COMPARISON AND PREDICTION OF RETENTION OF DISUBSTITUTED AROMATIC COMPOUNDS IN CAPILLARY ELECTROKINETIC CHROMATOGRAPHY

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การเปรียบเทียบและการทำนายรีเทนชันของสารประกอบแอโรแมติกที่มีหมู่แทนที่สองหมู่ในคะพิลลารีอิเล็กโทรไคเนทิกโครมาโทกราฟี

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จากการศึกษาพบว่าค่ารีเทนชันอินเดกมีความเหมาะสมมากกว่าค่ารีเทนชันแฟกเตอร์ในการใช้เป็นพารามิเตอร์สำหรับเปรียบเทียบค่าแอฟฟินิตีสัมพัทธ์ (relative affinity) ของอนุพันธ์เบนซีนที่มีหมู่แทนที่สองหมู่ ในไมเซลลาร์อิเล็กโทรไคเนทิกโครมาโทกราฟี (MEKC) และไมโครอิมัลชันอิเล็กโทรไคเนทิกโครมาโทกราฟี (MEEKC) เนื่องจากค่ารีเทนชันอินเดกไม่ขึ้นกับความเข้มข้นของสารลดแรงตึงผิวโซเดียมโดเดกซิลซัลเฟต ค่ารีเทนชันอินเดกใน MEKC และ MEEKC จะให้ค่าที่เหมือนหรือแตกต่างกันขึ้นกับชนิดของหมู่แทนที่ และค่า K_{ow} ของอนุพันธ์เบนซีนที่มีหมู่แทนที่สองหมู่ สามารถหาค่าได้จากค่ารีเทนชันอินเดกของสารมาตรฐานอัลคิลเบนซีนใน MEKC และ MEEKC โดย K_{ow} คือ ค่าคงที่ของการกระจายตัวของสารระหว่างออกทานอลกับน้ำ นอกจากนี้พบว่าเมื่อใช้หลักการเติม (additive approach) ค่ารีเทนชันอินเดกจากการทำนายของอนุพันธ์เบนซีนที่มีหมู่แทนที่สองหมู่ที่ต่างกัน สามารถหาได้จากค่าเฉลี่ยรีเทนชันอินเดกของอนุพันธ์เบนซีนที่มีสองหมู่แทนที่เหมือนกัน อย่างไรก็ตามผลทางอิเล็กทรอนิก และ/หรือ อันตรกิริยาภายในโมเลกุลของหมู่แทนที่อาจมีผลทำให้ค่าที่ได้จากการทดลองแตกต่างจากการทำนาย

ลายมือชื่อนิสิต.....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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Retention index (*I*), rather than retention factor (*k*) was found to be a more reasonable parameter for comparison of the relative affinity of disubstituted benzenes in MEEKC and MEKC, due to independent of *I* with the SDS surfactant concentration. MEKC and MEEKC may give similar or different *I* values, depending on types of moieties. With known *I* and K_{ow} for alkylbenzenes as references in MEKC and MEEKC, the values of log K_{ow} for disubstituted benzenes can be estimated from the observed *I* values, where K_{ow} is the octanol-water distribution constant. In addition, a group additive approach can be used to predict *I* for disubstituted benzenes with different moieties from the average observed *I* for the disubstituted benzenes with the same moieties. However, electronic effects and/or intramolecular interaction may result in the different observed *I* from prediction.

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LIST OF ABBREVIATIONS AND SYMBOLS

α_{CH2}	methylene selectivity
η	viscosity
μ	electrophoretic mobility
μ_{eo}	electroosmotic mobility
μ_{mc}	mobility of micelle or microemulsion phase
μ_{net}	total mobility
ζ	zeta potential
BGE	background electrolyte
BZ	alkylbenzenes
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
CIEF	capillary isoelectric focusing
CITP	capillary isotachophoresis
CMC	critical micelle concentration
CZE	capillary zone electrophoresis
DAD	diode array detector
DB	dodecylbenzene
EOF	electroosmotic flow
EKC	electrokinetic chromatography
i.d.	internal diameter
MEKC	micellar electrokinetic chromatography
MEEKC	microemulsion electrokinetic chromatography
PPN	propiophenone
SD	standard deviation
SDS	sodium dodecyl sulfate
v/v	volume by volume
E	electric field strength
e	electronic charge
I	retention index

K	distribution constant
K_{ow}	octanol-water distribution constant
k	retention factor
L	total capillary length
l	length of capillary to detector
l_{inj}	length of analyte injected
n_{aq}	amount of analyte in aqueous phase
n_{mc}	amount of analyte in micelle or microemulsion phase
ΔP	pressure difference across the capillary
Q	amount of analyte
Q_{inj}	quantity of sample injected
r_h	hydrodynamic radius
t	time
t_{eo}	migration time of EOF
t_{inj}	injection time
t_m	migration time
t_{mc}	migration time of micelle or microemulsion phase
t_R	retention time
V	applied voltage
V_{inj}	volume of sample injected
v_{eo}	electroosmotic velocity
v_{ep}	electrophoretic velocity
v_{net}	total electrophoretic velocity
x_{aq}	mole fraction of analyte in aqueous phase
x_{mc}	mole fraction of analyte in micelle or microemulsion phase
z	charge of an ion

CHAPTER I
INTRODUCTION

1.1 Introduction and Modes of Capillary Electrophoresis

Capillary electrophoresis (CE) is an electrophoretic technique for direct separation of charged compounds in a capillary containing a background electrolytes (BGE) solution, such as a phosphate or borate buffer, under the influence of applied electric field. The separation mechanism is based on the difference in electrophoretic mobility of analytes depending on the charge-to-size ratio [1]. CE is similar to chromatography in many ways, and most of the terms used in chromatography are also found in CE. For example, resolution and efficiency are defined in a similar way. However, some terms are different. A pump is used to drive the analytes through the column in chromatography, while the analytes in CE migrate due to their electrophoretic mobility and electroosmotic flow under the electric field.

CE is classified by the different separation mechanism [1, 2] to six basic modes, allowing CE to be used for a wide variety of substances. These modes include capillary zone electrophoresis (CZE), capillary electrochromatography (CEC), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP), capillary isoelectric focusing (CIEF) and micellar electrokinetic chromatography (MEKC). Recently, microemulsion electrokinetic chromatography (MEEKC) has been accepted to be another mode in CE.

CZE [3, 4] is the simplest and most widely used mode in CE. However, the primary limitation of CZE is its inability to separate neutral compounds, while MEKC and MEEKC can be used to separate neutral solutes [2]. Nowadays, both MEKC and MEEKC have become the important fields of research in CE offering a large range of applications, and used in this research. Therefore, these two modes are briefly discussed in this section. Figure 1.1 shows separation mechanism in MEKC and MEEKC.

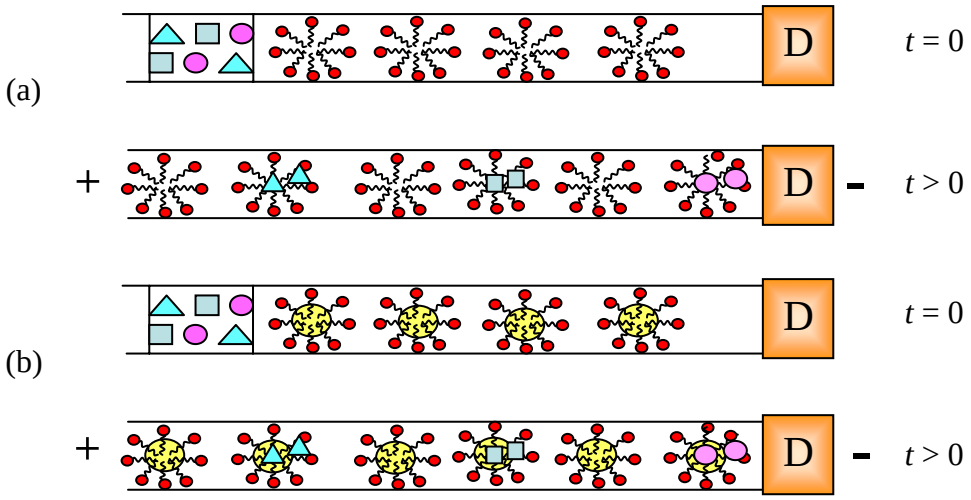


Figure 1.1 Separation mechanisms of (a) MEKC and (b) MEEKC. Adapted from Grossman and Colburn [2] and Altria [5].

1.2 Introduction to Micellar Electrokinetic Chromatography

MEKC was introduced by Terabe in 1984. It is a common mode of CE that can be applied for separation of both charged and neutral analytes [1, 6]. BGE used in MEKC for the separation of analytes is performed by adding a surfactant, such as sodium dodecyl sulfate (SDS), to form micelles or micellar phase. The molecules of surfactant exhibit both hydrophobic and hydrophilic characters, containing a long alkyl chain as the hydrophobic group and a polar or ionic head group as shown in Figure 1.2a. When the concentrations of the surfactant above its critical micelle concentration (CMC), the surfactant monomers aggregate to form micelles, as shown in Figure 1.2b, that have spherical in shape which the hydrophobic tails of the surfactants are oriented toward the center, while the polar head groups are faced outward into the electrolyte solution. Therefore, the micelles, charged media, can be acted as the separation medium for uncharged solutes. The micelles serve as the micellar phase as pseudostationary phase similar to a stationary phase in high-performance liquid chromatography (HPLC). Thus, the separation mechanism is based on the difference in partitioning of analytes between the micellar phase and aqueous phase, as shows in the Figure 1.1a.

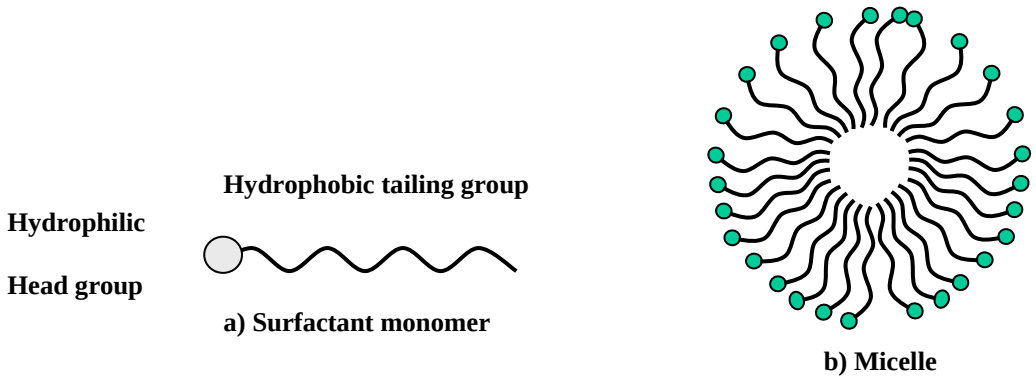


Figure 1.2 Schematic illustration of surfactant and micelle. Adapted from Khaledi [1].

1.3 Introduction to Microemulsion Electrokinetic Chromatography

MEEKC is a relative recent mode in CE, and is an electrophoresis technique related to MEKC. The BGE used in MEEKC is a microemulsion which containing dispersed nanometer-size oil droplets of an immiscible liquid. In particular the microemulsions are containing oil droplets surrounding the surfactant and co-surfactant suspended in aqueous buffer [5]. Typically, microemulsion in MEEKC is performed by adding oil droplets (such as *n*-octane and ethyl acetate), an emulsifier surfactant (such as SDS) to stabilize the microemulsion and to generate negatively charged oil droplets and co-surfactant (such as 1-butanol is short chain alcohol) to lower interfacial tension and to enhance stability of a microemulsion system, as shown in Figure 1.3.

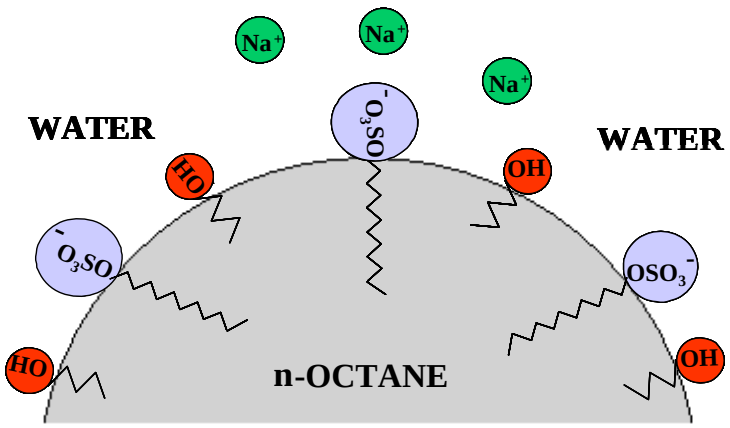


Figure 1.3 Schematic of microemulsion. Adapted from Altria [5].

The microemulsion serve as a pseudo stationary phase, similar to micellar phase in MEKC. Thus, the separation mechanism in MEEKC is similar to that in MEKC, which is based on the difference in partitioning of analytes between the aqueous phase and the pseudo stationary phase of charged oil droplets, as shown in 1.1b. Hydrophobic compounds favor partitioning into the oil droplet rather than hydrophilic compounds. MEEKC can be used as an electrodriven separation technique that offers the highly efficient separations of both charged and neutral analytes covering a wide range of water soluble and insoluble compounds [7-9].

1.4 Literature Review on Retention in MEKC and MEEKC

In chromatography and electrokinetic chromatography (EKC) such as MEKC and MEEKC, the retention factor (k), defined as the ratio of total moles of analytes in pseudo stationary phase versus those in aqueous phase, is one of the characteristics that indicates retention behavior of analytes in pseudo stationary phase [10-12]. The retention mechanism in EKC may be explained using quantitative structure-relationship and quantitative structure-property relationship between analytes and pseudo stationary phase [13-15]. Theoretically, the retention factor is related to K and the phase ratio (V_m/V_{aq}) as the equation $k = K(V_m/V_{aq})$ [8, 16, 17], where, K is the distribution constant of the analyte between two phases, and V_m and V_{aq} are the pseudo stationary phase volume and the aqueous phase volume, respectively. At the same concentration of SDS in the buffers, the higher values of log k in MEKC than MEEKC were found for six herbicides [16] bisphenol-A diglycidyl ether and its derivatives [17]. However, in comparison with MEKC at 50 mM SDS, MEEKC at 60 mM gave higher log k for test analytes [18]. Typically, the relative affinity of analytes in the same pseudo stationary phase and phase ratio under particular conditions can be compared using k. The higher values of the retention factor, the stronger the affinity or the partitioning of the analytes in pseudo stationary phase. In the case of the different phase ratios or pseudo stationary phase such as the microemulsion and the micelle [18], it is more reasonable to compare the relative affinity of the analytes between two systems in the term of K rather than k because k depends on the phase ratio, while K is independent of the phase ratio. However, with known k from experiment, the value of K in MEEKC cannot be calculated exactly because of an

inexact value for the microemulsion volume [12, 19]. In previous work on MEEKC, an decrease in *k* is affected by an increase in temperature [12, 20-22], Joule heating caused by high voltage [12, 16, 21] and the concentration of organic co-solvent [12, 20-22] was reported.

The retention index (*I*) or migration index is an another parameter to express retention behavior of analytes, compared with the reference standards such as homologous series of alkylbenzenes or alkyl aryl ketones [10, 12, 23]. The retention index is independent of the phase ratio and the concentration of the surfactant in MEKC [10, 23] and MEEKC [12], and therefore may be used for comparison of relative affinity of analytes in different pseudo stationary phase and/or phase ratio. However, in MEKC with the slightly dependence of temperature on *I* [10], whereas *I* of analytes in MEEKC dependent on temperature [12]. In previous work, in the MEKC buffer was added by organic solvents, some variations (RSD < 3%) of *I* were found at different concentrations of SDS [23], and the values of *I* in MEEKC with organic co-solvents were reported to decrease with an increase in the concentrations of organic co-solvent [12]. In addition, a good linear relationship between log K_{ow} and log *k* or log K_{ow} and *I* of analytes was obtained from EKC, where K_{ow} is the octanol-water distribution coefficient of analyte [10, 19, 24-26].

In our previous work on MEEKC, the retentions of bisphenol-A-diglycidyl ether derivetives (BADGEs) [17] and curcuminoids [21] containing two different moieties were observed to be between those containing two same moieties. In addition, the values of *k* and *K* for disubstituted benzenes in MEKC were predicted based on the solute structure of monosubstituted benzenes using a group additive approach [27]. However, the prediction of retention behavior for disubstituted benzenes with different moieties from those for disubstituted benzenes with same moieties in MEKC and MEEKC has not been reported previously.

1.5 Aim and Scope

Most of the previous work on EKC investigated factor affecting retention factor, retention index and resolution of hydrophobic aromatic compounds, and a few work

has been reported prediction of retention behavior of neutral aromatic compounds on MEKC. As previously mentioned, in MEEKC the retentions of compounds containing two different moieties were observed to be between those containing two of the same moieties, and in MEKC the predicted values of log *k* for positional isomers (such as *ortho*-, *meta*-, *para*-) of disubstituted benzenes were assumed to be equal, while the observed values of log *k* for these compounds are different. Therefore, it is interesting to extend previous work to predict the retention behaviors of disubstituted benzenes.

Therefore, the aims of this work are to investigate *I* for comparison of relative affinity of disubstituted benzenes between the micellar in MEKC and the microemulsion in MEEKC at either same or different SDS concentrations, and to establish an alternative of a group additive approach for prediction of *k* and *I* for disubstituted benzenes with different moieties from those for disubstituted benzenes with same moieties. The linear equation is developed for prediction of retention factor, retention index of disubstituted benzenes containing different moieties. Finally, the retention index is used to determine log K_{ow} of analytes.

MEKC and MEEKC will be carried out in this work using a 10 mM $Na_2B_4O_7$ solution at pH 9.2 as BGE to high electroosmosis, separation temperature 25 ^oC and voltage for separation 15 kV. Test analytes used are disubstituted benzenes with either same or different moieties of methyl ($-CH_3$), methoxy ($-OCH_3$), aldehyde (−CHO) and halogens (such as −F, −Cl −Br). In MEKC, SDS is used as pseudo stationary phase, while in MEEKC, ethyl acetate as oil, SDS as surfactant and 1-butanol as co-surfactant are used.

CHAPTER II
THEORY OF CE and EKC

2.1 CE Instrumentation

2.1.1 Basic apparatus of CE

The schematic diagram of a basic CE instrument is illustrated in Figure 2.1. A CE system consists of a high voltage power supply allowing voltages up between –30 and +30 kV, two electrodes (commonly platinum wire), two reservoirs of background electrolyte (BGE) at the inlet and outlet ends of the capillary, a capillary column with 10 to 200 μm I.D. and 20 to 100 cm in length, a detector mostly used UV-vis detector, injection system, and a cooling system for controlling temperature of the capillary and reducing Joule heating.

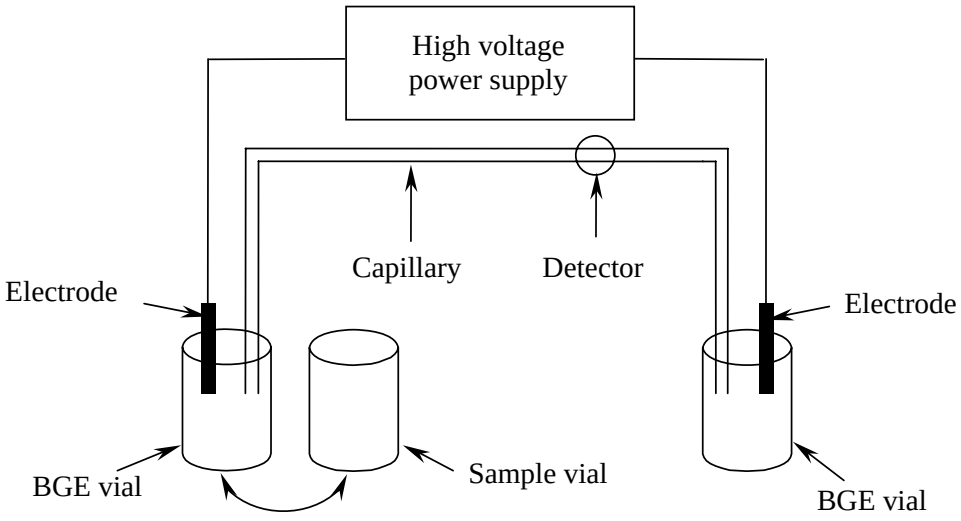


Figure 2.1 Schematic diagram of a basic CE instrument. Adapted from Weinberger [4].

For the simple analysis, BGE is filled into the capillary. Then, the inlet BGE vial is replaced by the sample vial, and a sample solution is introduced into the capillary. After that, the high voltage is applied to the capillary. Analytes migrate and are

separated by the difference in their electrophoretic mobilities. Finally, the analytes will migrate to the detector.

2.1.2 Sample introduction

Sample injection in CE [1] can be performed basically two common methods including electrokinetic and hydrodynamic injection. The hydrodynamic injection is the widely used in CE injection. It can be classified into gravity (rising the sample vial the outlet vial for a fixed interval time), vacuum (applying a vacuum to the outlet vial) or pressure (applying a pressure to the sample vial). The pressure injection is the most common used in CE. The length of sample plug injected into the capillary by pressure injection is given by

$$l_{\text{inj}} = \frac{\Delta P r^2}{8\eta L} t_{\text{inj}} \tag{2.1}$$

where t_{inj} is the injection time, ΔP the pressure difference between the inlet and outlet ends of capillary, r the radius diameter of capillary, η the liquid viscosity, and L the capillary length. Thus, the volume (V_{inj}) and quantity (Q_{inj}) of the sample injected can be calculated from the following equations:

$$V_{\text{inj}} = \frac{\Delta P \pi r^4}{8\eta L} t_{\text{inj}} \tag{2.2}$$

and

$$Q_{\text{inj}} = \frac{\Delta P \pi r^4}{8\eta L} t_{\text{inj}} c \tag{2.3}$$

where c is the concentration of analyte.

2.1.3 Detection [1, 28]

Detectors used in CE have been adapted from detection techniques used in HPLC, especially a UV-vis detector. For on-column UV-vis detection, a small section of

polyimide coating near the outlet of the capillary is removed to form a cylindrical window serving as the detector cell. The window is positioned in the path of the light beam, and the analytes are detected when they flow through this window. To enhance sensitivity of detection, an extension of the optical path length may be carried out by using a Z-shaped cell or bubble cell [2, 29]. 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, on-column UV-vis detection is used for analysis of disubstituted benzenes.

2.2 Basic Theories of CE

2.2.1 Electrophoretic mobility [2, 29]

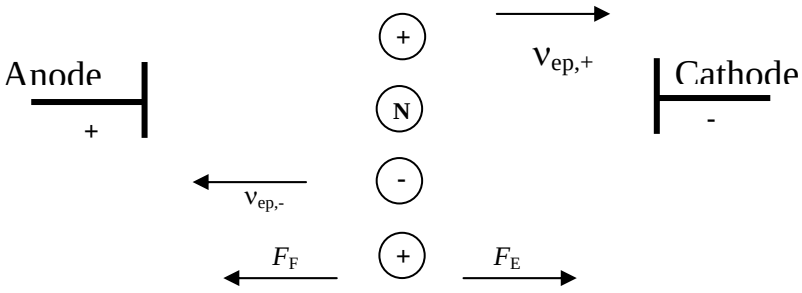


Figure 2.2 Migration behavior of each species. Adapted from Foret *et al.* [29].

Under the influence of electric field strength, E ($E = V/L$, where V is the applied voltage across L the total length of capillary) as shown in Figure 2.2, charged analytes migrate toward the electrode having opposite polarity with an electric force, F_E, which is proportional to the electric field strength and the charge on the ion, q, ($q = ze$, where z is the charge of an ion, and e the electronic charge):

$$F_E = zeE \tag{2.4}$$

Meanwhile, the migration of the ion is resisted by the frictional force, F_F, due to the viscosity of BGE, η (N s m^{-2})

$$F_F = 6\pi r_h \eta \nu_{ep} \quad (2.5)$$

where r_h is the hydrodynamic radius of an ion, and ν_{ep} is the electrophoretic velocity (m s^{-1}) of an ion in BGE. The acceleration of the ion will proceed until F_E is balanced by F_F, giving the equation:

$$zeE = 6\pi r_h \eta \nu_{ep} \quad (2.6)$$

The electrophoretic mobility, μ (m^2 V^{-1} s^{-1}) is defined as the electrophoretic velocity of the ion migrating in BGE under the influence of electric field strength of 1 V m^{-1}

$$\mu = \frac{\nu_{ep}}{E} = \frac{ze}{6\pi \eta r_h} \quad (2.7)$$

According to Equation 2.7, μ depends on the charge-to-size ratio of an ion, z/r_h. The electrophoretic mobility is the property of charged analytes under given conditions, depending on charge density of the analyte, ionic strength, viscosity of the electrolyte and temperature.

2.2.2 Electroosmotic flow [2, 29]

In CE, the migration of analytes in the presence of the electric field depends on their electrophoretic mobilities and electroosmotic flow (EOF). The EOF, as shown in Figure 2.3, is the movement of a medium toward the electrode when the voltage is applied. In the presence of BGE at pH > 2, silanol groups (-Si-OH) at the surface of an uncoated fused-silica capillary ionize, resulting in the negative charges of the capillary and excess positive ions in the solution as the equation:

$$\text{-SiOH } (s) + H_2O\ (l) \rightleftharpoons \text{-SiO}^- (s) + H_3O^+\ (aq) \qquad (2.8)$$

or

$$\text{-SiOH } (s) + OH^-\ (aq) \rightleftharpoons \text{-SiO}^- (s) + H_2O\ (l) \qquad (2.9)$$

These positive ions arrange themselves into a double electric layer as illustrated in Figure 2.4. Some positive ions are attracted at the negative surface of the capillary to form an imobilised layer, called the *Stern layer*, held by electrostatic forces, while some positive ions from the *diffusion layer* and the rest of the excess positive ions are in the bulk solution.

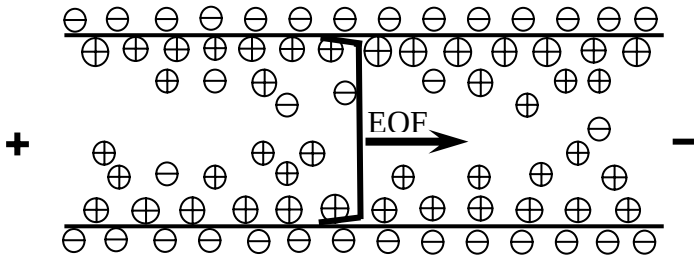


Figure 2.3 Electroosmotic flow (EOF). Adapted from Andrea *et al.* [30].

When the electric field is applied across the capillary, excess solvated positive ions in the diffuse layer and bulk solution migrate toward the cathode and carry water or solvent molecules to the same direction. This phenomenon is called *electroosmosis*, and the migration of water or solvent molecules is called *electroosmotic flow* (EOF).

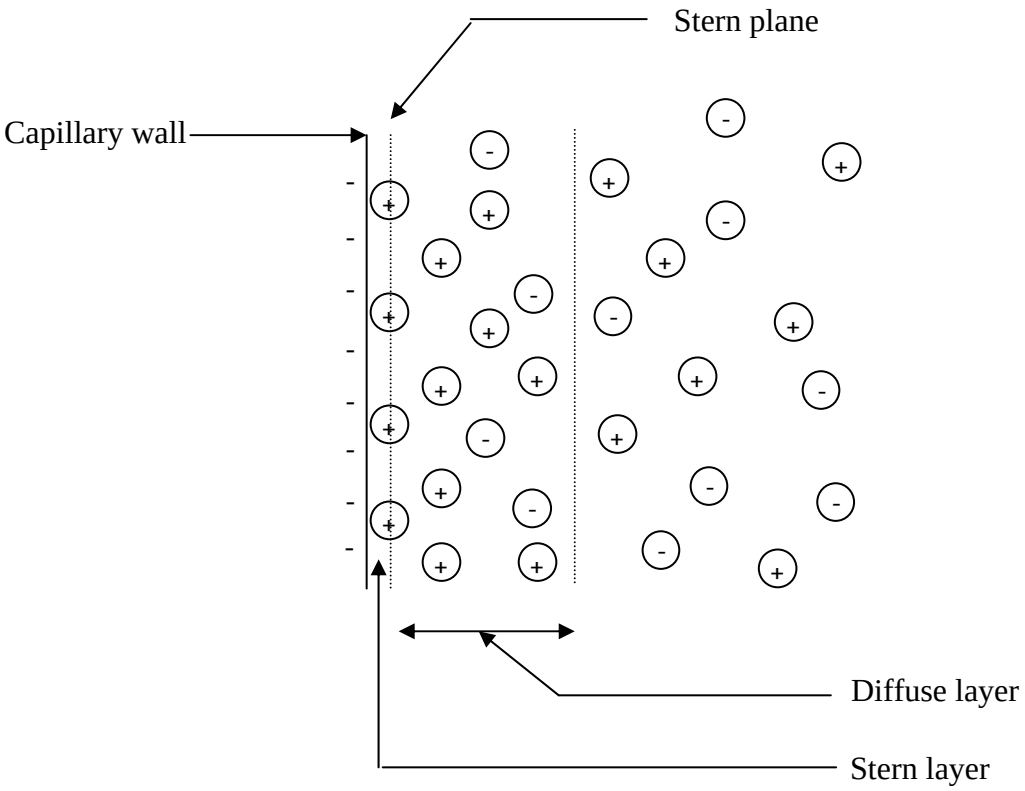


Figure 2.4 A model of a double electric layer and electrical potential. Adapted from Hiemenz *et al.* [31].

Because of silanol groups at the surface are weakly acidic, the degree of dissociation is dependent of pH. A change in 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 double layer. The velocity of electroosmotic flow (ν_{eo}) is proportional to the zeta potential, as given by

$$\nu_{eo} = -\frac{\varepsilon\zeta}{4\pi\eta}E \qquad (2.10)$$

where ε 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. From Equation 2.11, the electroosmotic mobility, μ_{eo} (m^2 V^{-1} s^{-1}), can be defined as the velocity of electroosmotic flow versus the applied electric field as the equation:

$$\mu_{eo} = \frac{\nu_{eo}}{E} = -\frac{\varepsilon\zeta}{4\pi\eta} \qquad (2.11)$$

The value of ζ is negative, and therefore, μ_{eo} has a positive sign for an uncoated fused silica. 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. 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.

2.2.3 Flow profile and migration behavior of analytes in CE

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 bulk solution and analytes in CE which is contrast to the parabolic profile generated by laminar flow driven by a pressure gradient in HPLC, as shown Figure 2.5. Thus, the peak width of analyte in CE is typically narrow, resulting in high efficiency and resolution in CE.

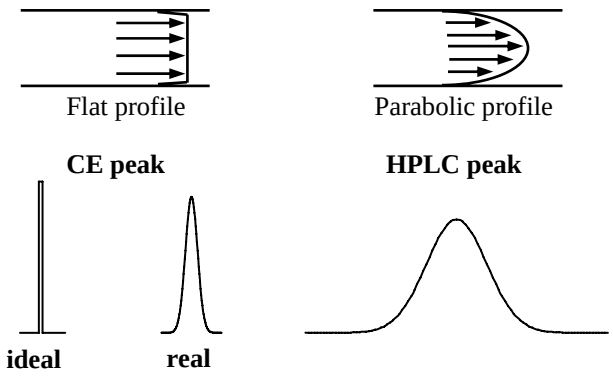


Figure 2.5 Flow profiles and peaks in CE and HPLC. Adapted from Chankvetadze [32].

In the presence of EOF, the net velocity, v_{net}, of the analyte is the sum of the electrophoretic velocity of the analyte and the electroosmotic velocity as Equation 2.12 and Figure 2.6

$$v_{net} = v_{ep} + v_{eo} \tag{2.12}$$

At 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 with the same velocity as the EOF.

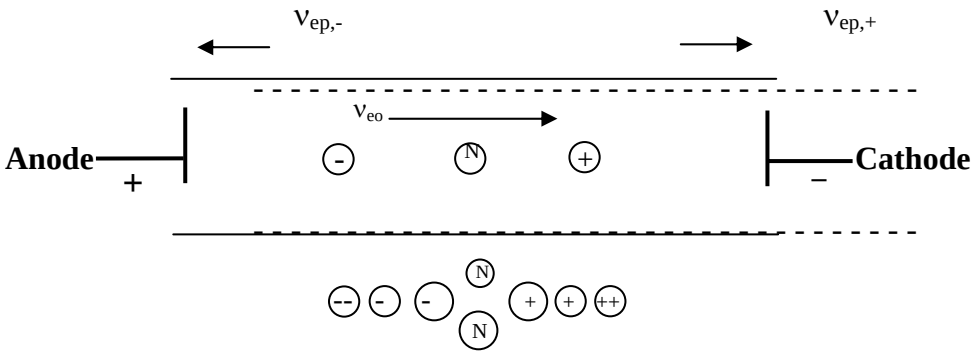


Figure 2.6 Migration behavior of analytes. Adapted from Li [3].

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

$$\mu_{net} = \frac{\nu_{net}}{E} = \frac{lL}{Vt_m} \tag{2.13}$$

$$\mu_{eo} = \frac{\nu_{eo}}{E} = \frac{lL}{Vt_{eo}} \tag{2.14}$$

$$\mu = \mu_{net} - \mu_{eo} = \frac{lL}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \tag{2.15}$$

where t_m and t_{eo} are the migration times of the analyte and the EOF marker, respectively, and l the length of the capillary to detector. Other parameters are previously mentioned.

2.3 Retention in MEKC and MEEKC

2.3.1 Retention factor in MEKC and MEEKC

From overview of EKC is given in the chapter I. In this section, more theoretical details of EKC are discussed. From section 1.2 and 1.3, sodium dodecyl sulfate (SDS)

is the common surfactant widely used in MEKC and MEEKC [5] due to no UV absorption, low CMC, available high purity, low cost and similar pseudostationary phase to C_8 and C_{18} stationary phase in HPLC. The SDS micelles have negative charge and migrate toward the positively charged electrode due to their electrophoretic mobility. Since the EOF is usually higher than the electrophoretic mobility of the micelles, eventually, the micelles are dragged by the EOF toward the detector (at cathode electrode). When analytes are injected into the capillary, they partition between the pseudo stationary phase, micellar or microemulsion and aqueous phase. The analytes that spend in the pseudo stationary phase for long time will reach to detector late. Therefore, in MEKC and MEEKC using SDS as surfactant, analytes will migrate between the EOF marker and the micelle or microemulsion.

A typical elution order is shown in Figure 2.7, where t_m, t_{eo} and t_{mc} are the migration times of solute, EOF marker and the micelle or microemulsion marker, respectively. A peak of EOF represents a neutral molecule that has no interaction with the pseudo stationary phase, thus it will migrate at the velocity of the EOF. A micelle or microemulsion peak is a peak of the analyte mostly retained in micellar or microemulsion phase. It can be seen that the separation mechanism in EKC is similar to that of the chromatographic technique. Therefore, the equations for the solute retention used in chromatography are also used in EKC.

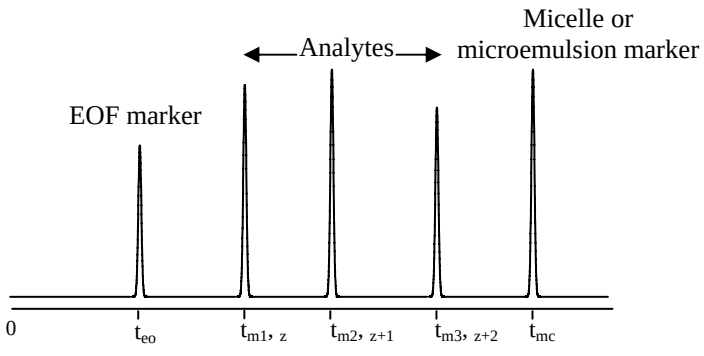


Figure 2.7 Typical elution order for analytes in EKC with high EOF. Adapted from Andrea and Brown [30].

As previously mentioned, the retention factor (k) is one of the characteristics that indicates retention behavior of analytes in chromatography and electrokinetic

chromatography such as MEEKC and MEKC. Similar to the concept of retention in chromatography and MEKC reported by [33], the retention factor in EKC is defined as the ratio of total moles of analyte in pseudo stationary phase as micellar phase in MEKC or microemulsion phase in MEEKC (n_m) versus those in the aqueous phase (n_{aq}) [34] as the equation:

$$k = \frac{n_m}{n_{aq}} = K\left(\frac{V_m}{V_{aq}}\right) \tag{2.16}$$

where K is the distribution constant between the two phase, the ratio of the concentration of the solute in pseudo stationary phase to that in aqueous phase, and (V_m/V_{aq}) the phase ratio, the pseudo stationary phase volume and the aqueous phase volume, respectively. The higher the retention factor, the stronger the retention or the partitioning of analytes in the pseudo stationary phase.

In EKC, the observed electrophoretic mobility of analyte A (μ) is a sum of the electrophoretic mobility of analyte A (μ_A) and the electrophoretic mobility of the micelle or microemulsion phase (μ_{mc}) as the equation:

$$\mu = x_{aq}\mu_A + x_{mc}\mu_{mc} \tag{2.17}$$

where x_{aq} and x_{mc} are the mole fractions of analyte in aqueous and micelle or microemulsion phase, respectively.

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

$$\mu = x_{mc}\mu_{mc} = \frac{n_{mc}}{n_{aq} + n_{mc}}\mu_{mc} \tag{2.18}$$

From Equations 2.16 to 2.18, μ can be expressed by

$$\mu = \frac{k}{1+k}\mu_{mc} \tag{2.19}$$

In EKC, with high EOF, the total mobility ($\mu_{net} = \mu + \mu_{eo}$) in Equation 2.20 is given by

$$\mu_{net} = \frac{k}{1+k}\mu_{mc} + \mu_{eo} \tag{2.20}$$

The retention time of neutral analyte, t_m, in EKC with high EOF is expressed by

$$t_m = \frac{(1+k)t_{eo}}{1+\left(\frac{t_{eo}}{t_{mc}}\right)k} \tag{2.21}$$

where t_{eo} is the migration time of the analyte due to electrophoretic mobility and EOF in BGE without micelle or microemulsion or unretained compound in pseudo stationary phase, t_{mc} the migration time of analyte fully partitioning into the pseudo stationary phase. Therefore, retention factor of the neutral analyte in MEKC and MEEKC with high EOF may be calculated from an electropherogram using the equation:

$$k = \frac{t_m - t_{eo}}{t_{eo}(1 - t_m/t_{mc})} \tag{2.22}$$

where t_m, t_{mc} and t_{eo} are the migration times of an analyte, a micelle or microemulsion maker and an EOF marker, respectively.

2.3.2 Retention index in MEKC and MEEKC

Retention index (I) is another characteristic used for describing retention behavior, where I is the number, obtained by interpolation (usually logarithmic), relating the adjusted retention time or the retention factor of the analyte to the adjusted retention times of two standards eluted before and after the analyte [10]. The retention index of

the analyte can be calculated by the logarithmic interpolation between the two neighboring standards of homologous series, according to the equation [23, 35]:

$$I = 100z + 100\frac{\log k_{\mathrm{A}} - \log k_{\mathrm{z}}}{\log k_{\mathrm{z+1}} - \log k_{\mathrm{z}}} \tag{2.23}$$

where k_{z} and $k_{\mathrm{z+1}}$ are the retention factors of the homologous with the number of carbon atoms z and z+1, respectively, and k_{A} the retention factor of the analyte. From Figure 2.7 and Equations 2.22 and 2.23, the retention index can be expressed as a function of the migration time as the equation

$$I = 100\mathrm{z} + 100\frac{\log\left(\frac{t_{\mathrm{m,A}} - t_{\mathrm{eo}}}{t_{\mathrm{mc}} - t_{\mathrm{m}}}\right) - \log\left(\frac{t_{\mathrm{z}} - t_{\mathrm{eo}}}{t_{\mathrm{mc}} - t_{\mathrm{z}}}\right)}{\log\left(\frac{t_{\mathrm{z+1}} - t_{\mathrm{eo}}}{t_{\mathrm{mc}} - t_{\mathrm{z+1}}}\right) - \log\left(\frac{t_{\mathrm{z}} - t_{\mathrm{eo}}}{t_{\mathrm{mc}} - t_{\mathrm{z}}}\right)} \tag{2.24}$$

According to the Martin's equation [10], the retention indices of homologous series compounds increase with an increase in the number of methylene groups, giving a linear equation between log k and z as the equation:

$$\log k = az + b \tag{2.25}$$

where a and b are the slope and the intercept, respectively. It follows from Equations 2.23 and 2.25 that the retention index may be calculated by interpolation and for the analyte with higher retention factor than the first homologue, can be expressed as [10, 12]:

$$I = \frac{100(\log k - b)}{a} \tag{2.26}$$

Compared to retention factors, the retention indices of analytes are not influenced by phase ratio, and are independent of the surfactant concentration [10, 23, 36]. Therefore, retention index provide more accurate qualitative data. However, in MEKC with addition of organic solvents in the buffer, some variations (RSD < 3%)

of retention index were found at different SDS concentrations [23]. The values of ΔI obtained with different surfactant systems as micellar phase in MEKC can be used to explain the functional group selectivity of these specific micellar systems and to classify pseudo stationary phases in MEKC [10].

2.3.3 Octanol-water distribution constant (log K_{ow})

The octanol-water distribution constant, log K_{ow}, has been the standard measure hydrophobicity of compounds used to predict biomembrane transport in living systems, bioaccumulation in plants and animals, and soil adsorption [37, 38]. Early direct measurement of log K_{ow} was generated using conventional shake-flask method. This method is time consuming (often in excess of 1 day/ solute), tedious and required large amounts of pure compounds [24]. Reverse-phase liquid chromatography (RPLC), attempt to avoid the disadvantage of shake-flask technique, has been used as a method for direct log K_{ow} determination through a linear relationship of retention factor and log K_{ow} [25, 39]. This approach is advantageous over the traditional direct measurement method including providing faster analysis times, improved reproducibility, and requiring less amounts of sample for testing. An alternative method, such as MEKC and MEEKC, has been recently used for the log K_{ow} indirect measurement [38, 40]. The relationship between log K_{ow} versus log k or log K_{ow} versus I in MEKC [19, 24] or MEEKC [25, 26] can be expressed by the following equation [12, 26]:

$$\log K_{ow} = c \log k + d \tag{2.27}$$

or

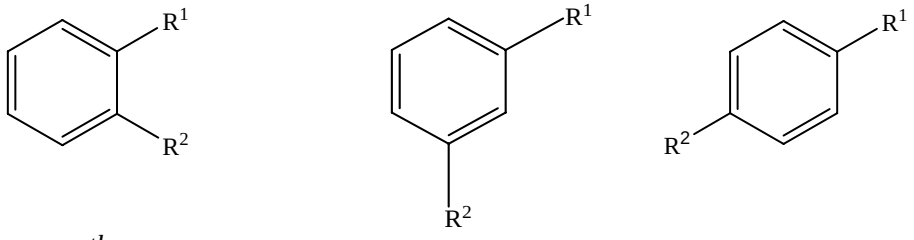
$$\log K_{ow} = e\, I + f \tag{2.28}$$

where c and d are the slope and intercept values, respectively, obtained from the linear plot between log K_{ow} and log k for standards; e and f are the slope and intercept values, respectively, obtained from the linear plot between log K_{ow} and nominal I for standards. Using standards with known log $K_{ow,}$ the linear plot between log K_{ow} and log k or log K_{ow} and I were obtained, and the values of log K_{ow} of analytes were predicted from log k and I.

CHAPTER III
EXPERIMENTAL

3.1 Chemicals

Disubstituted benzenes	R^1	R^2
Xylene (diMe)	CH_3–	$R^2 = R^1$
Dimethoxybenzene (diOMe)	OCH_3–	$R^2 = R^1$
Dibenzaldehyde (diCHO)	CHO–	$R^2 = R^1$
Difluorobenzene (diF)	F–	$R^2 = R^1$
Dichlorobenzene (diCl)	Cl–	$R^2 = R^1$
Dibromobenzene (diBr)	Br–	$R^2 = R^1$
Chlorofluorobenzene (F/Cl)	F–	Cl–
Bromofluorobenzene (F/Br)	F–	Br–
Bromochlorobenzene (Cl/Br)	Cl–	Br–
Fluorotoluene (Me /F)	CH_3–	F–
Chlorotoluene (Me /Cl)	CH_3–	Cl–
Bromotoluene (Me/Br)	CH_3–	Br–
Fluoromethoxybenzene (OMe/F)	OCH_3–	F–
Chloromethoxybenzene (OMe/Cl)	OCH_3–	Cl–
Bromomethoxybenzene (OMe/Br)	OCH_3–	Br–
Fluorobenzaldehyde (CHO/F)	CHO–	F–
Chlorobenzaldehyde (CHO/Cl)	CHO–	Cl–
Bromobenzaldehyde (CHO/Br)	CHO–	Br–
Methoxytoluene (Me/OMe)	CH_3–	OCH_3–
Tolualdehyde (Me/CHO)	CH_3–	CHO–
Methoxybenzaldehyde(OMe/CHO)	OCH_3–	CHO–



ortho- *meta-* *para-*

Figure 3.1 Chemical structures of disubstituted benzenes. R^1 and R^2 are the moieties.

Disodium tetraborate decahydrate ($Na_2B_2O_7.10H_2O$), 1-butanol and homologous series of alkylbenzenes (BZ), such as benzene (BZ6), toluene (BZ7), ethylbenzene (BZ8), propylbenzene (BZ9) and butylbenzene (BZ10), were obtained from Fluka (Buchs, Switzerland); SDS was from Sigma (St. Louis, MO, USA); ethyl acetate was from Merck (Darmstadt, Germany); propiophenone (PPN), dodecylbenzene (DB) and disubstituted benzenes were from Sigma-Aldrich (Steinheim, Germany): *o*-, *m*-, *p*-xylenes (diMe); *o*-, *m*-, *p*-dimethoxybenzenes (diOMe); *o*-, *m*-, *p*-dibenzaldehydes (diCHO); dihalogenated benzenes with same moieties (diX) such as *o*-, *m*-, *p*-difluorobenzenes (diF), *o*-, *m*-, *p*-dichlorobenzenes (diCl) and *o*-, *m*-, *p*-dibromobenzenes (diBr); dihalogenated benzenes with different moieties (X/Y) such as *o*-, *m*-, *p*-chlorofluorobenzenes (F/Cl), *o*-, *m*-, *p*-bromochlorobenzenes (Cl/Br) and *o*-, *m*-, *p*-bromofluorobenzenes (F/Br); halogenated toluenes (Me/X) such as *o*-, *m*-, *p*-chlorotoluenes (Me/Cl), *o*-, *m*-, *p*-fluorotoluenes (Me/F) and *o*-, *m*-, *p*-bromotoluenes (Me/Br); halogenated methoxybenzenes (OMe/X) such as *o*-, *m*-, *p*-chloromethoxybenzenes (OMe/Cl), *o*-, *m*-, *p*-fluoromethoxybenzenes (OMe/F) and *o*-, *m*-, *p*-bromomethoxybenzenes (OMe/Br); halogenated benzaldehyde (CHO/X) such as *o*-, *m*-, *p*-chlorobenzaldehydes (CHO/Cl), *o*-, *m*-, *p*-fluorobenzaldehydes (CHO/F) and *o*-, *m*-, *p*-bromobenzaldehydes (CHO/Br); *o*-, *m*-, *p*-methoxytoluenes (Me/OMe); *o*-, *m*-, *p*-tolualdehyde (Me/CHO); *o*-, *m*-, *p*-methoxybenzaldehydes (OMe/CHO).

3.2 Preparation of Buffers and Analysis

3.2.1 Preparation of micelle and microemulsion buffers

The MEKC buffer containing a pH 9.2 10 mM $Na_2B_2O_7$ and 50 mM SDS was prepared by pipeting 1mL of stock aqueous solution of 100 mM $Na_2B_2O_7$ and 500 mM SDS into a 10 mL volumetric flask. The final solution was made up to 10 mL with Milli-Q water. In the case of preparation of the MEEKC buffer was prepared to give a final solution containing a pH 9.2 10 mM $Na_2B_2O_7$, 50 or 60 mM SDS, 0.5% v/v ethyl acetate and 1-butanol 1.2% v/v. All buffers were sonicated for 30 min to obtain the clear solutions and to degas in the solutions. The EKC buffers were filtered through 0.45 μm PTFE membrane filters prior to CE analysis.

3.2.2 Preparation of analytes

Stock solutions of each analyte, thiourea (an EOF marker) and DB (a pseudo stationary phase marker) were separately prepared at a concentration of 10,000 ppm in methanol. In MEKC, the sample mixture containing desired analytes, thiourea and DB at 100 ppm each was obtained by pipeting each stock solution and then diluting the mixture with 50 mM SDS in water, while the sample mixture for MEEKC analysis was diluted with the solution containing 50 mM SDS in water, 0.5% v/v ethyl acetate and 1.2% v/v 1-butanol. All final test analytes solutions were filtered through 0.45 μm PTFE membrane filters prior to CE analysis.

3.3 CE conditions

All the CE separations were performed using a Beckman Coulter MDQ-CE system equipped with a photo-DAD scanning from 190 to 300 nm and monitoring at 214 or 200 nm. An uncoated fused-silica capillary used was 40.2 cm in length (30 cm to detector) × 50 μm id, thermostated at 25 °C. Voltage was set at 15 kV. A sample solution was introduced by 0.5 psi pressure injection for 3 s. Each experiment was carried out in triplicate. A new capillary was conditioned with methanol for 15 min, 1 M HCl for 15 min, 1 M NaOH for 15 min, 0.1 M NaOH for 15 min, water for 15 min and finally with the BGE for 15 min. Prior to CE analysis each day, the capillary was rinsed with methanol, 0.1 M NaOH, water, and the MEKC buffer or MEEKC buffer for 15 min each. Between consecutive run, the capillary was rinsed with methanol, 0.1 M NaOH for 2 min each and then with the buffer for 1 min. After analysis, each day, the capillary was rinsed with water and methanol for 5 min each, and then 0.1 M NaOH and water for 10 min each.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Choice of CE Conditions

4.1.1 Diameter and length of capillary

The most commonly used capillaries are 50 and 75 mm μm i.d. [32]. The 75 μm i.d. capillary provides higher sensitivity and lower interaction between analytes and capillary wall, but may cause low resolution due to greater peak broadening caused by Joule heating. In addition, it has limitation of the use of high BGE concentration and high voltage. Therefore, the 75 μm i.d. capillary is usually used to improve detection sensitivity and to decrease wall interaction when the separation has no problem of resolution. Typically, the 50 μm i.d. capillary is used for many applications due to its compromise among resolution, sensitivity, heat dissipation and wall interaction. This work does not involve analysis of trace levels of analytes, and therefore, in this work the 50 μm i.d. capillary was selected. The total length of capillary (L) is important for the electric field strength. In CE, the length of capillary is usually used in a range of 30 to 60 cm. The shorter capillary gives poorer resolution but faster analysis time. Thus, the length of 40.2 cm (30 cm to detector) capillary was used to give fast analysis time.

4.1.2 pH and concentration of buffer

In this work, the background electrolyte (BGE) for analysis of disubstituted benzenes was selected using a borate buffer at pH 9.2. Since high pH buffers such as borate or phosphate buffers are commonly used in MEKC and MEEKC and these buffers generate a high EOF when a voltage is applied across a capillary filled with the buffer [5, 9, 41]. The disubstituted benzenes are neutral compounds. Therefore, the BGE at basic pH was chosen to generate high EOF enough to carry neutral analytes which partition in micelles or microemulsions toward the detector. Both electrophoretic mobility and EOF affect the migration time of analyte and depend on pH of BGE, and

therefore the buffer is necessary for CE analysis to maintain the constant pH. $Na_2B_4O_7$ dissolved in water is widely used as the basic BGE since it provides a buffer at pH 9.2 without adjusting pH [42]. It can be explained by the equation

$$Na_2B_4O_7 + 7H_2O \longrightarrow 2Na^+ + 2H_3BO_3 + 2B(OH)_4^- \quad (4.1)$$

The pH of a solution containing weak acid (HA) and conjugated base (A^-) is given by the Henderson-Hasselbalch equation [43]

$$pH = pK_a + \log\frac{[A^-]}{[HA]} \quad (4.2)$$

In this case, HA is H_3BO_3 and A^- is $B(OH)_4^-$. As seen in Equations 4.1 and 4.2, the equal concentrations of H_3BO_3 and $B(OH)_4^-$ provide high buffering capacity. As a result, the equal concentration of the borate buffer prepared from $Na_2B_4O_7$ leads to precision in electrophoretic mobility, EOF and migration time. In addition, $Na_2B_4O_7$ is available in highly pure reagent grade, and the borate buffer is easy to prepare and stable for a long period of time [32].

At low ionic strength (5-10 mM) borate or phosphate buffers generates relatively low currents and a reasonably fast EOF. However, the too low concentrations of the buffer result in poor precision of migration times due to electrolysis effect [44], and poor peak shape due to high electromigration dispersion. On the other hand the too high concentrations of the buffer generate high current and Joule heating which may limit the use of high voltage and temperature. Therefore, a concentration of the borate buffer at 10 mM was chosen to use in this work for generates high EOF.

4.1.3 Surfactant type and concentration

As described in Section 2.3.1, SDS is a most widely used as anionic surfactant in EKC because it is cheap and available in highly purified form. Other anionic surfactants, such as sodium cholate and sodium tetradecyl sulfate etc., are also used [11]. However, they gave different selectivity compared to SDS. Cationic surfactants

such as cetyltrimethylammonium bromide (CTAB) are used in EKC [11, 45]. They produce positively charged oil droplets and also generate a positively charged surface bi-layer on the capillary wall, which reverses the EOF direction. In addition, CTAB produces high UV background at the wavelength in a range of 190 to 230 nm due to UV absorption of Br^-. Since the critical micellar concentration (CMC) of SDS is equal to 8.1 mM, the SDS concentration greater than CMC is required to obtain stable micellar phase in MEKC. SDS is usually used at a concentration of 3.3% w/w (typically 118 mM) for octane oil and at for ethyl acetate. In order to produce highly stable microemulsion in MEEKC, the C_{12} alkyl chain of the SDS surfactant penetrates into the oil droplet, while the negatively charged hydrophilic sulfate groups reside in the surrounding aqueous phase [11]. In EKC, the higher SDS concentration, the longer separation times [9, 46, 47]. It is caused by changing the PSP size-to-charged ratio. In addition, an increase in the surfactant concentration also increases the ionic strength of the buffer which reduces the EOF [5]. Therefore, 50 or 60 mM SDS in MEKC and MEEKC was chosen in this work for fast separations, and the effect of SDS concentration on *k* and *I* will be described in topic 4.2.

4.1.4 Oil droplet type and concentration

In MEEKC, hexane, *n*-heptane or *n*-octane is commonly used to form the oil droplet. In comparison with *n*-octane, *n*-heptane has lower toxicity, but faster vaporize [19] and gives poorer stability of the microemulsion [48]. In previous work, *n*-octane, used as the oil droplet, was reported to give more repeatable microemulsions with better peak resolution, efficiency and precision [11, 49, 50].

Other oil droplets used in MEEKC included ether, ester, alcohol and alkyl chloride groups such as diethyl ether, ethyl acetate, octanol and butyl chloride, respectively. However, these different types of oil droplet have been reported to give similar selectivity and migration time for separation and retention time for separation of neutral compounds [11]. High speed MEEKC using low interfacial oils as ethyl acetate has also been reported which requires a lower concentration of SDS to form the microemulsion, allowing high voltages to be applied [51, 52]. Therefore, ethyl

acetate at 0.5% (v/v) was chosen as oil droplet in this work, similar to that most commonly reported in literature [9].

4.1.5 Co-surfactant type and concentration

In MEEKC, 1-butanol is the most commonly used as a co-surfactant in order to reduce interfacial tension of a system to approach zero and to enhance stability of a microemulsion system [11, 47]. The co-surfactant lies between the surfactant molecules at the surface of oil droplet therefore reducing the repulsion between the surfactant head-group [9, 53]. Using higher co-surfactant concentration the longer migration times were observed due to higher the buffer viscosity and lower EOF [11]. In addition, the size of microemulsion droplets increase with increasing the co-surfactant concentration, result in a reduction of the charge density on the droplet, making the less ability of droplet to oppose EOF [9].

In previous work, other alcohols used as co-surfactant, 1-propanol, 1-pentanol, or 1-haxanol, have been shown to give broad peaks and poor resolution in MEEKC due to unstable microemulsion [21]. In comparison with 1-butanols, cyclohexanol gave the greater retention factor (k) of curcuminoids, but longer migration time due to the higher viscosity [21]. However, branched-chain alcohols such as 2-butanol attempt to use microemulsions formation, but they were completely unsuccessful to use [9]. A reason for this observation is that branched alcohols are unable to act as a co-surfactant between the oil and water interface because the chain length of the branched alcohol is too short to penetrate in to the oil [11]. In this work, 1-butanol at 1.2% (v/v) was chosen as the co-surfactant, which is typically used in MEEKC [9].

4.2 Retention and Retention Index in MEKC and MEEKC

In EKC, standard reference compounds for determination of retention index should satisfy a number of requirements [35]: (1) lowest homologue should be reasonably polar, in order to obtain a wide scale of retention index, covering the greater part of the elution window; (2) relationship between log k and the number of carbon atoms (z) in the molecule of homologues must be linear; (3) should contain a strong

chromophore to detect them spectrophotometrically, as the most CE instruments apply on-column UV detection; (4) should be uncharged; (5) should be chemically stable in common electrolyte systems. In addition, for anionic surfactant SDS, alkylbenzenes (BZ) were found to be suitable retention index standards in MEKC, while for cationic surfactants CTAB and DTAB, alkyl aryl ketones are more favorable [10]. Therefore, the homologous series of BZ were chosen as reference standard in this work because they have been widely used as standards for studying *k*, *I* and log K_{ow} in MEKC and MEEKC [12, 19, 35].

As described in Section 2.3.1, retention factor (*k*) for uncharged analytes can be calculated from an electropherogram of MEKC and MEEKC with high EOF. An example of electopherograms of homologous series of BZ (reference standards) is shown in Figure 4.1. In this work, DB, a highly hydrophobic compound, is used as a micelle or microemulsion maker for t_{mc} and thiourea is used as EOF marker for t_{eo}. An example of observed *k* of BZ, determined using electropherograms in Figure 4.1 and Equation 2.22, is shown in Table 4.1. It should be noted that BZ10 migrated close to DB, and inaccuracy in *k* may be obtained. Therefore, BZ6 to BZ9 were used as *I* standards. Propiophenone, PPN, was used as an *I* marker to check precision in *I* for each day.

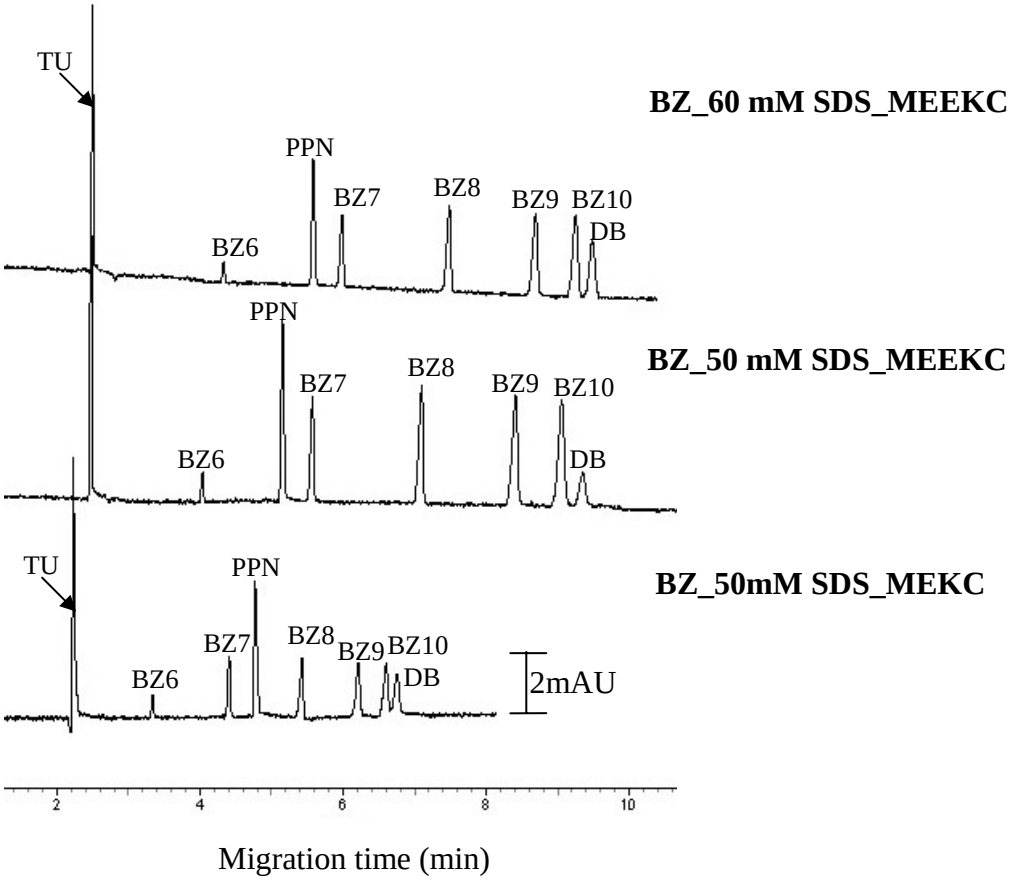


Figure 4.1 An example of electropherograms for homologous series of BZ in MEKC and MEEKC. An MEKC buffer contained 50 mM SDS and 10 mM NaB_4O_7 (pH9.2) and an MEEKC buffer contained 50 or 60 mM SDS in 10 mM NaB_4O_7 (pH9.2), 0.5% v/v ethyl acetate and 1.2% 1-butanol. CE conditions: uncoated fused silica capillary 50 µm i.d. × 40.2 cm (30 cm to detector), temperature 25 ºC, voltage +15 kV, 0.5 psi pressure injection for 3 s and UV detection at 214 nm. DB is a PSP marker and PPN is an *I* marker. TU (Thiourea) is unretained solute and also an EOF marker.

Table 4.1 Average migration times, and retention factor, with standard deviations (in parentheses) for alkylbenzenes in MEKC and MEEKC at 50 mM SDS. Each value is the average from three runs.

Compounds	MEKC		MEEKC	
	t_m (min)	k	t_m (min)	k
thiourea	2.052 (0.016)	0	2.417 (0.006)	0
BZ6	3.012 (0.040)	0.99 (0.01)	3.902 (0.018)	1.09 (0.01)
BZ7	3.883 (0.062)	2.81 (0.06)	5.367 (0.029)	3.06 (0.02)
BZ8	4.656 (0.094)	7.12 (0.16)	6.826 (0.040)	7.76 (0.04)
BZ9	5.255 (0.134)	22.27(0.44)	8.052 (0.049)	23.93(0.12)
DB	5.615 (0.187)	∞	8.921 (0.056)	∞

In MEKC and MEEKC, the retention index (I) of analytes can be estimated from the Equation 2.26. The slope (a) and the intercept (b) were obtained from a plot of log k versus z of the linear equation $\log k = az + b$. Using a homologous series of BZ (C6 to C9) as reference standard compounds, the good linear relationship between log k and z was obtained, as given in Figure 4.2 and Table 4.2. Similar methylene selectivity, where $\alpha_{CH2} = a$ [54], indicates that the microemulsion and micellar phases have similar hydrophobicity [12].

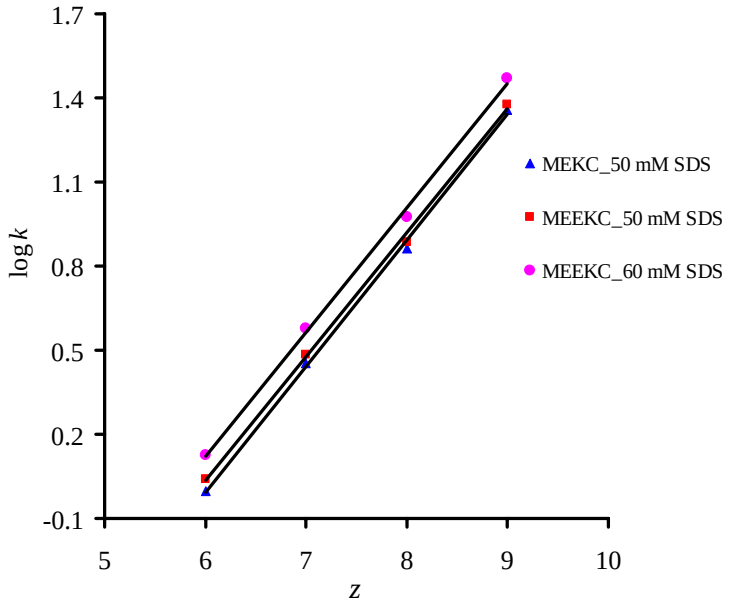


Figure 4.2 Relationship between log k and z of homologous series of BZ in MEKC and MEEKC. Other conditions are show in Figure 4.1.

Table 4.2 Linear relationship between the log k and z values obtained from a homologous series of BZ in MEEKC and MEKC

Mode	[SDS] mM	log $k = az + b$		
		a	b	r^2
MEKC	50	0.448±0.011	–2.698±0.084	0.9988
MEEKC	50	0.442±0.012	–2.615±0.087	0.9986
MEEKC	60	0.443±0.013	–2.534±0.096	0.9984

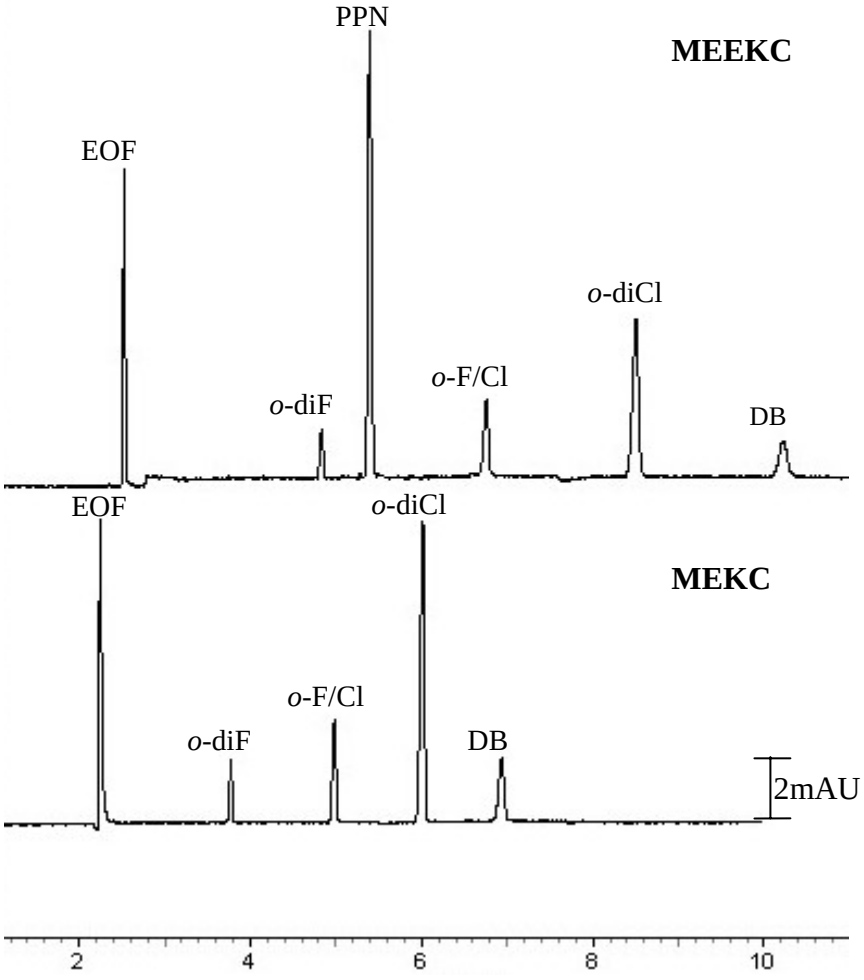


Figure 4.3 An example of electropherograms for disubstitued benzene in MEKC and MEEKC using 50 mM SDS. EOF = thiourea (unretained solute). Other conditions are shown in Figure 4.1.

Table 4.3 shows observed values of *I* for disubstituted benzenes in MEKC and MEEKC using 50 and 60 mM SDS. Figure 4.4 shows a comparison of the log *k* and *I* values for disubstituted benzenes, obtained from MEEKC and plotted against those obtained from MEKC, where *I* values were determined using BZ as standards. A dash line in each figure shows a linear plot of an equation $y = x$. The value above the dash line refers to the higher value in MEEKC than that in MEKC. Comparison of *I* and *k* values between the same and different concentrations of SDS revealed a similar relative *I* for each analyte in MEEKC against MEKC (Fig. 4.4b and 4.4d) and thus the independence of *I* with respect to the SDS concentration, whilst a different relative

log *k* value (Fig. 4.4a and 4.4c) was observed due to the dependence of *k* upon the SDS concentration. This implies that *I* can be used to compare the relative affinity of solutes in different pseudo-stationary phase (PSP) or phase ratios. Similar *I* values in MEKC and MEEKC were obtained for xylenes, dihalogenated benzenes and halogenated toluenes, while significantly smaller *I* values were observed in MEEKC than in MEKC for diCHO, diOMe and OMe/CHO. In addition, a slightly smaller *I* in MEEKC than in MEKC was observed for disubstituted benzenes containing a methoxy moiety or an aldehyde moiety, such as OMe/X, Me/OMe, CHO/X and Me/CHO. This may be explained as being due to the dominant hydrophobic interaction of the PSP-solute in the highly hydrophobic xylenes and dihalogenated benzenes resulting in a similar affinity of solutes in the micelles and the microemulsion. Since the methoxy and aldehyde moieties are more polar than the alkyl and halogenated moieties, free 1-butanol in the aqueous solution increases the solubility of polar compounds, and therefore decreases *K* [16], resulting in a smaller *I* value in MEEKC than in MEKC. It should be noted that the retention for disubstituted benzenes are in the order of (i) diCHO < diOMe < diMe < diF < diCl < diBr, and (ii) F/Cl < F/Br < Cl/Br.

Table 4.3 Observed values of *I* for disubstituted benzenes in MEKC and MEEKC

Compounds	*I*		
	MEKC	**MEEKC**	
	50 mM SDS[(a)]	50 mM SDS[(b)]	60 mM SDS[(c)]
o-dimethylbenzene	793 ± 14	789 ± 14	786 ± 22
m-dimethylbenzene	804 ± 14	802 ± 14	799 ± 22
p-dimethylbenzene	804 ± 14	802 ± 14	800 ± 22
o-difluorobenzene	641 ± 13	649 ± 13	652 ± 22
m-difluorobenzene	640 ± 13	646 ± 13	648 ± 22
p-difluorobenzene	626 ± 13	627 ± 13	629 ± 22
o-dichlorobenzene	848 ± 14	858 ± 14	858 ± 22
m-dichlorobenzene	857 ± 14	865 ± 14	865 ± 22
p-dichlorobenzene	834 ± 14	837 ± 14	837 ± 22
o-dibromobenzene	916 ± 14	917 ± 14	920 ± 23
m-dibromobenzene	934 ± 14	934 ± 14	938 ± 23
p-dibromobenzene	909 ± 14	905 ± 14	908 ± 23
o-chlorofluorobenzene	743 ± 14	753 ± 14	754 ± 22
m-chlorofluorobenzene	746 ± 14	752 ± 14	754 ± 22
p-chlorofluorobenzene	729 ± 14	731 ± 14	733 ± 22
o-bromofluorobenzene	779 ± 14	783 ± 14	785 ± 22
m-bromofluorobenzene	784 ± 14	787 ± 14	788 ± 22
p-bromofluorobenzene	766 ± 14	764 ± 14	768 ± 22
o-bromochlorobenzene	883 ± 14	889 ± 14	888 ± 22
m-bromochlorobenzene	896 ± 14	900 ± 14	901 ± 22
p-bromochlorobenzene	872 ± 14	872 ± 14	872 ± 22
o-fluorotoluene	741 ± 14	743 ± 14	743 ± 22
m-fluorotoluene	733 ± 14	734 ± 14	734 ± 22
p-fluorotoluene	731 ± 14	732 ± 14	732 ± 22
o-chlorotoluene	849 ± 14	855 ± 14	853 ± 22
m-chlorotoluene	842 ± 14	847 ± 14	849 ± 22
p-chlorotoluene	835 ± 14	839 ± 14	838 ± 22
o-bromotoluene	881 ± 14	879 ± 14	884 ± 22
m-bromotoluene	877 ± 16	875 ± 14	879 ± 22
p-bromotoluene	870 ± 14	869 ± 14	873 ± 22
o-methoxytoluene	761 ± 14	745 ± 14	742 ± 22
m-methoxytoluene	747 ± 14	725 ± 14	723 ± 22
p-methoxytoluene	753 ± 14	730 ± 14	731 ± 22
o-methylbenzaldehyde	710 ± 14	665 ± 13	665 ± 22
m-methylbenzaldehyde	722 ± 14	673 ± 13	671 ± 22
p-methylbenzaldehyde	716 ± 14	666 ± 13	666 ± 22
o-fluoroanisole	666 ± 13	643 ± 13	645 ± 22
m-fluoroanisole	694 ± 14	676 ± 13	678 ± 22
p-fluoroanisole	681 ± 13	654 ± 13	656 ± 22

Table 4.3 Observed values *I* for disubstituted benzenes in MEKC and MEEKC (continued)

Compounds	*I*		
	MEKC	**MEEKC**	
	50 mM SDS[(a)]	50 mM SDS[(b)]	60 mM SDS[(c)]
o-fluorobenzaldehyde	609 ± 13	566 ± 13	568 ± 22
m-fluorobenzaldehyde	625 ± 13	590 ± 13	592 ± 22
p-fluorobenzaldehyde	628 ± 13	590 ± 13	591 ± 22
o-chloroanisole	760 ± 14	744 ± 14	744 ± 22
m-chloroanisole	801 ± 14	795 ± 14	794 ± 22
p-chloroanisole	795 ± 14	787 ± 14	787 ± 22
o-chlorobenzaldehyde	710 ± 14	671 ± 13	675 ± 22
m-chlorobenzaldehyde	732 ± 14	699 ± 14	703 ± 22
p-chlorobenzaldehyde	739 ± 14	701 ± 14	707 ± 22
o-bromoanisole	794 ± 14	773 ± 14	774 ± 22
m-bromoanisole	832 ± 14	823 ± 14	823 ± 22
p-bromoanisole	829 ± 14	817 ± 14	818 ± 22
o-bromobenzaldehyde	744 ± 14	704 ± 14	710 ± 22
m-bromobenzaldehyde	766 ± 14	730 ± 14	736 ± 22
p-bromobenzaldehyde	773 ± 14	734 ± 14	734 ± 22
o-dimethoxybenzene	691 ± 14	637 ± 13	633 ± 22
m-dimethoxybenzene	716 ± 14	679 ± 14	675 ± 22
p-dimethoxybenzene	699 ± 14	658 ± 14	653 ± 22
o-methoxybenzaldehyde	681 ± 13	628 ± 13	622 ± 22
m-methoxybenzaldehyde	687 ± 14	635 ± 13	631 ± 22
p-methoxybenzaldehyde	688 ± 14	631 ± 13	627 ± 22
o-dibenzaldehyde	504 ± 13	446 ± 14	446 ± 22
m-dibenzaldehyde	597 ± 13	534 ± 13	531 ± 22
p-dibenzaldehyde	590 ± 13	534 ± 13	532 ± 22

I = (log k-b)/a: I = [(a)](log $k \pm \Delta k - 2.655 \pm 0.057$)/$0.443 \pm 0.003$, [(b)](log $k \pm \Delta k - 2.655 \pm 0.056$)/$0.447 \pm 0.003$, [(c)](log $k \pm \Delta k - 2.535 \pm 0.096$)/$0.443 \pm 0.003$.

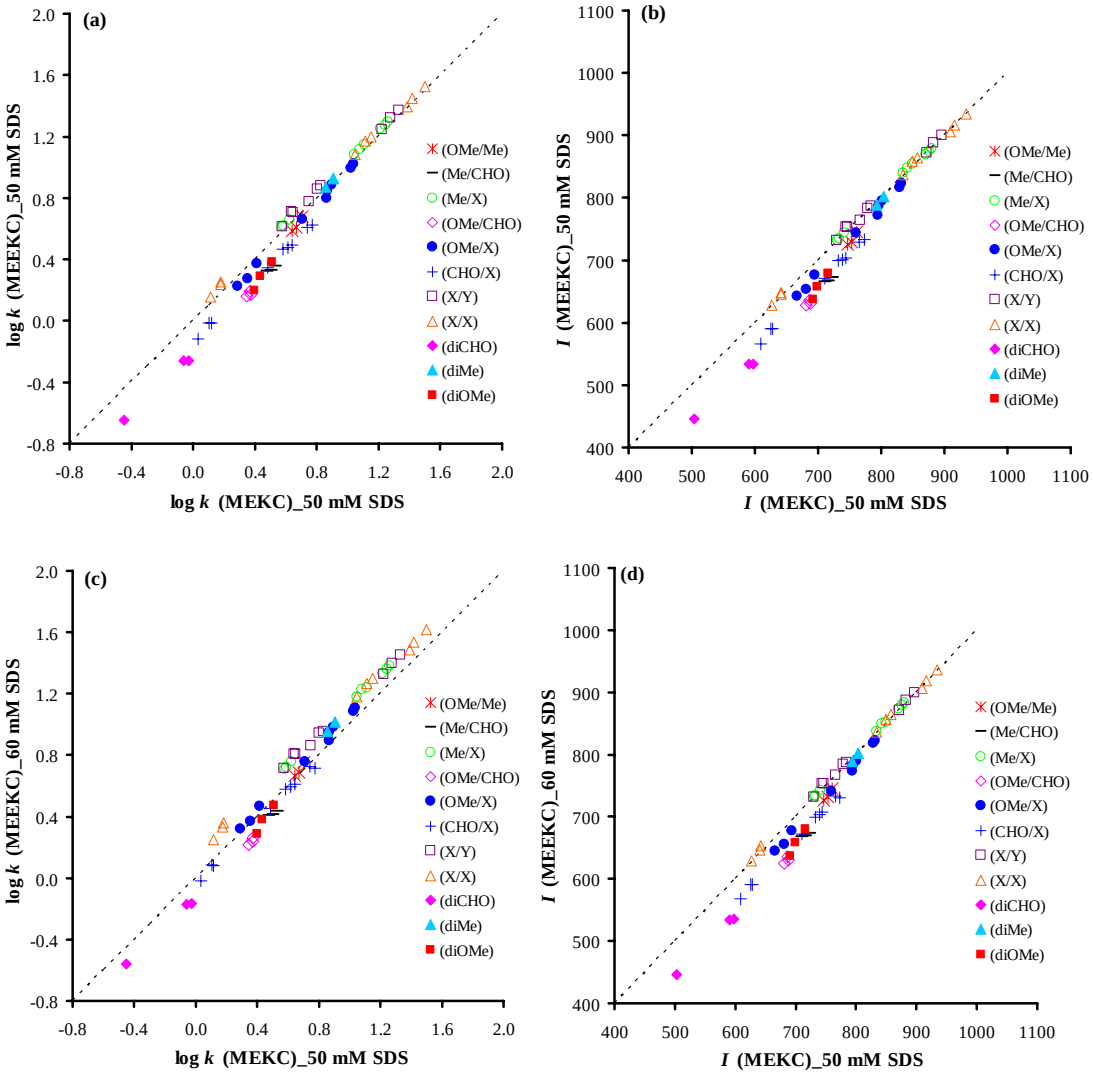


Figure 4.4 Plots of the observed (a) log *k* and (b) *I* values obtained for disubstituted benzenes in MEEKC against the same in MEKC. Each average value is obtained from three runs. The dash line refers to an equation of $y = x$. Other conditions are shown in Figure 4.1.

4.3 Octanol-water distribution constant (log K_{ow}) determined by MEKC and MEEKC

As previously described in Section 2.3.3, the value of log K_{ow} of analytes may be determined from the linear relationship between log K_{ow} and log k [25, 26, 55] or log K_{ow} and I according to Equations 2.27 and 2.28, respectively [12, 19]. In our previous work, no significant difference was found between log K_{ow} values obtained from log k or I. In addition, a calibration plot of log K_{ow} against I is simpler when known values of nominal I for BZ are used as I standards [12].

Using C6-C9 BZ as standards [12, 56], linear plots between log K_{ow} and nominal I are shown in Figure 4.5. The log K_{ow}-nominal I calibration gives the best fit with a linear equation (log K_{ow} = 0.00516(±0.0001)I – 0.970(±0.080)), with an r^2 value of 0.9992. The predicted values of log K_{ow} for the disubstituted benzenes obtained from MEKC and MEEKC are shown in Table 4.3, with the literature values of the same compounds where available. Excellent agreement of log K_{ow} from MEKC and MEEKC was found for xylenes, dihalogenated benzenes and halogenated toluenes, while in contrast a smaller log K_{ow} value was derived from MEEKC than from MEKC for the disubstituted benzenes containing a mono- or di-substituent of methoxy or aldehyde. This difference can be explained in a similar way as that of the differences in I values in Section 4.2. In addition, the predicted log K_{ow} from MEKC and MEEKC as shown in Figure 4.6 was found to be in good agreement with the available literature values of some compounds, except for *o*-methylbenzaldehyde, indicating that in most cases either MEKC or MEEKC can be used for determination of log K_{ow}. Furthermore, the log K_{ow} order is similar to the I order as described in Section 4.2.

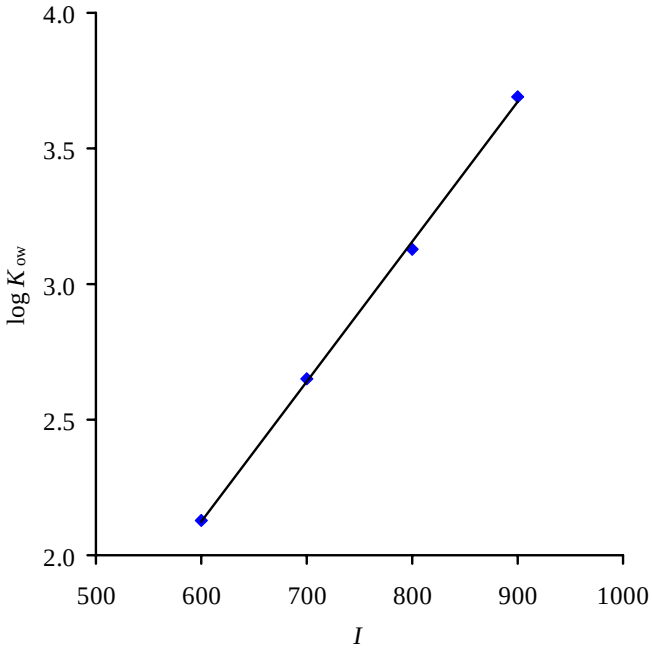


Figure 4.5 Linear relationship between log K_{ow} and I of BZ series standards in MEKC and MEEKC. The nominal I values are 600, 700, 800 and 900, and the log K_{ow} values are 2.13, 2.65, 3.13, and 3.69.

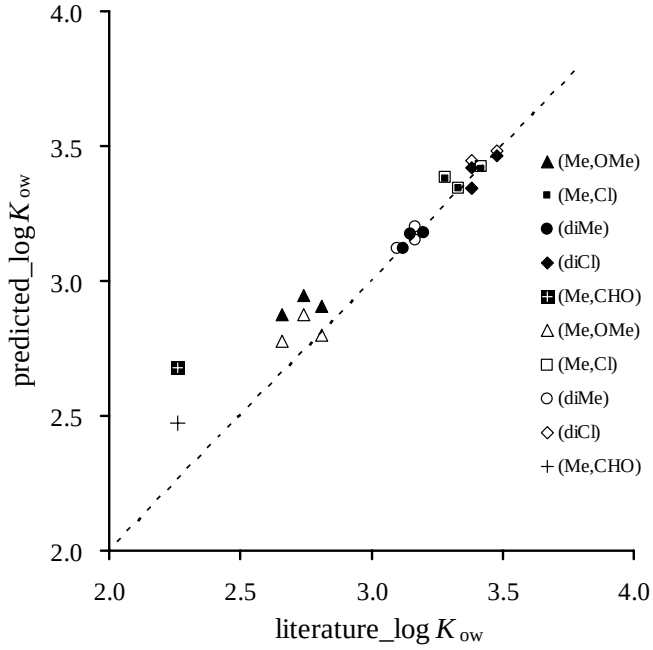


Figure 4.6 Relationship between literature values and predicted values of log K_{ow} of disubstituted benzene in MEKC (closed symbols) and MEEKC (open symbols). The dash line refers to an equation of $y = x$. Other conditions are shown in Figure 4.1.

Table 4.4 Predicted and literature values of log K_{ow} for the disubstituted benzenes in MEKC and MEEKC

Disubstituted benzene	log K_{ow}			Disubstituted benzene	log K_{ow}		
	Predicted[a)]		Literature [56]		Predicted[a)]		Literature [56]
	MEKC	MEEKC			MEKC	MEEKC	
o-diMe	3.12	3.10	3.12	*o*-Me/CHO	2.68	2.47	2.26
m-diMe	3.18	3.17	3.20	*m*-Me/CHO	2.74	2.51	NA
p-diMe	3.18	3.17	3.15	*p*-Me/CHO	2.71	2.48	NA
o-diF	2.33	2.38	NA	*o*-OMe/F	2.46	2.36	NA
m-diF	2.33	2.37	NA	*m*-OMe/F	2.61	2.53	NA
p-diF	2.26	2.27	NA	*p*-OMe/F	2.54	2.41	NA
o-diCl	3.42	3.45	3.38	*o*-OMe/Cl	2.69	2.50	NA
m-diCl	3.46	3.48	3.48	*m*-OMe/Cl	2.80	2.64	NA
p-diCl	3.34	3.34	3.38	*p*-OMe/Cl	2.84	2.64	NA
o-diBr	3.78	3.76	NA	*o*-OMe/Br	3.33	3.27	NA
m-diBr	3.87	3.85	NA	*m*-OMe/Br	3.32	3.24	NA
p-diBr	3.74	3.71	NA	*p*-OMe/Br	3.13	3.02	NA
o-F/Cl	2.87	2.91	NA	*o*-CHO/F	2.16	1.96	NA
m-F/Cl	2.88	2.91	NA	*m*-CHO/F	2.25	2.08	NA
p-F/Cl	2.79	2.80	NA	*p*-CHO/F	2.26	2.08	NA
o-F/Br	3.06	3.09	NA	*o*-CHO/Cl	2.95	2.86	NA
m-F/Br	3.09	3.11	NA	*m*-CHO/Cl	3.17	3.12	NA
p-F/Br	3.00	2.99	NA	*p*-CHO/Cl	3.14	3.08	NA
o-Cl/Br	3.61	3.62	NA	*o*-CHO/Br	2.87	2.66	NA
m-Cl/Br	3.68	3.68	NA	*m*-CHO/Br	2.99	2.80	NA
p-Cl/Br	3.55	3.54	NA	*p*-CHO/Br	3.03	2.82	NA
o-Me/F	2.85	2.86	NA	*o*-OMe/CHO	2.53	2.28	NA
m-Me/F	2.81	2.81	NA	*m*-OMe/CHO	2.56	2.32	NA
p-Me/F	2.80	2.80	NA	*p*-OMe/CHO	2.56	2.29	NA
o-Me/Cl	3.42	3.42	3.42	*o*-diOMe	2.59	2.33	NA
m-Me/Cl	3.38	3.38	3.28	*m*-diOMe	2.72	2.54	NA
p-Me/Cl	3.34	3.35	3.33	*p*-diOMe	2.63	2.42	NA
o-Me/Br	3.59	3.59	NA	*o*-diCHO	1.60	1.34	NA
m-Me/Br	3.55	3.56	NA	*m*-diCHO	2.09	1.80	NA
p-Me/Br	3.54	3.54	NA	*p*-diCHO	2.06	1.80	NA
o-Me/OMe	2.95	2.88	2.74				
m-Me/OMe	2.87	2.78	2.66				
p-Me/OMe	2.91	2.80	2.81				

NA = Not available, [a)]SD $\leq \pm$ 0.08.

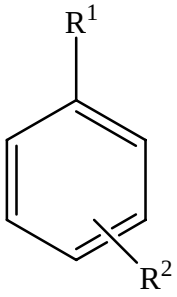
4.4 Predicted and observed retention for disubstituted benzenes with different moieties

The group additive approach is based on the assumption of additive-constitutive properties [23, 57, 58], and is applied for calculation of log K_{ow} as the equation [24, 25, 59]

$$\log K_{ow}(\mathrm{PR}) = \kappa(\mathrm{P}) + \sum \kappa(\mathrm{R}) \tag{4.3}$$

where κ(P) and κ(R) are the values of log K_{ow} for the parent P and substituent R, respectively.

The values of log K_{ow} for disubstituted benzenes with the same and different R-values are given by Equations. 4.4 to 4.6, where P in this work refers to C_6H_4.



$$\log K_{ow}(\mathrm{PR^1R^1}) = \kappa(\mathrm{P}) + 2\kappa(\mathrm{R^1}) \tag{4.4}$$

$$\log K_{ow}(\mathrm{PR^2R^2}) = \kappa(\mathrm{P}) + 2\kappa(\mathrm{R^2}) \tag{4.5}$$

$$\log K_{ow}(\mathrm{PR^1R^2}) = \kappa(\mathrm{P}) + \kappa(\mathrm{R^1}) + \kappa(\mathrm{R^2}) \tag{4.6}$$

It follows from Equation. 4.4 to 4.6 that:

$$\log K_{ow}(\mathrm{PR^1R^2}) = \frac{\log K_{ow}(\mathrm{PR^1R^1}) + \log K_{ow}(\mathrm{PR^2R^2})}{2} \tag{4.7}$$

Since log k and I are directly related to log K_{ow}, it follows from Equation. 2.25, 2.28 and 4.6 that:

$$I(\mathrm{PR^1R^2}) = \frac{I(\mathrm{PR^1R^1}) + I(\mathrm{PR^2R^2})}{2} \tag{4.8}$$

and so:

$$\log k(\mathrm{PR^1R^2}) = \frac{\log k(\mathrm{PR^1R^1}) + \log k(\mathrm{PR^2R^2})}{2} \tag{4.9}$$

Therefore, with known log K_{ow}, I and log k for the disubstituted benzenes with the same R moietes, the predicted values for disubstituted benzenes with a different R can

be obtained from the average value for PR^1R^1 and PR^2R^2, without having to know the values of κ.

From previous work, the retention of bisphenol-A-diglycidyl ether derivatives (BADGEs) [17] and curcuminoids [21] containing two different moieties were observed to be good between those containing the same two moieties. In addition, the good agreement was found between the predicted and observed log *k* values for curcuminoid and BADGE each containing two different moieties, where the predicted value was obtained from the average value of log *k* for the curcuminoid and BADGE with the same moieties, respectively.

Table 4.5 and Figure 4.7 compares the observed and predicted values of *I* for different disubstituted benzenes, using *I* data obtained from disubstituted benzenes with the same R. For example, the predicted *I* for *p*-chlorofluorobenzene is obtained from the average *I* for *p*-dichlorobenzene and *p*-difluorobenzene. An example of electopherograms of disubstituted benzenes in MEKC and MEEKC is shown in Figure 4.3. In general, the observed values of *I* were found to be consistent with the predicted ones, and especially for halogenated toluenes and dihalogenated benzenes. However, poor agreement between the observed and predicted *I* values was seen for the methoxyaldehydes and tolualdehydes, and particularly in the case of the *ortho*-isomers. This may be caused by electronic effects [60], such as the electron withdrawing by aldehyde groups and electron releasing by methoxy groups, and intramolecular interactions. Either the electronic effects or intramolecular interactions can change in the polarity of these compounds, depending on the substituted position. This implies that the group additive approach is suitable for the prediction of the reliable retention rates for disubstituted benzenes with small effects of electronic affecting groups and/or intramolecular interactions. It should be noted that, in previous work [27], the group additive approach was used for prediction of the micelle-water distribution constant (K_{mw}) in MEKC using a similar relationship to that of Equations 4.3. However, the known values of κ_{mw} for P and R were needed and, moreover, the predicted values of K_{mw} for *o*-, *m*- and *p*-PR^1R^2 were equal, yet the observed values of K_{mw} for these positional isomers are in fact different.

Table 4.5 Comparison of observed and predicted values of *I* for disubstituted benzenes in MEKC and MEEKC

Compounds	MEKC[(a)]		MEEKC[(b)]	
	observed	predicted	observed	predicted
o-chlorofluorobenzene	744± 14	744± 14	753±14	753± 14
m-chlorofluorobenzene	746± 14	748± 14	752±14	754± 14
p-chlorofluorobenzene	729± 14	729± 14	731± 14	731± 14
o-bromochlorobenzene	888± 14	888± 14	890±14	890± 14
m-bromochlorobenzene	901± 14	901± 14	901± 14	901± 14
p-bromochlorobenzene	876± 14	877± 14	873± 14	874± 14
o-bromofluorobenzene	781± 14	782± 14	787± 14	785± 14
m-bromofluorobenzene	786± 14	790± 14	791± 14	795± 14
p-bromofluorobenzene	769± 14	771± 14	768±14	770± 14
o-methylchlorobenzene	850± 14	822± 14	851± 14	822 ± 14
m-methylchlorobenzene	843± 14	831± 14	844±14	832 ± 14
p-methylchlorobenzene	836± 14	820± 14	836± 14	820 ± 14
o-methylfluorobenzene	740± 14	717± 14	742± 14	719 ±14
m-methylfluorobenzene	732± 14	722± 14	733± 14	724± 14
p-methylfluorobenzene	730± 14	715± 14	731± 14	715± 14
o-methylbromobenzene	884± 14	857± 14	884±14	854± 14
m-methylbromobenzene	876± 16	876± 15	879±14	868± 14
p-methylbromobenzene	874± 14	858± 14	874± 14	855± 14
o-methoxychlorobenzene	759± 14	770± 14	742±14	748± 14
m-methoxychlorobenzene	802± 14	788± 14	792± 14	771± 14
p-methoxychlorobenzene	796± 14	767± 14	784± 14	746± 14
o-methoxyfluorobenzene	665± 13	665± 13	645±13	644± 13
m-methoxyfluorobenzene	693± 14	677± 13	678±13	666± 13
p-methoxyfluorobenzene	680± 13	661± 13	656± 13	648± 13
o-methoxybromobenzene	795± 14	804± 14	773± 14	775± 14
m-methoxybromobenzene	834± 14	825± 14	822± 14	805± 14
p-methoxybromobenzene	831± 14	805± 14	817± 14	780± 14
o-methylmethoxybenzene	759± 14	740± 14	745± 14	714 ± 14
m-methylmethoxybenzene	745± 14	758± 14	726± 14	740 ± 14
p-methylmethoxybenzene	751± 14	749± 14	731±14	729 ± 14

Table 4.5 Comparison of observed and predicted values of *I* for disubstituted benzenes in MEKC and MEEKC (continued)

Compounds	MEKC[(a)]		MEEKC[(b)]	
	observed	predicted	observed	predicted
o-chlorobenzadehyde	709± 14	676± 13	672± 13	653± 13
m-chlorobenzadehyde	731± 14	727± 14	699±14	699± 14
p-chlorobenzadehyde	738± 14	713± 14	701±14	685± 13
o-fluorobenzadehyde	607± 13	571± 13	568± 13	548± 13
m-fluorobenzadehyde	623± 13	617± 13	591± 13	590± 13
p-fluorobenzadehyde	626± 13	632± 13	591± 13	581± 13
o-bromobenzadehyde	745± 14	709± 14	704± 14	681± 13
m-bromobenzadehyde	767± 14	767± 14	730± 14	733± 14
p-bromobenzadehyde	774± 14	751± 14	734± 14	720± 14
o-methylbenzadehyde	707± 14	644± 13	667±13	619 ± 13
m-methylbenzadehyde	719± 14	700± 14	674± 13	610 ± 13
p-methylbenzadehyde	714± 14	694± 14	668± 13	669 ± 13
o-methoxybenzaldehyde	678± 13	592± 13	630±13	546± 13
m-methoxybenzaldehyde	684± 14	652± 13	637± 13	609± 13
p-methoxybenzaldehyde	684± 14	641± 13	633±13	598± 13

$I = (\log k\text{-b})/a$: $I = {}^{(a)}(\log k \pm \Delta k - 2.655 \pm 0.057)/0.443 \pm 0.003$, ${}^{(b)}(\log k \pm \Delta k - 2.655 \pm 0.056)/0.447 \pm 0.003$.

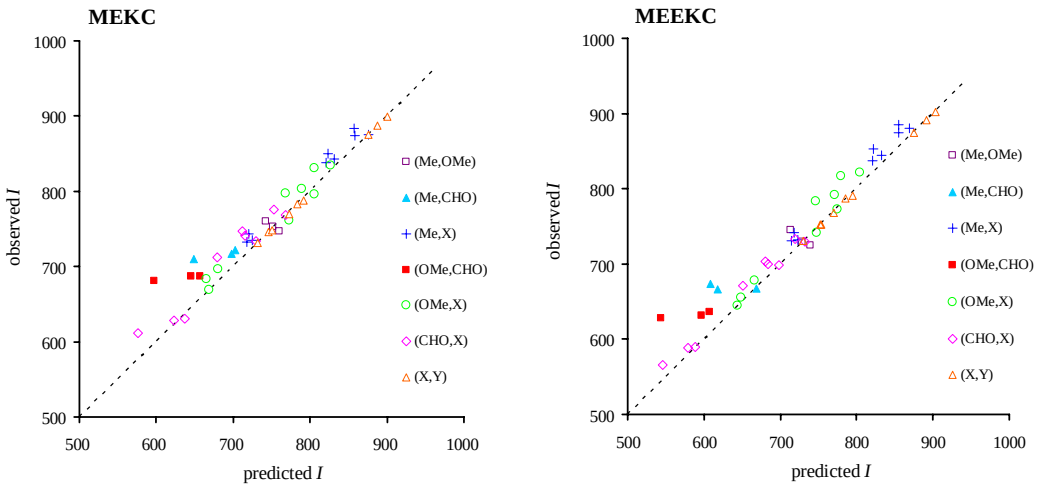


Figure 4.7 Plots of the observed and predicted *I* values obtained for disubstituted benzenes with different moieties in MEKC and MEEKC. The dash line refers to an equation of $y = x$. Other conditions are shown in Figure 4.1.

CHAPTER V
CONCLUSION

5.1 Conclusion

The values of retention factor (*k*) and retention index (*I*) for disubstituted benzenes in MEKC and MEEKC were determined. Comparing the relative *I* values for each solute in MEEKC against those in MEKC, revealed very similar values, and was independent of *I* with respect to the SDS concentration. In contrast, under the same comparisons, different relative *k* values were found. This implies that *I*, rather than *k*, can be used as the parameter to compare the relative affinity of compounds in MEEKC and MEKC that have different PSP and phase ratios. A similar *I* value in both the MEEKC and MEKC was observed for the disubstituted benzenes with $–CH_3$ and/or halogen moieties (–F, –Cl and –Br). In contrast, the same disubstituted benzenes with –CHO and $–OCH_3$ moieties revealed a smaller *I* value being obtained in MEEKC than in MEKC, presumably because free 1-butanol in the aqueous phase in MEEKC increases the solubility of the solutes and thereby, reduces the affinity or *I* of the solutes in the microemulsion.

The log K_{ow} between observed values and literature values from MEKC and MEEKC was found to be in good agreement. Therefore, MEKC and MEEKC are useful systems for estimate log K_{ow}.

In addition, in MEKC and MEEKC, the predicted log *k* and *I* values for disubstituted benzenes with different moieties of $–CH_3$ and halogens were found to be in excellent agreement with the observed values, when the predicted values were obtained from the average for disubstituted benzenes with same moieties, according to a group additive approach. However, a significant difference between the observed and predicted retention was found for disubstituted benzenes with at least one moiety of –CHO or $–OCH_3$, due to electronic effects of polar moieties causing a deviation in the actual affinity of solutes away from the predicted interactions.

5.2 Future Work

In the future work, the retention index could be studied for retention behaviors of other analytes in MEKC and MEEKC with or without electroosmotic flow. Our proposed equation, as in Equation 4.8, can be used for prediction of retention factor and retention index for other disubstituted compounds in MEKC and MEEKC.

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