CHAPTER II

THEORY

2.1 Gas chromatographic separation of enantiomers

Since the pioneer work of Gil-Av, Feibush and Charles-Sigler in 1966, the rapid advances in the development and comprehending of enantiomeric interactions and separations have made many analytical techniques. Among them, gas chromatography (GC) has been proven as the reliable and accurate analytical technique for separation and quantitation of volatile and thermally stable enantiomers. Due to the high efficiency, sensitivity, reproducibility and speed of separation of capillary GC, contaminants and impurities can be separated from the analytes and the simultaneous analysis of multi-component mixtures can also be possible in one analytical run. Thus, capillary GC has become the ideal choice for the analysis of enantiomers in complicated matrices from environmental, biological, agricultural, food, and essential oil samples [13-15].

Separation of enantiomers by capillary GC can be performed in two approaches: indirect and direct. The indirect approach involves the conversion of enantiomers into diastereomers by complete chemical reaction with a chiral derivatizing agent and subsequent separation of diastereomers on achiral stationary phase. Disadvantages of this method include the requirement of active functional group and high purity of chiral reagent. Furthermore, discrimination may occur as a result of incomplete recovery, decomposition or losses during work-up, isolation and sample handling. On the other hand, the direct approach relies on utilizing chiral selector as a stationary phase, which is coated as a thin film on a capillary wall, generating transient diastereomeric intermediates with analytes [13-16]. This approach requires no sample derivatization.

Among several classes of chiral selectors, cyclodextrins (CDs) and their derivatives were the most frequently used selectors to separate a large variety of chiral molecules with different geometries and functionalities.

2.2 Cyclodextrins and derivatives as gas chromatographic stationary phases

Cyclodextrins are cyclic oligosaccharides produced through degradation of starch by the cyclodextrin glucanotransferase (CGTase) enzyme. The most commonly used CDs composed of six, seven and eight D-glucose units linked by α-1,4-glycosidic bond; referred to as α-, β- and γ-CDs respectively. Some properties of three CDs are summarized in Table 2.1 [17, 20].

Table 2.1 Some properties of α-, β- and γ-CDs

CD	α	β	γ
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	23.20
decomposition temperature (°C)	278	299	267

Native CDs are crystalline, homogeneous and non-hygroscopic substances which are torus-like macromolecules. The CD ring consists of the glucopyranose unit in chair conformation (Figure 2.1a). 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 secondary hydroxyl groups of C2 and C3 atoms of each glucose unit are at the larger rim of CD. The primary hydroxyl groups of C6 atoms are located at the base or narrow edge of the torus (Figure 2.1b). CDs thus have relatively hydrophobic property inside the cavity and relatively hydrophilic character on the rim of the cavity. Due to their unique properties, CD molecules can easily form

inclusion complexes with a wide variety of analyte molecules. This complex-forming ability is an important characteristic for their applications [17-20].

(a) (b)

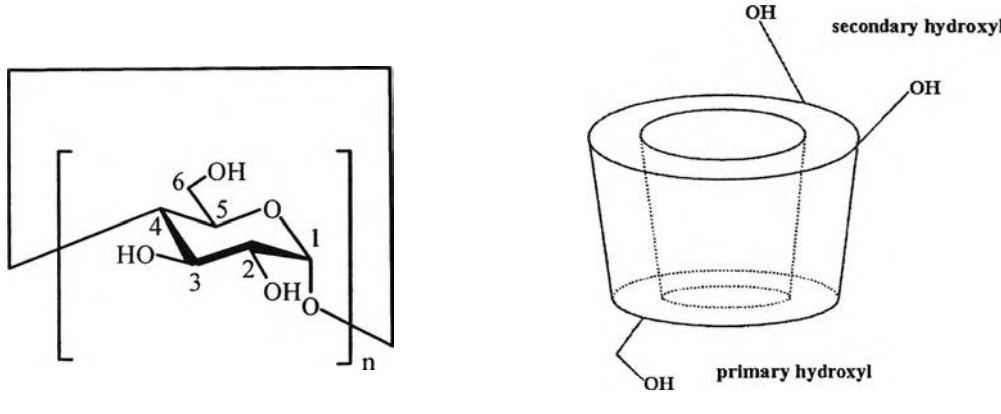

Figure 2.1 (a) Structure of a cyclodextrin molecule with n glucose unit; (b) Side view of cyclodextrin showing primary hydroxyls on a narrow rim and secondary hydroxyls on a larger rim of a ring

The three hydroxyl groups of each glucopyranose unit in CD ring can be modified through substituting with various functional groups. Several modified CDs have been used successfully for chiral separation in GC. 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 to increase their solubilities in polysiloxane.

The high crystallinity and insolubility of underivatized CDs in most organic solvents have made them unsuitable to coat onto GC column; therefore, functionalized CDs that can form viscous oils at the operating temperature were generated. However, some viscous CD derivatives cannot produce homogeneous film coating or cannot be used at high operating temperature otherwise column lifetime and column efficiency may decrease. Thus, dilution of derivatized CDs in polysiloxane is employed for GC analysis to improve the temperature stability and to

obtain high efficiency over a broad operating temperature range. The dilution of CDs derivatives is nowadays employed routinely and widely used in GC [7, 13-16, 21-27].

Schlenk *et al.* [28] firstly reported the use of CD derivatives in GC separation in 1962. The acetate, propionate, butyrate and valerate derivatives of β-CDs were used to separate the homologues of fatty alcohols, fatty esters, methyl esters, olefins, aldehyde esters and aldehydes. The result showed that the type of substituent of derivatized CDs play the important role on the polarity and heat stability of the stationary phases. The valerate modified β-CD offered the best resolution while the acetate modified β-CD showed the poorest resolution for the analytes.

Schurig [14] 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 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-homogeneous film coating of derivatized CDs, while maintaining high separation efficiency.

2.3 Parameters influencing the enantiomeric separations

While the formation of transient diastereomeric intermediate occurs during the direct enantiomeric separation of GC, the recognition process may involve with various forces such as hydrogen bonding, dispersion forces, dipole-dipole interactions and other forces [18]. The three hydroxyl groups of glucose unit of CD ring can easily be substituted with similar or different groups that can introduce or change the type of interactions associated between analyte and CD. The increase differential interaction between each enantiomer and stationary phase; thus, enhance the enantiomer resolution. Previous research had reported that there are several factors affecting the enantiomeric separation using CD derivatives as chiral selectors such as CD ring size, substitution pattern on the CD rings, concentration of CDs in

polysiloxane, polarity of polysiloxane matrix, separation temperature and structure of chiral analytes [21-35].

Some studies on enantiomeric separation by gas chromatography using cyclodextrin derivatives as chiral stationary phases (CSPs) are summarized as followed.

Nie *et al.* [30] 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.

Anderson *et al.* [31] separated seventeen 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. The results demonstrated that the size and polarity/electronegativity of sulfoxide substituents had affected on their enantioselectivities as well as the size and substituent pattern of all CD derivatives. 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.* [32] examined the influence of substituents in the C6 position of CDs. Three different acyl groups (valeryl, heptanoyl, and octanoyl) were substituted at the C6 position of 2,3-di-*O*-pentyl-β-cyclodextrin, respectively. The

chromatographic properties of these CDs as capillary GC stationary phases were also investigated. The result showed that the carbon chain length of acyl groups at the C6 position of CDs had many important effects on the enantioseparation of CDs. Among CD derivatives studied, 2,3-di-*O*-pentyl-6-*O*-valeryl-β-cyclodextrin exhibited the best enantioselectivity to 15 pairs of enantiomeric analytes. Moreover, results obtained on CDs with acyl groups at the C6 position were compared to those obtained on CDs with acyl groups at the C3 position [33]. Both types of CD derivatives possess enantioselectivity for some chiral compounds studied. But more baseline separations were obtained on CDs with acyl groups at the C3 position. However, the enantioseparation depends on the structure and property of analytes as well.

Skórka *et al.* [34] investigated both the influence of the size of CDs and the structure of monoterpenoids on enantioseparation. It was found that enantioseparation of monoterpenes by α- and β-CDs resulted from the formation of 1:2 stoichiometric complexes. Furthermore, thermodynamic data (enthalpy, entropy and free energy of the complexation process) from both α- and β-CDs displayed higher values for bicyclic than for monocyclic monoterpenoids.

The introduction of *tert*-butyldimethylsilyl (TBDMS) group at the C6 position of CDs derivatives not only improves their solubility in polysiloxanes but also impacts on the conformation of CDs toward blocking of the small rim of CD cavity that can greatly affect enantioselectivities as well. The 6-*O*- *tert*-butyldimethylsilyl substituted CDs have been widely used as CSPs for GC [21, 23, 24-27, 35].

Shitangkoon and Vigh [21] studied the influence of the size of substituents at C6 position of CDs on the enantioselectivities of various chiral test compounds from different chemical classes. Heptakis(2,3-di-*O*-methyl) derivatives of β-cyclodextrin with various size of 6-*O*-substituents (including deoxy-fluoro, methyl, *n*-pentyl, *n*-propyldimethylsilyl, *tert*-butyldimethylsilyl, and triisopropylsilyl) were prepared and then dissolved in OV-1701 to form useful gas chromatographic stationary phases. The result showed that all phases could operate at temperature as

high as 250 °C, the lower operating temperature decreased greatly as the size of substituent increased. Moreover, the solubility of CDs in polysiloxane (OV-1701) also increased as the size of substituent increased. Among all the substituents at C6 position of CDs, *tert*-butyldimethylsilyl group showed the best enantioselectivity for most of the test compounds.

Takahisa and Engel [24, 25] investigated the influence of the size of CDs on the enantioseparation of various flavor compounds from different chemical classes. The heptakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD (or 2,3-MOM-6-TBDMS-β-CD) and octakis(2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl)-γ-CD (or 2,3-MOM-6-TBDMS-γ-CD) were employed as CSPs for GC analysis. It was observed that 2,3-MOM-6-TBDMS-γ-CD could resolve a very broad spectrum of volatiles comprising various functional groups while the 2,3-MOM-6-TBDMS-β-CD showed better enantioselectivity towards several classes of compounds, except for secondary alcohols. Considering the result obtained from the enantioseparation of homologous series of esters of secondary alcohols that varying in chain length of acyl moieties (from acetate to hexanoate), the separation factor obtained from both phases decreased as the length of side chain increased. However, 2,3-MOM-6-TBDMS-γ-CD showed the higher separation factor for butanoates and hexanoates than 2,3-MOM-6-TBDMS-β-CD. The result showed that not only analyte structures significantly effect to the enantioseparation but also the size of CDs plays also the important role on the enantioseparation.

2.4 Enantiomeric separation of phenoxy acids by derivatized cyclodextrins

Derivatized cyclodextrins have been used extensively as chiral selectors for enantiomeric analyses in chromatographic and electrophoretic techniques for different fields of research such as essential oils, fragrances, alcoholic beverages, terpenoids, pharmaceutical, pesticides and herbicides [19]. Separation of chiral phenoxy herbicides were mostly performed by capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) using derivatized CDs as the chiral

selectors [6, 36-40]. Research related to enantiomeric analysis of phenoxy herbicides are as followed:

Schmitt *et al.* [6] separated four groups of chiral pesticide enantiomers: organophosphorus, DDT congeners, phenoxy acid methyl esters and metolachlor (Figure 2.2) using micellar electrokinetic chromatography (MEKC). 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 dodecyl sulfate buffer with and without organic modifier, to separate these pesticides and their enantiomers. The enantiomers of malathion, ruelene and dialifos 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 allowed the baseline separation of the three phenoxy acid methyl esters, the 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 dichlorprop 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.

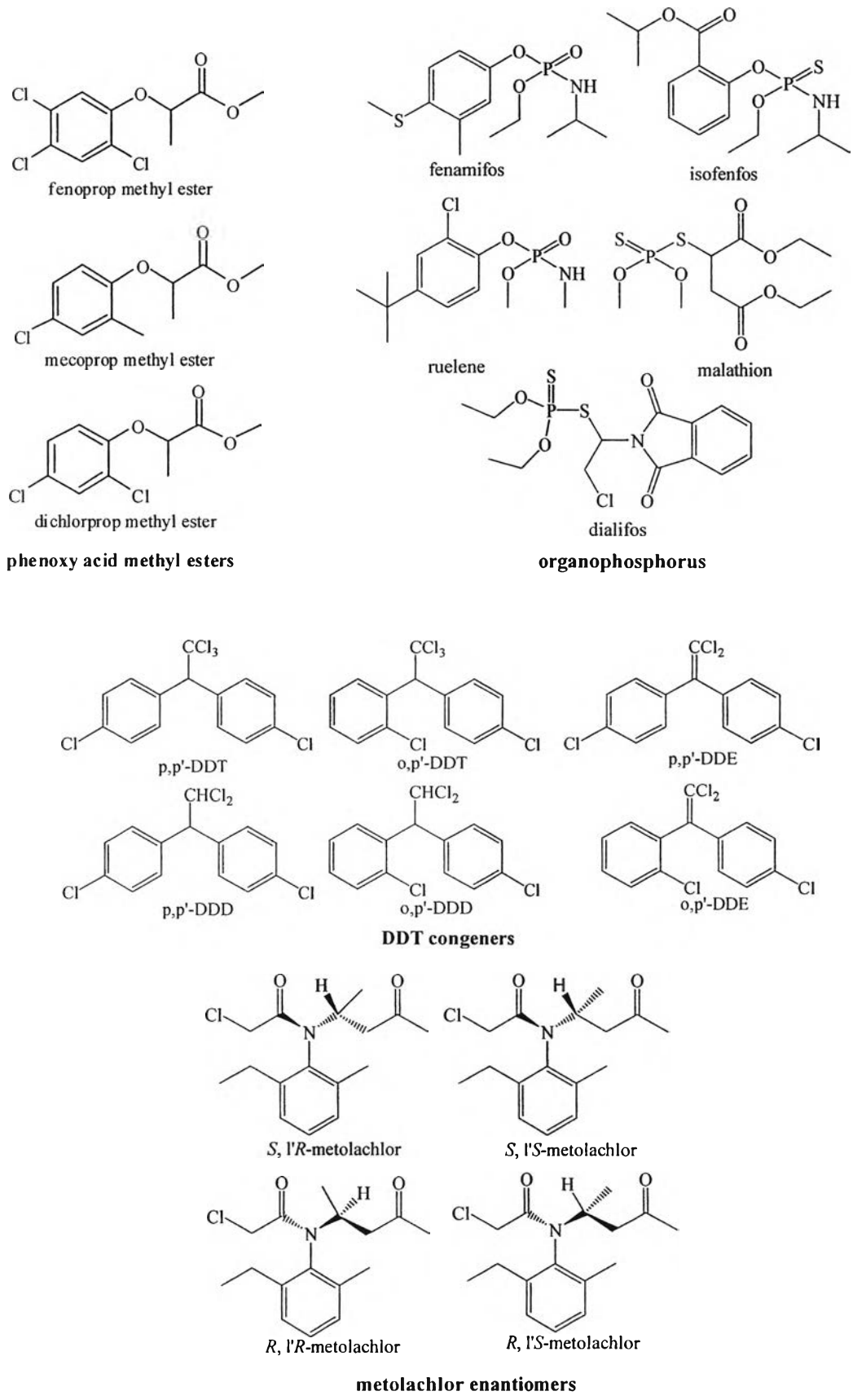

Figure 2.2 Structures of four classes of herbicides studied by Schmitt *et al.*

Zerbinati *et al.* [36] investigated 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 in 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 racemates. 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.* [37] studied mutual and chiral separations of nine phenoxy acid herbicides (Figure 2.3) 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. For the chiral separation of seven racemic phenoxy acid herbicides, unmodified and methylated-α-CDs exhibited higher enantioselectivities than the β- and γ-CD additives. 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.

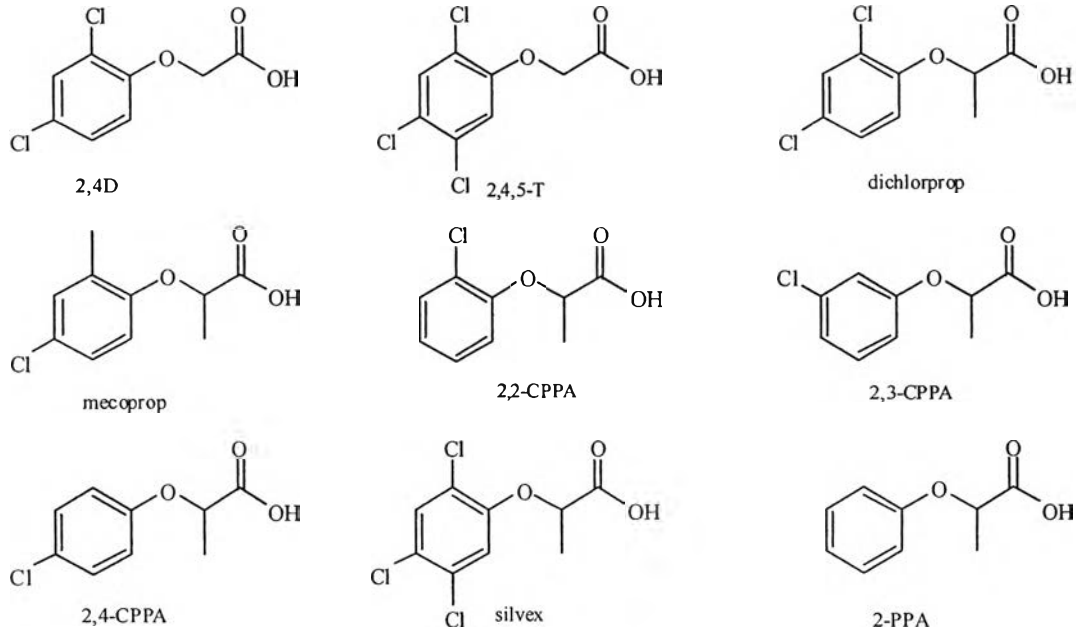

Figure 2.3 Structures of nine phenoxy acid herbicides studied by Miura *et al.*

Later, Tsunoi *et al.* [38] investigated the simultaneous (chiral and mutual) separation of nine phenoxy acid herbicides including seven pairs of phenoxy acid enantiomers by CE using CDs with negatively charged sulfonyl group as additive. The introduction of a sulfopropyl or a sulfonyl group to the neutral CDs produced a decrease in the enantioselectivities for the 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.

Martín-Biosca *et al.* [39] studied the enantiomeric resolution of six chiral phenoxy acid herbicides (Figure 2.4) by electrokinetic chromatography using cyclodextrin as chiral pseudophase (CD-EKC). Various chiral selectors such as native, neutral and charged CDs were optimized under several experimental conditions. Among several cyclodextrins tested, (2-hydroxy)propyl β-cyclodextrin (HP-β-CD) had demonstrated to be the most appropriate chiral selector for baseline resolution of the enantiomers of phenoxy acids studied. Among six chiral analytes, four of them (2-PPA, 3-CPPA, 4-CPPA, and 2,4-DCPPA) were enantioseparated by HP-β-CD. In addition, the influence of the chiral selector concentration, background electrolyte, pH

and temperature has been studied. It was found that 15 mM HP-β-CD in 50 mM ammonium formate at pH 5 and 40 °C was a suitable condition for baseline separation of four phenoxy acids. Furthermore, separation of multicomponent mixtures was also possible.

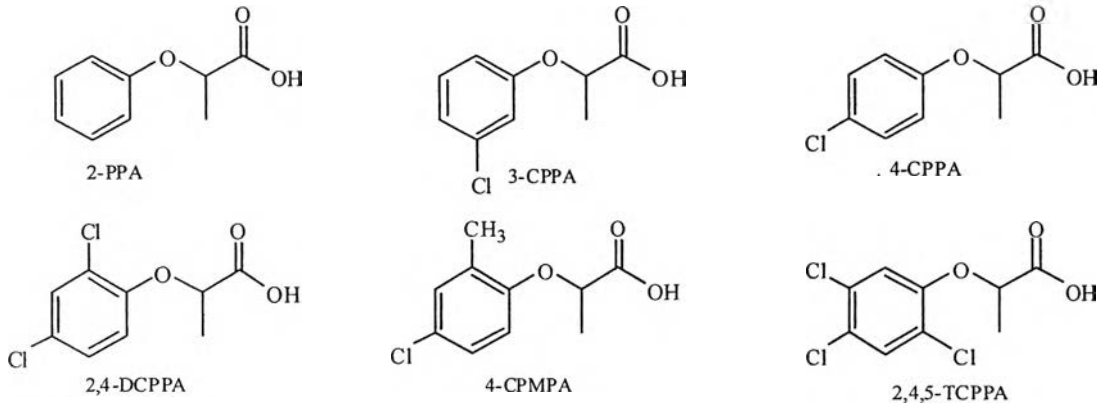

Figure 2.4 Structures of six herbicides studied by Martín-Biosca *et al.*

Darrouzain *et al.* [40] investigated the retention and complexation mechanisms of phenoxypropionic acid (PPA) herbicide series by reversed phase high performance liquid chromatography using hydroxyl-propyl-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 an entropically controlled process.

Phenoxy herbicides were widely produced to the market as the racemates in two forms; the acid form and the ester form. The acid forms of phenoxy herbicides have high polarity; therefore, they were usually derivatized into less polar form before GC analyses [41, 42]. Previously, there are only a few investigations on enantiomeric separations of phenoxy herbicides by GC technique. Some studies

related to enantiomer separation of phenoxy herbicides by GC using CD derivative as chiral selector were discussed below:

Weber *et al.* [7] 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 fenoprop-methyl, demonstrating that type, position and number of aromatic substituents play important roles on the enantioselective separation of phenoxypropionate analytes.

König *et al.* [29] separated enantiomers of three phenoxy acid herbicides (mecoprop, dichlorprop and fenoprop) by GC with electron capture detection (GC-ECD) employing three modified cyclodextrins as chiral stationary phases. The excellent enantioseparations toward three esters were obtained with columns containing a 1:1 mixture of liquid per-*O*-pentylated and solid per-*O*-methylated-β-CD. This column can only be used above 100 °C, below this temperature per-*O*-methylated-β-CD seems to crystallize and only broad peaks are obtained. Enantiomers of the methyl esters of mecoprop and dichlorprop were also resolved on octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin. On this phase, the reversal of elution order was observed.

Rodthongkum [23] firstly reported the systematic studies of enantiomeric separation of forty-six phenoxy acid methyl esters with different type, position and number of substitution by capillary GC. Heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (BSiMe) and heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (BSiAc) were employed as chiral selectors. The result showed that BSiMe phase exhibited higher degree of enantioseparation towards most analytes than BSiAc phase. However, the type, position and number of substituent on the aromatic ring of analyte strongly influence enantioseparation on both columns. Furthermore, the type of substituent on cyclodextrin molecule (BSiMe *vs.* BSiAc) also affects enantioseparation of phenoxy acid methyl esters as well.

As mentioned previously, the analyte structure and type of CD are the crucial factors affecting the enantioselectivities; nevertheless, these factors are 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.

2.5 Thermodynamic study of enantiomeric separation by gas chromatography

Although the chiral recognition mechanism of chromatographic separation using CDs has still been unclear, some mechanistic aspects can be derived from thermodynamic investigations. From the retention behaviors, the thermodynamic parameters (e.g. enthalpy, entropy, Gibbs free energy, etc.) associated with the enantiomers and CSP can be obtained. Generally, it is accepted that the direct enantiomeric separation is based on the formation of reversible diastereomeric complex associated 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 [14, 22].

In van't Hoff approach [22], the difference in Gibbs 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):

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

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).

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

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

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

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

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 α vs. 1/T plot. However, the calculations of thermodynamic parameters from plot of ln α 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 polysiloxane stationary phase [22].

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.

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

$$\Delta G = \Delta H - T \cdot \Delta S \quad \textbf{(6)}$$

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

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

$$\ln k' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} - \ln \beta \quad \textbf{(7)}$$

where K is the distribution coefficient of chiral analyte 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 enantiomeric separation.