CHAPTER II

THEORY

2.1 Gas chromatographic separation of enantiomers

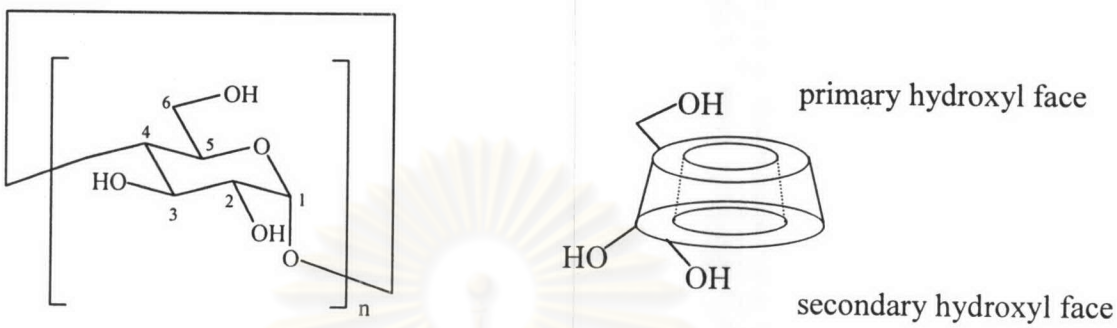
Enantiomer separation by gas chromatography (GC) can be attained by two methods: indirect and direct. With indirect separation, enantiomers are reacted with an enantiomerically pure resolving agent and then are converted into diastereomers, which differ in physical and chemical properties from each other. Subsequently, the mixture of diastereomers is separated by conventional gas chromatography. However, this method requires high enantiomeric purity and stability of chiral resolving agent [2]. Moreover, the derivatization process is frequently time-consuming and may cause discrimination due to kinetic resolution, incomplete recovery, decomposition, or loss during work-up, isolation, and sample handling [2, 21-22].

The direct enantiomer separation, a more commonly used and effective method, is based on the formation of reversible diastereomeric complexes between a chiral analyte and a chiral resolving agent, also acting as a stationary phase. Several types of chiral selectors have been formerly reported, i.e. amino acid and dipeptide derivatives, chiral organometallic chelate complexes, and derivatives of carbohydrate [2, 6, 21-23]. Normally, cyclodextrin derivatives are the preferred chiral selectors for direct gas chromatography.

2.2 Cyclodextrins and their derivatives

Cyclodextrins (CDs) are cyclic oligomers composed of D-glucose units linked together by α-1,4-glycosidic bonds (figure 2.1 (a)). Generally, CDs with six, seven, and eight glucose units, assigned with Greek letters as α-, β-, and γ- CDs, respectively, are used in analytical applications. The native CD has a shape of truncated cone having a cavity with primary hydroxyl groups (C6-OH) on its narrower opening and secondary hydroxyl groups (C2-OH and C3-OH) on the opposite, wider edge (figure 2.1 (b)). As a result of all hydroxyl groups directing outwards from the cavity, the outside of the molecule is hydrophilic while the inside

is relatively hydrophobic. Because of their macrocyclic, conical structure and inherent chirality, CDs (as host molecules) are able to form diastereomeric complexes with a wide variety of chiral guest molecules. Consequently, CDs have turned out to be very versatile chiral selectors for enantioseparation [6, 20, 22-24].



(a) (b)

Figure 2.1 (a) A native cyclodextrin molecule with *n* glucose units
(b) Schematic representation of CD structure, showing primary hydroxyl side and secondary hydroxyl side

Hydroxyl groups at position C2, C3, and C6 of glucose units can be easily modified by chemical reactions and accordingly yield numerous derivatives with different substitution patterns. As a rule, CD derivatives, pure or diluted in polysiloxane, are preferable to the native ones for the use as chiral selectors in gas-liquid chromatography since the native CDs decompose instead of melting at working temperature range [20]. For most derivatized CDs that are solids at room temperature and/or produce non-homogeneous film coatings, they are usually diluted in achiral polysiloxane in order to improve their properties as stationary phases. These stationary phases provide high column efficiency with broad operating temperature range [20].

2.3 Mechanistic aspects of chiral separation

As mentioned above, the enantiomer separation occurs via the formation of reversible diastereomeric complexes between the CD derivatives and each enantiomer of chiral analytes. The enantioselectivity of the separation results from the different stabilities of these complexes. Nonetheless, the mechanism of enantiomeric separation thus far has been still ambiguous.

Based on a number of previous publications [7, 10-14, 20, 22-23], CDs and their derivatives exhibit a wide spectrum of enantioselectivity for enantiomers of various classes of compounds. This can be attributed to some unique features of CD molecules: numerous chiral centers and induced fit mechanism. In each glucose unit, all chiral centers have different orientation and distance from one another; in turn, the shapes of the glucose units do not repeat themselves from unit to unit. Additionally, CD shape is changeable to interact closely with guest molecules (induced fit interactions) [20].

It is proposed that the possible mechanisms are concerned in the inclusion of analytes either with the whole molecules or with their hydrophobic parts into the CD cavity, and in the interaction of the analytes with the outside hydrophilic part of the CD molecule. Moreover, many types of interactions between CDs and analytes are important to the separation processes and mechanisms, for example inter alia inclusion, hydrogen bonding, dispersion forces, dipole-dipole interaction, electrostatic interaction, and hydrophobic interaction [6].

2.4 Parameters affecting enantioseparation

Based on previous works [7-15, 18-23, 25-36], the enantioseparation by GC using CD derivatives as CSPs is influenced by the type of CD, the substitution pattern on the CD ring, the polarity of polysiloxane matrix, the concentration of CD in polysiloxane, and the structure of analytes.

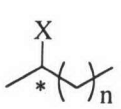
The CD ring size and the substituents of glucose units at C2, C3, and C6 positions considerably affect chemical, physical properties as well as enantioselectivity of CD derivatives. Owing to the differences in the number of glucose units in their structure, α-, β-, and γ-CDs possess different cavity size. This affects inclusion-complexation mechanism of some analytes, which can completely or partly accommodate in the CD cavity. In addition, the chiral recognition depends on the types of substituents and their positions on the CD molecules. Normally, substituents at chiral C2 and C3 positions (mainly alkyl or acyl groups) impact the enantioselectivity while those at nonchiral C6 position (mostly alkyl or bulky groups) generally affect polarity, melting point, and solubility in polysiloxane. However, it has been recently reported that bulky groups (e.g. *tert*-butyldimethylsilyl) at C6

position have an influence on the conformation of the CD ring, which in turn can impact on the enantioselectivity [6].

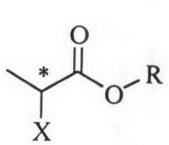
When modified CDs are used as solutions in polysiloxanes, the concentration of the CD derivatives and the polarity of the polysiloxane solvents affect the enantioselectivity as well. In general, in the usual temperature range of enantioselective GC, increasing the CD contents in polysiloxanes and/or decreasing the polarity of polysiloxanes improve enantioselectivity. However, at higher CD concentration, the enantioselectivity tends to level off, and the loss of efficiency of CSPs has been observed [20, 33, 37].

Nevertheless, the influence of all aforementioned parameters on enantioseparation is also dependent upon the structure of analytes such as the type, size, and position of substituent. In general, the studies concerning enantiomeric separation keep all contributions to the chiral recognition constant but one. Commonly, the easiest factor to vary is analyte structure. Some examples of the investigation into the effect of analyte structure on separation and enantioselectivity are as follows.

Venema et al. [7] investigated the separation of enantiomers of 2-substituted alkanes and alkanoic acid esters on perpentyl β-CD. By varying the analyte structure, it was concluded that alkyl chain length and type of substituent affected enantioselectivity. For example, for bromoalkane homologues (C_4-C_7), the maximum selectivity value was observed for 2-bromopentane. Besides, substitution of chloro with bromo or iodo caused enantioselectivity to increase. Similar to alkanes, increasing ester chain length or decreasing the size of halogen resulted in the loss of enantioselectivity of 2-alkanoic acid esters. The results also indicated that the main contribution to enantioseparation of these groups of analytes was hydrophobic interactions. It was assumed that the apolar part of these molecules, alkyl chain and ester chain, was accommodated in the CD cavity.



alkanes



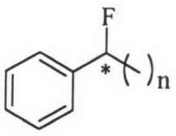
alkanoic acid esters

Smith and Simpson [8-9] studied the separations of several chiral alcohols as well as their acyl and fluoroacyl derivatives on a GC column coated with octakis(3-*O*-trifluoroacetyl-2,6-di-*O*-*n*-pentyl)-γ-CD. The chromatographic results for underivatized alcohols and their derivatives were rather different. Among two types of derivatives, only the fluoroacylated derivatives were separated into their enantiomers. In general, enantioselectivities of fluoroacyl derivatives are higher than those of underivatized alcohols. In some cases, derivatization caused the reversal of elution order for some alcohol analytes. It was also reported that the length of the longest carbon chain attached to the chiral center, the relative position of hydroxyl and methyl or fluoroacyl groups, multiple bonds in their molecules, and the size of the fluoroacyl group affected the enantioselectivity of both underivatized alcohols and their fluoroacylated derivatives in similar way. For example, among the group of 3-hydroxyalkanes and their derivatives, 3-hydroxyheptane exhibited the greatest enantioselectivity. The enthalpy-entropy compensation result also supported that the alcohols and trifluoroacetyl derivatives interacted with this phase by similar mechanism. Based on the data obtained, it was concluded that the hydrogen bonding and/or dipole-dipole interaction between the analytes and this phase seemed to be essential for stereoselective interactions.

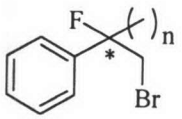
Berthod and co-workers [10] investigated the separation mechanism of various chiral compounds on 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl derivatized β- and γ-cyclodextrins (DP-TFA CDs). For many groups of compounds such as alkyl esters of 2-bromobutyric acid, by lengthening the alkyl side chain of homologous series the retention time increased, but enantioselectivity did not change. Based on thermodynamic data of these analytes on DP-TFA phase, it was proposed that there may be two chiral recognition mechanisms, one involving inclusion complex formation and the other concerning external association.

Reinhardt et al. [11] separated fluoroalkyl- and fluorobromoalkyl-substituted benzene by GC using permethylated CDs as chiral stationary phases. The results showed that the types and positions of the substituents as well as the size of the molecules influenced the chiral recognition. For instance, enantioselectivities of some fluoroalkyl-benzene derivatives decreased with increasing alkyl chain length. The results also suggested that various interactions, i.e. complete or partial inclusion,

hydrophobic and van der Waals interactions, contributed to the separation of enantiomers of the analytes.

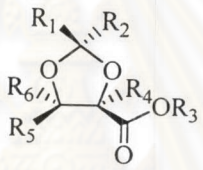


fluoroalkyl-substituted benzene



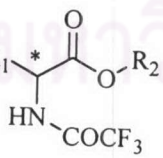
fluorobromoalkyl-substituted benzene

Jaques et al. [12-13] separated a series of 2,2-dialkyl-4-alkoxycarbonyl-1,3-dioxolane derivatives with some derivatized cyclodextrins. It was observed that the geometry of the analyte played an important role on the selectivity. Different substitution types and positions (R_1-R_6) resulted in different enantioselectivities.



2,2-dialkyl-4-alkoxycarbonyl-1,3-dioxolane derivatives

Spanik et al. [14] studied the gas chromatographic separations of enantiomers of seven *N*-TFA-*O*-alkyl amino acid derivatives on stationary phases containing CD derivatives. From chromatographic and thermodynamic data, it was observed that the retention, the interaction, and the enantioselectivity of enantiomers of these derivatives were dependent upon the length of the linear alkyl chain attached to the chiral center and to the ester part of the amino acid derivatives.



N-TFA-*O*-alkyl amino acid derivatives

2.5 Thermodynamic investigation of enantiomeric separation by gas chromatography

As mentioned above, even though chiral recognition can be accomplished by many chromatographic and electrophoretic methods, the complete separation mechanisms have been still inconclusive. However, some mechanistic aspects of enantiomeric separation can be derived tentatively from thermodynamic investigations. Experimental thermodynamic data provide information about enantioseparation in terms of the interactions and discrimination of two enantiomers towards a chiral stationary phase. These data are easily accessed from the measurement of gas chromatographic retention.

Enantiomer separation by GC on CSPs is based on fast kinetics and is controlled by thermodynamics [6]. In other words, the direct enantioselective GC relies on the different stabilities of diastereomeric complexes of enantiomers and a chiral selector which are formed rapidly and reversibly. This can be described by Gibbs-Helmholtz thermodynamic parameters (ΔG, ΔH, and ΔS) which are different for individual enantiomer in enantiomeric pairs.

Thermodynamic parameters responsible for enantioseparation by GC can be determined by two approaches. The first approach, *van't Hoff approach*, relies on the direct determination of thermodynamic parameters using the separation factor (α) or retention factor (k′) obtained at different constant temperatures on a single chiral column. Alternatively, described by Schurig et al. [37-39], the other approach is based on the determination of a retention increment accessible from the relative retention of enantiomers and a reference standard on two columns: *a reference column* containing only the nonchiral stationary phase and *a chiral column* containing a chiral selector in the same stationary phase.

2.5.1 van't Hoff approach

By using the separation factor (α) obtained from the enantiomer separation on a chiral column at a given temperature, the difference in Gibb's free energy, $\Delta(\Delta G)$, is directly calculated according to equation (1):

$$-\Delta(\Delta G) = RT \cdot \ln \alpha = RT \cdot \ln\left(\frac{k'_2}{k'_1}\right) \quad (1)$$

where α is the separation factor or selectivity and is calculated from the ratio of k′ of two enantiomers.

k′ is the retention factor or capacity factor of each enantiomer. k′ is calculated from solute retention time, $\frac{t_R - t_M}{t_M}$.

R is the universal gas constant (1.987 cal/mol·K)

T is the absolute temperature (K)

1,2 refer arbitrarily to the first eluted and the second eluted enantiomers, respectively

Combining equation (1) with the Gibbs-Helmholtz relationship, equation (2, leads to equation (3).

$$-\Delta(\Delta G) = -\Delta(\Delta H) + T \cdot \Delta(\Delta S) \quad (2)$$

$$RT \cdot \ln \alpha = -\Delta(\Delta H) + T \cdot \Delta(\Delta S) \quad (3)$$

From equation (3), the following equation can be rewritten

$$\ln \alpha = \frac{-\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \quad (4)$$

where $\Delta(\Delta H)$ is the difference in enthalpy values for enantiomeric pairs

$\Delta(\Delta S)$ is the difference in entropy values for enantiomeric pairs

According to equation (4), the relationship between ln α and 1/T should be linear; therefore, $\Delta(\Delta H)$ and $\Delta(\Delta S)$ can be evaluated from the slope and y-intercept of the plot. However, the calculations of thermodynamic parameters from van't Hoff plot of ln α versus 1/T is not possible, as a result of curvatures observed in

many cases. This is due to the nonlinear dependence of selectivity on concentration of selectors in diluted system. Thus, this method is only valid for undiluted chiral selectors [39].

Otherwise, thermodynamic parameters can be calculated from retention factors instead of separation factors. Combination of equation (5) and (6) results in equation (7), which shows that the relationship between ln k′ and 1/T is linear. Thermodynamic parameters of individual enantiomers together with the differences in enthalpy and entropy of two enantiomers can be obtained from van′t Hoff plot of ln k′ against 1/T.

$$-\Delta G = RT \cdot \ln K = RT \ln (k' \cdot \beta) \tag{5}$$

$$\Delta G = \Delta H - T \cdot \Delta S \tag{6}$$

$$-\Delta H + T \cdot \Delta S = RT \cdot \ln(k' \cdot \beta)$$

$$\frac{-\Delta H}{RT} + \frac{\Delta S}{R} = \ln k' + \ln \beta$$

$$\ln k' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} - \ln \beta \tag{7}$$

where K is the distribution constant of chiral analyte (selectand) between the gas and the liquid phases.

β is a constant called phase ratio (the ratio of mobile phase volume to stationary phase volume).

ΔH is enthalpy change resulting from the interaction of the enantiomer with the stationary phase. ΔH value describes the degree of the strength of the interaction. The more negative the ΔH value, the higher the strength of interaction and the larger the retention in the column.

ΔS is entropy change resulting from the interaction of the enantiomer with the stationary phase. ΔS value describes the degree to which the structure of the solute influences the interaction.

2.5.2 Schurig approach

This method, introduced by Schurig and co-workers [37-39], is based on the concept of a retention increment (R′), and thermodynamic parameters can be calculated according to equation (8).

$$-\Delta(\Delta G) = RT \cdot \ln\left(\frac{R'_2}{R'_1}\right) \tag{8}$$

The retention increment or chemical capacity factor is a quantitative measure of the increase in the retention of enantiomer resulted from interactions between the solute and the selector (CD in th is case), added to the achiral solvent (polysiloxane), and is defined as

$$R' = K \cdot m \tag{9}$$

where K is the association constant between chiral analyte (selectand) and a chiral selector in the stationary phase.

m is molality (mol/kg) of the selector in achiral solvent.

The determination of the retention increment relies on experimental values of relative adjusted retention data of the enantiomers and a reference standard (usually a small alkane) on an achiral reference column containing only polysiloxane (r_o) and a chiral column containing CD in polysiloxane (r). A relationship referring to the retention increment and relative retention data is defined by

$$R' = \frac{r - r_o}{r_o} \tag{10}$$

where $r = \frac{t'}{t'^*} = \frac{k'}{k'^*}$ for chiral column (cyclodextrin in polysiloxane)

$r_o = \frac{t'_o}{t'^*_o} = \frac{k'_o}{k'^*_o}$ for achiral reference column (only polysiloxane)

and t', t'^* are adjusted retention time of chiral analyte and a reference standard, respectively on the chiral column.

t'_o , t'^*_o are adjusted retention time of chiral analyte and a reference standard, respectively on the achiral reference column.

k', k'^* are retention factors of chiral analyte and a reference standard, respectively on the chiral column.

k'_o , k'^*_o are retention factors of chiral analyte and a reference standard, respectively on the achiral reference column.

By combining equation (8) with equation (2), $\Delta(\Delta H)$ and $\Delta(\Delta S)$ are obtainable from equation (11) by plotting $R \cdot \ln(\frac{R'_2}{R'_1})$ versus 1/T:

$$R \cdot \ln(\frac{R'_2}{R'_1}) = \frac{-\Delta(\Delta H)}{T} + \Delta(\Delta S) \qquad (11)$$

Furthermore, applying equation (9) to the thermodynamic relationship in (5) results in

$$-\Delta G = RT \cdot \ln(\frac{R'}{m}) \qquad (12)$$

$$\ln R' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln m \qquad (13)$$

Thus, the thermodynamic parameters of individual enantiomers can be accessible from the plot of $\ln R'$ against 1/T.

Contrary to *van't Hoff approach* which express enantioselectivity through separation factor obtained directly from the retention factors, *Schurig approach* relies upon the concept of the retention increment. This concept eliminates the effects of the achiral polysiloxane (use as diluent) which contribute to the overall retention and interaction, but not the enantioselectivity. Moreover, the retention increment is linearly related to the concentration of the chiral selector; thus, the thermodynamic parameters for diluted systems can be obtained from this method. Nevertheless, *n*-alkanes used as reference standards are not totally

inert. They probably interact with the cyclodextrin selector weakly; consequently, in some cases thermodynamic parameters cannot be calculated correctly.