qquad (58)$$

where A is a constant that is different for each molecule.

The attractive forces increases rapidly as molecules get closer until the molecules actually contact each other, at which point the interactional energy goes instantly to infinity. However, before the molecules try to occupy the same space, so-called repulsion forces occur between the electron clouds of the molecules as they approach each other. This

electron repulsion can be added to the formula for attractive interaction energy (Lennard-Jones potential):

$$U_{vdW} = -A/r6 + B/r^{12} \tag{59}$$

where A and B are constants that are different for each molecule.

Many results indicate that the van der Waals interactions also play a considerable role in many fields of chromatographic separations. As the measurement of the individual interacting forces discussed above is extremely difficult for such complicated molecular assemblies as analytes-eluent components-surface of stationary phase, little attention has been devoted to the exact determination of this type of interaction.

2.5 Hydrophobic Interactions

The importance of hydrophobic interactions has been well recognized. Hydrophobic interaction is an attraction in aqueous medium between apolar or amphipathic molecules by which some of the more hydrophobic sites, upon approaching one other, preferentially interact with each other, frequently by van der Waals interactions, which tend to be attractive between low surface energy materials immersed in high-energy solvents such as water As discussed above, van der Waals forces include a variety of component forces such as permanent dipole-permanent dipole, permanent dipole-induced dipole, and induced dipole-induced dipole interactions. The extent of involvement of these component forces in the chromatographic separation process is obviously different depending on the character of the interacting molecular species.