CHAPTER II

THEORY

2.1 Cyclodextrin

Cyclodextrins (CDs) are a homologous series of nonreducing cyclic oligosaccharides made up of six or more (α)-D-glucopyranose units linked together by α-1,4-glycosidic bond (figure 2.1). The macrocyclic conformation of the cyclodextrins corresponds to a torus shape with secondary hydroxyl groups (C2-OH and C3-OH) at the wider opening and primary hydroxyl groups (C6-OH) at the opposite narrower opening [11, 12]. Some important properties of cyclodextrins are summarized in table 2.1.

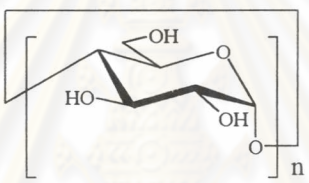

Figure 2.1 Schematic structure of cyclodextrin (n = 6, 7, 8 correspond to α-, β-, and γ-cyclodextrins, respectively)

Table 2.1 Molecular dimension and physical properties of cyclodextrins [12]

cyclodextrin	α	β	γ
number of glucose units	6	7	8
number of chiral centers	30	35	40
molecular weight	972.86	1135.01	1297.15
cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
volume of cavity (Å)	174	262	427
solubility in water (g/100 mL, 25 °C)	14.50	1.85	23.20
decomposition temperature (°C)	278	299	267

The CD molecule contains several hydroxyl groups, all directed outward; therefore, the cavity is relatively hydrophobic compared to its outer surface. This makes CD molecule favorable to inclusion with several types of nonpolar guest compounds. The hydroxyl groups presented on the rim of the CDs can be modified by chemical reaction to introduce substituents with specific functions in order to vary their solubility behavior, complexation properties and, subsequently, selectivities [13]. By formation of inclusion complex with CD, the stability, solubility, bioavailability, residence time, toxicity, and odor properties of guest molecule can be beneficially altered to facilitate the pharmaceutical and food industries [14, 15].

2.2 Cyclodextrin as Stationary Phase in GC

Natural underivatized CDs were proved to be unfavorable for use as stationary phases in capillary GC because they are solid at room temperature, have limited operating temperature range, and have low solubility in polysiloxane diluent. Therefore, they are unsuitable for coating capillary columns and give columns with very low efficiency.

Derivatized CDs with appropriate physical and chemical properties can be prepared by replacing reactive hydroxyl groups of CD with proper substituents. Randomly methylated and permethylated CDs were first utilized in high-resolution capillary GC in undiluted form. The disadvantage of using undiluted methylated CD is that they can only be employed as stationary phase in molten state at high temperature or at ambient temperature in a super cooled state [16]. To overcome this problem, Schurig and Nowotny dissolved solid permethylated-β-CD in moderately polar polysiloxane and used as stationary phase irrespective of its melting point or any phase transition [17]. Conversely, König and co-workers prepared liquid CD derivatives by substituting hydroxyl groups with hydrophobic moieties, such as long alkyl chain, to yield liquid CDs, e.g. per-*n*-pentylated CDs [18]. Armstrong and co-workers, on the other hand, prepared a mixture of isomers and homologues of derivatized CDs that differs in degree of substitution [19]. These liquid CDs can be coated directly, without dilution, onto capillary column and high efficiency GC columns can be obtained as well.

Several CD derivatives have been prepared and used as chiral GC stationary phases. Commonly, nonpolar alkyl (e.g. methyl, ethyl, pentyl) and bulky (e.g. *tert*-butyldimethylsilyl, *tert*-hexyldimethylsilyl) groups are introduced at the primary hydroxyls (C6-OH), while alkyl, acyl, or carbamate groups are placed at the secondary hydroxyls (C2- and C3-OH) [16-30]. These derivatives differ in their physical, chemical and chromatographic properties depending on the CD ring size and the type of substituents. Examples of CD derivatives are

- hexakis(2,6-di-*O*-pentyl-3-*O*-acetyl)cyclomaltohexaose [20]
- heptakis(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)cyclomaltoheptaose [24]
- heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)cyclomaltoheptaose [29]
- octakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltooctaose [30]
- octakis(2,6-di-*O*-*tert*-butyldimethylsilyl)cyclomaltooctaose [30]

2.3 Gas Chromatographic Separation of Enantiomer with Cyclodextrin Derivatives

Based on previous studies [20-34], it had been demonstrated that enantiomeric separation with derivatized CDs as chiral stationary phases was affected mainly by

- chemistry of CD selector such as ring size, type of derivatization, and CD concentration dissolved in polysiloxane matrix
- polarity of polysiloxane matrix
- chemical structure of enantiomers to be resolved
- separation temperature

Armstrong et al. [22] studied the effect of ring size on enantiomeric separation by using three types of CD derivatives: (2,6-di-*O*-pentyl) derivative of α-, β- and γ-CDs. Very different enantioselectivities of test compounds were observed. The largest number of compounds was separated on the derivatized β-CD column. However, both the dipentyl-γ-CD and particularly, the dipentyl-α-CD were clearly superior at resolving certain types of racemates.

The influence of CD ring size on chiral resolution was also investigated by Kobor and Schomburg [31]. The separation of different homologues of 1-phenylethanol on (2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl) derivative of

α- and β-CDs were performed. It was observed that short side chain analytes (e.g. 1-phenylethanol, 1-phenylpropanol) were separated with greater enantioselectivity with derivatized α-CD as chiral selector. While larger β-CD showed better enantioselectivity towards 1-phenyl-1-butanol and 1-phenyl-1-pentanol, which having longer side chain. Interestingly, no enantiomeric separation of any 1-phenyl-1-alkanol homologues was observed when γ-CD derivative was used. Similar results were achieved with 2-bromoalkanes.

Type of cyclodextrin derivatization on enantiomeric separation was examined by König et al. [20]. A nonpolar derivative, heptakis(2,3,6-tri-*O*-pentyl)-β-CD, and a polar derivative, heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-CD, of identical ring size were compared for their enantioseparation capability. It was found that polar enantiomers seem to be separated on polar CD (acetyl) better than on nonpolar CD (pentyl).

Effect of size of substituent on CD was also observed by Maas et al. [30]. By changing from small alkyl substituents (methyl) of octakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethysilyl)-γ-CD to longer alkyl chain (butyl), the enantioselectivity of several enantiomers increased with increasing chain length.

The position of substituents on the CD ring plays an important role on enantioselectivity as well. Although the C6 carbons on the primary face of CD molecule are nonchiral, the difference in enantioselectivity was observed when substituents at C6-hydroxyl were changed. Shitangkoon and Vigh [32] demonstrated that chiral selectivity of heptakis(2,3-di-*O*-methyl)-β-CD derivatives varied significantly with the size of the substituent at primary hydroxyls, ranging from small deoxy-fluoro, methyl, *n*-pentyl, *n*-propyldimethylsilyl, *tert*-butyldimethylsilyl, to bulky triisopropylsilyl groups. For most of the test compounds, chiral selectivity had a local maximum when the substituent was the *tert*-butyldimethylsilyl group.

Jung and Schurig studied the influence of CD weight percentage in polysiloxane on chiral separation factor [33]. They found that chiral selectivity of enantiomeric pairs increases with increasing amount of heptakis(2,3,6-tri-*O*-methyl)-

β-CD up to 30%. Their results were in agreement with those found by Kobor and Schomburg [31], who studied the influence of the concentration of heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethysilyl)-β-CD dissolved in polysiloxane SE-54 towards enantioselectivity. It was proved that increasing the concentration of the cyclodextrin derivative in a polysiloxane matrix led to a higher enantioselectivity. The use of selector concentration higher than 30% results in only a minor increase in enantioselectivity but lengthens analysis time. With a selector concentration higher than 50%, the cyclodextrin derivative becomes immiscible in polysiloxane, which leads to a serious loss in column efficiency and separation.

Kobor et al. [25] investigated the effect of chemical structure of analytes on selectivity. Two types of analytes, nonpolar (limonene) and polar (1-phenylethanol), were examined on two different stationary phases: heptakis(2,3,6-tri-*O*-methyl)-β-CD and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD. The results obtained, in combination with molecular modeling, indicated that the bulky substituted (*tert*-butyldimethylsilyl) cyclodextrin derivative offered better separation to nonpolar analyte (limonene) than the smaller substituted (methyl) derivative. However, enantioselectivities of both CD derivatives towards polar analyte (1-phenylethanol), which experienced stronger intermolecular interaction than limonene, are similar.

Effect of substitution size of enantiomer on enantioselectivity was also observed by Kobor et al. [25]. Separation of aromatic alcohols (1-phenylethanol, 1-phenyl-1-propanol, 1-phenyl-1-butanol and 1-phenyl-1-pentanol) was performed on heptakis (2,3,6-tri-*O*-methyl)-β-CD stationary phase. The decrease in enantioselectivity was observed when the analyte chain length increased.

Position of substituent on aromatic ring of enantiomer can influence in enantioselectivity as well [20]. The separation of racemic *o*-, *m*- and *p*-methyl substituted 1-phenylethanol was perform on a hexakis(2,3,6-tri-*O*-pentyl)-α-CD column. The methyl-substituted isomers and the unsubstituted derivative were separated except for the *o*-methyl derivative, which has the structure that is probably unfavorable for inclusion into the CD cavity.

Reiher et al. [21] separated aromatic alcohols with chiral center at different position using hexakis(2,3,6-tri-*O*-pentyl)-α-CD as chiral phase. Aromatic alcohols whose chiral center in alpha position to the phenyl group (e.g. 1-phenylethanol, 1-phenyl-1-propanol, and 1-phenyl-1-butanol) were separated. Nonetheless, alcohols with chiral center far from phenyl group (e.g. 1-phenyl-2-propanol, 1-phenyl-2-butanol, and 1-phenyl-3-butanol) were not resolved.

Kobor et al. [31] and Dietrich et al. [34] investigated the effect of polarity of polysiloxane matrix towards enantioselectivity. They found that enantioselectivity could be improved by decreasing the polarity of the silicon solvent.

Separation temperature is another important factor that can be easily adjusted and offers great impact on enantioselectivity. Usually the separation factor could be enhanced by decreasing the column temperature [31].

2.4 Thermodynamic of enantioseparation [35]

When a volatile solute B (selectand) migrates through a gas chromatographic column containing a dilute solution of the cyclodextrin derivative A (selector) in the nonchiral inert solvent S (polysiloxane), a 1:1 diastereomeric associate is formed rapidly and reversibly between A and B. Two separated equilibria can be drawn:

$$B_{(g)} \rightleftharpoons B_{(l)} \qquad (1)$$

$K_L^\circ = \frac{C_{B_{(l)}}}{C_{B_{(g)}}}$, is the distribution constant of B between the gas and the pure liquid phase S (i.e. the physical contribution to retention, neglecting the presence of A in S)

$$B_{(l)} + A \rightleftharpoons AB \qquad (2)$$

$K = \frac{C_{AB}}{C_A \cdot C_{B_{(l)}}}$, is the thermodynamic association constant of A and B in S (i.e. the chemical contribution to retention)

In direct enantiomer separation, the resolution is based on the formation of reversible diastereomeric associates or complexes through intermolecular interactions of enantiomers with a chiral selector dissolved in polysiloxane solvent. This formation process depends only on chemical contribution to retention (K) as seen in equation (2). This process can be characterized by Gibbs-Helmholtz thermodynamic parameters (ΔG, ΔH and ΔS), according to the general equation

$$\Delta G = \Delta H - T \cdot \Delta S \quad (3)$$

There are two known methods for the determination of thermodynamic parameters. The first method (method A) is based on direct determination from the chromatographic parameters, which are obtained from the separation of the enantiomers of analyte on a single chiral column at different temperatures. The other method (method B) relies on the determination of the relative retention of the enantiomers of analyte relating to an inert reference standard (*n*-alkane) on a reference column (containing only polysiloxane) and on a chiral column (containing cyclodextrin derivative dissolved in polysiloxane).

Method A

In this method, thermodynamic parameters are calculated from retention factors (k′) or separation factor (α) of enantiomers. The overall retention factor of the enantiomers in a diluted stationary phase is the combination of contribution from an achiral solvent and a chiral selector. Thermodynamic parameters of individual enantiomer can be obtained by employing the relationship between retention factors and Gibb's free energy as follow.

$$-\Delta G = RT \ln (k' \cdot \beta) \quad (4)$$

Combining equations (3) and (4) results in equation (5), which demonstrates that the relationship between ln k′ and 1/T is linear.

$$\ln k' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} - \ln \beta \quad (5)$$

where β is the ratio of mobile phase volume to stationary phase volume (or phase ratio)

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

T is the absolute temperature (K)

ΔH is enthalpy change resulting from the interaction of the enantiomer with the stationary phase.

ΔS is entropy change resulting from the interaction of the enantiomer with the stationary phase.

The difference in enthalpy, $\Delta(\Delta H)$, and entropy, $\Delta(\Delta S)$, values can be calculated from the corresponding thermodynamic values of each enantiomeric pair or can be acquired from the ln α vs. 1/T relationship.

$$RT \cdot \ln \alpha = -\Delta(\Delta H) + T \cdot \Delta(\Delta S) \quad (6)$$

$$\ln \alpha = -\frac{\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \quad (7)$$

Method B

This method is based on the determination of a retention increment (or a chemical capacity factor), R′. It is a measure of the increase in the retention of analyte caused by the addition of cyclodextrin derivative to the nonchiral polysiloxane solvent and is defined by

$$R' = K \cdot m \quad (8)$$

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

m is the molality of the chiral selector in achiral solvent

The retention increment can be attained experimentally from relative adjusted retention data of the enantiomers and reference standards on a chiral column (r) and on a reference column containing only nonchiral solvent (r_o).

$$R' = \frac{r - r_o}{r_o} \quad (9)$$

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

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

where t', t'^* are adjusted retention time of chiral analyte and reference standard respectively, on a chiral column (cyclodextrin in polysiloxane)

t'_o , t'^*_o are adjusted retention time of chiral analyte and reference standard respectively, on a reference column (only polysiloxane)

Thermodynamic parameters of individual enantiomer can be obtained from the ln R′ vs. 1/T plot, according to equation (10).

$$\ln R' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln m \qquad (10)$$

The difference in enthalpy and entropy values can be calculated from the corresponding thermodynamic values of each enantiomeric pair or can be obtained from the $\ln\left(\frac{R'_2}{R'_1}\right)$ vs. 1/T relationship.

$$\ln\left(\frac{R'_2}{R'_1}\right) = -\frac{\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \qquad (11)$$

where 1, 2 refer to the less and the more retained enantiomers, respectively

The approach in method B, as opposed to method A, allows the determination of thermodynamic parameters that are independent of selector concentration. Nevertheless, method A is still generally used as a first choice because of its simplicity and shorter analysis time.