CHAPTER II

THEORY

2.1   Gas chromatographic separation of enantiomers

Separation of enantiomers by gas chromatography (GC) on a chiral stationary phase (CSP) was discovered by Gil-Av *et al.* in 1966 [19]. According to Chirbase data, a wide variety of chiral compounds have been analyzed and approximately 2,200 publications related to chiral GC analyses have been published [19].

Gas chromatography has been proven a reliable analytical method for separation of chiral compounds that can be vaporized without decomposition. Its advantages include speed, reproducibility, sensitivity and limit of detection. Due to the enormous separation power of capillary GC, innumerable compounds can be separated without derivatization. In addition, several volatile components in complicated sample matrix can be directly analyzed from the vapor phase by GC coupled with headspace technique and the simultaneous analysis of multicomponent mixtures is also possible [10, 19].

Chiral separations using GC can be performed indirectly or directly. The indirect approach can be achieved by derivatization the racemic analytes with a chiral derivatizing agent (CDA), resulting in formation of diastereomers that can be separated on achiral stationary phase. On the other hand, the direct approach utilizes a non-racemic chiral stationary phase (CSP) as a selector, generating transient diastereomeric intermediates with analytes, whereby the interaction occurs both rapidly via fast kinetics and reversibly via distinct thermodynamics. The direct enantiomeric separation using CSPs is a simple and effective method to obtain pure enantiomers. In chiral GC, the chiral selector is chemically immobilized or coated as a thin film on a capillary wall or deposited on a packing material [10].

Among different chiral selectors, cyclodextrins (CDs) and their derivatives have been utilized extensively in GC because they can discriminate between positional isomers, functional groups, homologous compounds and enantiomers. Because of these properties, they become the outstanding CSPs for enantiomeric separations of various compounds. Furthermore, the thermal stability at reasonable temperature range of CDs and their derivatives makes them one of the most versatile tools for GC [10-11, 19-21].

2.2 Cyclodextrins and their derivatives

Cyclodextrins are natural cyclic oligosaccharides consisting of α-1,4-linked D-glucopyranose units. They are produced through degradation of starch by the enzyme cyclodextrin glycosyltransferases (CGTase). The major products of prolonged action comprise 6, 7 and 8 glucopyranose units usually referred to as α-, β- and γ-CDs respectively. Some characteristics of three native CDs are compared in table 2.1.

Table 2.1　　Some characteristics of native α-, β- and γ-CDs [21-23].

CD	α-CD	β-CD	γ-CD
number of glucopyranose units	6	7	8
number of chiral centers	30	35	40
anhydrous molecular weight (g/mol)	972.85	1134.99	1297.14
internal diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
cavity depth (Å)	7.9	7.9	7.9
cavity volume (Å)^3	174	262	427
water solubility (g/100 mL, 25 °C)	14.50	1.85	73.20
decomposition temperature (°C)	278	299	267

Native CDs are crystalline, non-hygroscopic, homogeneous substances which are torus-like macromolecules. Considering CD structures, the sugar adopt the

chair conformation (figure 2.1a) and orient themselves so that the molecule forms a truncated cone structure (figure 2.1b). Because of their torus-like geometry, relatively hydrophobic surface of the internal cavity and the hydrophilic character of external hydroxyl groups, CDs molecules can easily form inclusion complexes with a wide variety of organic and inorganic molecules. This complex-forming ability is an important characteristic for their applications [6, 24-29].

Every glucopyranose unit of CD ring has three free hydroxyl groups (-OH), two of which are secondary hydroxyls and the rest is primary hydroxyl. The mouth of the torus-shaped CD molecule has a larger circumference than the base and is linked to secondary hydroxyls of the C2 and C3 atoms of each glucose unit. The primary hydroxyls are located at the base of the torus, on the C6 atoms. The size of the cavity enlarges with increasing amount of glucose units. Because of the availability of multiple reactive hydroxyls, the functionality of CDs is greatly increased by chemical modification. Through modification, the applications of CDs become expanded [14, 21-23, 30].
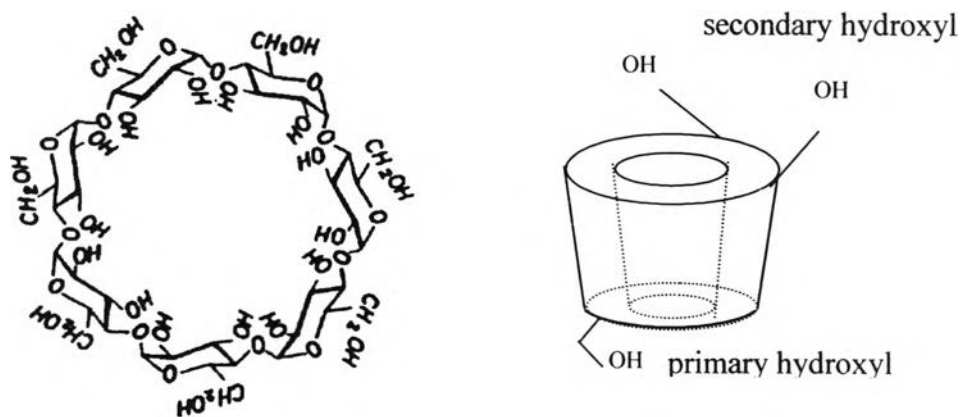

Figure 2.1   (a)   A top view of underivatized β-cyclodextrin comprising 7 glucopyranose units

(b)   The side view of cyclodextrin showing primary and secondary hydroxyls on both edges of a ring

CDs are modified through substituting various functional groups on the primary and/or secondary faces of molecule. Modified CDs are useful as chiral selectors in separation science because the selectivities of modified CDs are usually different from native CDs. Generally, the C2 and C3 positions of glucopyranose unit are modified with small alkyl or acyl groups to affect the enantioselectivities, whereas the substituents at the non-chiral C6 position are the longer alkyl or bulky groups. The introduction of bulky group, e.g. *tert*-butyldimethylsilyl (TBDMS), at the C6 of CDs not only improves their solubility in polysiloxanes but also impacts on the conformation of the CDs toward blocking of the entrance of the cavity at the smaller rim that can influence the enantioselectivities as well. The C6-TBDMS-modified CDs have been proven as one of the most useful CSPs for GC [10, 19].

2.3   Mechanistic considerations of chiral separations and complexation of modified cyclodextrins

As described above, most of enantiomeric resolutions occur by generating a transient diastereomeric intermediate between chiral analyte and CSP. During the separation processes, various types of interactions are associated, such as electrostatic interaction, hydrophobic interaction, dispersion force, dipole-dipole interaction and hydrogen bonding [14, 30]. Derivatization of free hydroxyls of CD molecule can introduce or change the type of interactions associated between analyte and CSP. This increases the differential interaction between each enantiomer and the stationary phase; thus, increasing the enantiomeric separation ratio and the resolution. The binding of guest molecules within the host CDs is not fixed or permanent but rather is a dynamic equilibrium. Binding strength depends on how well the host-guest complex fits together and on specific local interactions between surface atoms. Although the chiral recognition mechanism has still been abstruse, some mechanistic aspects can be obtained from thermodynamic investigations [23, 30].

CD modifications involve shape, size and other properties of the CD annuli and surfaces [23-30]. Some practical useful applications of modified CDs are discussed below.

Easton and Lincoln [21, 25] reported that modification of CDs often leads to improved stereoselectivities and complexation abilities with the racemic guests. The difference of stability constants deriving from ^{1}H-NMR and induced circular dichroism spectra in aqueous solution between the complexes of (R)-2-phenylpropanoate and (S)-2-phenylpropanoate with 6-amino-β-CD is two times larger than that of these analogous complexes with underivatized β-CD. This result indicates that substituents can enhance asymmetric property of CD and bring about the higher complexation selectivity.

Mitchell *et al.* [26] evaluated the enantioselectivities of native and derivatized CDs used as CSPs for separation of aromatic and aliphatic chiral sulfoxides by high performance liquid chromatography (HPLC). Many sulfoxide enantiomers could be baseline-resolved with 2,3-dimethyl-β-cyclodextrin as CSP in the reversed phase mode. The most important factor influencing enantioselectivity is the presence of steric bulk at α-position to the chiral center of analytes. The 2,3-dimethyl-β-cyclodextrin stationary phase exhibited the broadest enantioselectivity for neutral chiral sulfoxides. Native β-CD and hydroxypropyl-β-CD were much less effective in separating these compounds. In this case, substituents on an aromatic ring bonded to the sulfoxide decrease the enantioselectivity.

Bergeron *et al.* [27] studied the factors effecting the stability of the complexes deriving from ^{1}H-NMR and induced circular dichroism spectra in aqueous solution between phenols and phenolate ions using α-CD as chiral selectors. It was found that position of analyte substituents and charges of analytes highly influence the complex formation. Because of the steric hindrance, the 3, 5-dimethyl-4-nitrophenol forms a less stable complex with α-CD than 2, 6-dimethyl-4-nitrophenol. Furthermore, the phenolate ions combined with α-CD stronger than phenol derivatives because the

dipole moment of analytes also effects on the alignment and the stability of complexes.

2.4 Cyclodextrins and derivatives as gas chromatographic stationary phases

Both the high crystallinity and insolubility in most organic solvents of native CDs make them difficult to formulate into GC stationary phases; therefore, some functionalized CDs that can form viscous oils at the operating temperature were generated. Moreover, decomposition at high working temperature and the high melting point of some CDs derivatives can produce non-homogenous film coating that limits the column lifetime and separation efficiency. Consequently, dilution of derivatized CDs in polysiloxanes is employed routinely for GC analysis to improve the stationary phase properties and to obtain high separation efficiency with broad operating temperature range. The commercial availability of these phases provided the tremendous growth of GC uses [19-20, 24].

Schlenk *et al.* [31] firstly reported the use of CD derivatives in GC separation in 1962. The acetate, propionate, butyrate and valerate derivatives of β-CDs were employed to separate the homologues of fatty alcohols, fatty esters, methyl esters, olefins, aldehyde esters and aldehydes. The substituent types of derivatized CDs greatly affected the polarity and stability of the stationary phases. The valerate modified β-CDs offered the best resolution while the acetate modified β-CDs showed the poorest resolution for the analytes.

Smolková-Keulemansová *et al.* [32] were the first group that systematically investigated cyclodextrins for GC separations. They used the solutions of underivatized α- and β-CD deposited on a solid support. The homologues and positional isomers were separated, but the results showed poor efficiency due to the non-homogeneous stationary phase. However, many descriptions of the structural, positional, and steric preference for the formation of inclusion complexes were first detailed in this study.

Li *et al.* [33] used a series of liquid cyclodextrin derivatives comprising 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl α-, β- and γ-cyclodextrins as highly selective chiral stationary phases for capillary gas chromatography. It was found that more than 150 enantiomeric pairs of different compounds were resolved; particularly 80% of enantiomeric pairs were successfully separated by 2, 6-di-*O*-pentyl-3-*O*-trifluoroacetyl-γ-cyclodextrin, whereas, 60 and 30% of analyte enantiomers were resolved by derivatized β- and α-CDs respectively. This was the first report revealing the superior applications of γ-CD than β-CD analogue. Furthermore, they assumed that several factors played roles on enantioselectivity, such as carbon chain length of analyte, analyte functionality, CD substituent and size of CD cavity.

König *et al.* [34] separated three phenoxypropionic acid herbicide enantiomers (mecoprop, dichlorprop and fenoprop) by gas chromatography with electron capture detection (GC-ECD) employing modified cyclodextrins as chiral stationary phase. The excellent enantioseparations were obtained with columns containing a 1:1 mixture of liquid per-*O*-pentylated and solid per-*O*-methylated-β-CD. Nonetheless, this column can be used only above 100°C, below this temperature the per-*O*-methylated-β-CD seems to crystallize and only broad peaks are obtained.

Schurig [19] dissolved solid permethylated β-CD in moderately polar polysiloxane (OV-1701) and employed for gas chromatographic separation of 17 chiral analytes of different classes. The results still provide the reasonable enantioselectivities, symmetric peak shape and stable baseline over the operating temperature range (70-150°C). These indicate that polysiloxanes help to overcome the problems of degradation and non-homogenous film coating of derivatized CDs, while maintaining high separation efficiency of CD.

2.5   Parameters influencing the enantiomeric separations

Based on preceeding research, the enantioseparation by GC using CD derivatives as chiral selectors was influenced by several factors, such as CD ring size, substitution patterns on the derivatized CD rings, concentration of CDs in polysiloxane, polarity of polysiloxane matrix, separation temperature and structure of chiral analytes [6, 11, 14, 28-39].

Takahisa *et al.* [13] investigated the influence of the size of CDs on the enantiodifferentiation of various flavor compounds from different chemical classes. The heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD and octakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-γ-CD were employed as CSPs for GC analysis. It was generally observed that derivatized β-CD showed better enantioselectivity towards several classes of compounds, except for secondary alcohols. Furthermore, the separation factors determined for δ-lactones were similar in both columns, while the separation factor of sotolone, a γ-lactone possessing an enol-structure in the ring, was significantly higher on the derivatized β-CD than on the corresponding γ-CD analog.

Anderson *et al.* [11] separated 17 chiral sulfoxides and eight chiral sulfinate esters by GC on four types of CD derivatives composing of 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl-γ-CD (G-TA); 2,6-di-*O*-pentyl-3-*O*-propionyl-γ-CD (G-PN); 2,6-di-*O*-pentyl-3-*O*-butyryl-γ-CD (G-BP) and 2,6-di-*O*-methyl-β-CD (B-DM). Each derivatized-γ-CD and B-DM showed different enantioselectivity. Among all derivatized-γ-CD stationary phases, G-TA exhibited superior enantioselectivity for most sulfoxides and sulfinate esters; nevertheless, B-DM provided the best enantioselectivity for most of sulfinate esters. The size and polarity of sulfoxide substituents also affected their enantioselectivities on all CSPs. Moreover, G-TA and B-DM CSPs commonly provide opposite elution order and this order appears to be a function of both the size of CDs and the substituents on CDs.

Chen *et al.* [40] studied the influences of substituent at primary hydroxyls of CDs. Various acyl groups (valeryl, heptanoyl, and octanoyl) at C6 of 2,3-di-*O*-pentyl-β-cyclodextrin were employed as CSPs for GC and the chromatographic properties of these three stationary phases were investigated. It was found that the carbon chain length of acyl groups at the C6 position of CDs had many important effects on the enantioseparation abilitites of CDs. Among CD derivatives studied, 2,3-di-*O*-pentyl-6-*O*-valeryl-β-cyclodextrin showed the best enantioselectivity towards 15 pairs of enantiomeric analytes. Furthermore, they observed that enantioseparation is related to the structures and properties of the solutes, though the three CDs possessed the similar structures.

Shi *et al.* [17] examined the effects of substituent types and positions to the enantiomeric separation of some esters and epoxides by GC on four derivatized β-CD stationary phases: 2,6-di-*O*-pentyl-3-*O*-allyl-β-CD; 2,3-di-*O*-pentyl-6-*O*-allyl-β-CD; 2,6-di-*O*-pentyl-3-*O*-propyl-β-CD and 2,3-di-*O*-pentyl-6-*O*-propyl-β-CD. All CSPs could separate enantiomers of allethrone acetate, propargyllone acetate, 2-bromopropionic acid methyl ester, 2-chloropropionic acid methyl ester and epoxides. All selected CSPs provided the same elution order, enantioselectivities and peak characteristics indicating both the position of allyl group or propyl group on C3 or C6 and the double bond of allyl groups did not play any role in enantiomeric separation.

Nie *et al.* [28] separated enantiomers of amines, alcohols, diols, carboxylic acids, amino acids, epoxides, halohydrocarbons and ketones by GC using three derivatized-β-CDs as CSPs: heptakis-(2,6-di-*O*-nonyl-3-*O*-trifluoroacetyl)-β-CD (DNTBCD); heptakis-(2,6-di-*O*-dodecyl-3-*O*-trifluoroacetyl)-β-CD (DDTBCD) and heptakis-(2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl)-β-CD (DPTBCD). The results showed that DNTBCD can separate various types of enantiomers as broad as DPTBCD; however, DNTBCD showed superior enantioselectivities for analytes studied. In addition, thermodynamic data of racemic α-phenylethylamine derivatives demonstrated that enantioseparations on both CSPs were dominantly directed by

the same mechanism but DNTBCD had a stronger chiral recognition effect than DPTBCD.

McGachy *et al.* [12] studied the influence of ester alkyl group of 12 pairs of *N*-trifluoroacetyl-*O*-alkylnipecotic acid ester enantiomers on the interaction with permethylated β-CD (Me-CD) and enantioselectivities. The *n*-alkyl esters have stronger interactions with Me-CD than the esters containing branched alkyl groups. The α-branched alkyl esters exhibited higher enantioselectivities than the corresponding *n*-alkyl or β-branched isobutyl esters. Thermodynamic data also indicated that the α-branched alkyl esters have different types of interactions and/or stronger interactions with Me-CD than the C_1-C_5 *n*-alkyl esters or β-branched isobutyl ester.

Shitangkoon *et al.* [15] studied the relationship between the enantioresolution and the analyte structures of 19 enantiomers of 1-phenylethanol derivatives by GC using heptakis-(2,3-di-*O*-methyl-6-*O*-tert-butyldimethylsilyl)-β-CD in OV-1701 as CSP. The retention factors, enantioselectivities, and thermodynamic parameters exhibited that only small changes in the analyte structures such as size, polarity and position of the substituents considerably effected the separation selectivities. Moreover, substitution on the aromatic ring of the alcohol seems to promote the enantioseparation, whereas substitution on the side chain tends to decrease the enantiodifferentiation of investigated analytes.

2.6   Enantiomeric separation of phenoxy acids by derivatized cyclodextrins

Derivatized cyclodextrins have been used as chiral selectors for enantiomeric analysis in various chromatographic and electrophoretic techniques for different fields of contemporary research such as the authenticity control of essential oils, fragrances, alcoholic beverages, terpenoids, pharmaceutical and medicinal compounds, fertilizers, pesticides and herbicides. Nonetheless, there are small numbers of research on enantiomeric separations of phenoxy herbicides by capillary GC technique. Most works were done by capillary electrophoresis (CE) and HPLC

using derivatized CDs as the chiral selectors [10, 19-35]. Research related to enantiomeric analysis of phenoxy herbicides are as followed:

Zerbinati *et al.* [35] examined eight CDs, namely α-CD; β-CD; γ-CD; methyl-β-CDs; hydroxypropyl-β-CDs; C_6-capped-β-CD; ethylcarbonate-β-CD and ethylcarbonate-γ-CD as chiral resolving agents for the capillary zone electrophoretic resolution of the racemic dichlorprop (or 2-(2',4'-dichlorophenoxy)propionic acid). Among eight CDs investigated, ethylcarbonate-β-CD provided the best enantiomeric resolution, while native β-CD and C_6-capped-β-CD gave no resolution of the racemate. Although, complexation constants of derivatized β-CDs were found to be lower than that of the native β-CDs, efficient resolution was possible due to the large relative difference between the complexation constants of two enantiomers.

Miura *et al.* [36] studied mutual and chiral separations of nine phenoxy acid herbicides (figure 2.2) including seven pairs of phenoxy acid enantiomers, by capillary zone electrophoresis (CZE) using native and selectively methylated α-, β- and γ-CD derivatives as additives. The selective methylation of the secondary hydroxyls of the CDs pronounced remarkable selectivity changes for the phenoxy acid herbicides. Hexakis(2,3-di-*O*-methyl)-α-CD (or 2,3-DM-α-CD) exhibited high enantioselectivity at 10 mM for all seven pairs of phenoxy acids. For the nine phenoxy acids, α-CD at 5 mM produced complete separation of analytes, but could not resolve all pairs of enantiomers. The simultaneous (chiral and mutual) separation could be improved by mixing α-CD with 2,3-DM-α-CD.
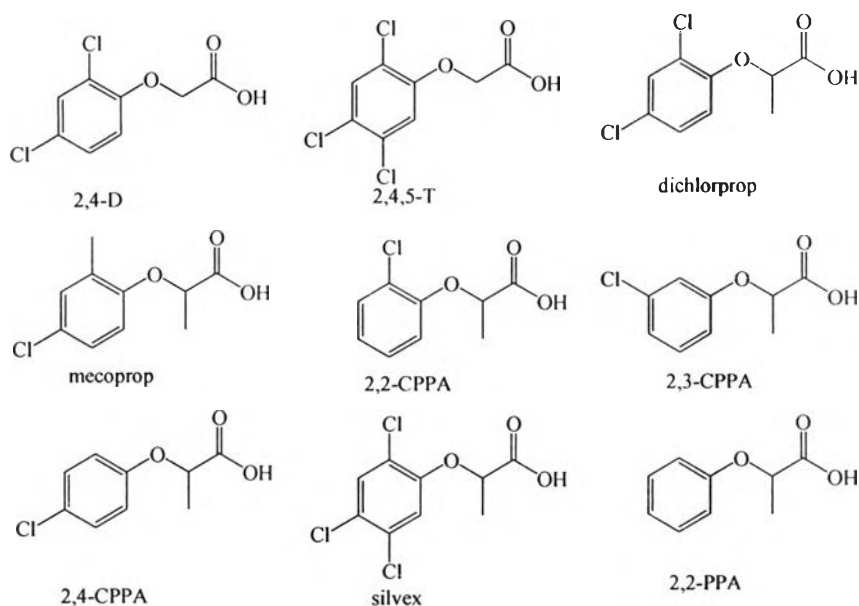
Figure 2.2   Structures of nine phenoxy acid herbicides [36, 37]

Later, Tsunoi *et al.* [37] investigated the simultaneous (chiral and mutual) separation of nine phenoxy acid herbicides including seven pairs of phenoxy acid enantiomers by CE using seven negatively charged sulfonylated CDs as additives. The introduction of sulfopropyl or a sulfonyl group to the neutral CDs produced a decrease in the enantioselectivities for seven racemic phenoxy acid herbicides. The dual CD system using 5 mM of 2,3-DM-α-CD and 2.5 mM of sulfonyl propylether-α-CD as the separation additive in CE provided the first complete simultaneous separation of nine phenoxy acid herbicides used in this study.

Schmitt *et al.* [6] used micellar electrokinetic chromatography (MEKC) to separate four groups of chiral pesticide enantiomers: organophosphorus, DDT congeners, phenoxy acid methyl esters and metolachlor (figure 2.3). Each of six CDs (α-CD, β-CD, γ-CD, hydroxypropyl-β-CD, dimethyl-β-CD and trimethyl-β-CD) was then added to the borate-sodium dodecylsulfate buffer with and without organic modifier, to separate these pesticides and their enantiomers. The enantiomers of malathion, ruelene and dialiphos were separated by hydroxypropyl-β-CD, β-CD or γ-CD, while the enantiomers of isofenfos and fenamifos could not be separated. The γ-CD with methanol modifier could separate three phenoxy acid methyl esters,
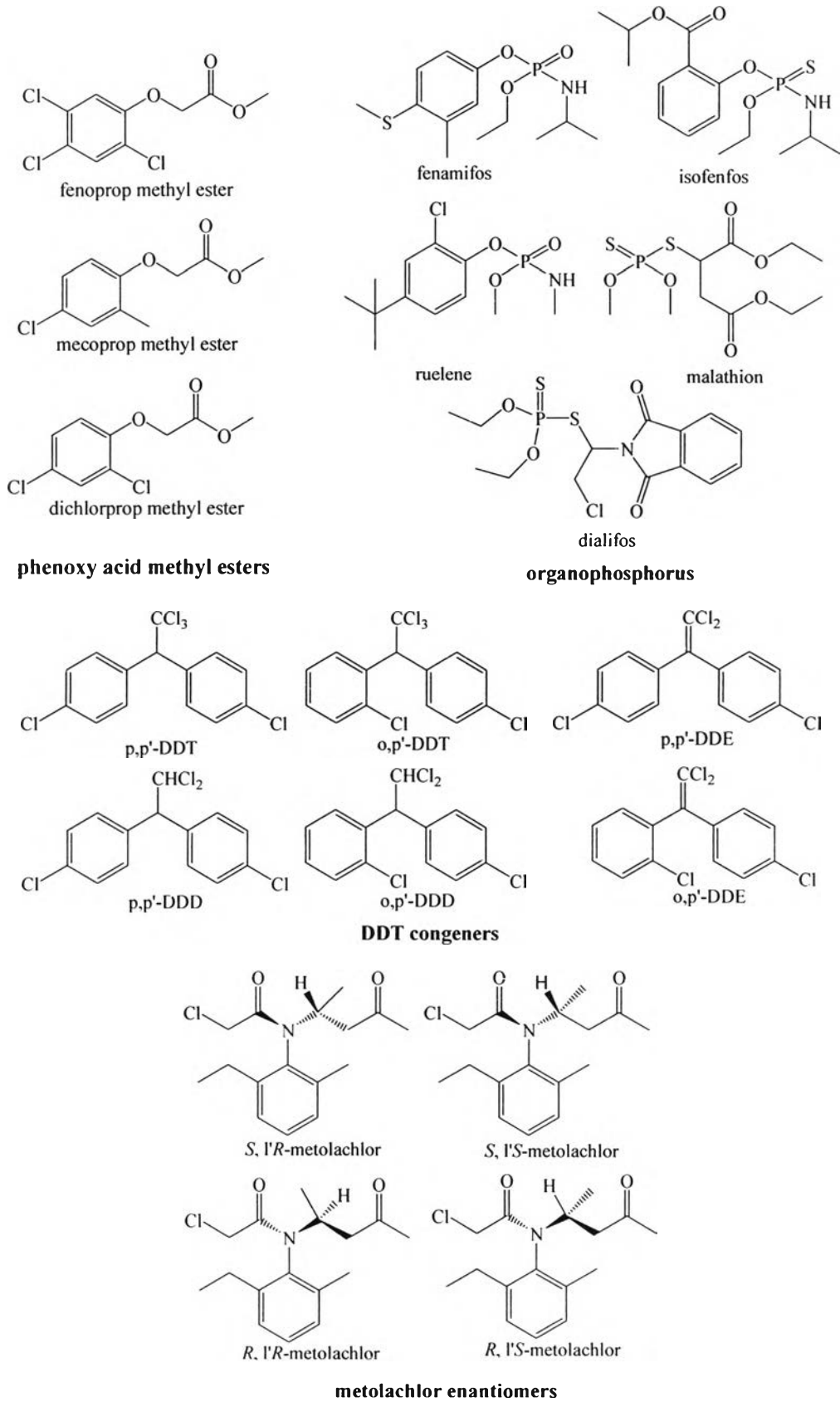
phenoxy acid methyl esters

organophosphorus

DDT congeners

metolachlor enantiomers

Figure 2.3   Structures of four classes of herbicides in Schmitt *et al*. study [6].

enantiomers of fenoprop methyl ester and three enantiomers of metolachlor, while γ-CD with acetonitrile modifier showed excellent separation of six DDT congeners and their enantiomers. However, none of the CDs separated the enantiomers of mecoprop and dichloprop methyl esters. These results indicated that type of CDs, types of organic modifiers and structure of analytes play important roles in enantiomeric separation of pesticides investigated.

Darrouzain *et al.* [29] investigated the retention and complexation mechanisms of phenoxypropionic acid (PPA) herbicide series by reversed phase HPLC using hydroxypropyl-β-CD (HP-β-CD) as mobile phase additive. The effects of organic content and the HP-β-CD concentration in mobile phase were analyzed at various column temperatures. It was shown that the retention mechanism was led by free PPA at low HP-β-CD concentration and by PPA/HP-β-CD complex at high concentration. In addition, thermodynamic results demonstrated that solute retention depends on the organic content in the mobile phase and the PPA/HP-β-CD complexation mechanism was entropically controlled process.

Weber *et al.* [9] investigated the influence of aromatic substituents on the enantioseparation of chiral phenoxypropionates by GC using permethylated β-CD as a CSP. The enantioselectivities and separation efficiency tend to decrease by three fold from mecoprop-methyl and dichlorprop-methyl to chlorinated fenoprop-methyl, demonstrating that type, position and number of aromatic substituents play important roles on the enantioselective separation of phenoxypropionate analytes.

Since all synthesized phenoxy acid methyl esters are thermally stable compounds and have high vapour pressure, the direct capillary GC is considered as one of the most appropriate techniques to separate their enantiomers. As mentioned previously, the analyte structure is one of the crucial factors affecting the enantioselectivities; nevertheless, this factor is difficult to predict and there is a lack of basic information from prior research. Therefore, systematic investigation of the relationship between analyte structure and enantioseparation are focused in this research. Thermodynamic parameters related to the interactions between analytes and stationary phases will be investigated as well.

2.7   Thermodynamic investigation of enantiomeric separation by gas chromatography

Temperature is the important factor influencing the retention factor, enantioselectivity and resolution; therefore, a change in temperature can be used to optimize the enantioseparation. However, the enantioselectivity greatly depends on the interaction mechanism. Although the chiral recognition mechanisms obtained from chromatographic method has still been abstruse, some mechanistic aspects can be derived from thermodynamic investigations. From the retention behavior and chromatographic measurements, the thermodynamic parameters (e.g. enthalpy, entropy, Gibbs free energy, etc.) associated with the enantiomers and CSP can be obtained [19, 38-39].

Generally, it is accepted that the direct enantiomeric separation is based on the formation of reversible diastereomeric associates created by intermolecular interaction of enantiomers with a chiral selector. This process for the individual enantiomer can be characterized by thermodynamic data using the Gibbs-Helmholtz equation [19].

van't Hoff approach is commonly used as the first method to determine enantioseparation by GC because of its simplicity and fast analysis. In this approach, the dependencies of the natural logarithms of capacity factor (k') or separation factor (α) on the inverse of temperature $(1/T)$ are commonly used to determine thermodynamic parameters on a single chiral column that characterize the enantiomeric separation, which allow the study of some mechanistic aspects of chiral recognition processes. If the chiral selector were diluted in a medium, the calculated values would represent the interaction between analytes and the overall stationary phase [38-39].

In van't Hoff approach [39], the difference in Gibb's free energy, $\Delta(\Delta G)$, is calculated from the separation factor (α) obtained from chiral separation on a chiral column at given temperature according to equation (1):

$$-\Delta(\Delta G) = RT \cdot \ln \alpha = RT \cdot \ln(\frac{k_2'}{k_1'}) \qquad (1)$$

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 and is calculated from solute retention time according to

$$k' = \frac{t_R - t_M}{t_M}$$

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

       T is the absolute temperature (K)

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

      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

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

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

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

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

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

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), $\Delta(\Delta H)$ and $\Delta(\Delta S)$ could be evaluated from the slope and y-intercept of the $\ln \alpha$ vs. $1/T$ plot. However, the calculations of thermodynamic parameters from plot of $\ln \alpha$ versus $1/T$ are not possible, as a result of curvatures observed. This is due to the nonlinear dependence of selectivity on concentration of selectors in diluted stationary phase; therefore, this method is only valid for undiluted chiral selectors [39].

Alternatively, thermodynamic parameters can be calculated from retention factors instead of separation factors. Combination of equations (5) and (6) results in equation (7), demonstrating the relationship between $\ln k'$ and $1/T$ is linear. Thermodynamic parameters of individual enantiomers can be obtained from van't Hoff plot of $\ln k'$ against $1/T$. Subsequently, the differences in enthalpy and entropy of two enantiomers can be achieved.

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

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

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

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

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

where   K   is the distribution coefficient 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 the interaction and the larger retention in the column.

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

Thermodynamic parameters acquired in this research through van't Hoff approach would bring greater insight about the interaction between phenoxy acid methyl ester analytes and CD derivatives. Hopefully, the interpretation of the data obtained from this work will clarify some mechanistic knowledge about the influence of analyte structure on enantioselective selector-analyte binding interaction.