CHAPTER II

THEORY

2.1 Phenoxy acids

Phenoxy acids are the most widely used herbicides. They have been commercially accessible for more than sixty years. In 1970s, there was a study in safety of using phenoxy acids by Lennart Hardell. The outcome indicated that there was not enough evidence to support any risk from 2,4-dichlorophenoxyacetic acid (2,4-D), the most general herbicide, to human health. Therefore, U.S. Environmental Protection Agency (EPA) categorized 2,4-D as a D group which was not classifiable as to human carcinogenicity. Also, U.S. EPA decided that 2,4-D was acceptable for prolonged usage [13].

Phenoxy herbicides are mostly used in the ester forms, phenoxy acid methyl esters (PAMEs), because of their higher herbicidal activity than the acid forms. In some cases, (*R*)-enantiomers of PAMEs demonstrate greater herbicidal activity than (*S*)-enantiomers. Hence, there is great necessity to produce, purify and analyze single enantiomer. To obtain purely single enantiomer, there are two methods; asymmetric synthesis and enantiomeric separation. Presently, asymmetric synthesis using chiral reagents is an outstanding way. It is vigorously improved. For enantiomeric separation, chromatographic and electrophoretic techniques are used. Gas chromatography (GC) is one of separation techniques that can be used to analyze phenoxy herbicides in the ester forms [13-15].

2.2 Gas chromatographic separation of enantiomers

GC has been recognized as a reliable analytical method for separation of volatile and thermally stable organic compounds. Capillary GC is a technique with high efficiency, reproducibility, sensitivity, simplicity and short analysis time. Owing to several advantages of capillary GC, many compounds can be separated without

derivatization. Therefore, capillary GC has become the good option for the analysis of enantiomer in several samples [8, 9].

Enantiomeric separation using capillary GC can be demonstrated in both indirect and direct ways. The indirect method can be obtained by derivatization of the racemic analytes with a chiral derivatizing agent, resulting in diastereomers which can be separated on most achiral stationary phases. In contrast, the direct method uses a CSP as a selector to analyze samples without changing to diastereomers. The direct enantiomeric separation using CSP is a common and effective method to analyze enantiomers. In chiral capillary GC, the chiral selector is coated as a thin film on a capillary wall. Among several chiral selectors, CD derivatives are the most generally used selectors to separate various chiral molecules [16-18].

2.3 CDs and derivatives as gas chromatographic stationary phases

CDs are cyclic oligosaccharides formed through degradation of starch by the enzyme cyclodextrin glycosyltransferase (CGTase). The three most generally used CDs consisted of six, seven and eight D-glucose units linked by α-1,4-glycosidic bond. They are α-, β- and γ-CDs respectively. Some properties of these CDs are concluded in Table 2.1.

Table 2.1 Some properties of α-, β- and γ-CDs [19, 20]

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	23.20
decomposition temperature (°C)	278	299	267

Native CDs are non-hygroscopic, homogeneous and crystalline materials which look like torus. The CD ring contains glucopyranose unit in chair conformation. Each glucopyranose unit of CD ring has three free hydroxyl groups (-OH), two of them are secondary hydroxyls and the rest is primary hydroxyl. The secondary hydroxyls 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 narrow edge of the torus (Figure 2.1). Thus, CDs are hydrophobic inside the hole and hydrophilic on the edge of the hole. CDs can simply form inclusion complexes. This is a significant character for the applications [21, 22].

(a) (b)

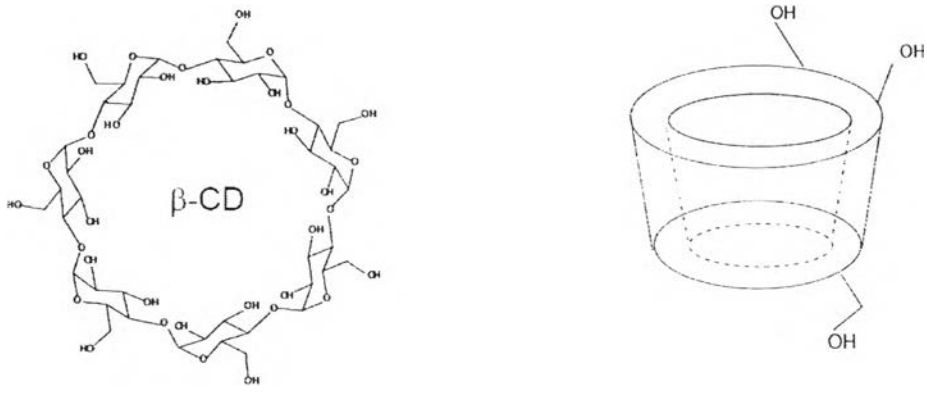

Figure 2.1 (a) Structure of β-CD molecule with 7 glucose units; (b) Side view of CD showing primary hydroxyl on a narrow rim and secondary hydroxyls on a larger rim of a ring

CDs are substituted with various types of functional groups on the primary and/or secondary hydroxyl groups to change their selectivities in separation. Normally, chiral C2 and C3 atoms of glucopyranose unit are changed using small alkyl or acyl groups which influence the enantioselectivity. On the contrary, C6 atom of glucopyranose unit, a non-chiral atom, is changed using the longer alkyl or bulky groups which improve the solubility in polysiloxanes. Nevertheless, substitution at nonchiral carbon affects on the conformation of the CDs toward blocking of the entrance of the hole at the narrow edge which can impact the enantioselectivity as well [21-23].

2.4 Parameters influencing the enantiomeric separation

Previous publication had shown that there are numerous factors affecting the enantiomeric separation by GC using CD derivatives as chiral selectors such as CD ring size, substitution patterns on the CD rings, concentration of CD in polysiloxane, polarity of polysiloxane matrix, separation temperature and structure of chiral analytes [24-28].

Some investigations on enantiomeric separation by GC using CD derivatives as CSPs are concluded below.

Nie *et al.* [26] analyzed enantiomers of amines, alcohols, diols, carboxylic acids, amino acids, epoxides, halohydrocarbons and ketones by GC using three derivatized β-CDs with different alkyl chain length as CSPs. They were heptakis-(2,6-di-*O*-pentyl-3-*O*-trifluroacetyl)-β-CD (DPTBCD); heptakis-(2,6-di-*O*-nonyl-3-*O*-trifluroacetyl)-β-CD (DNTBCD) and heptakis-(2,6-di-*O*-dodecyl-3-*O*-trifluroacetyl)-β-CD (DDTBCD). The outcome indicated that DNTBCD could separate the wide range of enantiomers with good enantioselectivity for most of the racemates investigated.

Chen *et al.* [24] examined the effect of substituents at primary hydroxyls of CDs. Three acyl groups with different alkyl chain length (valeryl, heptanonyl and octanonyl) were substituted at the C6 position of 2,3-di-*O*-pentyl-β-CD. Chromatographic properties of these CDs as capillary GC stationary phases were studied. The result showed that 2,3-di-*O*-pentyl-6-*O*-valeryl-β-CD demonstrated the best enantioselectivity for fifteen pairs of enantiomers studied.

Shi *et al.* [27] studied the effects of substituent types and positions of some selected ester and epoxide enantiomers by GC using four derivatized β-CDs as CSPs. They were 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. These CSPs were able to separate enantiomers of allethrone acetate, propargyllone acetate, 2-bromopropionic acid methyl ester, 2-chloropropionic acid methyl ester and epoxides. Elution order, enantioselectivity and peak characteristic were the same in all selected CSPs demonstrating that position of allyl group or propyl group on C3 or C6 did not influence in enantiomeric separation.

McGachy *et al.* [25] studied the effect of ester alkyl group of twelve enantiomers of *N*-trifluoroacetyl-*O*-alkylnipecotic acid esters on the interaction with permethylated-β-CD (Me-CD) and enantioselectivity values. The result showed that the *n*-alkyl analytes had stronger interaction with Me-CD than the branched alkyl analytes. Also, the α-branched alkyl esters showed greater enantioselectivity than the *n*-alkyl or β-branched isobutyl esters.

Shitangkoon *et al.* [28] studied the correlation between nineteen analyte structures and enantioseparation. This research analyzed enantiomers of 1-phenylethanol derivatives by GC using heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD mixed in OV-1701 as CSP. Retention factors, enantioselectivity and thermodynamic factors illustrated that only trivial changes in analyte structures such as position, polarity and size of substituents significantly affected the separation selectivity. Furthermore, substitutions on aromatic ring of alcohols tend to enhance the enantiomeric separation. Nonetheless, substitutions on side chains seem to reduce the enantiomeric separation of selected analytes.

2.5 Enantiomeric separation of phenoxy acids by derivatized CDs

CD derivatives have been used broadly as chiral selectors for enantiomeric separation in chromatographic and electrophoretic techniques for dissimilar field of research such as fragrances, essential oils, pharmaceutical compounds, fertilizers and herbicides [22]. Most separation of chiral phenoxy herbicides were accomplished by gas chromatography (GC) [6, 11, 12], high performance liquid chromatography (HPLC) [29] and capillary electrophoresis (CE) [30, 31] using derivatized CDs as chiral selectors. Research related to enantiomeric analysis of phenoxy herbicides are listed below.

Weber *et al.* [6] considered the effect of aromatic substituents on enantiomeric separation of phenoxypropionates (Figure 2.2) by GC using permethylated β-CD as a CSP. Enantioselectivity and separation efficiency seem to be declined by three fold from mecroprop-methyl and dichlorprop-methyl to fenoprop-methyl, illustrating that type, position, and number of aromatic

substituents play an important role on enantioselective separation of phenoxypropionate analytes.

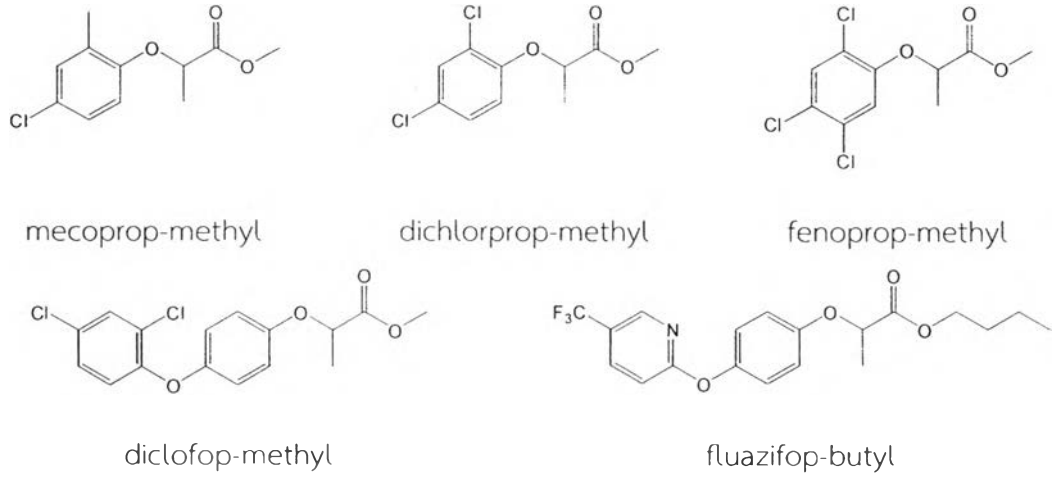

Figure 2.2 Structures of five phenoxypropionates studied by Weber *et al.* [6]

Zerbinati *et al.* [31] separated racemic of 2,4-DCPPA (Figure 2.3) by capilly zone electrophoretic using eight CDs. They were α-CD, β-CD, methyl-β-CDs, hydroxypropyl-β-CDs, C_6-capped-β-CD, ethylcarbonate-β-CD and ethylcarbonate-γ-CD as chiral resolving agents. The result showed that α-CD, methyl-β-CD, hydroxypropyl-β-CDs, ethylcarbonate-β-CD and C_6-capped-β-CD could separate the racemates. Among all CDs studied in this research, ethylcarbonate-β-CD gave the best enantiomeric resolution. While, native β-CD and C_6-capped-β-CD did not gave resolution for the racemic 2,4-DCPPA.

Martin-Biosca *et al.* [30] studied the enantiomeric resolution of six phenoxy acid herbicides by CE using (2-hydroxy)propyl-β-CD (HP-β-CD) as chiral selector. Six analytes were 2-PPA, 3-CPPA, 4-CPPA, 2,4-DCPPA, 4-CPMPA and 2,4,5-TCPPA (Figure 2.3). The result showed that HP-β-CD could separate enantiomers of 2-PPA, 3-CPPA, 4-CPPA and 2,4-DCPPA. 4-CPPA gave the best enantioselectivity (α=1.34). Moreover, CD concentration, background electrolyte, pH and temperature were also studied. A suitable condition for baseline resolution of four phenoxy acids was 15 mM HP-β-CD in 50 mM ammonium formate at pH 5 and 40 °C.

Darrouzain *et al.* [29] studied the retention and complexation mechanisms of four phenoxypropionic acid (PPA) herbicides by reversed phase HPLC using HP-β-CD

as mobile phase additive. The analytes were 2-PPA, 2-CPPA, 3-CPPA and 2,4,5-TCPPA (Figure 2.3). The influence of organic component and the HP-β-CD concentration in mobile phase were analyzed at several column temperatures. At low HP-β-CD concentration, retention mechanism was led by free PPA and elution order was 2-PPA < 2-CPPA ≈ 3-CPPA < 2,4,5-TCPPA respectively. At high HP-β-CD concentration, retention mechanism was led by free PPA/ HP-β-CD complexation and elution order was 2,4,5-TCPPA < 2-PPA < 2-CPPA ≈ 3-CPPA respectively.

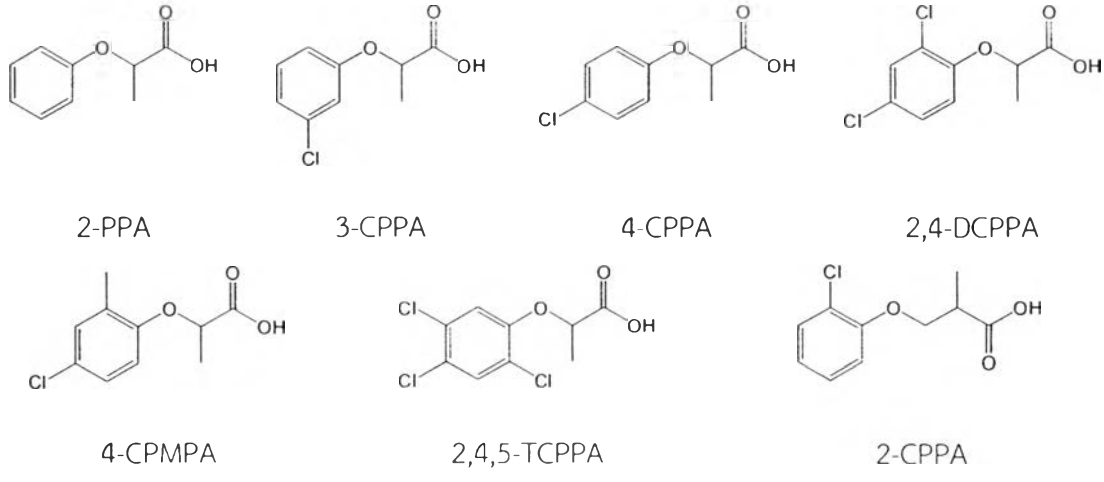

Figure 2.3 Structures of seven phenoxy acids studied by Zerbinati *et al.* [31], Martin-Biosca *et al.* [30] and Darrouzain *et al.* [29]

Rodthongkum [12] systematically studied GC enantiomeric separation of fourty-six PAMEs with different type (F, Cl, Br, Me, OMe, CF_3, CN, NO_2), position (*ortho*-, *meta*-, *para*-) and number (1 to 3) of substitution. Heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD (BSiMe) and heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-CD (BSiAc) were used as chiral selectors. The results showed that BSiMe phase could separate fourty-three pairs of enantiomeric PAMEs, while BSiAc phase (having different type of substitution on CD) could separate thirty-three pairs of enantiomers. On BSiMe column, *ortho*- and *para*-substituted analytes tended to improve enantioselectivity. In addition, type of substituent played a key role in separation. Methyl 2-(4'-(trifluoromethyl)phenoxy) propanoate showed the highest degree of enantioseparation. On BSiAc column, *meta*-substituted analyte seem to improve enantioselectivity. Considering type of substituent, methyl-substituted analytes increased the enantioselectivity on this column.

Maha-ard [11] systematically studied enantiomeric separation of fourty-six PAMEs with different type (F, Cl, Br, Me, OMe, CF_3, CN, NO_2), position (*ortho*-, *meta*-, *para*-) and number (1 to 3) of substitution by capillary GC. Hexakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-α-CD (ASiMe) and octakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-γ-CD (GSiMe) were used as chiral selectors. The result showed that ASiMe phase could separate thirty-two enantiomers of PAMEs, while GSiMe phase could separate seventeen enantiomers. On ASiMe column, *meta*-substituted analyte tended to improve enantioselectivity. In addition, type of substituent played a key role in separation. Methyl 2-(3',5'-dichlorophenoxy)propanoate showed the highest degree of enantioseparation. On GSiMe column, *meta*-substituted analyte also seem to improve enantioselectivity. Furthermore, type of substituent played a significant effect to separation. Only all three isomers of trifluoromethyl- and cyano-substituted PAMEs could be enantioseparated.

2.6 Thermodynamic study of enantiomeric separation by gas chromatography

Even though chiral recognition mechanism of enantiomeric separation by chromatographic techniques using CDs has been still uncertain, some mechanistic features can be obtained from thermodynamic calculation. Thermodynamic parameters such as enthalpy, entropy, Gibbs free energy, etc. related to enantiomers and CSP can be obtained from chromatographic values [32, 33].

Normally, it is known that direct enantiomeric separation depends on formation of reversible diastereomer and intermolecular interaction of enantiomers with a chiral selector. This process for each enantiomer can be characterized by thermodynamic data using the Gibbs-Helmholtz equation [33].

According to van't Hoff equation [33], the different in Gibbs free energy, ΔΔG, is calculated from the separation factor (α) achieved from chiral separation on a chiral column at particular temperature from equation (1)

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

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 of 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

In relation to equation (4), $\Delta\Delta H$ and $\Delta\Delta S$ could be evaluated from the slope and y-intercept of ln α versus 1/T plot. Nevertheless, calculations of thermodynamic parameters from the plot of ln α versus 1/T are impossible because of curvatures.

This is a non-linear dependence of selectivity on concentration of selectors in diluted polysiloxane stationary phase. Thus, this process is only available for undiluted chiral selectors [33].

Otherwise, thermodynamic parameters can be calculated from retention factors instead of separation factors. Combination of equation (5) and (6) results in equation (7), indicating the correlation between k' and 1/T which is linear. Thermodynamic parameters of each enantiomer can be done from ln k' versus 1/T plot. Then, the differences in enthalpy and entropy of two enantiomers can be obtained.

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

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

$$-\Delta H + T \cdot \Delta S = RT \cdot \ln (k' \cdot \beta) \quad (7)$$

$$\frac{-\Delta H}{RT} + \frac{\Delta S}{R} = \ln k' + \ln \beta \quad (8)$$

$$\ln k' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} - \ln \beta \quad (9)$$

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 interaction of the enantiomer with 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 interaction of the enantiomer with stationary phase. ΔS value describes the degree of which the solute structure influences the interaction.

Thermodynamic parameters learned in this research would bring great vision about interaction between PAMEs and CD derivatives. Expectedly, the interpretation of the data found from this work will simplify some mechanistic knowledge about the effect of analyte structure on enantiomeric separation.