CHAPTER I

INTRODUCTION

1.1 Introduction and Modes of CE

Capillary electrophoresis (CE) is a modern analytical technique for separation of charge compounds in a small capillary containing an electrolyte solution under electric field. Applications of CE are, for example, separations of proteins and peptides, DNA sequencing, determination of organic and inorganic ions, and chiral separation. CE provides efficiency up to two orders of magnitude greater than high-performance liquid chromatrograpy (HPLC). [Chankvetadze 1997]

Six basic modes in CE include capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP) and capillary isoelectric focusing (CIEF). The first three modes are commonly used for separation and analysis of small molecules, including enantiomeric separation.

Capillary Zone Electrophoresis (CZE) is the simplest and most popular mode of CE. The background electrolytes (BGE) used in CZE are typically buffers such as phosphate, borate and acetate. The separation mechanism is based on the different electrophoretic mobilities of the analytes due to the difference in the charge-to-size ratios of the analytes.

Micellar Electrokinetic Chromatography (MEKC) is the second most commonly used mode of CE. The BGE in MEKC contains a surfactant such as sodium dodecyl sulphate (SDS), which forms micelles. The separation mechanism is based on the different partitioning of the analytes between micelle and aqueous phases. The most

important feature of MEKC is that it allows the separation of neutral compounds and is sometimes beneficial for charged analytes.

Capillary Electrochromatography (CEC) is a hybrid of CE and HPLC. The CEC column contains HPLC packing materials, and analytes migrate due to electric field, instead of pressure as in HPLC. The separation mechanism in CEC is based on either difference in the partitioning of analytes between mobile phase and stationary phase or the difference in electrophoretic mobilities of analytes. Using the same stationary phase in a capillary column, CEC gives higher efficiency due to flat flow profile in CEC [Dittmann *et al.* 1995], while parabolic flow profile in HPLC.

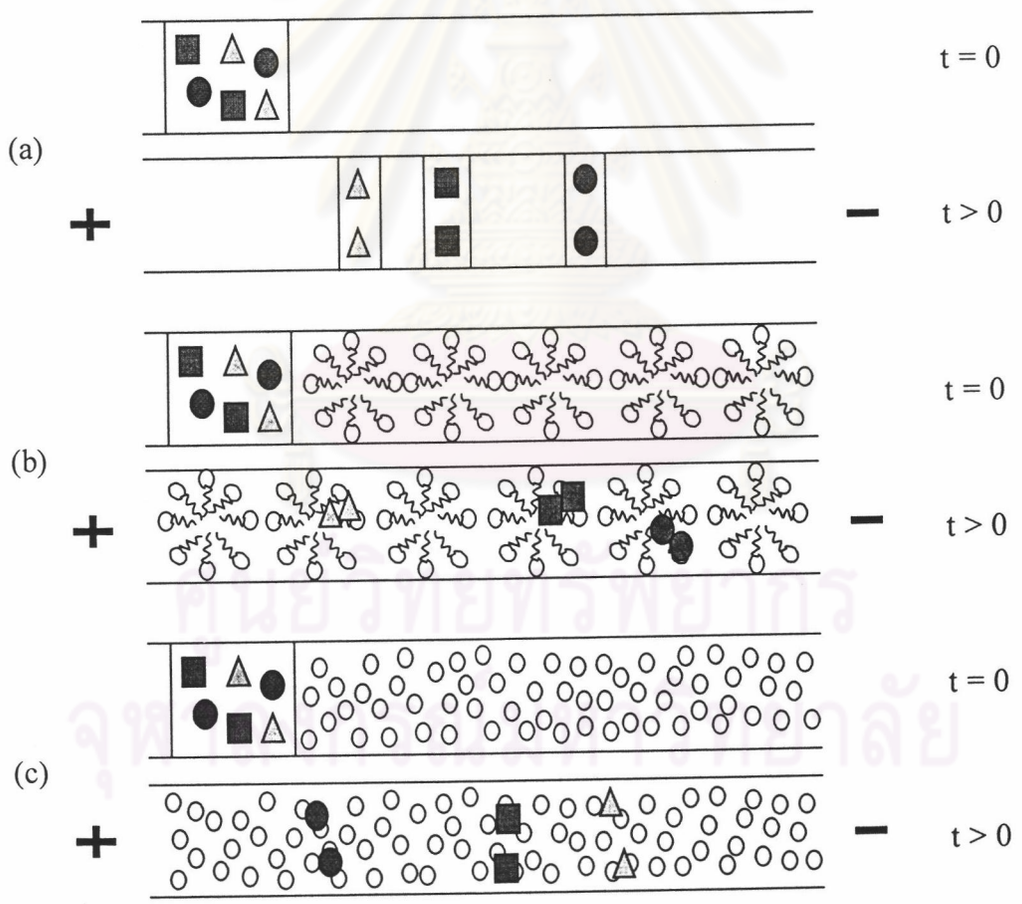


Figure 1.1 Separation mechanism in (a) CZE, (b) MEKC and (c) CEC. Adapted from Andrea and Brown [1997].

1.2 Fundamental of CE and CZE

1.2.1 Electrophoretic Mobility [Grossman and Colburn 1992, Foret *et al.* 1993]

When a voltage V is applied across a capillary of length L containing the BGE, each ionic species is accelerated by a constant electric force F_E

$$F_E = zeE \tag{1.1}$$

where z is the charge of an ion, e the fundamental electronic charge, and E the electric field

$$E = \frac{V}{L} \tag{1.2}$$

The acceleration will proceed until the electric force is balanced by the frictional force F_F due to the viscosity of the medium.

$$F_E = F_F \tag{1.3}$$

For a spherical ion, F_F is given by

$$F_F = 6\pi\eta r_h v_{ep} \tag{1.4}$$

where η is the viscosity of the medium, r_h the hydrodynamic radius of the ion, and v_{ep} the electrophoretic velocity of the ion. It follows from Equations 1.1–1.4 that

$$v_{ep} = \frac{ze}{6\pi\eta r_h} E \tag{1.5}$$

Electrophoretic mobility, μ, is defined as the electrophoretic velocity of an ion when an electric field of 1 V m^{-1} is applied.

$$\mu = \frac{v_{ep}}{E} = \frac{ze}{6\pi\eta r_h} \tag{1.6}$$

It follows from Equation 1.6 that, in a medium of fixed viscosity, the values of electrophoretic mobility depend on the charge-to-size ratios of the ions, *z/e*. The electrophoretic mobility at zero ionic strength of the BGE is called the absolute mobility or limiting mobility, μ^o.

1.2.2 Electroosmotic Flow [Grossman and Colburn 1992, Foret *et al.* 1993]

In CE, the migration of analytes in the presence of the electric field depends on not only their electrophoretic mobilities, but also electroosmotic flow (EOF). The EOF, as shown in Figure 1.2, is the movement of a medium toward the electrode when the voltage is applied. In the presence of the background electrolyte (BGE) at pH > 2, silanol groups at the surface of the fused-silica capillary ionize as the equation below, resulting in the negative charges of the capillary and excess positive ions in the solution.

$$\text{-SiOH}\ (s) \rightleftharpoons \text{-SiO}^-\ (s) + \text{H}^+\ (aq)$$

These positive ions arrange themselves to form a double electric layer. Some positive ions are attached at the negative surface of the capillary to form an immobilized layer, called *the compact or Stern layer*, held by electric forces. Some positive ions form *the diffusion layer*, and the rest of the excess positive ions is in the bulk solution.

When the electric field is applied across the capillary, excess solvated positive ions in the diffuse layer and bulk solution migrate toward the cathode. This results in a flow of water molecules in the same direction. This phenomena is called *electroosmosis*, and the movement of water or solvent is called *electroosmotic flow*.

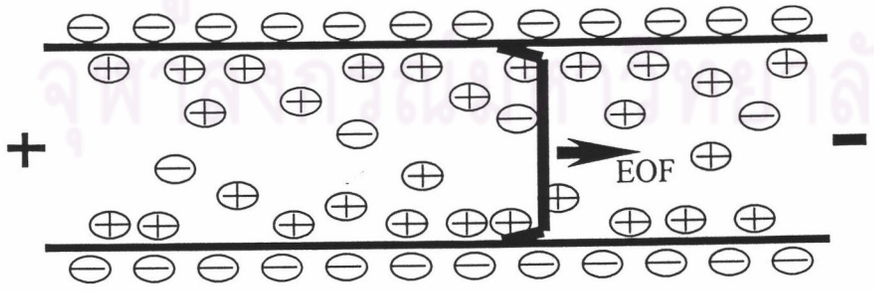


Figure 1.2 Electroosmotic flow (EOF). Adapted from Andrea and Brown [1997].

From the internal capillary surface, the electroosmotic velocity increases with increasing distance, and is constant at the distance of approximately 15 nm from the wall [Grossman and Colburn 1992]. Typically, the capillary used in CE has 20 to 100 μm I.D. (20000 to 100000 nm). Thus, it can be said that the electroosmotic velocity is constant throughout the capillary radius. The electroosmotic velocity (v_{eo}) is proportional to the zeta potential, as given by

$$v_{eo} = -\frac{\varepsilon\zeta}{4\pi\eta}E \qquad (1.7)$$

where ξ and η are the permittivity and the viscosity of the liquid in the double layer. These values may be different from those in the bulk solution [Kenndler 1998].

Since EOF is generated at the capillary wall, and driving force of EOF is uniformly distributed along the capillary, there is no pressure drop within the capillary. This results in a flat profile of the bulk flow which does not directly contribute to the zone broadening. The flat flow profile in CE contrasts to the parabolic profile generated by laminar flow driven by a pressure gradient in HPLC (Figure 1.3), resulting in high efficiency and resolution in CE than HPLC.

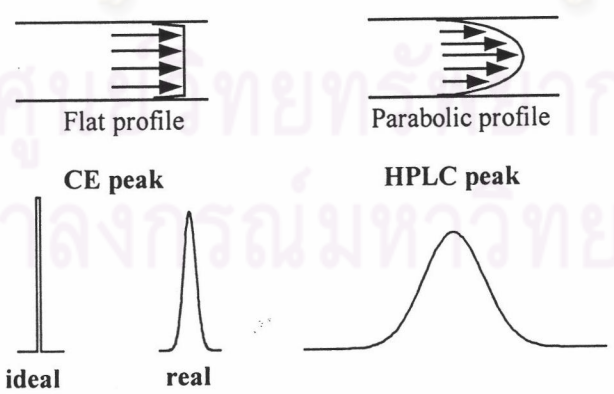


Figure 1.3 Flow profiles in CE and HPLC. Adapted from Chankvetadze [1997].

The net velocity, v_{net}, of the analyte is the sum of the electrophoretic velocity of the analyte and the electroosmotic velocity as Equation 1.8 and Figure 1.4

$$v_{net} = v_{ep} + v_{eo} \tag{1.8}$$

In the presence of high EOF, both anions and cations migrate to the detection window. For cations, $v_{ep,+}$ and v_{eo} have the same direction to the cathode at the detection window. The higher the ion charges and the smaller the ion size, the faster the migration toward the cathode. For anions, $v_{ep,-}$ has the direction toward the anode. In the case where $v_{eo} > v_{ep,-}$, the anions can migrate to the cathode. The higher the ion charges and the smaller the ion size, the smaller the net velocity. Neutral molecules migrate toward the cathode only due to EOF, and cannot be separated.

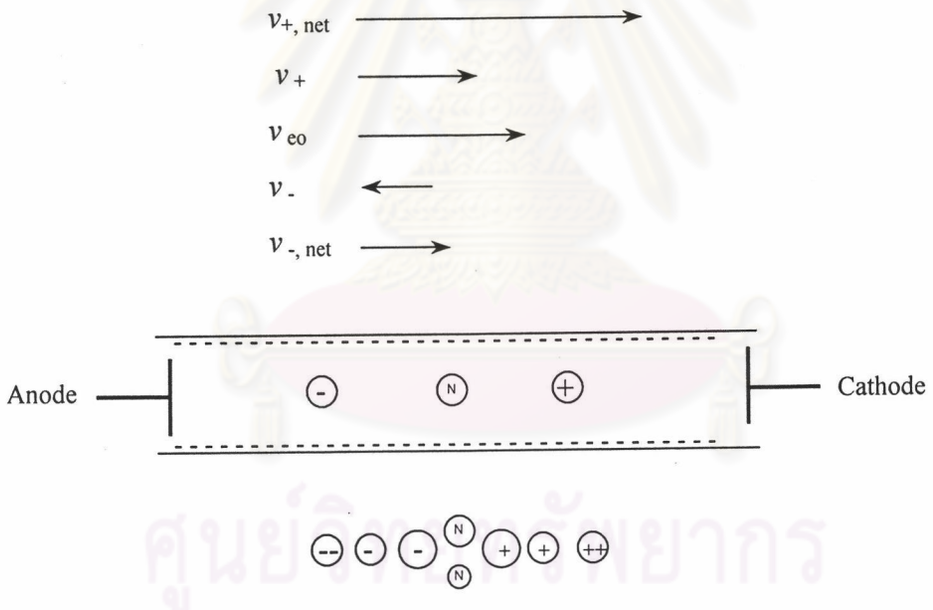


Figure 1.4 Migration behavior of analytes. Adapted from Li [1992].

1.2.3 Peak Efficiency and Resolution

The parameters of peak efficiency and resolution used in CE are characterized similarly to those in chromatography. Theoretically, when the solute migrates from the injection end to the detector, both chromatographic and electrophoretic peak are assumed to have a Gaussian peak with standard deviation, σ in the distance units and τ in time units, as shown in Figure 1.5. The width of the peak at base, w_b, may be obtained by drawing lines at tangents to the points of inflection, and measuring the separation between the points of intersection with the baseline.

$$w_b = 4\sigma \text{ or } w_b = 4\tau \tag{1.9}$$

and the peak width at half height, w_h, is give by [Giddings 1991]

$$w_b = 2.354\,\sigma \text{ or } w_b = 2.354\,\tau \tag{1.10}$$

Peak efficiency may be expressed by the number of theoretical plates, N. N can be calculated directly from the electropherogram by

$$N = 16\left(\frac{t_m}{w_b}\right)^2 = 5.54\left(\frac{t_m}{w_h}\right)^2 \tag{1.11}$$

where t_m is the migration time. N is also related to the standard deviation by

$$N = \left(\frac{l}{\sigma}\right)^2 = \left(\frac{t}{\tau}\right)^2 \tag{1.12}$$

The resolution, R_s, between peaks of two analytes, 1 and 2, is defined as the ratio of the difference in their migration times to the average of their peak width at base.

$$R_s = \frac{t_{m2} - t_{m1}}{0.5(w_{b1} + w_{b2})} \tag{1.13}$$

In CZE, it can be shown that Equation 1.13 allows resolution to be related to the mobilities and the average number of theoretical plates, $\overline{N}$, according to the equation

$$R_s = \frac{1}{4}\left(\frac{\Delta\mu}{\overline{\mu} + \mu_{eo}}\right)\sqrt{\overline{N}} \qquad (1.14)$$

where $\Delta\mu$ and $\overline{\mu}$ are the difference in the mobility and the average electrophoretic mobility of the analytes. Equation 1.14 shows that, when it is required to improve resolution, the most important parameter to enhance is the mobility difference between the analytes. Resolution also scales with the square root of the efficiency.

1.2.4 Sources of Peak Broadening [Grossman and Colburn 1992, Khaledi 1998]

Peak broadening in CE is caused by both the physical processes fundamental to the migration of analytes, e.g. longitudinal diffusion, Joule heating, electromigration dispersion and solute-wall interaction, and the effect due to instrument design, e.g. detector aperture width and detector time constant. The overall peak broadening can be expressed by the peak variance, σ^2.

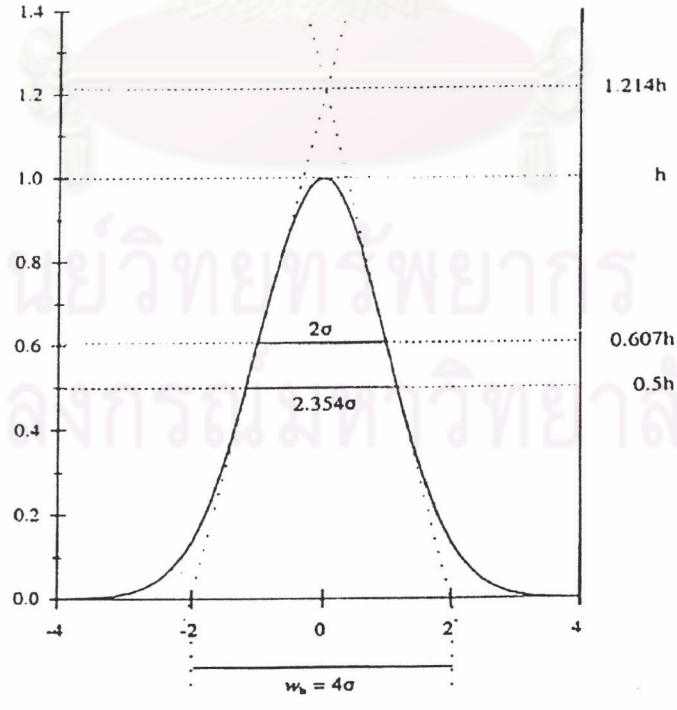


Figure 1.5 Gaussian peak. Adapted from Dyson [1990].

1.2.4.1 Longitudinal Diffusion

When the sample solution is introduced into one end of the capillary with an infinitely thin plug length, the concentration of the solute c is a function of its position in the capillary, x, at any given time, t, where diffusion is the only source of peak broadening

$$c(x,t) = \frac{Q_{inj}}{(4\pi Dt)^{1/2}} \exp(\frac{-x^2}{4Dt}) \qquad (1.15)$$

where Q_{inj} is the amount of sample injected, and D the diffusion coefficient of the analyte. This is the formula for a Gaussian peak with the special variance $2Dt$, in accordance with the random walk theory and given by the Einstien-Smoluchowski equation

$$\sigma^2_{diff} = 2Dt \qquad (1.16)$$

Here t is the time available for the solute particles in the sample zone to move and is thus the migration time, t_m in CZE. The diffusion coefficient is given by Nernst-Eistien equation

$$D = \frac{\mu^\circ kT}{ze} \qquad (1.17)$$

where μ° is the absolute mobility at zero ionic strength, k the Boltzmann constant, and T the absolute temperature.

1.2.4.2 Thermal Dispersion

Joule heating caused by the passage of current through an electrolyte leads to a parabolic temperature profile in the capillary. As mobility is a function of temperature, the parabolic temperature profile also results in a parabolic velocity profile for the analyte in an analogous fashion to profile caused by pressure-driven laminar flow in a capillary. The analytes in the center of the capillary travel faster than those at the capillary wall. Thermal peak broadening is then expressed analogously to the Einstien-Smoluchowski equation for one-dimensional diffusion by

$$\sigma_{th}^2 = 2D_{th}t_m \tag{1.18}$$

where D_{th} is the thermal dispersion coefficient

$$D_{th} = \frac{f_T^2 \kappa_c^2 E^6 r_c^6 \mu^2}{3072 \lambda_s^2 D} \tag{1.19}$$

where f_T is the temperature factor, $(1/\mu)(d\mu/dT)$. κ and λ_s are the electric conductivity and thermal conductivity of the BGE, respectively.

1.2.4.3 Electromigration Dispersion (EMD)

Electromigration dispersion (EMD) occurs due to lower conductivity of the BGE zone than that of the sample zone and mismatch of the electrophoretic mobilities between the analyte ion A and the BGE co-ion C. The BGE co-ion has the same charge as the analyte. For example, when the cationic analyte has higher μ than does the BGE co-ion, the cationic analyte in the leading edge of the sample zone will migrate faster into the BGE zone which has lower conductivity or higher local electric field strength ($\nu_{ep} \propto E$ as shown in Equation 1.5), resulting in the fronting triangular distribution in the distance domain, as shown in Figure 1.6a. When the cationic analyte has lower μ than does the BGE co-ion, the BGE co-ion will migrate faster into the tailing edge of the sample zone, resulting in the slower migration of the analyte in the tailing zone and the tailing triangular distribution of the analyte. In the presence of higher EOF and normal polarity, this situation is reversed when anions are analyzed instead of cations [Chankvetadze 1997, Nhujak 2001]. Figure 1.7 shows the effect of electromigration dispersion on the analyte concentration distribution in the presence of diffusion. It is noted that the peak in the time domain is the mirror image of that in the distance domain.

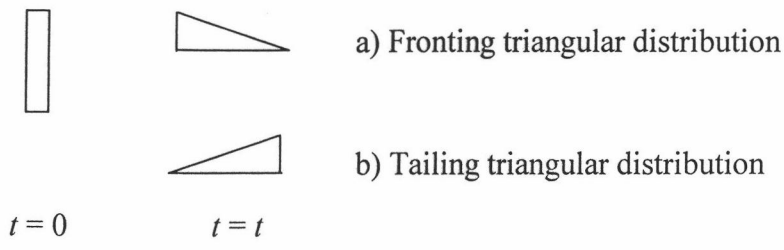


Figure 1.6 Concentration distribution of EMD in the distance domain. Adapted from Chankvetadze [1997].

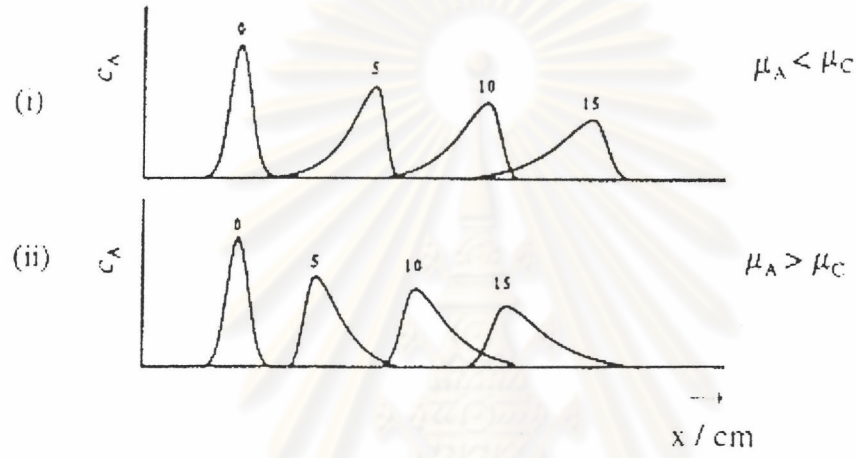


Figure 1.7 Schematic diagram illustrating concentration distribution in the presence of EMD and diffusion. Evaluation (i) tailing and (ii) fronting peaks in the distance domain as a function of time (min). Reproduced from Kuhn and Hoffstetter-Kuhn [1993].

The peak variance from EMD is given by [Erny *et al.* 2001]

$$\sigma^2_{EMD} = \left|\frac{2}{9} l l_{inj} c_A a_A\right| \left|\frac{\mu_A}{\mu_A + \mu_{eo}}\right| \tag{1.20}$$

with

$$a_A = \frac{\left(\frac{\mu_A}{\mu_C} - \frac{\mu_B}{\mu_C}\right)\left(1 - \frac{\mu_A}{\mu_C}\right)}{\frac{\mu_A}{\mu_C}\left(1 - \frac{\mu_B}{\mu_C}\right)c_C} \tag{1.21}$$

where subscripts A, B, and C are the analyte ion, the BGE counter-ion and the BGE co-ion, respectively, c_i the concentration of species i, and l_{inj} the injection length.

1.2.4.4 Detection Cell Width

The peak dispersion σ^2_{det} caused by the detection path length can be expressed as follow

$$\sigma^2_{det} = \frac{l^2_{det}}{12} \tag{1.22}$$

where l_{det} is the length of the zone in which detection is performed. As this length increases, the separation efficiency may be decreased.

1.2.4.5 Injection Plug

The peak variance due to a rectangular distribution of the sample of plug length l_{inj} is given by

$$\sigma^2_{inj} = \frac{l^2_{inj}}{12} \tag{1.23}$$

When a sample solution with lower ionic strength or conductivity than that of the BGE is injected, and the voltage is then applied, the electric strength in the sample zone dramatically increases. This results in a large increase of electrophoretic mobility of the analytes. The analytes move rapidly and stack up, leading to reduce the effective of analyte zone. Therefore, the peak variance due to rectangular profile of the sample is small in our experiment.

1.3 The Basic Instrument in CE

The schematic of CE instrument is illustrated in Figure 1.8. A CE system consists of a high voltage power supply allowing voltages up to 30 kV, two electrodes (commonly platinum wire), a capillary column with 10-200 μm I.D. and 20-100 cm in length, a detector mostly used UV-Vis detector, and a cooling system for controlling temperature of the capillary and reducing Joule heating.

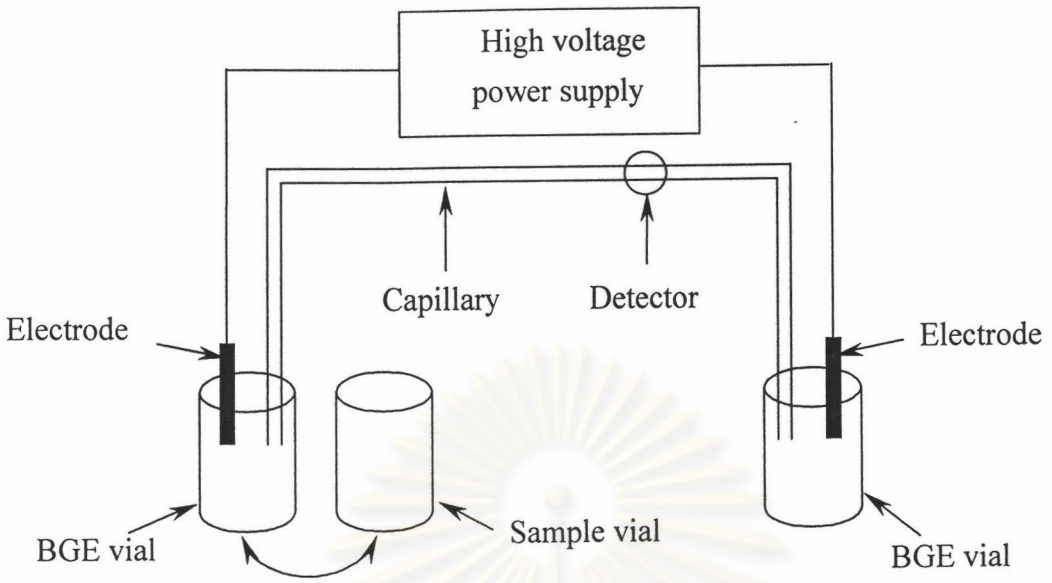


Figure 1.8 Schematic diagram of a basic CE instrument. Adapted from Weinberger [1993]

1.3.1 Sample Injection [Khaledi 1998]

There are basically two different methods of sample introduction into the capillary either hydrodynamic injection or electrokinetic injection. *The hydrodynamic method* is based on pressure differences between the inlet and outlet ends of the capillary. This pressure difference can be achieved by various methods such as gravimetric, overpressure, and vacuum. The length of the sample zone injected into the capillary by the hydrodynamic method is

$$l_{inj} = t_{inj} v_{hf} \tag{1.24}$$

where t_{inj} the total injection time and v_{hf} the hydrodynamic flow velocity.
For pressure injection in a capillary with a fixed length (L) and radius diameter (r), the hydrodynamic flow is proportional to the viscosity of the liquid in the capillary, η. The flow velocity is described by the Poisseuille equation:

$$v_{hf} = \frac{\Delta P r^2}{8\eta L} \tag{1.25}$$

where ΔP is the pressure difference between the inlet and outlet ends of capillary. Thus, the length (l_{inj}), the volume (V_{inj}) and quantity (Q_{inj}) of the sample injected can be calculated from the following equations

$$l_{inj} = \frac{\Delta P r^2}{8\eta L} t_{inj} \tag{1.26}$$

$$V_{inj} = \frac{\Delta P \pi r^4}{8\eta L} t_{inj} \tag{1.27}$$

and

$$Q_{inj} = \frac{\Delta P \pi r^4}{8\eta L} t_{inj} c \tag{1.28}$$

where c is the analyte concentration. In general, pressure injection has better reproducibility and greater control over the amount of sample injected into the capillary. Since the injection is based on the pressure difference, it is universally applied to all kinds of sample matrices without any bias on the sample compounds.

In electrokinetic injection, one end of the capillary and the injecting electrode are removed from the separation buffer reservoir and placed in the sample vial. High voltage is then applied for a short period of time. Analyte ions then migrate into the capillary due to the combination of electrophoretic migration of the ions and electroosmotic flow of the sample solution. The length of sample zone introduced into the capillary during electrokinetic injection :

$$l_{inj} = (v_{eo} + v_{ep}) t_{inj} \tag{1.29}$$

where v_{eo} is the electroosmotic velocity of the bulk solution and v_{ep} is the electrophoretic velocity.

1.3.2 Detector

The inherent feature of CE of using small diameter capillaries is very favorable with regard to separation efficiency but at the same time it causes substantial problem with the sensitivity in the optical detection systems due to shall path length. Among optical detectors, the UV-Vis absorption detector is most commonly used in CE. It is almost universally applicable to organic compounds and additionally it can be used for

inorganic ions in the indirect detection mode. The main limitation of the UV-Vis detector is the relatively low sensitivity.

According to Beer's law, the optical absorbance of a sample is directly proportional to the optical pathlength through the absorbance measurement is performed. Therefore, an extension of the optical path length may lead to an increase in detection sensitivity. Increasing the inner diameter of the capillary is simple, but this results in increased joule heating and a loss of peak resolution. Sensitivity in CE may be improved by using a Z-cell or a bubble cell [Grossman and Colburn 1992, Foret *et al.* 1993], which gives a longer path length, but worse resolution.

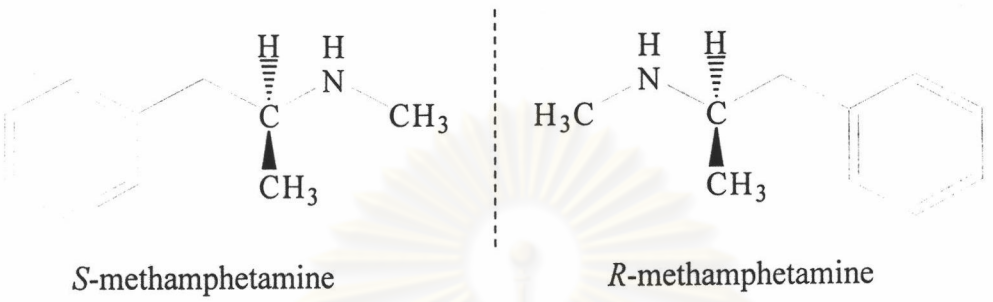
Fluorescence detection is also used in CE due to its high sensitivity. Since not all compounds possess high fluorescence, the derivatization must be required for fluorescence detection. In addition, other types of detectors used in CE include electrochemical detectors, mass spectrometry (CE-MS), and nuclear magnetic resonance (CE-NMR). In this research, UV detection is used for analysis of amphetamine drugs.

1.4 Introduction to Chiral Separation

1.4.1 Chirality and Enantiomers

Chirality is a characteristic of compounds which lack any symmetry element, which may be a center, an axis or a plane, in the molecule. Enantiomers are isomeric forms of two chiral compounds that are mirror images. An example of enantiomers is shown in Figure 1.9. Enantiomers have identical physical and chemical properties in an achiral environment, but this is not the case in a chiral environment. When plane-polarized light is passed through a solution of either enantiomer, the plane of polarization is rotated by equal but opposite angles. Clockwise rotation is designated as (+) and anti-clockwise rotation as (-). Traditionally, the (+) and (-) isomers have also been designated as dextro (*d* or *D*) and levo (*l* or *L*), respectively; this terminology is used particularly with carbohydrates, hydroxy-acids and amino acids. In chemistry, the symbols (*R*) and (*S*) (rectus and sinister) are normally used to designate the enantiomers and to provide structural information. If the decrease in

priority order is clockwise, the isomer is defined as (*R*), and if anti-clockwise, the isomer is (*S*). A mixture of (*R*) and (*S*) enantiomers in equal proportions is called a racemate and is optically inactive (non-rotation of plane polarized light). The racemate is designated as (±).



S-methamphetamine *R*-methamphetamine

Figure 1.9 Structure of *S*- and *R*-methamphetamine

1.4.2 Importance of Chiral Separation

The chiral separation is of great importance in the pharmaceutical field, where a wide number of drugs have one or more chiral centers and are used as racemic mixtures. Very often, the pharmaceutical activity and metabolism of the two enantiomers are different. The development of pure enantiomeric drugs requires not only the synthesis or isolation of the substances but also powerful techniques for the analysis of the chiral entities, including their pharmacokinetic and pharmacodynamic fate in an organism (i.e. absorption, excretion and metabolism).

Separation methods are, therefore, required to discriminate between the two isomeric forms. Since the 1980s, a range of chiral stationary phases (CSPs) for both GC and HPLC have become available commercially. GC is limited to the analysis of volatile compounds, therefore has not been used as much as the solution-based techniques of HPLC and CE. In comparison with HPLC, chiral separation in CE has the advantages of higher separation efficiency, speed of analysis, and flexibility of rapid incorporation of various chiral selectors.

1.4.3 Mechanism of Chiral Separations

In HPLC the separation of enantiomers is carried out by using a chiral stationary phase, or by having the chiral selector as a mobile phase additive. In CE, the chiral selector is normally used as an additive to the BGE. The CEC mode can use immobilized chiral selectors, as in HPLC with a CSP.

Dalgliesh [1952] proposed a model for enantiomeric resolution based on the "three-point interaction" theory. Figure 1.10 shows the interaction of each enantiomer with a chiral selector. Differences in diastereomeric association lead to a retention factor difference in HPLC and an apparent mobility difference in CE. Therefore, the resolution of enantiomers can occur. The main processes involved in chiral interactions include co-ordination to transition metals, charge-transfer interaction, hydrogen bonding and inclusion phenomena.

For charge-transfer interaction, both enantiomeric analytes and a chiral selector have π-electron sites, such as aromatic groups. An example of this is when A and B groups of the chiral selector in Figure 1.10 are π-electron and acidic sites, respectively, and C is a basic or steric interaction site, whilst X and Y groups of the enantiomers are π-electron and basic sites, respectively, and W and Z are acidic, basic, small or large sites [Pirkle *et al.* 1984]. Enantioselectivity is based on an aromatic π-π interaction, together with additional polar interactions (hydrogen bonding, dipole interaction) or steric interaction.

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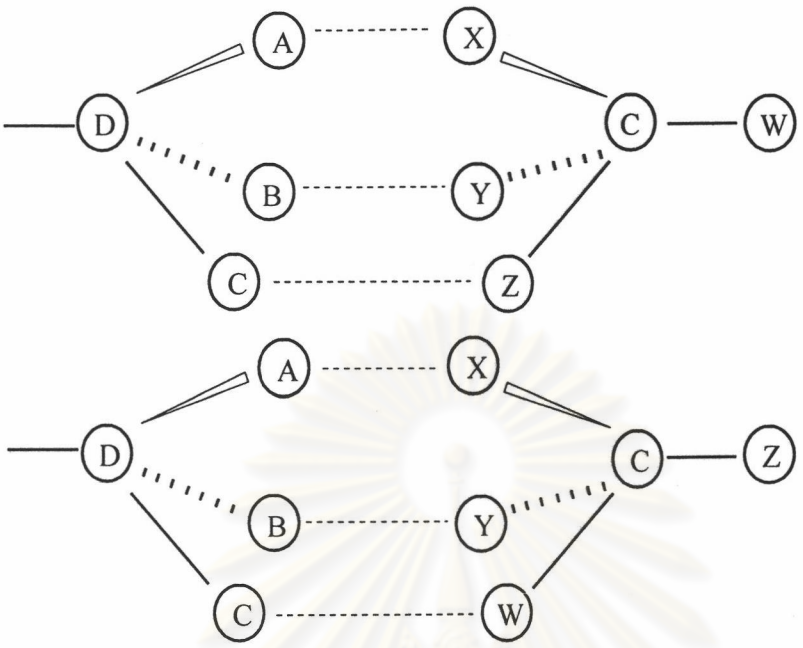

Figure 1.10 The three point interaction between a chiral selector and two enantiomers. Adapted from [Pikle et al. 1984]

Inclusion phenomena can occur when chiral selectors such as crown ethers and cyclodextrins have a cavity and act as hosts in host-guest equilibria. Selectivity entails differential inclusion of the enantiomers as guest molecules. The stability of inclusion complexes depends on the size and shape of the analyte, hydrophobic and hydrophilic interaction, hydrogen bonding and solvent effects.

1.5 Chiral Separation in CE Using Cyclodextrin as Chiral Selector

The classes of chiral selectors used in CE include cyclodextrins, macrocyclic antibiotics, crown ethers, chiral metal complexes and chiral surfactants. Work in this thesis involves cyclodextrins.

1.5.1 Cyclodextrins (CDs)

Cyclodextrins (CDs) are cyclic oligosaccharides composed of different numbers of *D*-glucose units, which connect a ring structure through α-(1,4)-glucosidic bonds. Native CDs with six, seven and eight glucose units, corresponding to α-, β- and γ-CD. Figure 1.11 shows the geometric structure of CD. Their major physical and chemical properties are listed in Table 1.1.

The key characteristics of CDs for chiral separations are the unique arrangement of the glucose units and the fact that each glucose unit contains five chiral centers. In three dimensions, the structure of a CD molecule resembles a truncated cone with a non-polar cavity. The rim at the top of the cone is lines with secondary (C2,C3) hydroxyl groups, and at the bottom with primary (C6) hydroxyl groups. The central hydrophobic cavity selectively includes a variety of guest molecules to form inclusion complexes.

The first application of CD for CE chiral separation was reported by Snopek [1988]. Fanali [1989] first reported the use of CDs as chiral selectors in CZE. In a review covering the lecture up to 1994, nearly 90% of all work on CE chiral separation was found to be based on CDs and derivatives [Arnstrong and Nair 1997]. Advantages of CDs over other CE chiral selectors include their stability over a wide pH range (2-12), their lack of absorption in the UV region typically associated with detection of analytes and the enhancement of enentioselectivity possible using either neutral or charged CD derivatives [Okafo and Camilleri 1993].

Table 1.1 Properties of Native CDs [Wang and Khaledi 1998]

Characteristics	α	β	γ
Number of glucose units	6	7	8
Molecular weight / g mol^{-1}	972	1135	1297
External diameter / nm	1.37	1.53	1.69
Internal diameter / nm	0.57	0.78	0.95
Torus height / nm	7.9 ± 0.1	7.9 ± 0.1	7.9 ± 0.1
[α] / deg dm^{-1} ml g^{-1}	+150.5	+162.0	+177.4
pK_a of hydroxyl groups	12.1-12.6	12.1-12.6	12.1-12.6
Solubility in water / % w/v	14.5	1.8	23.2
Typical guest molecules	Benzene	Naphthalene	Anthracene

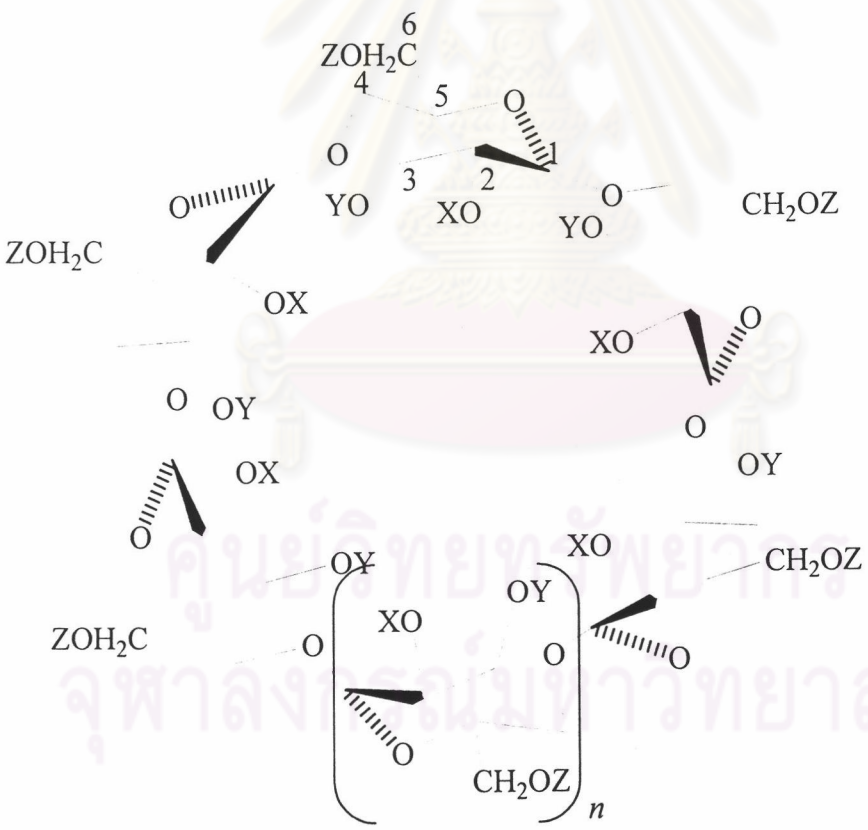

Figure 1.11 Structure of cyclodextrins (CDs), when n = 1, 2, 3 for α-, β- and γ-CD, respectively, and X, Y and Z = H or CH_3 for native or methyl ether derivatives.

1.5.2 Inclusion Complexation of Cyclodextrin and Enantiomers

Inclusion complexation of cyclodextrin and analyte is shown in Figure 1.12. In the presence of cyclodextrin (CD) in the BGE, for CD and enantiomers A, the formation of a complex with 1:1 stiochiometry can be expressed as follows:

$$\begin{aligned} A1 + CD &\overset{K_1}{\rightleftharpoons} CDA1 \\ A2 + CD &\overset{K_2}{\rightleftharpoons} CDA2 \end{aligned} \qquad (1.30)$$

where K is the binding constant, which can be expressed as

$$K = \frac{[CDA]}{[CD][A]} \qquad (1.31)$$

The electrophoretic mobility, μ, of A in presence of selector in BGE is given by

$$\mu = x_A \mu_o + x_{CDA} \mu_\infty \qquad (1.32)$$

where μ_o and μ_∞ are μ at zero and infinite concentration of CD, respectively. x_i is the mole fraction. It follows that [Wren and Rowe 1992a]

$$\mu = \frac{\mu_o + KC\mu_\infty}{1 + KC} \qquad (1.33)$$

where C is the free concentration of cyclodextrin, [CD], and may be assumed to be the initial concentration of CD ([S]) when [S] >> [A]. With known μ_o and C, the binding constant may be calculated by fitting the electrophoretic mobilities of the analyte as a function of CD concentration.

The relationship between the difference in electrophoretic mobility of enantiomers ($\Delta\mu$) and CD concentration can be expressed by [Wren and Rowe 1992a, Penn *et al.* 1993, 1994]

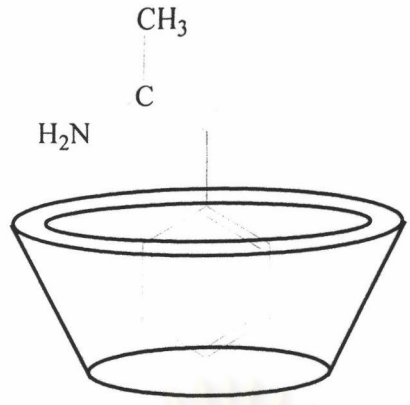

Figure 1.12 Inclusion complexation of cyclodextrin and methamphetamine

$$\Delta\mu = \frac{\Delta K(\mu_o + \mu_\infty)C}{(1 + \bar{K}C)^2} \qquad (1.34)$$

where ΔK is the binding constant difference, $\bar{K}$ average binding constant ($\bar{K} = (K_1 + K_2)/2$ or $\bar{K} = \sqrt{K_1 K_2}$). Therefore, it can be seen from Equation 1.34 that $\Delta\mu$ for enantiomers depends on ΔK for each enantiomer to CD, leading to enantiomeric resolution term.

1.5.3 Previous work on Chiral Separation Using CD as Chiral Selector

1.5.3.1 Single Cyclodextrin

Much of previous work on chiral CE separation using single CD as selector has reported the effects of variation the following parameters: CD types and structure, CD concentration, organic additive, temperature, pH of BGE, nature and concentration of buffer and capillary dimensions and inner surface [Chankvetadze, 1997]. Some work will be reviewed in the following paragraph.

In the first paper on use of CD as a chiral selector in CZE, Fanali [1989] reported that, in comparison with β-CD and TM-β-CD, DM-β-CD gave better separations of sympathomimetic drug, e.g. ephedrine, epinephrine and related compounds. Comparison of CD types for enantiomeric separation of other chiral compounds have

subsequently been reported [Nielen 1993a, Nishi *et al.* 1994, Rogan *et al.* 1994, Bechet *et al.* 1994 and Guttman *et al.* 1995].

The CD concentration plays an important role on chiral separation. Wren and Rowe [1992a], Shibukawa *et al.* [1993] and Penn *et al.* [1993] reported that the electrophoretic mobility difference reached a maximum value as the chiral selector concentration was increased, and then decreased at higher chiral selector concentration. The CD concentration at maximum electrophoretic mobility difference, $C_{\Delta\mu,max}$ correlates with the average binding constant of enantiomers and CD, $C_{\Delta\mu,max} = 1/\bar{K}$. Penn *et al.* [1994] showed that the CD concentration gives maximum resolution was slightly higher than the CD concentration which needed to obtain maximum mobility difference. The work by these groups is very useful for prediction of optimum CD concentration needed for enantiomeric separation and also to explain the effects of change of solvent concentration and temperature on enantiomeric separation.

Wren and Rowe [1992b] and Penn *et al.* [1994] reported that addition of organic solvent in the BGE containing CD resulted in a decrease of binding constants, leading to higher $C_{\Delta\mu,max}$ for BGE with organic solvent. Therefore, whether the addition of organic solvent will result in an increase or a decrease in $\Delta\mu$ and resolution will depend on whether the CD concentration is below or above $C_{\Delta\mu,max}$ for BGE without organic solvent. If CD concentration above $C_{\Delta\mu,max}$, the addition of organic solvent will increase $\Delta\mu$ and resolution, while if it is below $C_{\Delta\mu,max}$, the addition of organic solvent will decrease $\Delta\mu$ and resolution.

Shibukawa *et al.* [1993] studied the effect of change in temperature of the BGE on enantiomeric resolution. The resolution of some chiral compounds increased with an increase in temperature, while opposite effect occurred for other compounds. Penn *et al.* [1994] reported that the binding constants of tioconazole and β-CD decreased with an increase in temperature. If a given CD concentration is lower than the optimum CD concentration for particular separation, an increase in temperature will lead to a decrease in resolution. In contrast, if a given CD concentration is higher than the optimum CD concentration, the resolution will improve at higher temperature.

1.5.3.2 Dual Cyclodextrins

Typically, separation of one pair of enantiomers is carried out using a suitable single CD. In some cases, simultaneous separation of several pairs of enantiomers, cannot be achieved by using only single CD. One approach to enhance the simultaneous separation of all analytes is the use of dual CDs, a combination of two CD types, in the BGE. The following dual neutral CDs have shown to improve simultaneous separation of several pairs of enantiomers: β- and γ-CDs for Dns-*DL*-amino acids [Terabe *et al.* 1994], α- and TM-β-CDs for binaphthyl compounds [Nishi *et al.* 1995], β- and TM-β-CDs or γ- and TM-β-CDs for phenoxy acid herbicides [Mechref *et al.* 1996], β- and γ-CDs for polychlorinated biphenyls [Marina *et al.* 1996], and β- and DM-β-CDs for amphetamine drugs [Chinaka *et al.* 2000]. In comparison with a single chiral selector as charged CD, dual chiral selectors as neutral and charged CDs have been reported to provide benefit of better resolution and peak shape [Lurie *et al.* 1994]. A review, covering the literature up to 2000, of enantiomeric separation of basic drugs using dual charged and neutral CDs has been reported by Fillet *et al.* [2000].

1.5.3.3 Enantiomeric Separation of Amphetamine Drugs in CE

In this work, five amphetamine drugs as shown in Figure 1.13, amphetamine (AP), methamphetamine (MA), pseudoephedrine (PE), ephedrine (EP) and norephedrine (NE), are used as test analytes for enantiomeric separation using β-CD and DM-β-CD as dual chiral selectors. Therefore, chiral CE separation of amphetamine drugs is reviewed in this section.

Runge *et al.* [1995] studied CE enantiomeric separation of AP, MA and other basic drugs using single CD such as β-CD, methylate-β-CD, hydroxypropyl (HP)-β-CD and carboxymethylate (CM)-β-CD. Results showed that the BGE containing single CD as β-CD or CM-β-CD gave better resolution of AP and MA enantiomers. In comparison with DM-β-CD, HP-β-CD was shown to be a better selector for enantiomeric separation of a mixture of eleven EP enantiomers [Flurer *et al.* 1995]. Using single CD as β-CD, DM-β-CD, TM-β-CD and HP-β-CD, Chinaka *et al.* [2000] reported that

β-CD gave higher resolution for AP and MA enantiomers, while DM-β-CD gave higher resolution for EP and NE enantiomers. Szoko *et al.* [1996] studied the effect of methanol on enantiomeric separation of AP, MA, PE, EP, NE and other phenylalkylamine derivatives using DM-β-CD. The better and worse resolution of some enantiomers was obtained. Their results can be explained using theory reported by Wren and Rowe [1992b] and Penn *et al.*[1994] as previously mentioned in Section 1.5.3.1. In comparison with single CD, a dual CDs system containing DM-β-CD and sulfobutyl ether-β-CDs [Lurie *et al.* 1994] or β-CD and DM-β-CDs [Chinaka *et al.* 2000] was found to improve resolution of amphetamine drugs.

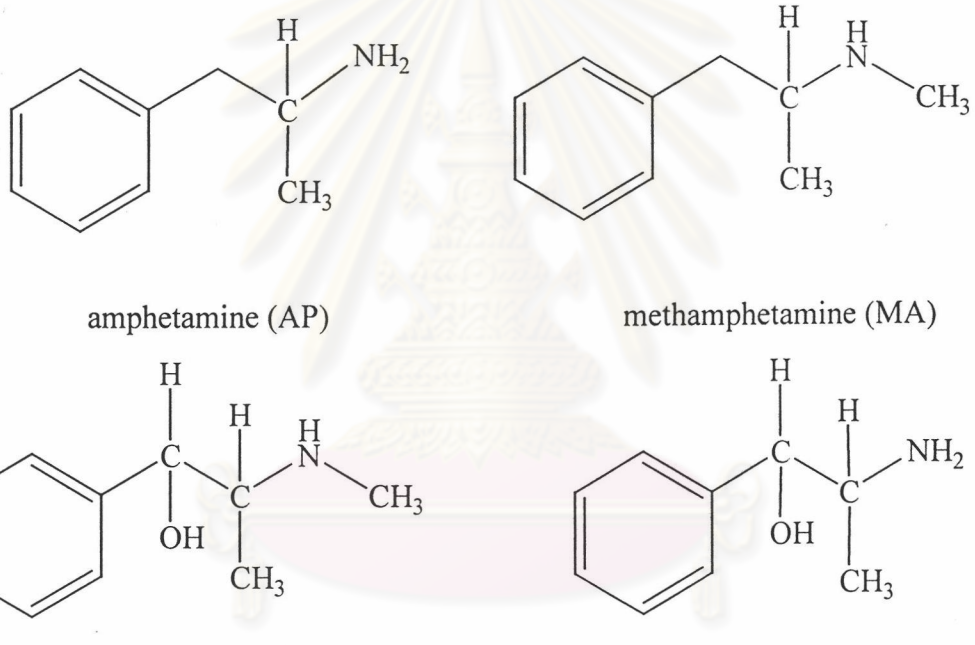

Figure 1.13 Structures of amphetamine drugs: amphetamine, methamphetamine, pseudoephedrine, ephedrine and norephedrine.

1.6 Aim, Scope and Advantages

Most previous work on dual CDs for chiral CE separation involves resolution optimization carried out by random variation of CD concentrations. The use of dual CDs may result in improvement of resolution for some enantiomers, while loss of resolution for other enantiomers. In dual CDs system, difference models, based on enantioselectivity (α) for a pair of enantiomers, have been proposed to explain a change of $\Delta\mu$, where α is defined as the ratio of migration times [Fillet *et al.* 1999] and electrophoretic mobilities [Abushoffa *et al.* 2002]. However, the enantioselectivity defined by these concepts depends on the CD concentration. Penn *et al.* [1994] reported the enantioselectivity defined as the ratio of binding constants (K) of enantiomers and CD in a single CD. The latter concept can be well used to describe a change of $\Delta\mu$ influenced by temperature, organic solvent and CD concentration and to accurately predict CD concentrations at maximum $\Delta\mu$ and maximum R_s [Penn *et al.* 1994, Ferguson *et al.* 1996 and 1997]. Recently, Nhujak [2001] has also reported this concept for prediction of maximum resolution and enantiomeric resolution over a wide range of concentrations single of CD.

It is interesting to investigate theoretical models to explain a change in $\Delta\mu$ of enantiomers in dual CDs based on enantioselectivity which is defined as the ratio of binding constants. Therefore, the aims of this work are to develop theoretical models of $\Delta\mu$ of enantiomers in capillary electrophoresis using dual cyclodextrins and to compare theory and practice of enantiomeric separation. The experiment will be carried out using test analytes as five amphetamine drugs and BGE as dual CDs containing β-CD and DM-β-CD in a triethanolammonium-phosphate buffer at pH 3.0.

One objective is to extend the treatment of Wren and Rowe [1992a] and Penn *et al.* [1994], involving separation of one pair of enantiomers using single CD, in order to compare experimental and predicted $\Delta\mu$ of several pairs of enantiomers over a wide range of concentrations of dual CDs. In initial work, the binding constant (K) of each isomer and each CD will be determined by CE using a single CD system. Equations and theoretical models for $\Delta\mu$ of enantiomers in a wide range for concentrations of dual CDs will be first proposed in this work, based on enantioselectivity and

dimensionless quantity of KC, where C is the CD concentration. It is expected that the proposed $\Delta\mu$ models could be used to explain a change, an increase or a decrease, of $\Delta\mu$ when the second CD (CD2) is added into the BGE containing the first CD (CD1).

A further objective is to extend Nhujak's work [2001], moving from one pair enantiomers in single CD to several pairs of enantiomers in dual CDs, in order to compare observed and predicted peak variance and resolution over a wide range of dual CDs concentrations. Two major contributions, diffusion and EMD, causing peak variance are taken into account for. Equations involving dimensionless quantity of KC will be developed for prediction of peak variance, efficiency and resolution of enantiomers in dual CDs. This prediction is expected to be useful to choose CD concentration giving optimum resolution of each pair of enantiomers.

A final objective is to optimize simultaneous separation of several pairs of enantiomers using data from binding constants, enantioselectivity, predicted $\Delta\mu$ and μ. At a fixed concentration of CD1, $\Delta\mu$ and μ will be plotted as a function of CD2 enantiomers. The experiments of simultaneous separation of several pairs of enantiomers will be compared with prediction. It is expected to be very useful to achieve resolution of simultaneous separation of all analytes.

It is expected that this whole work will be very useful for explanation of a change of electrophoretic mobility difference and resolution of enantiomers in capillary electrophoresis using dual cyclodextrins and for optimization of simultaneous separation of several pairs of enantiomers. In addition, this work may be applied for forensic evidence in order to determine enantiomeric profiles of amphetamine drug tablets and also urine samples. The former samples link to batch and source of drug production. The latter relates to legal or illegal drugs taken. It should be noted that *S*-MA and its main metabolite *S*-AP in urine samples is evidence of illegal use of *S*-MA, while *R*-MA and its main metabolite *R*-AP for legal use of *R*-MA medicines.