CHAPTER III
THEORY

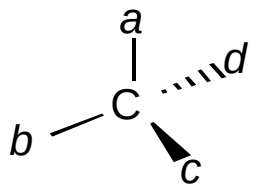
3.1 Diastereomeric Analyte-CSP Complexes Formation

A chiral molecule (from the Greek word "cheir" for hand) has a nonsuperimposable mirror image, called an enantiomer. Enantiomers have the same internal energies and possess similar physical and chemical properties in an achiral environment. The chirality[224] of a molecule can be attributed to any of the following: (1) by virtue of a chiral center, e.g., an sp^3-C covalently bonded to four different groups of atoms; (2) it lacks an axis of symmetry; and (3) it has no plane of symmetry (Fig. 6). The chiral analytes, 2-arylmethyl-propionic acids, that are being studied in this research all possess a chiral center.

In order for enantiomers to be chromatographically separated on a CSP, two conditions must be fulfilled. As noted in Chapter II, each enantiomer must form transient diastereomeric analyte-CSP complexes and these diastereomeric complexes must adequately differ in their stability. Three separate interactions are required between the enantiomers and the CSP, and these interactions may be attractive or repulsive and they may be single point (e.g., hydrogen bonding) or multipoint (e.g., dipole stacking and π-π interactions). At least one of these interactions must be stereochemically dependent. Figure 7 shows the formation of diastereomeric analyte-CSP complexes which follows the Three Point Rule[225]: three simultaneous interactions between the analyte enantiomers and CSP.

Diastereomeric complexes are also formed by diffusion of analyte enantiomers into a chiral matrix[226]. Even though no bonding interactions are invoked, the analyte enantiomers are in a chiral environment. Here, chiral recognition should it occur, would be entirely steric in origin. Such conditions are equivalent to a stationary phase containing chiral cavities, for example cyclodextrin. In such a situation, enantiomer separation may occur because one analyte enantiomer is better able to enter the cavities than the other.

(a)



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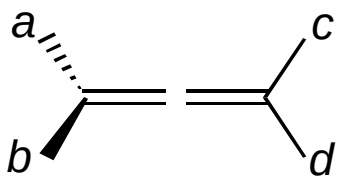

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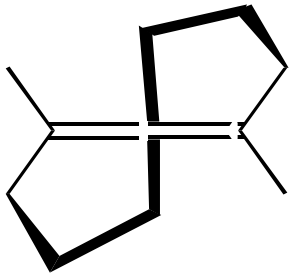


Figure 6. Three possible categories for chirality: (a) chiral center; (b) chiral axis; and (c) chiral plane.

THREE POINT MODEL
(Diastereomeric Analyte-CSP Complexes)

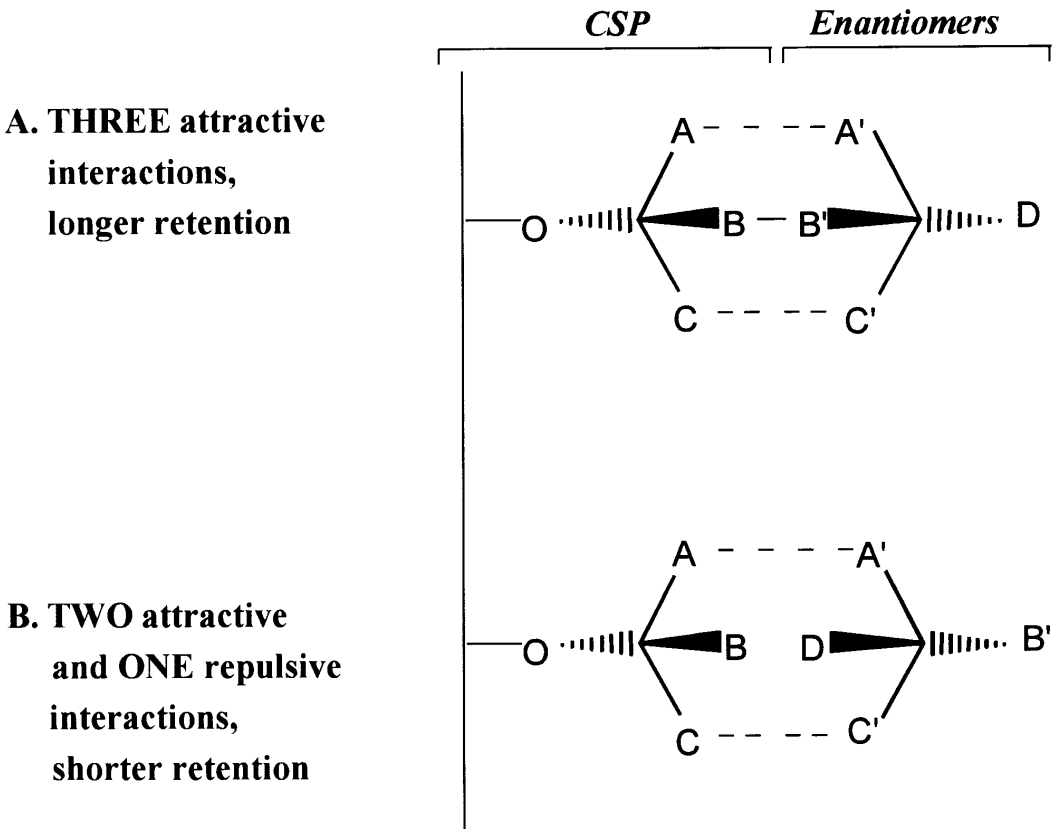


Figure 7. Three Point Model for the formation of the diasteromeric analyte-CSP complexes: (a) more stable and more retained and (b) less stable, hence elutes first.

The Three Point Rule is still in effect during enantioselective discrimination of the least retained enantiomer.[227] Combination of attractive forces and chiral cavities are also possible for diastereomeric complexes formation, and are presumably encountered in polymeric and protein-derived CSPs.[228] The amylose tris(3,5-dimethylphenylcarbamate) and cellulose tris(3,5-dimethylphenylcarbamate) CSPs that are being investigated in this research are known to exhibit both attractive interaction and steric fit into chiral cavities.[229-232]

It was noted in Chapter I that a CSP is composed of a chiral selector that is immobilized, either adsorbed or chemically bonded, to a silica support. The chiral selector consists of active adsorption sites where the interactions between analyte and CSP takes place. The analyte-CSP interactions[230], leading to the formation of diastereomeric complexes are:

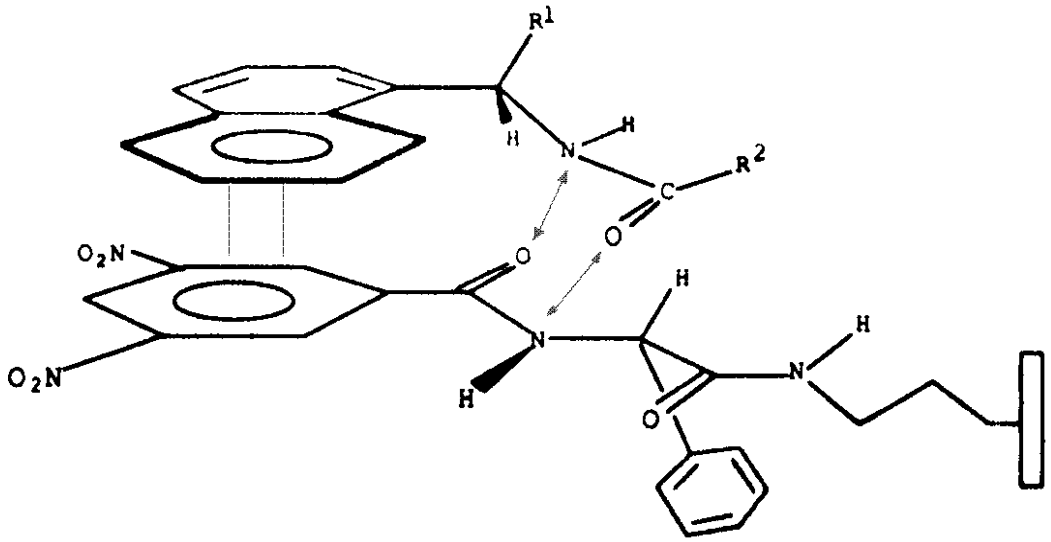
1. Hydrogen Bonding
2. Ion - Dipole
3. Dipole - Dipole (Keesom)
4. Dipole - Induced Dipole (Debye)
5. London Dispersion Forces
6. Electron Pair Donor-Acceptor (π-π interactions)

Some analyte-CSP interactions are illustrated in Fig. 8.

Hydrogen bonding (a high energy form of dipole-dipole interaction) is the most important interaction for the majority of the CSPs. A hydrogen bond is formed when an acidic proton is in close proximity with an electron pair donor or a hydrogen bond acceptor group such as oxygen (in alcohols, carboxyl, carbonyl, and ethers), a nitrogen atom (primary and secondary amines), a halogen, and a sulfur atom (thiol groups). Hydrogen bonds are nearly linear, asymmetrical and equal to the distance between the proton donor and acceptor atoms. Hydrogen bond energies can vary between 20 kJ/mol for O-H--O to as high as 150 kJ/mol, depending on the distance of interaction and type of the hydrogen donor and hydrogen acceptor groups.

Ion-dipole interaction is a Coulombic attraction that exist between an ionic and polar species. Compared to dipole-dipole, dipole-induced dipole, and induced dipole-induced

(a) π-π interaction and dipole-dipole stacking



(b) inclusion and hydrogen bonding

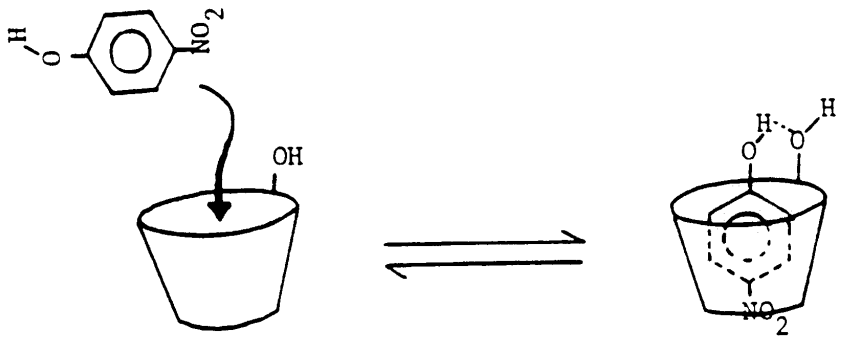


Figure 8. Analyte-CSP interactions: (a) π-π interactions between the phenyl moieties of the analyte (donor) and CSP (acceptor) (-----) and dipole-dipole stacking (↔); and (b) inclusion and hydrogen bonding formation between analyte and cyclodextrin CSP.

dipole interactions, ion-dipole attraction has the greatest Coulombic content. The potential energy of the ion-dipole interaction, as defined by Kaliszan, is given by

$$\mathbf{E_{i\text{-}d} = -W^2 (z \mu \cos \alpha / \varepsilon r^2)} \qquad (3.1a)$$

where **z** is the ion's charge, μ the dipole moment of the polar molecule, α the angle between a line **r** between the ion and the dipole's center. Relative electric permititivity, ε, characterizes the ability of the solvent to pass a charge, and is high for dissociated solvents (81 F/m for water) and lower for less polar solvents (27 for ethanol). **W** is a constant that depends on the system used. The dipole moment[234] μ is equal to

$$\mu = \mathbf{q R} \qquad (3.1b)$$

where **q** is the charge magnitude and **R** the separation between charges.

In practical terms, the addition of a less polar solvent to a mobile phase in which the analyte-CSP interaction is dependent on the ions in the mobile phase (such as Cu^{+2} in ligand exchange chromatography) would decrease the ion-dipole energy ($\mathbf{E_{i\text{-}d}}$) and thus decrease retention.

Dipole-dipole interactions occur between two polar molecules that possess permanent dipole moments. The degree of attraction is dependent on the orientation of the two molecules, and the population of the various orientations is controlled by the Boltzmann distribution, which in turn is highly influenced by temperature. The dipole-dipole interaction energy, $\mathbf{E_{d\text{-}d}}$, is given by

$$\mathbf{E_{d\text{-}d} = -W^2 (2/3 KT) (\mu_1^2 \mu_2^2 / \varepsilon r^6)} \qquad (3.1c)$$

where W is the system constant, **K** is the Boltzmann constant, **T** is the absolute temperature, μ_1 and μ_2 are the two dipole moments, ε is electric permittivity, and **r** is the distance between dipoles.

A high attractive dipole-dipole interaction energy is favored when the molecules are oriented so that the positive dipole is near the negative dipole. At high temperatures, all dipole orientations are equally populated, and at low temperatures high dipole-dipole interaction is favored.

From the above relationship, it can be inferrred that the enantioseparation on a CSP would be favored at low temperature if dipole-dipole interactions are critical for chiral discrimination.

Dipole-induced dipole interactions exist between polar and nonpolar molecules. The nonpolar molecule is polarized, inducing a dipole moment, due to the presence of the polar molecule that possesses a permanent dipole. The induced dipole moment, in this situation, is not affected by temperature since it cannot be disoriented from the direction of the inducing moment[235]. The interaction energy, $\mathbf{E_{d\text{-}id}}$, is

$$\mathbf{E_{d\text{-}id} = -W^2\ [0.5\ (\alpha_1\, E_2^{\,2} + \alpha_2\, E_1^{\,2})]} \qquad (3.1d)$$

where **W** is the system constant, α is the polarizability of molecules 1 and 2, $\mathbf{E_2}$ and $\mathbf{E_1}$ are the electric fields generated by the polar and nonpolar molecules, respectively.

London dispersion forces exist between all types of polar and nonpolar molecules. These dispersion forces arise from the imbalance concentration of electron cloud around a molecule that induces a temporary dipole moment on a nearby molecule. The energy associated with dispersion forces, $\mathbf{E_{disp}}$, is

$$\mathbf{E_{disp} = -W^2\ (3/2)\ (I_1\, I_2 / I_1 + I_2)\ (\alpha_1\ \alpha_2 / \varepsilon\ r^6)} \qquad (3.1e)$$

where $\mathbf{I_1}$ and $\mathbf{I_2}$ are the ionization potentials for the two interacting molecules. There is no temperature dependence of $\mathbf{E_{disp}}$ since molecular orientation does not affect the random instantaneous dipoles.

Dipole-dipole, dipole-induced dipole, and London dispersion interactions are non-specific, low energy attractive forces. The total potential energy for dipole-dipole, dipole-induced dipole, and London forces are

$$\mathbf{E_{total} = E_{d\text{-}d} + E_{d\text{-}id} + E_{disp}} \qquad (3.1f)$$

The decreasing strength of these interactions is: dipole-dipole > dipole-induced dipole > induced dipole-induced dipole.

The last possible attractive interactions between the analyte and CSP are the electron donor-acceptor interactions. These involve a transfer of an electron pair residing in a higher energy orbital (the donor) to a vacant, lower energy orbital (the acceptor). Generally, in the analyte-CSP interactions, the π electrons are involved, e.g. π-π

interactions between phenyl moieties. Kaliszan notes that the energy for electron donor-acceptor interactions involving neutral species, such as benzene-1,3,5-trinitrobenzene, is weaker (~-8 kJ/mol) compared to those formed between charged species (~-188 kJ/mol).

Another important interaction between analyte and CSP is negative interaction or repulsion. This arises from the proximity of like charges. Huheey[236] notes that at very short distances the electron clouds begin to repel one another (Pauli exclusion priciple). The relationship between repulsive energy, $\mathbf{E_R}$ and distance is

$$\mathbf{E_R = + \mathit{k} / r^n} \qquad (3.1g)$$

where ***k*** is a constant and **r** is the internuclear distance. The Leonnard-Jones function that describes attractive-repulsive interactions between two molecules as given by Kaliszan is

$$\mathbf{E_{(r)} = - (a / r^6) + (b / r^m)} \qquad (3.1h)$$

where $\mathbf{(a / r^6)}$ is the attractive term, and $\mathbf{(b / r^m)}$ is the repulsive term with a value for **m** between 9 and 12.

Table III summarizes the all the possible analyte-CSP interactions, relative strengths, and distances between the interacting molecules.

3.2 Thermodynamics of Enantioseparation

The separation of the R and S enantiomers on a chiral stationary phase involves the reversible formation of a pair of reversible diastereomeric analyte-CSP complexes[238], **[R•CSP]** and **[S•CSP]**:

$$\mathbf{R_M + CSP = [R \bullet CSP]} \qquad (3.2a)$$

$$\mathbf{S_M + CSP = [S \bullet CSP]} \qquad (3.2b)$$

where $\mathbf{R_M}$ and $\mathbf{S_M}$ represents the R and S analytes in the mobile phase, before interacting with the CSP. The differences in the stability between these diastereomeric complexes leads to a difference of retention time. The enantiomer that forms the less stable complex

Table III. A summary of intermolecular attractions between analyte and CSP for diastereomeric complexes formation. (After Huheey, Ref 237.)

Interaction Type	Relative Strength	Working Distances
Hydrogen bond	Very Strong	Long range
π-Electron Donor-Acceptor	Strong	Moderately long range
Ion - Dipole	Strong	$1/r^2$, short range
Dipole - Dipole	Moderately Strong	$1/r^3$, short range
Dipole - Induced Dipole	Weak	$1/r^6$, very short range
London Dispersion Forces	Very Weak	$1/r^6$, extremely short range

will be eluted first. These diastereomeric complexes must therefore differ adequately in free energy for an enantiomer separation to be observed.

In chromatography, the magnitude of solute retention is expressed by the retention factor, ***k***, which is a measure of the stoichiometric mass distribution of analyte between the stationary and mobile phases[239].

k = analyte mass in stationary phase / analyte mass in mobile phase (3.2c)

For the enantiomers R and S, their respective retention factors $\boldsymbol{k_R}$ and $\boldsymbol{k_S}$ expressions are

$$\boldsymbol{k_R} = \mathbf{(R{\bullet}CSP)_S} \;/\; \mathbf{R_M} \qquad (3.2d)$$

$$\boldsymbol{k_S} = \mathbf{(S{\bullet}CSP)_S} \;/\; \mathbf{S_M} \qquad (3.2e)$$

where $\mathbf{(R{\bullet}CSP)_S}$, $\mathbf{(S{\bullet}CSP)_S}$, $\mathbf{R_M}$, and $\mathbf{S_M}$ are the masses of R and S in the stationary phase and mobile phase, respectively.

Expressing k_R and k_S in terms of molar concentrations

$$\boldsymbol{k_R} = \mathbf{[R\text{-}CSP]_S\ V_S} \;/\; \mathbf{[R]_M\ V_M} \qquad (3.2f)$$

$$\boldsymbol{k_S} = \mathbf{[S\text{-}CSP]_S\ V_S} \;/\; \mathbf{[S]_M\ V_M} \qquad (3.2g)$$

where $\mathbf{[R\text{-}CSP]_S}$, $\mathbf{[S\text{-}CSP]_S}$, $\mathbf{[R]_M}$, and $\mathbf{[S]_M}$ are the molar concentrations of R and S in the stationary phase, and $\mathbf{V_S}$ and $\mathbf{V_M}$ are the volumes of the stationary phase and mobile phase respectively. In a given column the volume ratio of the stationary and mobile phases, $\boldsymbol{\varphi}$, is fixed so that the mass distribution ratio is simply given by

$$k_R = \varphi \ ([R\text{-}CSP]_S / [R]_M) \qquad (3.2h)$$

$$k_S = \varphi \ ([S\text{-}CSP]_S / [S]_M) \qquad (3.2i)$$

Considering the equilibrium distribution of the R and S enantiomers between the stationary and mobile phases, the respective equilibrium constants denoted by K_R and K_S are:

$$K_R = [R\text{-}CSP]_S / [R]_M \qquad (3.2j)$$

$$K_S = [S\text{-}CSP]_S / [S]_M \qquad (3.2k)$$

Eqn. 3.2j and 3.2k can be rewritten as

$$k_R = \varphi K_R \qquad (3.2l)$$

$$k_S = \varphi K_S \qquad (3.2m)$$

The enantioselectivity, α_{RS}, which describes the selective interaction of the enantiomers with the chiral stationary phase is

$$\alpha_{RS} = k_R / k_S \qquad (3.2n)$$

when $k_R > k_S$.

Because retention factor is directly related to its equilibrium distribution constant, and from the relationship of the Gibbs free energy change and the distribution equilibrium constant, K,

$$\Delta G = - RT \ln K = - RT \ln (k / \varphi) \qquad (3.2o)$$

Eqn. 3.2p becomes

$$\alpha_{RS} = \exp[\sum(-\Delta G_R / RT)] / \exp[\sum(-\Delta G_S / RT)] \qquad (3.2p)$$

where the $\sum\Delta G_R$ and $\sum\Delta G_S$ are the total molecular free energies of adsorption of the R and S enantiomers, respectively.

In the case of enantiomers, all analyte-stationary phase interactions except chiral interactions, $\sum\Delta G$, are identical and cancel.[240]

$$\alpha_{RS} = \exp\sum[(-\Delta G_R - \Delta G_S) / RT] \qquad (3.2q)$$

Rearrangement of Eqn. 3.2s gives the relationship between α_{RS} and $\Delta(\Delta G)$, the difference in the molar free energy of the interaction for the two enantiomers with a chiral stationary phase.

$$\alpha_{RS} = \exp\sum[-\Delta(\Delta G) / RT] \qquad (3.2r)$$

The $\Delta(\Delta G)$ difference is solely responsible for enantioselectivity. It must be remembered that the enantiomers have equal internal energies, their solvation enthalpies are equal in a given mobile phase. The larger the value of $\Delta(\Delta G)$ the better is the enantiomeric separation. Since $\Delta(\Delta G)$ determines enantioselectivity, the designer of a CSP should maximize $\Delta(\Delta G)$ while minimizing the adsorption energies, ΔG_R and ΔG_S. Large ΔG_R and ΔG_S lead to long retention times and broad chromatographic peaks.

Applying the Gibbs-Helmholtz , G = H - TS, Eqn. 3.2t becomes

$$R \ln \alpha_{RS} = - \Delta(\Delta H) / T + \Delta(\Delta S) \qquad (3.2s)$$

where R is a gas constant, $\Delta(\Delta H)$ and $\Delta(\Delta S)$ are the differences in the enthalpy and entropy of the interactions of R and S enantiomers with the stationary phase, respectively.

The $\Delta(\Delta H)$ and $\Delta(\Delta S)$ values can be obtained by measuring the α_{RS} of an enantiomeric pair at different temperatures and plotting $R \ln \alpha_{RS}$ versus $1 / T$ (Fig. 9). If $\Delta(\Delta H)$ is a constant within the temperature range, a straight line should be obtained. In chromatography, this means that the retention mechanism is invariant under the temperature range being studied is invariant. The slope is $\Delta(\Delta H)$ and the intercept is $\Delta(\Delta S)$.

In addition, as follows from the Gibbs-Helmholtz relationship, there exists a certain temperature, called isoenantioselective temperature, T_{iso}, at which $\Delta_{RS}(\Delta G)$ is zero with no enantiomer resolution.[241-242]

$$T_{iso} = \Delta_{RS}\Delta H / \Delta_{RS}\Delta S \qquad (3.2t)$$

At temperatures higher than T_{iso}, the enantiomer elution order should be reversed. Evidence for the reversal of enantioselectivity has been found in gas chromatographic

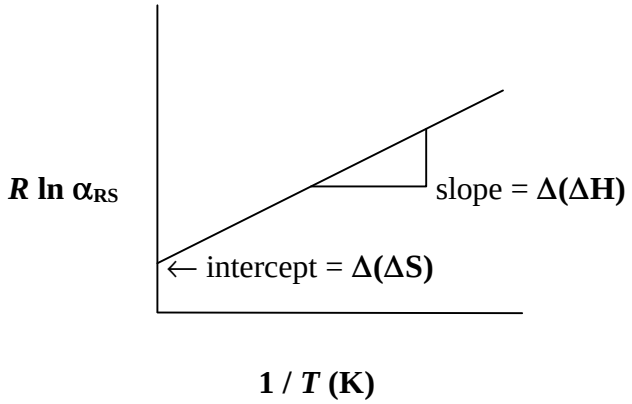


Figure 9. Van't Hoff type plot. $\boldsymbol{R \ln \alpha_{RS}}$ versus $\boldsymbol{1 / T}$: slope is $\boldsymbol{\Delta(\Delta H)}$ and intercept is $\boldsymbol{\Delta(\Delta S)}$.

enantiomer separations.[243-248] Below the isoenantioselective temperature, enantioselectivity is governed by $\boldsymbol{-\Delta_{RS} \Delta H}$ and above it by $\boldsymbol{\Delta_{RS} \Delta S}$[249].

Another way of determining the $\boldsymbol{\Delta(\Delta H)}$, $\boldsymbol{\Delta(\Delta S)}$, and $\boldsymbol{\Delta_{RS}}$ $\boldsymbol{(\Delta G)}$ is by plotting the logarithm of the retention factor, $\boldsymbol{\ln k}$, against the reciprocal of absolute temperature, $\boldsymbol{1/T}$ (van't Hoff plots) for both R and S enantiomers (Fig. 10).

From Eqn. 3.2q

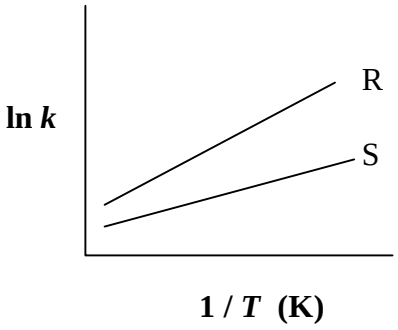
$$\boldsymbol{\ln k = - \Delta H / RT + \Delta S / R} + \ln \boldsymbol{\varphi} \qquad (3.2u)$$

To a first approximation ,

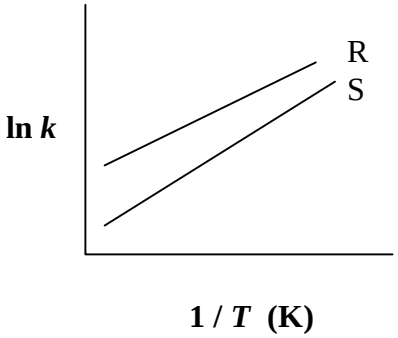
$$\boldsymbol{\ln k = - \Delta H / RT + \Delta S / R} \qquad (3.2v)$$

where the slope is $\boldsymbol{\Delta(\Delta H)}$ and the intercept, $\boldsymbol{\Delta(\Delta S)}$. A linear van't Hoff curve indicates that the retention mechanism under the temperature range being studied is invariant. At the $\boldsymbol{T_{iso}}$, the two ln k vs. 1/T lines cross each other (Fig. 10c).

(a) Enthalpy Controlled



(b) Entropy Controlled



(c) T_{iso}

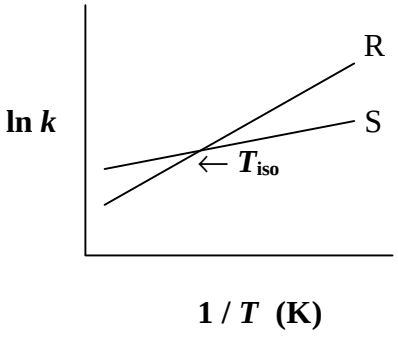


Figure 10. Plots of **ln *k*** agains **1 / *T* (K)** (van't Hoff plots): (a) enthalpy-controlled enantioseparation; (b) entropy-controlled enantioseparation; and (c) isoenantioselective temprature, T_{iso}.

As noted in Chapter 1, the influence of temperature on retention is almost always unidirectional. An increase in temperature usually decreases the retention of analytes due,

on a molecular level, to the faster migration of analyte molecules through the chromatographic column. However, the enantioselectivity, α_{RS}, may increase or decrease with an increase in temperature.

According to Schurig and others[249-250], the separation of enantiomers on a CSP is said to be enthalpy driven when the enthalpy term, **Δ(ΔH)** dominates over the entropy term, **Δ(ΔS)**. In this case, lowering the temperature improves enantioselectivity and thus, the enantioseparation. Considering the vant Hoff plots of the R and S enantiomers (Fig 10a), this is manifested by a large difference in slopes that corresponds to **Δ(ΔH)**. That is, the two lines are diverging from each other.

For an entropy controlled enantioseparation, **Δ(ΔS)** dominates over **Δ(ΔH)**. This means a decrease in temperature decreases enantioselectivity. The vant Hoff plots of the R and S enantiomers (Fig 10b) shows a large difference in the intercepts of the two lines, which corresponds to **Δ(ΔS)**. From the method development point of view, increasing the temperature helps optimize the entropy controlled enantioseparation.

3.3 Enthalpy-Entropy Compensation

As noted in Chapter II, the enthalpy-entropy compensation method was used by Melander, Campbell and Horvath[251] in 1978 in reversed phase HPLC to study hydrophobic interactions and separation mechanisms. This is an extra-thermodynamic approach to the analysis of physico-chemical data, such as chromatographic retention data. The term extra-thermodynamic denotes the fact that they fall short of a rigorous thermodynamic foundation but they can still be very helpful in interpreting the data. In the enthalpy-entropy compensation analysis, a similarity in values for the compensation temperature suggests that the analytes are retained by the stationary phase through essentially identical interaction mechanisms, thus, the compensation study is a useful tool for comparing retention mechanisms in different chromatographic systems.[252]

Enthalpy-entropy compensation is expressed by

$$\Delta H^\circ = \beta \Delta S^\circ + \Delta G^\circ_\beta \qquad (3.4a)$$

where $\Delta G°_{\beta}$ is the Gibbs free energy of a physico-chemical interaction at the compensation temperature β, and $\Delta H°$ and $\Delta S°$ are the corresponding standard enthalpy and entropy, respectively. The above equation means that in the vicinity of β changes in $\Delta H°$ are offset by changes in $\Delta S°$ so that the Gibbs free energy change is independent of temperature. When enthalpy-entropy compensation is observed with a family of compounds in a particular chemical transformation, then the values of β and $\Delta G°_{\beta}$ are invariant.

Using the Gibbs-Helmholtz relation, G = H - TS, Eqn. 3.1a can be rewritten to express the free enrgy change, $\Delta G°_{T}$, measured at a fixed temperature, T, for the compensation process.

$$\Delta G_T° = \Delta H° (1 - T / \beta) + T \Delta G°_{\beta} / \Delta G_T° / \beta \qquad (3.4b)$$

According to Eqn. 3.4b, a plot of $\Delta G_T°$ against the corresponding $\Delta H°$ yields a straight line and the compensation temperature can be evaluated from the slope. When this behavior is observed, the species involved in the transformations are believed to share common physico-chemical properties that play an important role in determining the energetics of the process.[253] Conversely, if a particular chemical transformation fails to conform to the common compensation pattern observed with the related process, it is assumed to have different physico-chemical properties.

Chromatographic retention is conveniently measured by the dimensionless retention factor, k, which is related to the thermodynamic equilibrium constant K, for the analyte association with the stationary phase by

$$k = \varphi K \qquad (3.4c)$$

where φ is the volume ratio of the stationary and mobile phases. The free energy change for the process is expressed by

$$\Delta G° = - RT \ln K = - R T \ln (k / \beta) \qquad (3.4d)$$

Using the Gibbs-Helmholtz equation, G = H - TS, and rearranging Eqn. 3.4d yields

$$\ln k = - \Delta H° / RT + \Delta S° / R + \ln \varphi \qquad (3.4e)$$

If the mechanism of the retention process is invariant over the temperature range investigated and the enthalpy is constant, a plot of ln k against 1/T, which is commonly referred to as a van't Hoff plot, yields a straight line.

Combination of Eqn. 3.4b and 3.4d give

$$\ln k_T = - \Delta H^\circ / R (1 / T - 1/ \beta) - \Delta G_\beta^\circ / Rb + \ln \varphi \qquad (3.4f)$$

where k_T is the retention factor at temperature T. According to Eqn 3.4f, plots of the capacity factors of various solutes measured at a given temperature under different conditions against the corresponding enthalpy change are linear, when the compensation occurs, e.g., the reversible binding of analyte by the stationary phase involves only one mechanism.[254]

3.4 Generalized Chiral Separation Mechanism

As noted in Chapter II, chiral separation on a CSP is an adsorption-desorption process involving diastereomeric complexes possessing different internal energies.[255] Pirkle and Welch[256] described the series of adsorption-desorption equilibria taking place during enantioseparation. Icons were used to show the interactions of analyte enantiomers, CSP, and mobile phase for the formation of diastereomeric complexes leading to the separation of enantiomers. For the enantiomers to be completely separated, the difference in the Gibbs free energy of the diastereomeric complexes must be great enough. Thirteen possible schemes were outlined, and a summary of the essential adsorption and desorption steps are shown in Fig. 11.

In the first stage, the solvent molecules are adsorbed onto the active sites of both CSP and analyte enantiomers. The solvent molecules referred to, in this case, are the modifier molecules. Usually the mobile phase in HPLC consists of a binary mixture of solvents: e.g., an apolar solvent and a polar solvent (the modifier) such as alcohol in the normal phase mode. The modifier is the solvent that compete with the analyte for the interaction sites of the stationary phase. As the analyte band moves through the column,

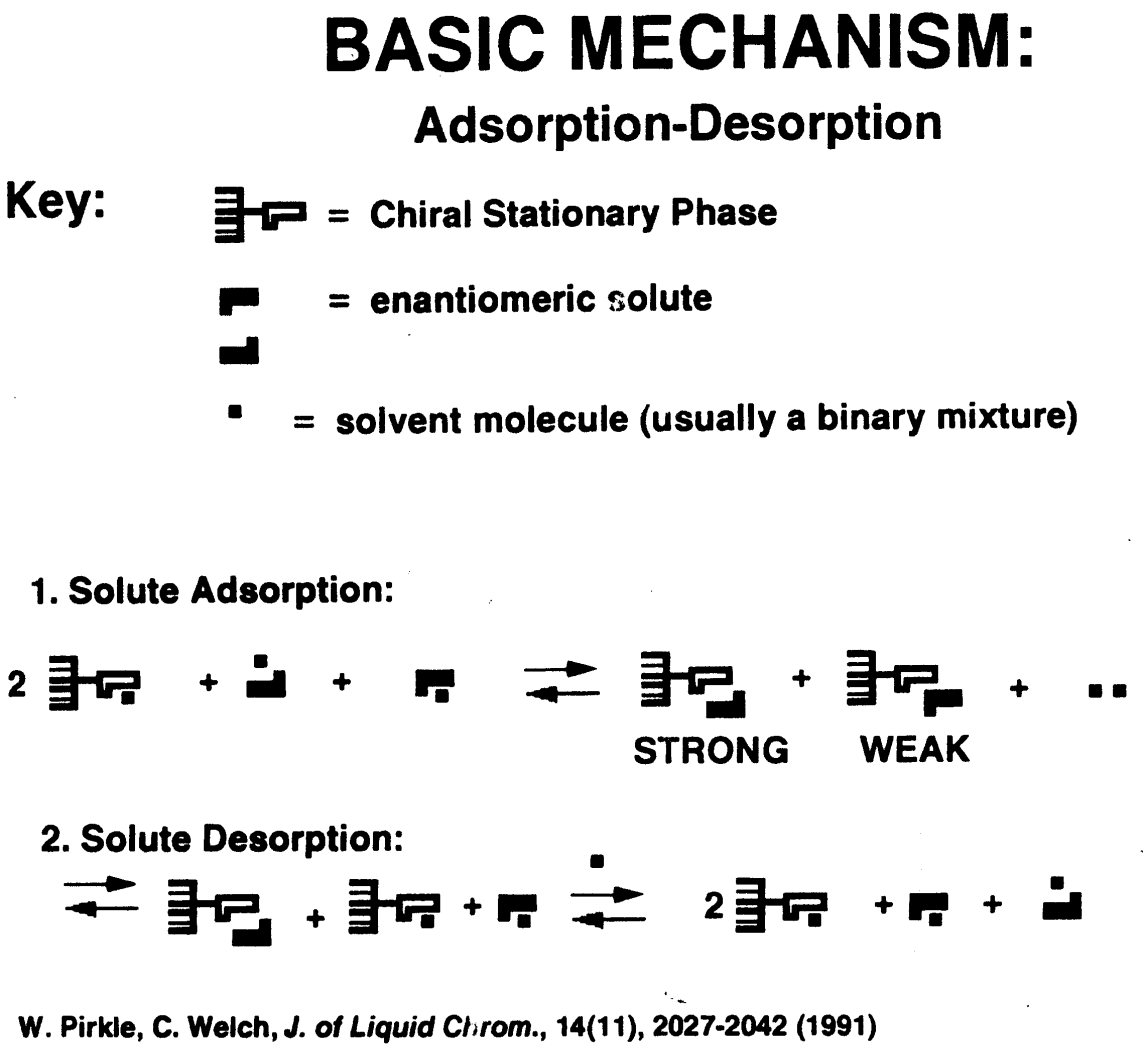


Figure 11. Adsorption-desorption equilibria for the interaction of enantiomer analytes, CSP, and solvent molecule during the enantioseparation process.

the first of the many analyte adsorptions takes place as the analyte displaces a solvent molecule from the CSP. Stronger and weaker diastereomeric complexes possessing different internal energies are formed. Another solvent molecule then selectively resolvates the weaker analyte-CSP complex, and sometime later the stronger complex will be solvated. The difference in the Gibbs free energy of fomation for the two diastereomeric complexes determines the difference in total time each complex is in existence, and hence the difference in retention times. The ratio of the retention of these complexes, expressed as capacity factors, determines enantioselectivity.

3.5 Chiral Recognition Mechanisms for Derivatized Amylose and Derivatized Cellulose CSPs

Amylose and cellulose, as mentioned in Chapter 2, are biopolymers consisting of D-glucose units but are linked differently, i.e., the former has an α-(1,4)-D-glucose linkage and the latter is a β-(1,4)-D-glucose linkage. The different glucose linkages give rise to the different higher order structures of these polymers in which the polymer chains are held by intramolecular and intermolecular hydrogen bonds. Chirality of these polymers reside on the individual glucose residues (each glucose unit has three chiral centers) and the helical grooves or cavities formed between the D-glucose units.

Derivatized amyloses and derivatized celluloses as CSPs display better chiral recognition properties than the corresponding native forms.[257-258] Examples are amylose tris(3,5-dimethylphenylcarbamate), Chiralpak AD, and cellulose tris(3,5-dimethylphenyl-carbamate), and Chiralcel OD. Both CSPs consist of tris(3,5-dimethylphenylcarbamate)-D-glucose units as chiral adsorbing sites. It has been reported that the arrangements of the tris(phenylcarbamate)-D-glucose units along the polysaccharide chain differ between amylose and cellulose. On the basis of X-ray analysis, the structures of the tris(phenylcarbamate)s of amylose and cellulose (Fig. 12) are left-handed fourfold (4/1) and three fold (3/2) helices[259-260], respectively. Both derivatized polysaccharides, therefore, form a chiral helical cavity of tris(phenylcarbamate)-D-glucose

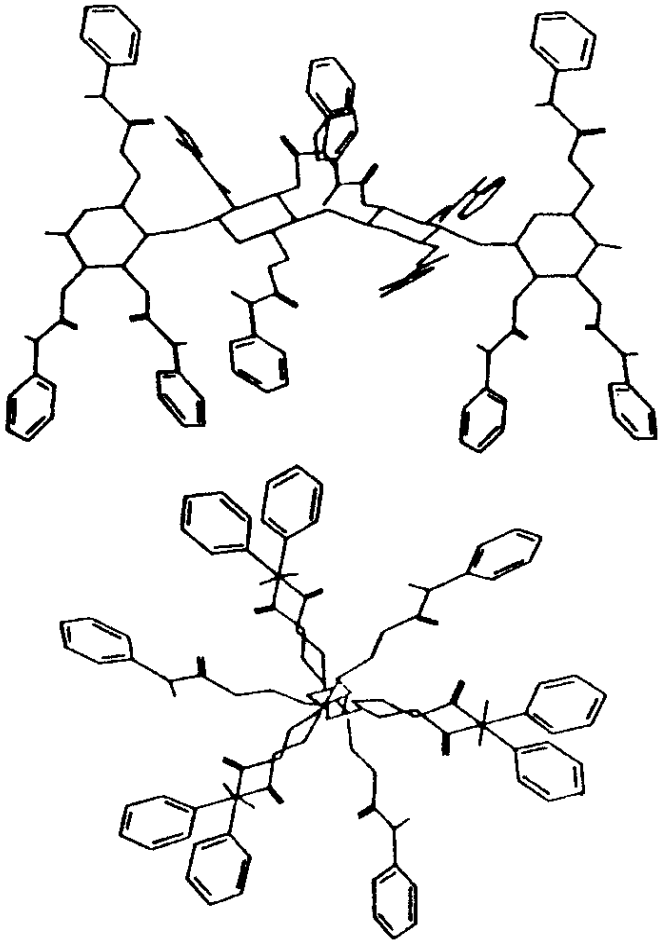


Figure 12. Structure of cellulose trisphenylcarbamate[261]. Top, along the chain axis; bottom, perpendicular to the chain axis.

units such that inclusion or steric fit of a racemate may be efficiently discriminated into enantiomers.

The general mechanism for the chiral recognition of Chiralpak AD or Chiralcel OD involves attractive interactions (Type 1 mechanism) and inclusion[263-265] of analytes to the chiral cavities (Type 3 mechanism). Attractive interactions between the analyte and these Type 2 CSPs occur mainly at the 3,5-dimethylphenylcarbamate moiety of the CSP. The possible interactions of the analyte with the CSP include: (a) hydrogen bonding by the carbonyl oxygen and amide proton; (b) dipole interaction that is inherent of the polar carbamate moiety; and (c) π interactions by the phenyl ring. Because of these combined mechanisms (attractive-repulsive interactions and inclusion) operating in Chiralpak AD and Chiralcel OD, these CSPs offer a great versatility for separating a wide range of chiral compounds.

At the start of this research in 1995, there were only a few in-depth studies for the chiral recognition mechanisms of Chiralpak AD and Chiralcel OD. In 1992, Witte *et al.*[266] studied the chromatographic behavior of twenty one racemic amino tetralins on Chiralcel OD at different temperatures and two mobile phase compositions to make conclusions on the chiral recognition mechanism. The serious shortcomings of this mechanistic study, however, are: (a) the authors did not take into account the inclusion mechanism, only attractive-repulsive interactions were considered; (b) the mechanism was correlated with resolution, which is dependent on three chromatographic parameters of retention factor, efficiency (theoretical plates), and enantioselectivity; and (c) only one mobile phase modifier, ethanol, was considered.

The mechanism suggested by Witte *at al.*[267] for the enantiomeric separation of the amino tetralins on Chiralcel OD was that hydrogen bonding is the strongest attractive force between the analyte and CSP, and the π-π interaction forces are secondary. Further, according to the author, the possibility of solute-CSP "free" π-π interactions should greatly enhanced the enantioresolution, and this π-π interaction depends greatly on temperature. The conclusion that a decrease of temperature does not improve the resolution for racemic aminotetralins with a primary amine, which are dependent on

hydrogen bonding, is not proven by their data. Their results show only one racemic aminotetralins which exhibited improved resolution at high temperatures.

Wainer *et al*. in 1996[268], as noted in Chapter II, studied the chiral recognition mechanism for the separation of chiral 2-alkylaryl propionic acids on Chiralpak AD using quantitative structure-enantioselective retention relationships (QSERR) including hydrogen bonding ability and aromaticity of the analytes. However, in this study, only one mobile phase composition was used, 95/5/0.1% hexane/2-propanol/TFA. Moreover, as what has been noted in Section 2.4.3, the QSERR analysis does not involve descriptors for the mobile phase molecules, i.e., only analyte-CSP interactions are considered. Nevertheless, the authors proposed that the chiral recognition mechanism involves hydrogen bonding as the dominant interaction, insertion to chiral cavities and further attractive interaction (a secondary one), and the mechanism is conformationally driven. Steric fit to the chiral cavities is not critical for the chiral recognition process in their mechanism.

Wainer *et al*.[269] in the same year studied the enantioselective mechanism for the separation of mexiletine and related compounds on Chiralpak AD using QSERR and linear free energy relationships. Again, this study did not consider the influence of mobile phase composition. Only one mobile phase composition was used, 95/5/1% hexane/ethanol/TFA. Nonetheless, the results of the study indicated that there were two separate retention mechanisms, which are based on the presence or absence of the secondary hydrogen-bonding groups.

From the above discussion, there is still a need to determine the enantioselective mechanism for Chiralcel OD and Chiralpak AD. Enantioseparations on Chiralpak AD and Chiralcel OD CSPs are difficult to predict because their higher order structures greatly influence enantioselectivity. Moreover, there are two main type of chiral recognition mechanisms that may exist: (a) attractive interactions followed by steric fit; and (b) inclusion followed by attractive interactions, in which steric fit to the chiral cavities is critical. Other possible types of chiral recognition may also exist, such as that concluded by Wainer *et al*. for Chiralpak AD.

In addition, according to Grinberg *et al.*[270], the enantioselectivity for derivatized cellulose CSPs, which can be applied to derivatized amylose CSPs, is dependent upon a number of factors. Among these factors are the type of derivatized functional group, the derivatization conditions, the morphology of the support, the temperature, and eluent composition. It is presumed that these factors bring changes in the higher order structures that provide a multitude of structurally different cavities.[271] For the adsorption sites that are readily accessible, the adsorption/desorption process is fast. For those sites that are less accessible for bulky analytes, the adsorption/desorption process is considerably slower. The restricted sites seem to be critical for chiral recognition.[272]

It was reported by Ichida *et al.*[273] that chiral recognition depends on the type of functional groups present on the amylose and cellulose derivatives. The enantioselectivity of a chiral molecule on a derivatized CSP with different chiral selectors, e.g., an ester and carbamate, is different due to the different interactions between enantiomers and CSP. Furthermore, it was shown that, for CSPs with the same derivative, enantioselectivity depends on the derivatization process.[274]

The morphology of support have also been shown to affect the enantioselectivity. It was reported that a reversal of enantioselectivity was observed for the same enantiomeric pair separated on amorphous and crystalline CTA.[275] Moreover, CTA coated on a silica gel support showed to have better chiral selectivities than when it is in the pure form.[278]

Variation of temperature also affects the higher order structure of the derivatized cellulose and amylose CSPs. Conformational changes from a semirigid form to a flexible form were reported for cellulose tricarbanilate with increasing temperature.[279] The conformational changes were attributed to a change in the helical structure brought on by hydrogen bond breakage.[280] Such conformational change affects the geometry and/or size of the chiral cavities, and consequently, enantioselectivity.

The eluent composition also influences enantioseparating abilities of the derivatized amylose cellulose and derivatized cellulose. The effect is mainly attributed to the solvent molecules (mobile phase modifiers) that interact with the CSP. It was reported that nonswollen CTA shows only weak ability for chiral recognition, whereas solvent-swollen CTA shows increased enantioselectivity.[281] The degree of swelling of the CSP depends on

the dielectric constant and hydrogen bonding capability of the solvent, with lower alcohols inducing greater swelling than ethers or alkanes. The alcohols with higher dielectric ocnstants interact strongly with the acetoxy group of CTA through hydrogen bonding.[282] Consequently, changes in the adsorption sites through modification of the size and geometry of the chiral cavities affect retention and enantioselectivity. It must be noted here that that the effect of the mobile modifiers is very important in the separation of chiral compounds.

Considering all these factors that can bring changes to the higher order structures of derivatized amylose and cellulose CSPs, there is an utmost need in defining the chiral recognition mechanism for the CSP. A complete understanding of the chiral recognition process is the key for a successful separation of racemic compounds on an HPLC CSP. For a given CSP, this requires: (a) a full knowledge of the chemistry of the racemic analytes and CSP, including the higher order stucture of the CSP, (b) the influence of mobile phase, specifically the modifier, on retention and enantioselectivity, and (c) the dependence of retention and enantioselectivity on temperature.

In this dissertation, most of the studies for the chiral separations of 2-methylarylpropionic acids on Chiralpak AD and Chiralcel OD, temperature dependence and mobile phase effects were already performed before Wainer *et al*. in 1996[283] reported the possible chiral recognition mechanism for Chiralpak AD based on QSERR and enthalpy-entropy compensation studies. Similar results to Wainer's were obtained in this research for the enantioseparation of 2-methylaryl propionic acids on Chiralpak AD.