CHAPTER II

THEORY

2.1 Gas chromatographic separation of enantiomers

Gas chromatography (GC) is usually considered as an ideal method for the separation of volatile and thermally stable organic compounds. For enantiomeric separation by GC, two strategies can be employed. The first one involves the conversion of the enantiomers into diastereomeric derivatives with an appropriate auxiliary, enantiomerically pure derivatization reagent prior to GC analysis. Disadvantages associated with this indirect approach include the requirement for an active functional group for the formation of diastereomeric derivatives; the difficulties in obtaining optically pure reagents; the requirement for chemical and stereochemical stabilities of the derivatives under GC conditions; chiral discrimination in the reaction rates of enantiomers with the chiral derivatizing agent; and loss due to decomposition and incomplete recovery during work-up, isolation, and sample handling[1, 8].

Another method, the direct enantiomeric separation, takes advantage of chiral stationary phases (CSPs) which can rapidly and reversibly form diastereomeric association complexes with chiral analytes. Principally, there are three types of CSPs distinguished by selector-selectand interaction: chiral amino acid derivatives *via* hydrogen-bonding, chiral metal coordination compounds *via* complexation, and cyclodextrin derivatives *via* (*inter alia*) inclusion. However, cyclodextrin derivatives are the most frequently used as chiral selectors for direct gas chromatography [1, 8, 16].

2.2 Cyclodextrin and their derivatives

Cyclodextrins (CDs) are cyclic oligosaccharides obtained from enzyme degradation of starch. The three most common CDs are composed of six, seven, or eight D-glucose units (named α-, β-, and γ-CD, respectively) linked by α-1,4-glycosidic bonds (figure 2.1 (a)). The native CD is a toroidal cone having axial cavity

with primary C6 hydroxyl groups around its narrow rim and secondary hydroxyl groups at C2 and C3 positions on the opposite, wider edge (figure 2.1 (b)). As a result, the exterior of the molecules is hydrophilic while the interior shows hydrophobic property. Because of their hollow structure, they are able to host a large variety of guest molecules, including isomers and enantiomers. In addition, cyclodextrins could be chemically modified to improve their properties as chiral selectors for enantiomeric separation [8, 9, 16-18].

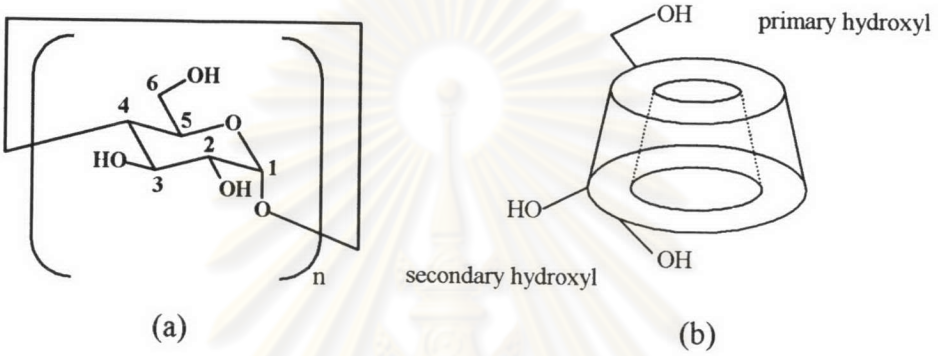


Figure 2.1 (a) A structure of cyclodextrin molecule with n glucose units and (b) side-view of cyclodextrin showing primary and secondary hydroxyl groups

The hydroxyl groups at C2, C3, and C6 positions of each glucose unit in CD can be modified by chemical reactions to provide numerous derivatives with different substitution. Generally, pure or diluted CD derivatives in polysiloxane or silicone are preferable to the native ones for the use as chiral stationary phases in gas-liquid chromatography since the native CDs decomposed at high working temperature. At room temperature, most derivatized CDs are solid which can cause non-homogenous film coating problem. Therefore, they are usually diluted in achiral polysiloxane in order to improve their properties and to obtain high efficiency stationary phases with broad operating temperature range [16].

2.3 Parameters affecting enantioseparation

Based on previous studies [1, 7, 10, 16, 19-25], it had been demonstrated that the enantioseparation by GC using CD derivatives as stationary phases was affected by the type of CD, the derivatization of hydroxyl groups on the CD rings, the polarity of polysiloxane matrix, the concentration of CD in polymer, and the chiral solutes of different molecular structure.

Considering the structure of CD, the substituents on glucose units at C2, C3, and C6 positions have an impact on physical, chemical properties as well as enantioselectivity of CD derivatives. The CD ring size, the differences in number of glucose units of CDs structure (α-, β-, and γ-CDs), posses different cavity size that affects inclusion-complexation mechanism between CD derivatives and some analytes. Furthermore, the chiral recognition depends upon the type and position of substituents on CD molecules. Generally, substituents at chiral C2 and C3 positions, mostly small alkyl or acyl groups, affect the enantioselectivity whereas substituents at the nonchiral C6 position have an effect on polarity, melting point, and solubility of CD in polysiloxane matrix. Nonetheless, an overwhelming proportion of publications described the use of 6-*O*-*tert*-butyldimethylsilyl substituent because of its influence on the conformation of CD ring that can impact on enantioselectivity [7, 20]. Studies associated with the use of CD derivatives and the separation of alcohol enantiomers by GC are summarized as follow.

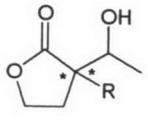
Kobor and Schomburg [21] studied the effect of ring size on enantiomeric separation of different homologues of 1-phenylethanol by using three different types of CD derivatives: 6-*t*-butyldimethylsilyl-2,3-dimethyl derivatives of α-, β-, and γ-cyclodextrins. It could be seen that the analytes with short alkyl chain length, e.g. 1-phenylethanol and 1-phenylpropanol were resolved with better enantioselectivity by α-CD derivative selector whereas larger β-CD derivative provided greater enantioselectivity for the analytes with longer alkyl chain length such as 1-phenyl-1-butanol and 1-phenyl-1-pentanol. However, no enantiomeric pairs of 1-phenyl-1-alkanol homologues could be resolved on γ-CD derivative.

Effect of derivatization of hydroxyl groups on the CD rings was observed by Maas et al. [22]. By changing the chain length of substituents at C2 and C3 positions of alkyl groups, the enantioselectivity was extremely affected. While changing chain length of acyl groups at the same position slightly altered enantioselectivity.

When mixtures of modified CDs dissolved in polysiloxanes are used as stationary phases, the concentration of CD derivatives and the polarity of polysiloxane solvents are also factors influencing enantioselectivity. Increasing CD contents and/or decreasing polarity of polysiloxanes could improve the enantioselectivity. Nevertheless, a leveling off of selectivity and a decrease in efficiency of chiral stationary phases have been observed [16, 21, 23].

Kobor et al. [20] studied effect of analyte structures on two β-cyclodextrin derivatives possessing identical substituents on the secondary hydroxyl groups: permethyl-β-CD (PMCD) and 2,3-dimethyl-6-*tert*-butyldimethylsilyl-β-CD (TBCD). For the separation of 1-phenyl-1-alkanol (C2-C5) homologues, it was observed that the enantiomers could be resolved at higher enantioselectivity with the TBCD. For the isomeric phenylpropanols, it could be seen that the enantioselectivity of the PMCD decreased with the distance of the polar hydroxyl group from the asymmetry center of alcohol analytes. For several groups of analytes, the less flexible TBCD seems to be more suitable for chiral analysis than PMCD.

Ramos et al. [24] separated 2-alkyl-2-keto-γ-butyrolactone derivatives and their alcohol analogs by using 2,3-di-*O*-methyl-6-*O*-*tert*.-butyldimethylsilyl-β-cyclodextrin (DIMETBCD) as a chiral selector. By molecular modeling calculations, the chiral recognition for DIMETBCD depends more on the geometry than on the polarity of the alkyl substituent on the butyrolactones. Moreover, hydrogen bonds and alkyl group steric effects could affect the chiral recognition.



γ-butyrolactone derivatives

Berthod et al. [19] investigated enantioselective retention mechanisms of various chiral compounds on 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl (DP-TFA) derivatized β- and γ-cyclodextrins. By lengthening the alkyl side chain of homologous series of many compounds, the retention time increased whereas enantioselectivity remained constant. Based on thermodynamic studies, it was believed that there might be two different chiral recognition mechanisms with this type of stationary phases: one involves inclusion complex formation and the other involves external interaction.

Li et al. [1] studied enantiomeric separation of numerous groups of compounds by gas chromatography using 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl derivatives of α-, β-, and γ-cyclodextrins (DP-TFA α-, β-, and γ-CD). It was observed that the DP-TFA γ-CD exhibited wider chiral selectivity and usefulness than the others. It was reported that the selectivity values of the larger homologous compounds were independent of carbon chain length, only part of the carbon chain contributed significantly to chiral recognition. The longer carbon chains increased the retention but did not improve the enantioselectivity. The substituent group on CD and the size of CD cavity can affect the orientation of the analyte molecules. Additionally, the analyte functionality played an important role on chiral recognition.

2.4 Mechanistic considerations on enantioseparation

As mentioned above, most of enantiomer resolutions by GC occur from the formation of reversible diastereomeric association complexes between chiral stationary phases (CSPs) and chiral analytes. The enantioselectivity of the separation depends on different stabilities of association complexes. However, the mechanism of enantiomeric separation still has been unclear.

To date, a number of studies have exhibited the broad spectrum of cyclodextrins and their derivatives for their chiral recognition of various classes of compounds [1, 7-8, 16, 18-20, 26]. Their unique characteristics could arise from their numerous chiral centers, the different orientation of each chiral center, and the unidentical shape of each glucose unit. Many types of interactions associated between

CDs and analytes, e.g. *inter alia* inclusion, hydrogen bonding, dipole-dipole interaction, dispersion forces, electrostatic interaction, and hydrophobic interaction, play an important role on the separation processes and mechanisms [7-8, 18]. The "induced fit" is another proposed mechanism for enantiomeric separation by cyclodextrin-based gas chromatographic stationary phases since CDs have a flexible shape and can change their shape to accommodate several types of analytes [16].

2.5 Thermodynamic investigation of enantiomeric separation by GC

As mentioned above, although chiral recognition mechanisms obtained from chromatographic method has been still ambiguous, some mechanistic aspects can be derived from thermodynamic investigations of reliable experimental parameters. Such data, providing information about enantioseparation, may easily be accessed from gas chromatographic retention measurement.

Gas chromatographic separation of enantiomers on CSPs is based on fast kinetics and is governed by thermodynamics [7]. Likewise, the direct enantioseparation by GC relied on the different stabilities of rapidly and reversibly diastereomeric complex formation and could be explained by Gibbs-Helmholtz thermodynamic parameters (ΔG, ΔH, and ΔS) which are different for each enantiomer of enantiomeric pairs. The van't Hoff approach is used to determine thermodynamic parameters from retention factor (k') and separation factor (α) acquired at different temperatures on one single chiral column.

In van't Hoff approach, the difference in Gibb's free energy, $\Delta(\Delta G)$, is calculated from the separation factor (α) derived from chiral separation on a chiral column at given temperature according to equation (1) [13].

$$-\Delta(\Delta G) = RT \cdot \ln \alpha \qquad (1)$$

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

where α is the separation factor or selectivity calculated from the ratio of k′ of an enantiomeric pair

k′ is the retention factor or capacity factor of each enantiomer calculated from the retention time according to $k' = \frac{t_R - t_M}{t_M}$

t_R is the retention time of an enantiomer or analyte

t_M is the time for mobile phase or unretained compound to travel at the same distance as analyte

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

Equation (3) could be derived from equation (1) and the Gibbs-Helmholtz equation (2) as shown below:

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

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

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

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

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

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

According to equation (4), $\Delta(\Delta H)$ and $\Delta(\Delta S)$ could be evaluated from the slope and y-intercept of the ln α vs. 1/T plot. Unfortunately, the calculations of thermodynamic parameters from these plots are not always possible because of the nonlinear behavior of chiral selector concentration in diluted stationary phase.

Alternatively, thermodynamic parameters could be calculated from retention factor. The linear relationship between ln k′ and 1/T could be derived as shown in equation (5). Thermodynamic parameters of individual enantiomers including the differences in enthalpy and entropy of enantiomer pair can be obtained from plots of ln k′ against 1/T.

$$
\begin{aligned}
-\Delta G &= RT \cdot \ln K \\
&= RT \cdot \ln (k' \cdot \beta) \\
RT \cdot \ln (k' \cdot \beta) &= -\Delta H + T \cdot \Delta S \\
\ln k' &= \frac{-\Delta H}{RT} + \frac{\Delta S}{R} - \ln \beta
\end{aligned}
\qquad (5)
$$

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 interaction strength. The large negative ΔH value indicates high strength of interaction between analyte and stationary phase.

ΔS is entropy change resulting from the interaction of the enantiomer with the stationary phase. ΔS value describes the degree of interaction sites associated with the interaction.