CHAPTER II

THEORY

2.1 Introduction and Modes of Capillary Electrophoresis [Camilleri 1993]

Capillary Electrophoresis (CE) is a novel analytical technique for separation of charged analytes in a small capillary containing an electrolyte solution under the influence of electric field. CE has proved to be a rapid and versatile analytical technique that combines simplicity with high reproducibility. Nowadays, CE is commonly used as an alternative separation method providing short analysis time, high efficiency and low consumption of solvent.

Typically, CE can be classified into six basic modes of separation including 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 mode, CZE, is commonly used for separation of charged analytes whereas MEKC can be applied for separation of both charged and uncharged analytes. This work involves a relatively new mode called *microemulsion electrokinetic chromatography* (MEEKC), which is developed from MEKC. Figure 2.1 shows separation mechanism of CZE, MEKC and MEEKC. Other modes are not mentioned in this thesis.

Capillary Zone Electrophoresis (CZE) is the most popular mode of CE. A buffer solution is typically used as the background electrolyte (BGE). The separation mechanism is based on the difference in electrophoretic mobility of analytes that depend on the charge-to-size ratio of the analytes.

Micellar Electrokinetic Chromatography (MEKC) is also commonly used mode of CE. The BGE used in MEKC contains a surfactant such as sodium dodecylsulphate (SDS) to form micelles. The separation mechanism is based on the difference in electrophoretic mobility and the distribution constant of analytes between micellar phase and aqueous phase.

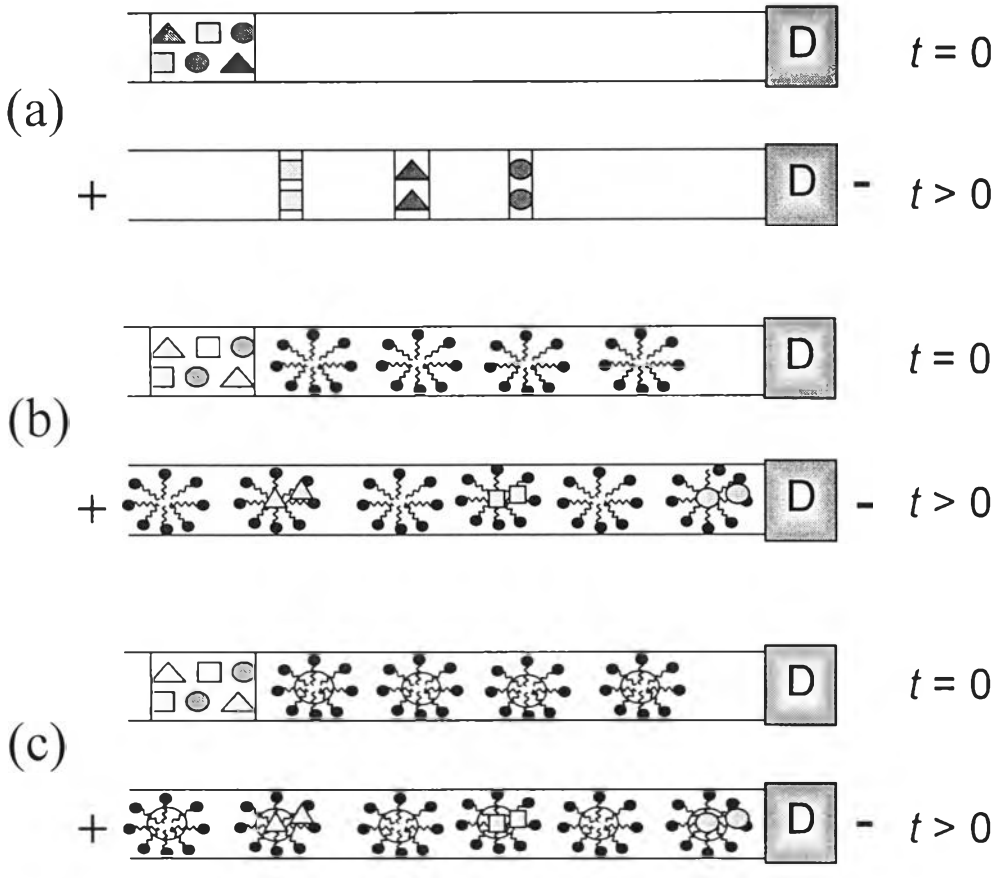

Figure 2.1 Separation mechanisms of (a) CZE, (b) MEKC and (c) MEEKC. Adapted from Grossman *et al.* [1992] and Altria [2000a].

Microemulsion Electrokinetic Chromatography (MEEKC) was introduced by Watarai [1991]. MEEKC has developed from MEKC, where pseudo-stationary phase is microemulsion in MEEKC instead of micelle in MEKC. Microemulsion is a solution containing nanometer-size droplets of an immiscible liquid. In MEEKC, the microemulsion used contains oil droplets dispersed in an aqueous buffer. The composition of an oil-in-water microemulsion consists of oil, a surfactant, and a co-surfactant. The separation mechanism in MEEKC is similar to that in MEKC. More details of MEEKC are discussed in Section 2.5.

2.2 Electrophoretic Mobility [Camilleri 1993]

The electrophoretic mobility, μ, is defined as the electrophoretic velocity (v_{ep}) of an ion migrating in BGE under the influence of an applied electric field (E).

$$\mu = \frac{v_{ep}}{E} \tag{2.1}$$

For a spherical ion, v_{ep}, is given by the equation

$$v_{ep} = \frac{ze}{6\pi\eta r_h} E \tag{2.2}$$

where z is the charge of the ion, e is the fundamental electronic charge, η is the viscosity of the BGE, and r_h is the hydrodynamic radius. According to Equations 1.1 and 1.2, μ of the analyte is given by

$$\mu = \frac{v_{ep}}{E} = \frac{ze}{6\pi\eta r_h} \tag{2.3}$$

At a fixed BGE viscosity, it follows from Equation 2.3 that μ of the analyte depends on the charge-to-size ratio of the ion, z/r_h.

2.3 Electroosmosis [Grossman *et al.* 1992, Camilleri 1993]

For an uncoated fused capillary containing a BGE solution having pH higher than 2, the silanol groups on silica surface ionize to be negatively charged, as shown in Equation 2.4.

$$\text{-SiOH (s)} \rightleftharpoons \text{-SiO}^- \text{ (s)} + \text{H}^+ \text{ (aq)} \tag{2.4}$$

Some positive BGE ions are attached at the negative surface of the capillary to form an imobilised layer, called Stern layer, whereas some positive BGE ions form

diffusion layer. The rest of the excess positive ions is in the bulk solution. When an electric field is applied, a large number of positive ions in the solution migrate toward cathode, resulting in the solution flows from anode to cathode. This phenomenon is called *electroosmosis*, and the resulting flow of liquid or solution in the capillary is called *electroosmotic flow* (EOF).

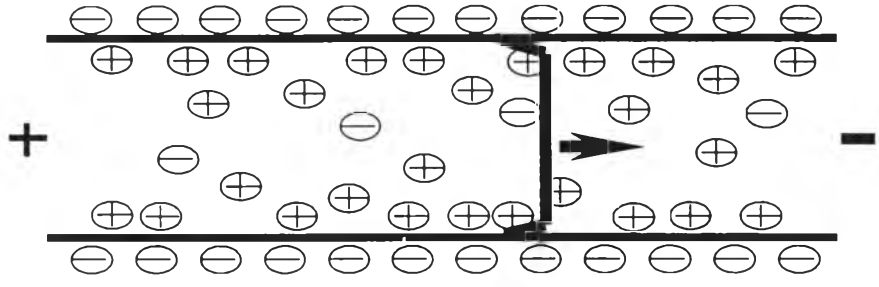

Figure 2.2 Electroosmotic flow (EOF). Adapted from Andrea *et al.* [1997].

Because of the silanol groups at the solid surface are weakly acidic, the degree of dissociation is pH dependent. A change of pH alters the degree of dissociation of silanol groups, resulting in a change of zeta potential (ζ), the electric potential at the shear plane of the double layer. This is directly related to the velocity of electroosmotic flow, v_{eo}, as shown in the following equation,

$$v_{eo} = -\frac{\varepsilon\zeta}{4\pi\eta}E \qquad (2.5)$$

where ε is the permittivity and η is the viscosity of the liquid in the double layer. These values may be different from those in the bulk solution [Khaledi 1998]. The electroosmotic mobility, μ_{eo}, can be defined as

$$\mu_{eo} = \frac{v_{eo}}{E} = -\frac{\varepsilon\zeta}{4\pi\eta} \qquad (2.6)$$

Since the value of ζ is negative, μ_{eo} has a positive sign for an uncoated fused silica. From the internal capillary surface, v_{eo} increases with increasing distance, and is constant at the distance of approximately 15 nm from the wall [Grossman *et al.*

1992]. Typically, the capillary used in CE has 20 to 100 μm I.D. (20,000 to 100,000 nm). Thus, it can be said that v_{eo} is constant throughout the capillary radius.

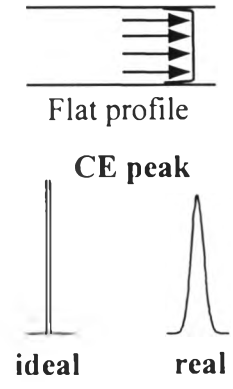

Figure 2.3 Flow profile and peak shape in CE. Adapted from Chankvetadze [1997].

Since EOF is generated at the capillary wall and driving force of EOF is uniformly distributed along the capillary, this result in a flat profile of the bulk flow which does not directly contribute to the zone broadening, as seen in Figure 2.3. For this reason, high efficiency and good resolution can be obtained in CE.

2.4 Migration Behaviour of Analytes in CE

In the presence of the EOF, the net velocity, v_{net}, of the analyte is the combination of the electrophoretic velocity of the analyte and the electroosmotic velocity as seen in Equation 2.7 and Figure 2.4.

$$v_{net} = v_{ep} + v_{eo} \qquad (2.7)$$

At high EOF, both anions and cations migrate to the detection end. For cations, $v_{ep,+}$ and v_{eo} have the same direction to the cathode at the detection end. The ion with higher charges and the smaller size migrates faster 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 ion charges and the smaller ion size, the smaller the net velocity. Neutral molecules migrate toward the cathode only due to EOF, and cannot be separated.

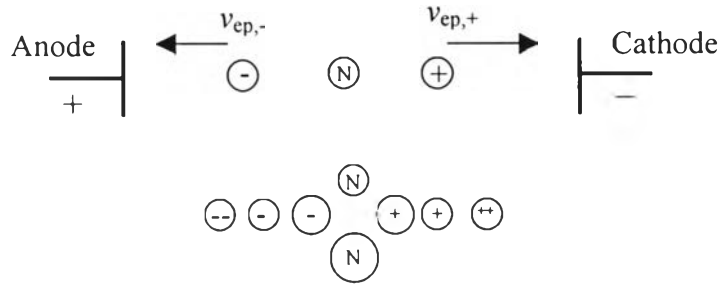

Figure 2.4 Migration behaviour of the analytes. Adapted from Li [1992].

The net electrophoretic mobility ($\mu_{net} = \mu + \mu_{eo}$), μ_{eo} and μ can be calculated from an electropherogram using the following equations:

$$\mu_{net} = \frac{v_{net}}{E} = \frac{lL}{Vt_m} \tag{2.8}$$

$$\mu_{eo} = \frac{v_{eo}}{E} = \frac{lL}{Vt_{eo}} \tag{2.9}$$

$$\mu = \mu_{net} - \mu_{eo} = \frac{lL}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \tag{2.10}$$

where t_m and t_{eo} are the migration times of the analyte and the EOF marker, respectively, l is the length of the capillary to detector, L is the total length of capillary and V is the applied voltage .

2.5 Microemulsion Electrokinetic Chromatography (MEEKC)

Figure 2.5 shows schematic of oil-in-water microemulsion. Generally, the oil and water are totally immiscible due to high surface tension between two phases. Addition of a surfactant reduces the surface tension because the hydrocarbon tails of the surfactant molecules penetrate into the oil droplet whilst the polar head groups reside in the surrounding aqueous phase. The surface tension is further lower by addition of a short-chain alcohol such 1-butanol, in order to obtain stable and optically transparent microemulsion.

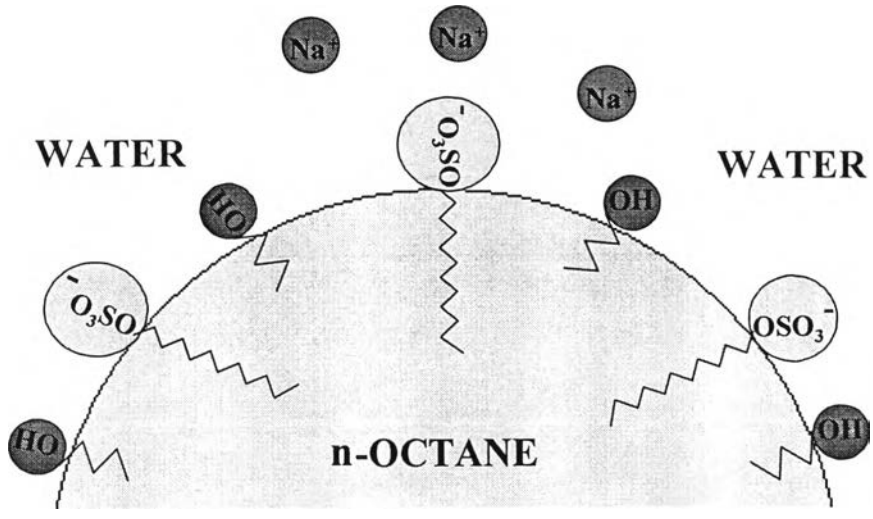

Figure 2.5 Schematic of microemulsion. Adapeted from Altria [2000a].

Typically, the oils used in MEEKC are, for example heptane or octane, while sodium dodecylsulphate (SDS), anionic surfactant, is widely used as emulsifier in MEEKC.

Much of previous work on MEEKC involved analysis of readily and moderately water soluble compounds, and was carried out using the basic buffer, such as phosphate and borate, to obtain high EOF, as shown in Figure 2.6a. Since μ_{eo} is higher than μ of the negatively charged oil droplets, analytes partitioning into the oil, which have the direction of μ to the cathod at the capillary inlet, can migrate toward the capillary outlet. Migration time order of analytes, a microemulsion marker and an EOF marker is compared in Figure 2.7a. The analyte with stronger partitioning in the oil, or higher retention factor (k), has greater mobility and longer migration time.

In the case of highly hydrophobic analytes, the long analysis time is obtained with MEEKC at high EOF. In order to reduce the analysis time, MEEKC may be carried out using suppressed EOF and reversed polarity of applied voltage, as shown in Figure 2.6b. The suppressed EOF maybe performed using a low pH buffer or a coated capillary. The former is simple and cheap, while the latter is expensive and has short lifetime of coating. In MEEKC with no or suppressed EOF, the higher the retention

factor of analyte, the higher the electrophoretic mobility, and therefore the faster the migration time, as shown in Figure 2.7b.

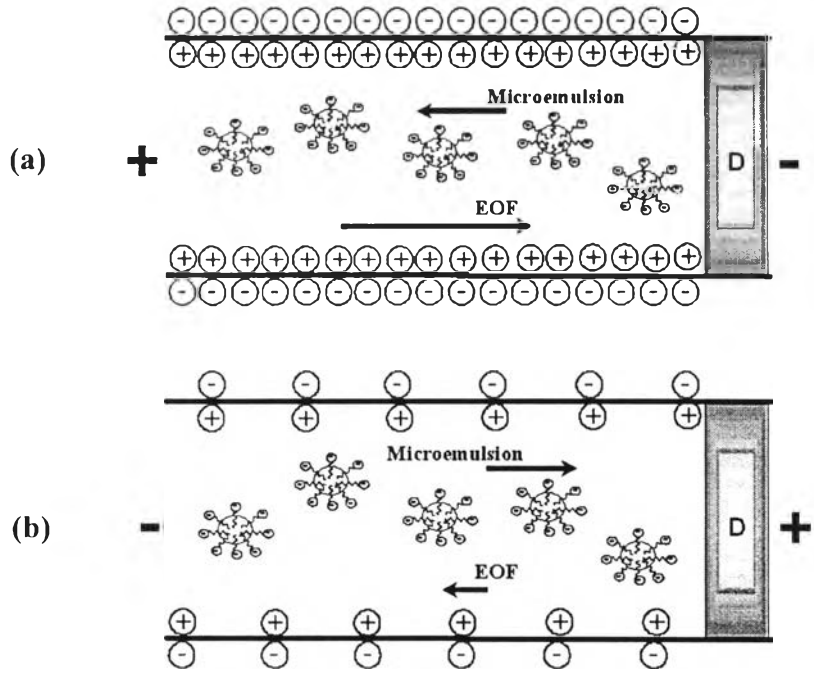

Figure 2.6 Schematic of the separation principles of MEEKC: (a) high EOF and (b) low EOF. Adapted from Altria [2000a].

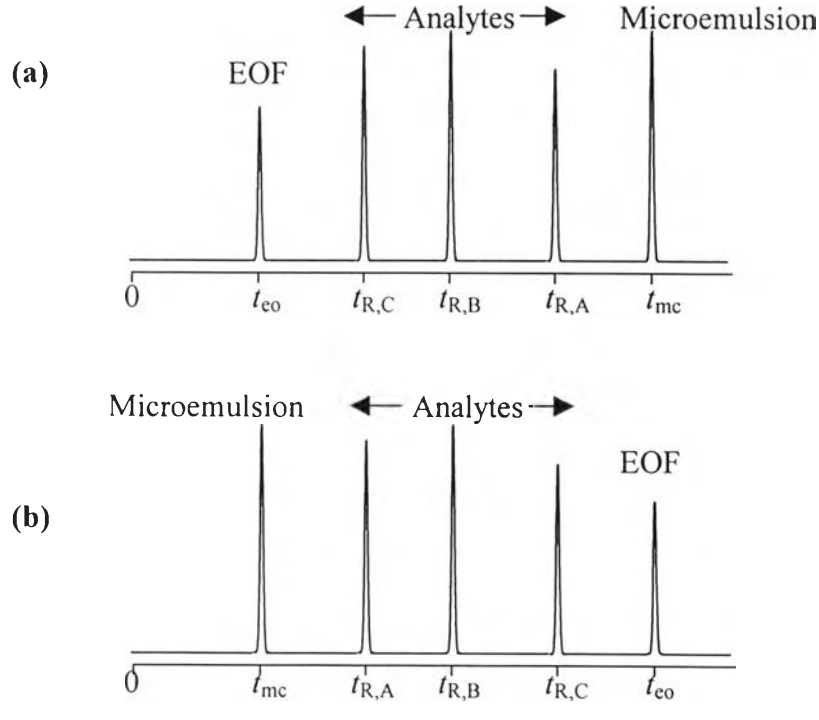

Figure 2.7 Typical elution order for analytes in MEEKC: (a) high EOF and (b) low EOF, where $k_A > k_B > k_C$. Adapted from Miola *et al.* [1998].

2.6 Retention in MEEKC [Terabe *et al.* 1985]

According to theory of chromatography and MEKC, the concept of retention in MEEKC is similary defined the retention factor, k, as the ratio of total moles of analyte in the microemulsion phase (n_{mc}) to those in the aqueous phase (n_{aq}) as shown in Equation 2.11 [Miola *et al.* 1998]:

$$k = \frac{n_{mc}}{n_{aq}} = K\phi \qquad (2.11)$$

where K is the distribution constant and ϕ is phase ratio, the ratio of the volume of the microemulsion phase to that of the aqueous phase.

Typically, the electrophoretic mobility of the complex between analyte and microemulsion is assumed to be equal to the electrophoretic mobility of the charged microemulsion (μ_{mc}). Therefore, the observed electrophoretic mobility (μ) of analyte, A, is expressed by

$$\mu = x_{aq}\mu_A + x_{mc}\mu_{mc} \qquad (2.12)$$

where x_{aq} and x_{mc} are the mole fractions of analyte in aqueous and microemulsion phase, respectively, μ is electrophoretic mobility of analyte.

In case of neutral analyte, A, μ_A is zero and μ is given by

$$\mu = x_{mc}\mu_{mc} = \frac{n_{mc}}{n_{aq} + n_{mc}}\mu_{mc} \qquad (2.13)$$

From Equations 2.11 to 2.13, μ can be expressed by

$$\mu = \frac{k}{1+k}\mu_{mc} \qquad (2.14)$$

Instead of using migration time (t_m) in CZE, MEEKC which combines electrophoretic term and chromatographic term from partitioning of analyte into the microemulsion usually define as the retention time (t_R). Therefore, it should be noted that t_m reported and discussed in this work is similar to t_R. From Equations 2.8 and 2.14, t_R for the neutral analyte in MEEKC without EOF is given by

$$t_R = \left(\frac{1+k}{k}\right) t_{mc} \tag{2.15}$$

where t_{mc} is the retention time of the analyte fully partitioning into the microemulsion droplet. By rearrangment of Equation 2.15, the value of k may be calculated from an electropherogram using the following equation:

$$k = \frac{t_{mc}}{t_R - t_{mc}} \tag{2.16}$$

On the other hand, the total mobility presence of EOF, ($\mu_{net} = \mu + \mu_{eo}$), in MEEKC is given by

$$\mu_{net} = \frac{k}{1+k}\mu_{mc} + \mu_{eo} \tag{2.17}$$

Therefore, t_R for the analyte in MEEKC with high EOF is expressed by

$$t_R = \frac{(1+k)t_{eo}}{1+\left(\frac{t_{eo}}{t_{mc}}\right)k} \tag{2.18}$$

where t_{eo} is the migration time of an EOF marker.

2.7 Peak efficiency, Peak broadening and Resolution [Grossman *et al.* 1992, Khaledi 1998]

2.7.1 Peak efficiency

Theoretically, an electrophoretic peak is assumed to have a Gaussian shape with standard deviation, σ in the distance unit and τ in time unit, as shown in Figure 2.8.

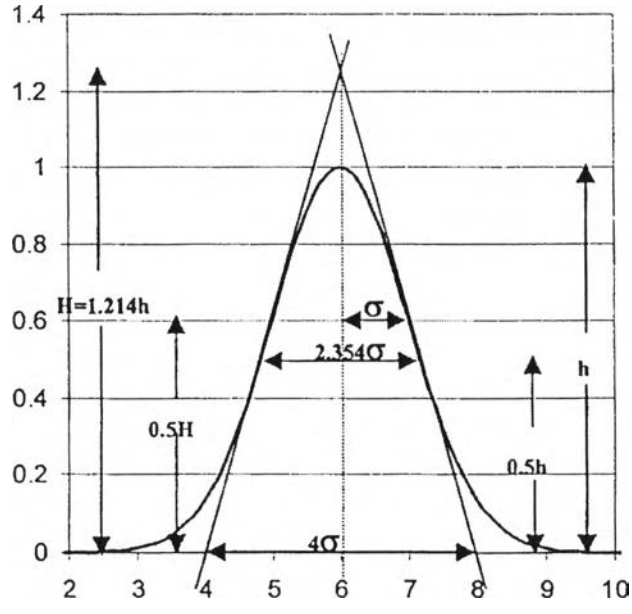

Figure 2.8 Gaussian peak.

Peak width at the base, w_b, can be obtained by drawing lines at tangent to the points of reflection and measuring the separation between these two points. The w_b is given by

$$w_b = 4\sigma \quad \text{or} \quad w_b = 4\tau \tag{2.19}$$

and the peak width at half height, w_h, is equal

$$w_h = 2.354\sigma \quad \text{or} \quad w_h = 2.354\,\tau \tag{2.20}$$

From the peak in an electropherogram, peak efficiency is usually expressed in terms of theoretical plate, N, given by the equation

$$N = \left(\frac{l}{\sigma}\right)^2 = \left(\frac{t}{\tau}\right)^2 \tag{2.21}$$

N may be calculated from the electropherogram using Equation 2.14, where t_m is the migration time.

$$N = 16\left(\frac{t_m}{w_b}\right)^2 = 5.54\left(\frac{t_m}{w_h}\right)^2 \tag{2.22}$$

2.7.2 Peak broadening

In MEKC and MEEKC, peak broadening can be expressed by peak variance (σ^2) or theoretical plate height (H) as similarly explained in chromatography and electrophoresis (Equation 2.16).

$$\sigma^2 = Hl \tag{2.23}$$

In this work, peak broadening will be discussed in term of H. The total H can be described as the summation of plate height caused by five main in-column effects as given in the following equation

$$H = H_l + H_{aq} + H_{mc} + H_t + H_{pd} \tag{2.24}$$

where H_l, H_{aq}, H_{mc}, H_t, and H_{pd} are the plate heights from longitudinal diffusion, intermicelle mass transfer in the aqueous phase, sorption-desorption kinetics in microemulsion solubilisation, thermal dispersion, and polydispersity of micelles, respectively. Each term of H contributed to band broadening is briefly discussed in the next part.

2.7.2.1 Longitudinal diffusion

According to the Fick's second law describing the change of the concentration as a function of time and space, concentration gradient in the axial direction causes a diffusional mass flux along the direction. Therefore, at any given time t, the solute concentration c can be determined as a function of its position x by

$$c(x,t) = \frac{Q_{inj}}{(4\pi Dt)^{1/2}} \exp(-\frac{x^2}{4Dt}) \qquad (2.25)$$

where Q_{inj} is the amount of analyte injected, and D is the diffusion coefficient of the analyte. In accordance with the Einstein-Smoluchowski equation and random walk theory, peak variance of Gaussian-shaped curve can be formulated to

$$\sigma^2_{diff} = 2Dt \qquad (2.26)$$

For Equation 2.19, t is the migration time in MEEKC and D is described by the Nernst-Einstein equation.

$$D = \frac{\mu^\circ k^* T}{ze} \qquad (2.27)$$

From equation 2.20, μ° is the absolute mobility at zero ionic strength, k^* is the Boltzmann constant, and T is the absolute temperature.

From Equations 2.8, 2.16 and 2.19, the plate height from longitudinal diffusion is derived as in Equation 2.21.

$$H_l = \frac{2D}{\mu_{net} E} \qquad (2.28)$$

Considering of analytes in both aqueous and microemulsion phase, H_l in Equation 2.21 can be divided into two components and expressed by the following equation

$$H_1 = \frac{2}{\mu_{eo} E}\left(\frac{D_{aq} + kD_{mc}}{1 + (t_{eo} / t_{mc})k}\right) \tag{2.29}$$

where D_{aq} and D_{mc} are the diffusion coefficients of the solute in the aqueous phase and the microemulsion phase, respectively [Terabe *et al.* 1989]. Since this work is carried out in the MEEKC condition regarding as absence of EOF, thus, H_l plays in this research can be given by

$$H_1 = \frac{2}{\mu_{mc} E}\left(\frac{D_{aq} + kD_{mc}}{k}\right) \tag{2.30}$$

2.7.2.2 Intermicelle mass transfer in the aqueous phase [Grossman *et al.* 1992, Terabe *et al.* 1989]

In MEEKC, intermicelle mass transfer is assumed to be the discrimination of analyte concentration distributing among microemulsions, similar to the resistance to mass transfer in the mobile phase term for packed column LC. Thus, H_{mc} can be derived from random walk theory [Terabe *et al.* 1989] and is given by

$$H_{aq} = \left(\frac{k}{1+k}\right)^2 \left(\frac{\left(1 - \frac{t_0}{t_{mc}}\right)^2}{1 + \left(\frac{t_0}{t_{mc}}\right)k}\right)\left(\frac{d^2 v_{eo}}{4D_{aq}}\right) \tag{2.31}$$

where t_0, t_{mc}, and d are the migration time of the insolubilised analyte, the migration time of microemulsion and the average intermicelle distance, respectively. From the relation of H_{aq} equation, the resistance to mass transfer increases with increasing distances between microemulsions ($H_{aq} \propto d^2$). Therefore, plate height H_{aq} should decrease with increasing surfactant concentration. Besides, theoretical plate height resulting from H_{aq} also arises from the slow diffusion rate between micelles.

2.7.2.3 Sorption-desorption kinetics in microemulsion solubilisation [Terabe *et al.* 1989]

Considering of the kinetics analogue of the distribution of molecule of analyte between aqueous phase and micellar or microemulsion phase, as shown in the equation

$$A_{aq} \underset{k_d}{\overset{k_s}{\rightleftharpoons}} A_{mc} \qquad (2.32)$$

where A_{aq} and A_{mc} represent the analyte molecule in the aqueous and the microemulsion phase, respectively, k_s and k_d are the sorption and desorption rate constants, respectively. Molecules or ions having the contribution of sortion-desorption kinetics will migrate through the capillary with a distribution of velocities resulting in H_{mc}, as given by

$$H_{mc} = \frac{2\left(1 - \frac{t_0}{t_{mc}}\right)^2 k}{\left[1 + \left(\frac{t_0}{t_{mc}}\right)k\right](1+k)^2}\left(\frac{v_{eo}}{k_d}\right) \qquad (2.33)$$

According to the nonequilibrium theory, the relation of H_{mc} was reported in Equation 2.33 and described that this dispersion is small unless there are electrostatic forces or ionic interactions between analytes and microemulsion [Terabe *et al.* 1989]. Therefore, H_{mc} may be negligible in this work.

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2.7.2.4 Thermal dispersion [Grossman *et al.* 1992, Khaledi 1998]

When the voltage is applied and heat is generated, parabolic temperature gradient occurs along the capillary in the radial direction. Since the mobility is a function of temperature, the parabolic temperature profile results in a parabolic velocity profile for the analyte. Like the longitudinal diffusion, peak variance from thermal dispersion can be expressed by replacing diffusion coefficient by thermal dispersion coefficient, D_{th}, analogously to the Einstein-Smoluchowski equation for one-dimensional diffusion,

$$\sigma_{th}^2 = 2D_{th}t_m \tag{2.34}$$

where D_{th} is given by

$$D_{th} = \frac{f_T^2 \kappa^2 E^6 r^6 \mu^2}{3072\lambda_s^2 D} \tag{2.35}$$

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, and r is the capillary radius. According to Equations 2.16, 2.27 and 2.28, H_t is expressed by

$$H_t = \frac{(1-\mu_{mc}/\mu_{eo})k}{24(D_{aq}+kD_{mc})} \frac{B^2 I_A^4 \mu_{eo} E}{64\kappa^2 \pi^4 r^2 \lambda_s^2 T^4} \tag{2.36}$$

where B is the constant value (2400 K), I_A is the electric current, and T is the temperature [Terabe *et al.* 1989]. In the case of no EOF, H_t is rearranged to be the equation

$$H_t = \frac{k}{24(D_{aq}+kD_{mc})} \frac{B^2 I_A^4 \mu_{mc} E}{64\kappa^2 \pi^4 r^2 \lambda_s^2 T^4} \tag{2.37}$$

It can be seen from Equation 2.37 that H_t directly depends on κ, r, and E.

2.7.2.5 Micellar polydispersity [Grossman *et al.* 1992, Khaledi 1998]

Micelle aggregation number is defined as the average number of surfactant monomers per micelle (microemulsion) presented in a solution. The different aggregation number of micelle in the bulk solution causes the variation in the electrophoretic mobility of micelle and the retention of analytes. This effect produces the dispersion of analyte zones causing band broadening which is called micellar polydispersity. Plate height generated from micellar polydispersity (H_{pd}) can be expressed from the distribution of electrophoretic mobilities of micelles having different aggregation numbers.

$$H_{pd} = \left[\frac{\sigma_{\mu mc}}{\mu_{mc}}\right]^2 \frac{\mu_{mc}^2 (1-R) t_{mc}^{*} E}{\mu_{net}} \qquad (2.38)$$

where $\sigma_{\mu mc}$ is the standard deviation of electrophoretic mobility of micelles, R is the retardation factor (or retention ratio, i.e., the fraction of analyte in the aqueous phase), and t_{mc}^{*} is the mean life-time of analyte in the micelle, which can vary from nanoseconds to milliseconds.

It can be concluded that H_{mc} is negligible for analytes having strong partitioning into the micelle (microemulsion). H_l increases with increasing retention time and high diffusion coefficient of analytes. H_{aq} and H_{pd} decrease with increasing surfactant concentration. H_t increases with increasing the conductivity of BGE, capillary radius and applied voltage.

2.7.3 Resolution

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

$$R_s = \frac{t_{m2} - t_{m1}}{0.5(w_{b1} + w_{b2})} \tag{2.39}$$

From the relationship between migration time and electrophoretic mobility of analyte combines with Equation 2.10, R_s can be related to the mobility and the average number of theoretical plates, $\overline{N}$, according to the equation

$$R_s = \frac{1}{4}\left(\frac{\Delta\mu}{\overline{\mu} + \mu_{eo}}\right)\sqrt{\overline{N}} \tag{2.40}$$

where $\Delta\mu$ is the difference in the mobility, and $\overline{\mu}$ is the average electrophoretic mobility of the analytes. In MEKC and MEEKC with high EOF, R_s of two analytes is expressed by [Terabe *et al.* 1985]

$$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)\left(\frac{1 - \frac{t_0}{t_{mc}}}{1 + \left(\frac{t_0}{t_{mc}}\right)k_1}\right) \tag{2.41}$$

where t_0 is the migration time of analyte in CE without charged oil droplet. In the case of neutral analyte, t_0 refers to the retention of an EOF marker or an unretained compound in the charged oil droplet.

In MEKC or MEEKC without EOF, it follows from Equation 2.40 that the resolution of two analytes is given by

$$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\Delta\mu}{\overline{\mu}}\right) \tag{2.42}$$

Recently, Nhujak *et al.* has reported a simple equation for R_s in MEKC and MEEKC without EOF [Nhujak *et al.* 2006]. Substituting the value of μ (Equation 2.14) relating to k into Equation 2.42 and assuming that $k_1+k_2 = 2k_1$, the resolution of analytes in MEEKC without EOF may be expressed by

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{1 + k_2}\right) \tag{2.43}$$

2.8 Instrumentation

2.8.1 Basic apparatus of CE

Figure 2.9 illustrates the schematic of CE instrument. Typically, a CE system consists of a high voltage power supply allowing voltages up between -30 and +30 kV, two electrodes commonly made of platinum wire, a capillary column with 10 to 200 μm I.D. and 20 to 100 cm in length, a detector mostly used a UV-Vis detector, and a cooling system for controlling temperature of the capillary and reducing Joule heating.

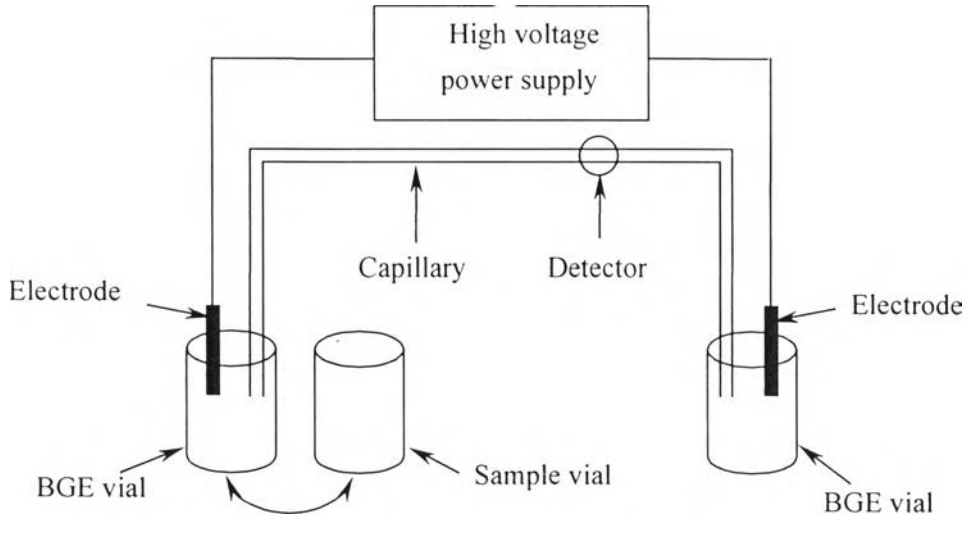

Figure 2.9 Schematic diagram of a basic CE instrument. Adapted from Weinberger [1993].

2.8.2 Injection system [Khaledi 1998]

Analytes can be introduced into the capillary by either hydrodynamic injection, accomplished by using pressure difference, or electrokinetic injection, which analytes move under the influence of applied electric field. This work involves hydrodynamic injection, and therefore the electrokinetic injection is not mentioned in this section.

Hydrodynamic injection is commonly used due to the proper amount of sample entered. This pressure difference can be performed by various methods such as gravimetric, vacuum and overpressure. The latter is called pressure injection and widely used in CE. According to the cylindrical shape of a capillary column, the length of sample injected (l_{inj}) depends on injection time (t_{inj}) and is given by the equation

$$l_{inj} = \frac{\Delta P r^2}{8\eta L} t_{inj} \tag{2.44}$$

where ΔP is the pressure difference between the inlet and outlet ends of capillary; r is the radius diameter of capillary; η is the liquid viscosity, and L is the capillary length. The volume (V_{inj}) and the amount (Q_{inj}) of analyte injected are determined using the following equations

$$V_{inj} = \frac{\Delta P \pi r^4}{8\eta L} t_{inj} \tag{2.45}$$

and

$$Q_{inj} = \frac{\Delta P \pi r^4}{8\eta L} t_{inj} C \tag{2.46}$$

where C is the analyte concentration.

2.8.3 Detection system

A detection method commonly used in CE is UV-Vis detection. This method is useful for a large number of compounds containing a chromophore. UV-Vis detection can be used with non-chromophore analytes by using indirect detection or a derivatization technique with direct detection. The main limitation of the UV-Vis detector is the relatively low sensitivity. The second most widely used detector is based on fluorescence due to its high selectivity and sensitivity. These advantages are especially important in biological application. Electrochemical detections have been reported as detection in CE, such as conductometry [Buchberger 1999], amperometry [Buchberger 1999], and potentiometry [Buchberger 1999]. Mass spectrometry (MS) hyphenated with CE is a useful method to provide structural information of analytes [Buchberger 1999]. In this work, the UV-Vis detection is used for analysis of avermectins.

2.9 Quantitative Analysis in CE

2.9.1 Peak area and corrected peak area [Mayer 2001]

For the quantitative analysis in CE, peak area, rather than peak height, is typically used because the peak area shows less variation and has a wide range of linearity. The amount of analyte (Q) found is proportional to peak area of analyte (A), integrated and converted from the natural units of AU s into concentration unit (mol/m^3), as described in equation

$$Q\ (\text{mol}) = \frac{A\ (\text{AU s}) \times V_F\ (\text{m}^3\text{s}^{-1})}{\text{Response factor (AU mol}^{-1}\ \text{m}^{-3})} \qquad (2.47)$$

where V_F is the volume flow of the analyte passing the detector.

The volume flow rate of an analyte in CE is expressed by

$$V_F = \frac{\pi r^2 l}{t_m} \tag{2.48}$$

where l is the length of the capillary to the detector.

Unlike in HPLC, where the residence time in the detector is equal for all analytes and driven by the constant flow rate of the mobile phase, the time required for injected analytes to reach the detector in CE is governed by their apparent mobilities. Analytes pass the detector with the different electrophoretic mobilities and identical EOF. The analyte that migrates with slower velocity remains longer in the detector cell and gives detector response for longer time, resulting in larger peak area and quantitative bias. From Equation 2.47 and 2.48, the peak area of analyte injected in CE is proportional to V_F, and Q is proportional to peak area divided by t_m, as shown in the equation

$$Q \propto \frac{A \text{ (AU s)}}{t_m \text{ (s)}} \tag{2.49}$$

Therefore, this effect in CE will be compensated by the use of corrected peak area, $A_{corr.}$ which is the ratio of peak area (A) to migration time (t_m).

$$A_{corr} = \frac{A}{t_m} \tag{2.50}$$

2.9.2 Internal standard [Mayer 2001]

In CE, an internal standard is used in a same way as in chromatography. The internal standard is mostly used to correct for errors of sample introduction caused by instrumental imprecision such as pressure, injection time and variation in the solution viscosity. In addition, the variation of sample levels also results in the difference in the quantity of the analyte injected. The same concentration of the internal standard is added into both sample and standard solutions. The calibration plot is established from the ratio of A_{corr} of the standard to that of internal standard against the concentration of the standard.