ปัจจัยที่มีผลต่อรีเทนชันและค่าการแยกของสารประกอบแอโรแมติกชนิดไฮโดรโฟบิก ในไมโครอิมัลชันอิเล็กโทรไคเนทิกโครมาโทกราฟี

นางสาวเกียรติสุดา ปูอุตรี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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FACTORS AFFECTING RETENTION AND RESOLUTION OF HYDROPHOBIC AROMATIC COMPOUNDS IN MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY

Miss Kieatsuda Poouthree

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Thesis Title	Factors Affecting Retention and Resolution of Hydrophobic Aromatic Compounds in Microemulsion Electrokinetic Chromatography
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เกียรติสุดา ปุอุตรี: ปัจจัยที่มีผลต่อรีเทนชันและค่าการแยกของสารประกอบแอโรแมติกชนิดไฮโดรโฟบิกในไมโครอิมัลชันอิเล็กโทรไคเนทิกโครมาโทกราฟี (FACTORS AFFECTING RETENTION AND RESOLUTION OF HYDROPHOBIC AROMATIC COMPOUNDS IN MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY) อาจารย์ที่ปรึกษา: ผู้ช่วยศาสตราจารย์ ดร. ธรรมนูญ หนูจักร, อาจารย์ที่ปรึกษาร่วม: ผู้ช่วยศาสตราจารย์ ดร. ณัฐชนัญ ลีพิพัฒน์ไพบูลย์ 127 หน้า.

ได้ศึกษาพฤติกรรมรีเทนชัน ได้แก่ รีเทนชันแฟกเตอร์ (k) และรีเทนชันอินเดก (I) ของสารประกอบประเภทอนุกรมโฮโมโลกัสในเทคนิคไมโครอิมัลชันอิเล็กโทรไคเนทิกโครมาโทกราฟี (MEEKC) ในภาวะที่กำจัดอิเล็กโทรออสโมซิส เมื่อใช้อัลคิลเบนซีน (BZ) เป็นสารมาตรฐาน พบว่าค่ารีเทนชันอินเดกของสารประกอบประเภทอนุกรมโฮโมโลกัสอื่นๆ ไม่ขึ้นกับความเข้มข้นของโซเดียมโดเดกซิลซัลเฟต ([SDS]) และอุณหภูมิ แต่จะขึ้นกับชนิดและความเข้มข้นของตัวทำละลายอินทรีย์ (φ) รีเทนชันแฟกเตอร์มีค่าเพิ่มขึ้นเมื่อเพิ่ม [SDS] แต่จะมีค่าลดลงเมื่อเพิ่มอุณหภูมิ หรือ φ ที่ [SDS] และ φ ใดๆ พบว่าค่า log k ของสารที่ได้จากการทดลองและการทำนายมีความสอดคล้องกันมาก โดยที่ค่าจากการทำนายได้มาจากสมการที่ได้ดัดแปลงในงานวิจัยนี้ เมื่อใช้ BZ เป็นสารมาตรฐาน พบว่าค่า log K_{ow} ที่ได้จากการทำนายสอดคล้องกับค่าที่มีผู้รายงานไว้ โดยที่ K_{ow} คือ ค่าคงที่ของการกระจายตัวของสารในออกทานอลและน้ำ

นอกจากนี้ได้เปรียบเทียบค่าการแยกของสาร (R_s) ใน MEEKC และไมเซลลาร์อิเล็กโทรไคเนทิกโครมาโทกราฟี (MEKC) โดยใช้บิสฟีนอล-เอ-ไดไกลซิดิลอีเธอร์และอนุพันธ์เป็นสารทดสอบ พบว่าสามารถเปรียบเทียบสเกลของการแยกสารโดยใช้สมการค่าการแยกของสาร คือ $R_s = (\sqrt{N}/4)(\alpha-1)/(1+k_2)$ โดยที่ α คือ ค่าการเลือกเฉพาะ (α เท่ากับ k_2/k_1 สำหรับ $k_2 \geq k_1 > 0$) และ $\overline{N}$ คือ ค่าเฉลี่ยของประสิทธิภาพการแยก เมื่อทำการเปรียบเทียบกับ MEKC ที่ [SDS], φ และอุณหภูมิใดๆ พบว่า MEEKC ให้ค่าการแยกของสารที่ดีกว่า เนื่องมาจาก MEEKC ให้ค่า k ที่น้อยกว่าอย่างมีนัยสำคัญแต่ไม่ให้ค่า α ที่สูงกว่า ในขณะที่ให้ค่า $\overline{N}$ ที่พอๆ กัน

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....เกียรติสุดา ปุอุตรี.....

สาขาวิชา.....เคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2549..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

457 38032 23: MAJOR CHEMISTRY
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KIEATSUDA POOUTHREE: FACTORS AFFECTING RETENTION AND RESOLUTION OF HYDROPHOBIC AROMATIC COMPOUNDS IN MICROEMULSION ELECTROKINETIC CHROMATOGRAPHY. THESIS ADVISOR: ASSISTANT PROFESSOR THUMNOON NHUJAK, Ph.D., THESIS COADVISOR: ASSISTANT PROFESSOR NATCHANUN LEEPIPATPIBOON, Dr.rer.nat, 127 pp.

Retention behaviors, such as retention factor (k) and retention index (I), of homologous series compounds in microemulsion electrokinetic chromatography (MEEKC) with suppressed electroosmosis were investigated. Using alkylbenzenes (BZ) as standard, the retention indices of other homologous series compounds are independent of the concentration of sodium dodecyl sulphate ([SDS]) and temperature, while dependent on the type and concentration of organic co-solvents (φ). The retention factor increases with increasing [SDS], while decreases with increasing temperature or φ. At a given [SDS] and φ, excellent agreement was found between the observed and predicted values of log k of analytes in MEEKC, where the predicted values were obtained from our modified equations. Using BZ as standards, excellent agreement is found between predicted and literature values of log K_{ow} of the test analytes, where K_{ow} is the octanol-water distribution constant of the analyte.

In addition, the resolution (R_s) in MEEKC and micellar electrokinetic chromatography (MEKC) was compared using bisphenol-A-diglycidyl ether and its derivatives as test analytes. Separation scales were compared using a resolution equation, $R_s = (\sqrt{\overline{N}}/4)\,(\alpha\text{-}1)/(1+k_2)$, where α is the selectivity ($\alpha = k_2/k_1$ for $k_2 \geq k_1 > 0$), and $\overline{N}$ the average efficiency. At a given [SDS], φ and temperature, in comparison with MEKC, MEEKC was found to give better resolution of analytes, mainly due to the significantly smaller k in MEEKC, but not the greater α in MEEKC, while a comparable range of $\overline{N}$.

Department.....Chemistry..... Student's signature.....Kieatsuda Poouthree.....
Field of study.....Chemistry ..... Advisor's signature.....Thumnoon Nhujak.....
Academic year.....2006..... Co-advisor's signature.....Natchanun Leepipatpiboon.....

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LIST OF ABBREVIATIONS AND SYMBOLS

ACN	acetonitrile
BA	alkylbenzoates
BADGE	bisphenol-A-diglycidyl ether
BADGE·2H_2O	bisphenol-A-bis(2,3-dihydroxypropyl) ether
BADGE·2HCl	bisphenol-A-bis(3-chloro-2-hydroxypropyl) ether
BADGE·H_2O	bisphenol-A-(2,3-dihydroxypropyl) glycidyl ether
BADGE·HCl·H_2O	bisphenol-A-(3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl) ether
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
EtOH	ethanol
HPLC	high-performance liquid chromatography
i.d.	internal diameter
M	pseudo stationary phase marker
MEEKC	microemulsion electrokinetic chromatography
MEKC	micellar electrokinetic chromatography
MeOH	methanol
PB	alkylparabens
PN	alkylaryl ketones

PrOH	propanol
RPLC	reversed-phase liquid chromatography
RSD	relative standard deviation
SD	standard deviation
SDS	sodium dodecyl sulphate
[SDS]	concentration of sodium dodecyl sulphate
$[A_{aq}]$	concentration of analyte in aqueous phase
$[A_{mc}]$	concentration of analyte in micelle or microemulsion phase
B	constant value (2400 K)
c	concentration of analyte
D	diffusion coefficient
D_{aq}	diffusion coefficient of solute in aqueous phase
D_{mc}	diffusion coefficient of solute in micelle or microemulsion phase
D_{th}	diffusion coefficient of solute by thermal dispersion
E	electric field strength
e	electronic charge
F	Faraday's constant
ΔG	free energy
H	plate height
H_{aq}	plate height due to intermicelle mass transfer in aqueous phase
H_l	plate height due to longitudinal diffusion
H_{mc}	plate height due to sorption-desorption kinetics in micelle or microemulsion phase
H_{pd}	plate height due to polydispersity of micelle or microemulsion phase
H_t	plate height due to thermal dispersion
ΔH^o	standard enthalpy
I	ionic strength
I_A	electric current
K	distribution constant
K_{ow}	octanol-water distribution constant

k	retention factor
k_d	desorption rate constants
k_s	sorption rate constants
k_w	retention factor extrapolated to pure water as mobile phase
k^*	boltzmann constant
L	total capillary length
l	length of capillary to detector
l_{inj}	length of analyte injected
N	number of theoretical plate, or peak efficiency
$\overline{N}$	average efficiency
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	gas constant
R_s	resolution
r	internal capillary radius
r_h	hydrodynamic radius
ΔS^o	standard entropy
T	absolute temperature
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
t^*_{mc}	mean life-time of analyte in micelle or microemulsion phase
V	applied voltage
V_F	volume flow of analyte passing the detector
V_{inj}	volume of sample injected
v_{eo}	electroosmotic velocity

v_{ep}	electrophoretic velocity
v_{net}	total electrophoretic velocity
$\bar{v}$	partial molar volume of micelles
w_b	peak width at base
w_h	peak width at half height
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
α	selectivity
α_{CH2}	methylene selectivity
ε	permittivity
ϕ	volume of aqueous phase to pseudo stationary phase
η	viscosity
κ	electrical conductivity
φ	concentration of organic co-solvent
λ_s	thermal conductivity
μ	electrophoretic mobility
μ_{eo}	electroosmotic mobility
μ_{obs}	observed mobility
μ_{mc}	mobility of micelle or microemulsion phase
μ_{net}	total mobility
μ^o	absolute mobility at zero ionic strength
$\bar{\mu}$	average efficiency electrophoretic mobility
σ	standard deviation of peak in distance unit
$\sigma_{\mu,mc}$	standard deviation of electrophoretic mobility of micelle or microemulsion phase
σ^2	peak variance
σ^2_{diff}	peak variance due to longitudinal diffusion
σ^2_{th}	peak variance due to thermal diffusion
τ	standard deviation of peak in time unit
ζ	zeta potential

CHAPTER I

INTRODUCTION

1.1 Introduction and Modes of Capillary Electrophoresis

Capillary electrophoresis (CE) is a process for separation of analytes in a capillary containing an electrolyte solution under the influence of applied electric field. Separation mechanism is based on the difference in electrophoretic mobility of analytes depending on the charge-to-size ratio [Khaledi 1998, Camilleri 1993]. Nowadays, CE can be used for the separation of charged and neutral analytes covering a wide range of water soluble and insoluble compounds. Advantages of CE include high efficiency, simple sample preparation, short analysis time and low consumption of sample and solvent [Hansen *et al.* 2003, McEvoy *et al.* 2007].

Based on separation mechanism [Grossman *et al.*1992, Khaledi 1998], CE can be classified into six basic modes 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 two modes are commonly used for CE separation, therefore, CZE and MEKC will be mentioned briefly in this section.

CZE is basic for others modes that commonly used for separation of charged analytes. The background electrolytes (BGE) used in CZE is a typical buffer, such as phosphate or borate. Separation mechanism in CZE is based on the difference in electrophoretic mobility of analytes only depending on the charge-to-size ratio of the analytes, as shown in Figure 1.1a.

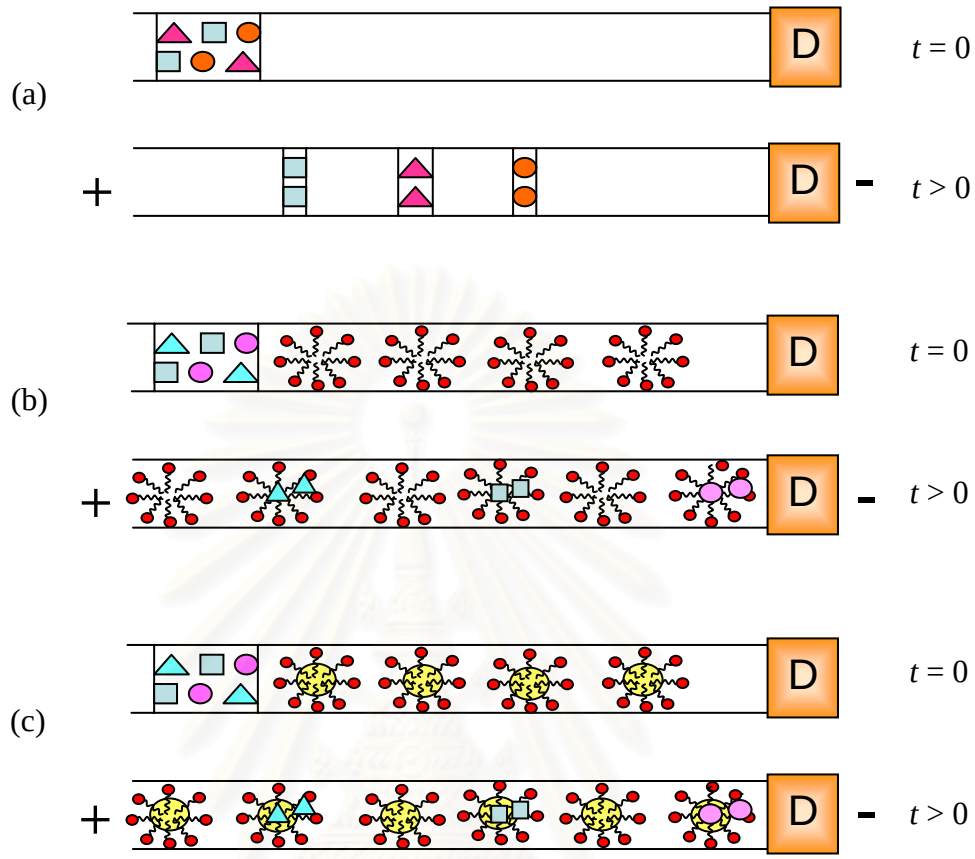


Figure 1.1 **Separation mechanisms of (a) CZE, (b) MEKC and (c) MEEKC. Adapted from Grossman *et al.* [1992] and Altria [2000a].**

MEKC is another mode of CE that can be applied for separation of both charged and neutral analytes. The BGE used in MEKC contains a surfactant, such as sodium dodecyl sulphate (SDS), to form micelles or a micellar phase served as a pseudo stationary phase, similar to a stationary phase in high-performance liquid chromatography (HPLC). Separation mechanism in MEKC is based on the difference in partitioning of analytes between an aqueous phase and a pseudo stationary phase of micelles, as shown in Figure 1.1b.

1.2 Introduction to Microemulsion Electrokinetic Chromatography

Microemulsion electrokinetic chromatography (MEEKC) is a new mode in CE. The BGE used in MEEKC is a microemulsion, a solution containing nanometer-size droplets of an immiscible liquid, containing oil droplets dispersed in an aqueous

buffer in the presence of a surfactant and co-surfactant [Watarai 1991]. Separation mechanism in MEEKC is similar to that in MEKC which is based on the difference in partitioning of analytes between an aqueous phase and a pseudo stationary phase of charged oil droplets, as shown in Figure 1.1c. More theoretical details of MEEKC are discussed in Section 1.2.

Typically, MEEKC is performed using *n*-octane as oil droplets, SDS as emulsifier surfactant to stabilize the microemulsion and to generate negatively charged oil droplets, and 1-butanol as co-surfactant to lower interfacial tension and to enhance stability of a microemulsion system, as shown in Figure 1.2.

The oil droplets surrounding the surfactant and co-surfactant 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. Hydrophobic compounds favor partitioning into the oil droplet rather than into the aqueous phase, and therefore they have stronger retention in the oil droplet than do hydrophilic compounds. MEEKC can be used as a method for separation of charged and neutral analytes covering a wide range of water soluble and insoluble compounds [Altria *et al.* 2003, Hansen 2003, McEvoy *et al.* 2007].

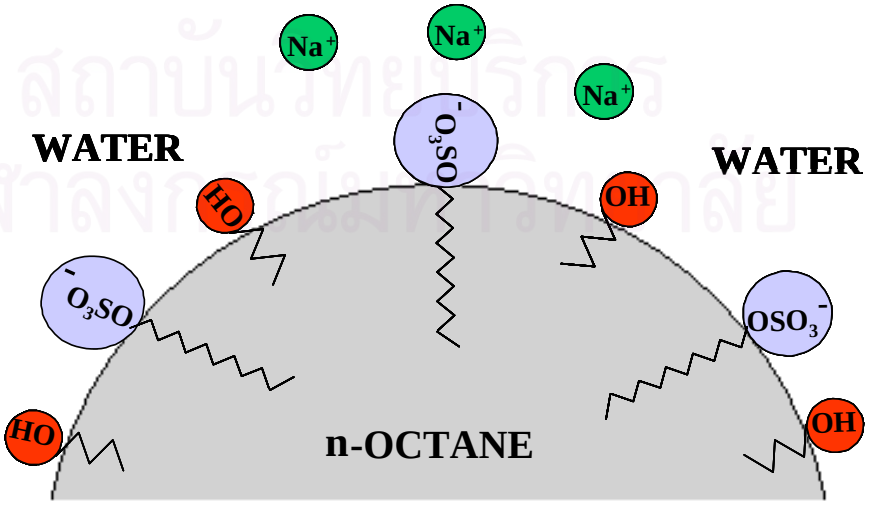


Figure 1.2 Schematic of microemulsion. Adapted from Altria [2000a].

1.3 Previous Work on Retention and Resolution in MEEKC

Retention factor (*k*) is one of the characteristics that indicates retention behavior of analytes in chromatography and electrokinetic chromatography such as MEEKC and MEKC. In MEEKC and MEKC, the retention factor is defined as the ratio of total moles of analyte in the pseudo stationary phase versus those in the aqueous phase [Altria *et al.* 2000a]. The higher the retention factor, the stronger the retention or the partitioning of analytes in the pseudo stationary phase. An increase in the concentration of surfactant results in an increase in *k* in MEEKC [Altria *et al.* 2000a, Nhujak *et al.* 2006, Seelanan *et al.* 2006, Ishihama *et al.* 1996, Song *et al.* 1995, Harang *et al.* 2004], while a decrease in *k* is affected by an increase in temperature [Nhujak *et al.* 2006, Seelanan *et al.* 2006, Harang *et al.* 2004], Joule heating caused by high voltage [Nhujak *et al.* 2006, Song *et al.* 1995] and the concentration of organic co-solvent [Nhujak *et al.* 2006, Seelanan *et al.* 2006, Harang *et al.* 2004, Gong *et al.* 2004]. Types of surfactant [Altria *et al.* 2000a], co-surfactant [Altria *et al.* 2000a] and organic co-solvent [Altria *et al.* 2000a, Nhujak *et al.* 2006, Ishihama *et al.* 1996] also influence a change in *k*. The relationship between log *k* of analytes in MEEKC and log K_{ow} was found to give a linear equation, where K_{ow} is the octanol-water distribution constant of the analyte [Gong *et al.* 2004, Klotz *et al.* 2001]. Using standards with known log K_{ow}, log K_{ow} of the analyte may be determined using log *k* of the analyte obtained from MEEKC. Most of previous MEEKC work above-mentioned was carried out using high EOF [Altria *et al.* 2000a, Hansen 2003, Ishihama *et al.* 1996, Song *et al.* 1995, Harang *et al.* 2004], except for a few work involving MEEKC with suppressed EOF [Nhujak *et al.* 2006, Seelanan *et al.* 2006].

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 [Muijselaar *et al.* 1994]. In MEKC with EOF and using homologous series of alkylbenzenes (BZ) or alkyl arylketones (PN) as retention index standards, the retention indices of analytes were obtained to be independent of the surfactant concentration, whereas slightly

dependent on the temperature [Muijselaar *et al.* 1994]. However, in MEKC with addition of organic solvents in the buffer, some variations (RSD < 3%) of retention index were found at different SDS concentrations [Ahuja *et al.* 1994]. The retention index can be used for identification of neutral analytes [Muijselaar *et al.* 1994]. In addition, a good linear relationship between I and log K_{ow} was obtained [Muijselaar *et al.* 1994]. Using the different surfactants as micellar phase in MEKC, the ΔI values, obtained from different micellar systems, can be used to elucidate the functional group selectivity of these specific micellar systems and to classify pseudo stationary phases in MEKC [Muijselaar *et al.* 1994]. Up-to-date, factors affecting retention index of analytes in MEEKC have not been reported previously.

Typically, the separation of two analytes can be expressed by selectivity (α) and resolution (R_s). In MEKC and MEEKC the selectivity is defined as the ratio of retention factor, i.e. $\alpha = k_2/k_1$ where $k_2 \geq k_1 > 0$. To obtain high separation, the high difference in k or high selectivity is required. In addition, the higher the resolution, the better the separation. In MEKC and MEEKC, R_s relates to α, k and efficiency (N). Typically, the greater the selectivity, the greater the resolution. In MEEKC, factors affecting resolution include the microemulsion buffer components, such as types and concentrations of buffer, oil, surfactant, co-surfactant and organic solvent, and parameters of CE instrument, such as temperature and voltage [Altria *et al.* 2000a and 2000b].

In previous work, the better separation in MEEKC than MEKC was reported for curcuminoids [Nhujak *et al.* 2006], biphenyl nitriles [Gong *et al.* 2004], catechins [Pomponio *et al.* 2003], benzophenones [Huang *et al.* 2005], vitamins [Sánchez *et al.* 2002], and xanthones [Bo *et al.* 2003]. However, separation scales of α, k and N have not been compared.

1.4 Aim and Scope

Much of previous work on MEEKC involved water soluble compounds, and a few work has been reported on MEEKC employing suppressed electroosmosis. Therefore, the aim of this work is to investigate factors affecting retention and resolution of hydrophobic aromatic compounds in MEEKC employing suppressed electroosmosis. A change in retention scales and resolution is explained using the theory of liquid chromatography and MEKC.

Since a few work has been reported on MEEKC of hydrophobic compounds, one objective is to investigate retention behaviors, such as retention factor and retention index of hydrophobic homologous series compounds, such as alkylbenzenes (BZ), alkylaryl ketones (PN), alkylbenzoates (BA) and alkylparabens (PB), in MEEKC with suppressed electroosmosis. MEEKC will be carried out using a 50 mM phosphate buffer at pH 2.5 to suppress electroosmosis. The following parameters affecting retention behaviors will be studied: the SDS concentration in a range of 100 to 200 mM, temperature in a range of 15 to 40^{o}C, voltage in a range of -10 to -20 kV, and types and concentrations of organic co-solvents such as acetonitrile (ACN), methanol (MeOH), ethanol (EtOH) and 2-propanol (2-PrOH) in a range of 0 to 30% v/v.

As previously mentioned, the better separation in MEEKC than MEKC was reported, and therefore, further objective is to compare and clarify the resolution of hydrophobic analytes in MEEKC and MEKC with suppressed electroosmosis. The separation scales of α, k and N in MEEKC and MEKC is explained using the resolution equation recently reported in previous work of our group [Nhujak *et al.* 2006]. MEEKC and MEKC will be carried out using a 50 mM phosphate buffer at pH 2.5 to suppress electroosmosis, and the resolution of test analytes will be compared at given MEEKC and MEKC conditions used: the SDS concentrations of 100 to 180 mM, temperatures of 15 to 40^{o}C, and organic co-solvents as ACN, MeOH, EtOH and 2-PrOH at levels of 20 to 30% v/v. The test analytes used are bisphenol-A-diglycidyl ether (BADGE) and its derivatives. BADGE is starting material for preparation of epoxy resins commonly used as protective coating for interior of metal food cans.

Residual BADGE and it derivatives in the coatings may migrate into the food upon contact.

Final objective is to predict the retention factor and log K_{ow} of analytes. The linear equation is developed for prediction of retention factor of homologous series compounds in MEEKC in a wide range of the concentrations of SDS ([SDS]) and organic co-solvents (φ), using the simple linear relationship between log k and the number of carbons of analyte, log k and [SDS] and log k and φ. The structure-retention relationship is also used to explain and predict the retention factor of compounds containing different disubstituting groups. In addition, the retention factor and retention index are used to determine log K_{ow} of analytes.

It is expected that this theoretical study could be used to explain and predict retention behaviors of hydrophobic compounds in MEEKC with suppressed electroosmosis. In addition, the results of better resolution in MEEKC than MEKC will be clarified.

CHAPTER II

THEORY OF CE AND MEEKC

2.1 CE Instrumentation

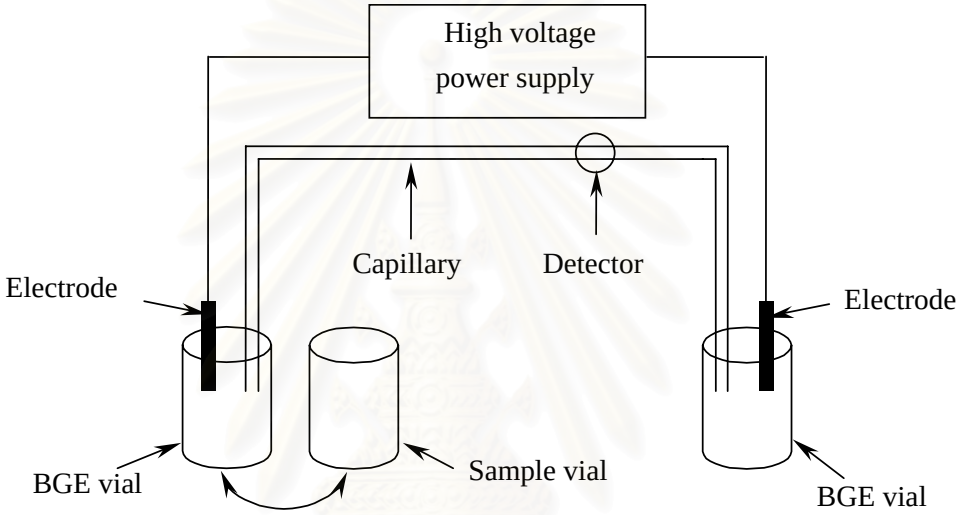


Figure 2.1 Schematic diagram of a basic CE instrument. Adapted from Weinberger [1993].

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

Sample injection in CE can be performed basically two modes including hydrodynamic and electrokinetic injection. The hydrodynamic injection is accomplished by using pressure difference between the inlet and outlet ends of the capillary. This pressure difference can be achieved by various methods such as

gravimetric, vacuum and overpressure. The latter is called pressure injection and widely used in CE. In the capillary column with cylindrical shape, the length of sample injected (l_{inj}) by application of pressure is given by the equation [Khaledi 1998]

$$l_{inj} = \frac{\Delta P r^2}{8\eta L} t_{inj} \qquad (2.1)$$

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

$$V_{inj} = \frac{\Delta P \pi r^4}{8\eta L} t_{inj} \qquad (2.2)$$

and

$$Q_{inj} = \frac{\Delta P \pi r^4}{8\eta L} t_{inj} C \qquad (2.3)$$

where C is the analyte concentration.

2.2 Electrophoretic Mobility [Grossman *et al.* 1992, 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) and relates to parameters as the equation

$$\mu = \frac{v_{ep}}{E} = \frac{ze}{6\pi\eta r_h} \qquad (2.4)$$

where z is the charge of the ion, e the fundamental electronic charge, η the viscosity of BGE, and r_h the hydrodynamic radius.

According to Equation 2.4, 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 analytes, ionic strength and viscosity of electrolyte, and temperature.

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

When silanol groups (-Si-OH) at the surface of an uncoated fused silica capillary contact with an electrolyte solution or BGE, especially a basic buffer, they ionize to be negatively charged ($-Si-O^-$) as the equation

$$-SiOH\ (s) + H_2O\ (l) \rightleftharpoons -SiO^-\ (s) + H_3O^+\ (aq) \qquad (2.5)$$

or

$$-SiOH\ (s) + OH^-\ (aq) \rightleftharpoons -SiO^-\ (s) + H_2O\ (l) \qquad (2.6)$$

Some BGE cations are attracted to the negative surface of the capillary, to form an electrical double layer, as shown in Figure 2.2. One layer is tightly bound by electrostatic force, called the Stern layer, while other BGE cations are more loosely bound, called the diffusion layer. Moreover, the rest of the excess BGE cations are in the bulk solution. When the voltage is applied, the excess BGE cations both in the diffusion layer and bulk solution migrate towards 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).

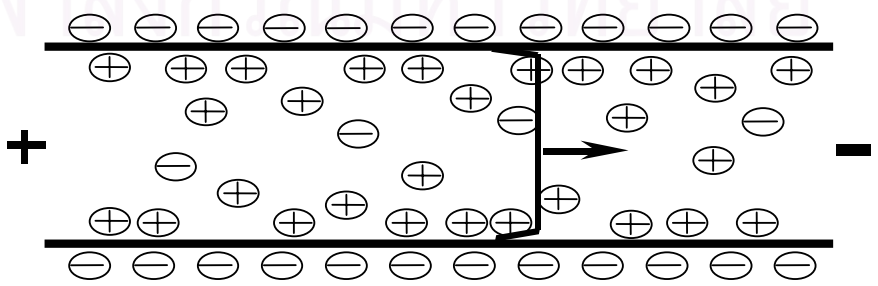


Figure 2.2 Electroosmotic flow (EOF). Adapted from Andrea *et al.* [1997].

Because of the silanol groups at the solid surface are weakly acidic, the degree of dissociation is pH dependent. A change 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 the double layer. This is directly related to the velocity of electroosmotic flow, v_{eo}, as the equation

$$v_{eo} = -\frac{\varepsilon\zeta}{4\pi\eta}E \qquad (2.7)$$

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

$$\mu_{eo} = \frac{v_{eo}}{E} = -\frac{\varepsilon\zeta}{4\pi\eta} \qquad (2.8)$$

The value of ζ is negative, and therefore, 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. Typically, the capillary used in CE has 20 to 100 μm i.d. (20000 to 100000 nm). Thus, it can be said that v_{eo} is constant throughout the capillary radius [Grossman *et al.* 1992].

Since EOF is generated at the capillary wall, and driving force of EOF is uniformly distributed along the capillary, there is no pressure drop within the capillary. This results in a flat profile of the bulk flow which does not directly contribute to the zone broadening, as shown in Figure 2.3. Thus, the peak width of analyte in CE is narrow, resulting in high efficiency and resolution in CE.

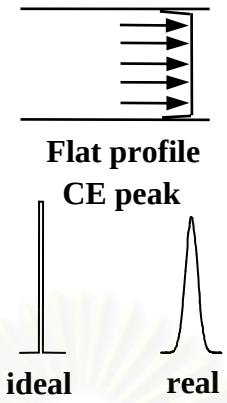


Figure 2.3 Flow profile and peak shape in CE. Adapted from Chankvetadze [1997].

In the presence of the EOF, the net velocity, v_{net}, of the analyte is the sum of the electrophoretic velocity of the analyte and the electroosmotic velocity as shown in Equation 2.9 and Figure 2.4.

$$v_{net} = v_{ep} + v_{eo} \qquad (2.9)$$

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, and the higher ion charges and the smaller ion size, the higher the net velocity. In contrast to anions, $v_{ep,-}$ has the direction toward the anode. In the case where $v_{eo} > v_{ep,-}$, the anions can migrate to the cathode, and thus 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.

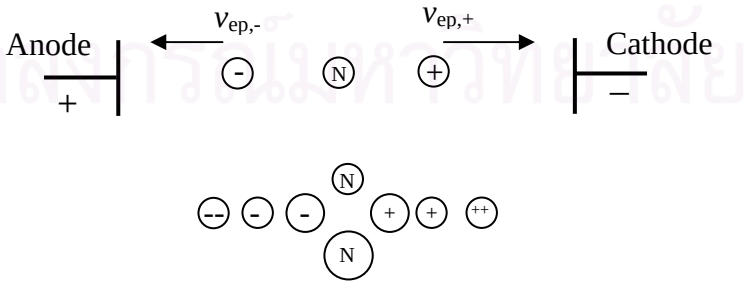


Figure 2.4 Migration behavior of the analytes. Adapted from Li [1992].

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

$$\mu_{net} = \frac{v_{net}}{E} = \frac{lL}{Vt_m} \quad (2.10)$$

$$\mu_{eo} = \frac{v_{eo}}{E} = \frac{lL}{Vt_{eo}} \quad (2.11)$$

$$\mu = \mu_{net} - \mu_{eo} = \frac{lL}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (2.12)$$

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

2.4 Retention in MEEKC

Overview of MEEKC is given in Section 1.2. In this section, more theoretical details of MEEKC are discussed. As previously mentioned in Section 1.3, retention factor or capacity 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 Terabe *et al*. [1985], the retention factor in MEEKC is defined as the ratio of total moles of analyte in the charged oil droplet or microemulsion phase (n_{mc}) versus those in the aqueous phase (n_{aq}) [Miola *et al*. 1998] as the equation

$$k = \frac{n_{mc}}{n_{aq}} = K\phi \quad (2.13)$$

where K is the distribution constant between the two phases, the ratio of the concentration of the solute in the microemulsion phase to that in the aqueous phase, and ϕ the phase ratio, the ratio of the volume of the aqueous phase to that of the

microemulsion phase. The higher the retention factor, the stronger the retention or the partitioning of analytes in the microemulsion phase.

In MEKC and MEEKC, 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}) [Camilleri 1993] as the equation

$$\mu = x_{aq}\mu_A + x_{mc}\mu_{mc} \qquad (2.14)$$

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

$$\mu = x_{mc}\mu_{mc} = \frac{n_{mc}}{n_{aq} + n_{mc}}\mu_{mc} \qquad (2.15)$$

From Equations 2.13 to 2.15, μ can be expressed by

$$\mu = \frac{k}{1+k}\mu_{mc} \qquad (2.16)$$

From Equations 2.12 and 2.16, t_m for neutral analyte in MEEKC without EOF is given by

$$t_m = \left(\frac{1+k}{k}\right)t_{mc} \qquad (2.17)$$

where t_{mc} is the migration time of the analyte fully partitioning into the microemulsion phase. By rearrangement of Equation 2.17, the retention factor of the neutral analyte in MEEKC without EOF can be calculated from an electropherogram using the equation

$$k = \frac{t_{mc}}{t_m - t_{mc}} \qquad (2.18)$$

In the presence of high EOF, the total mobility ($\mu_{net} = \mu + \mu_{eo}$) in Equation 2.16 is given by

$$\mu_{net} = \frac{k}{1+k}\mu_{mc} + \mu_{eo} \qquad (2.19)$$

Therefore, t_m of the neutral analyte in MEEKC with high EOF is expressed by

$$t_m = \frac{(1+k)t_{eo}}{1+\left(\frac{t_{eo}}{t_{mc}}\right)k} \qquad (2.20)$$

2.5 Efficiency and Band Broadening in MEEKC

Like in chromatography, an ideal peak in CE is assumed to have a Gaussian shape with standard deviation, σ in a distance unit and τ in a time unit, as shown in Figure 2.5.

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

$$w_b = 4\sigma \quad \text{or} \quad w_b = 4\tau \qquad (2.21)$$

and the peak width at half height, w_h, is given by

$$w_h = 2.354\sigma \quad \text{or} \quad w_h = 2.354\,\tau \qquad (2.22)$$

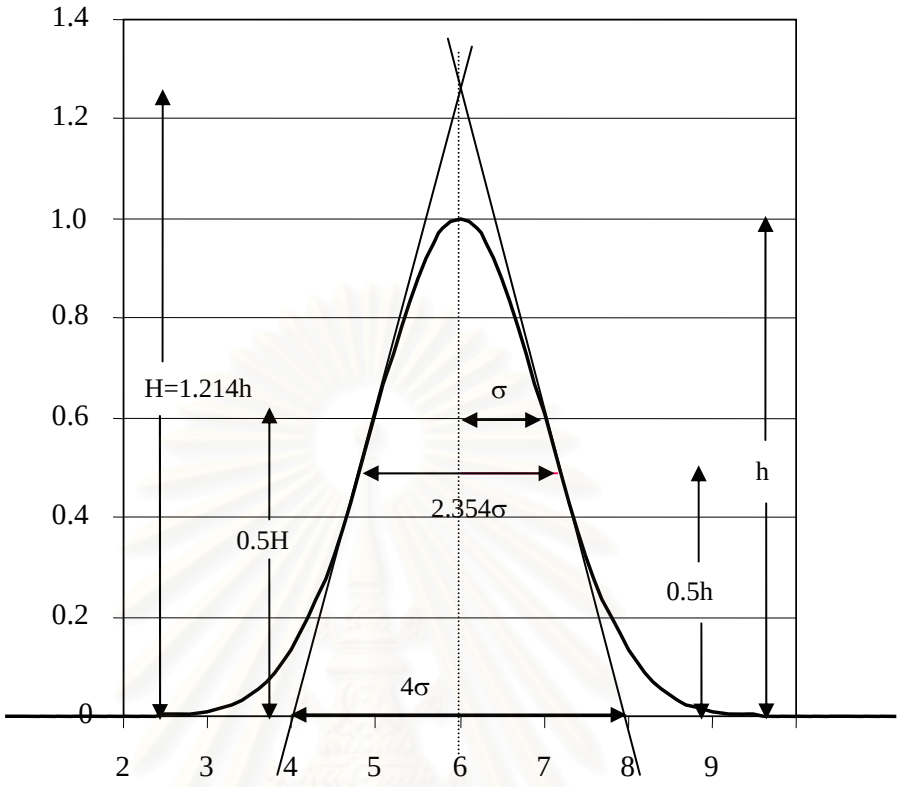


Figure 2.5 Gaussian peak.

Peak efficiency (*N*) is also related to σ and τ as the equation

$$N = \left(\frac{l}{\sigma}\right)^2 = \left(\frac{t}{\tau}\right)^2 \qquad (2.23)$$

It follow from Equations 2.21 to 2.23, *N* can be calculated from an electropherogram, according to the equation

$$N = 16\left(\frac{t_m}{w_b}\right)^2 = 5.54\left(\frac{t_m}{w_h}\right)^2 \qquad (2.24)$$

In MEEKC, peak broadening can be expressed by peak variance (σ^2) or theoretical plate height (*H*) as similarly explained in chromatography and MEKC as the equation [Terabe *et al.* 1989]

$$\sigma^2 = Hl \qquad (2.25)$$

Analogous to chromatography and MEKC, H in MEEKC also relates to N and l as the equation [Khaledi 1998]

$$H = \frac{l}{N} \tag{2.26}$$

According to Equation 2.26, the higher the efficiency, the smaller the plate height or peak broadening. In this work, peak broadening will be discussed in term of H. The total H can be described as the sum of plate height caused by five main contributions as the equation

$$H = H_{\mathrm{l}} + H_{\mathrm{mc}} + H_{\mathrm{aq}} + H_{\mathrm{t}} + H_{\mathrm{pd}} \tag{2.27}$$

where H_{l}, H_{mc}, H_{aq}, H_{t} and H_{pd} are the plate height generated by longitudinal diffusion, sorption-desorption kinetics in the pseudo stationary phase solubilisation, intermicelle mass transfer in the aqueous phase, thermal dispersion and polydispersity of the pseudo stationary phase, respectively.

2.5.1 Longitudinal diffusion

When the analyte solution is introduced into one end of the capillary, concentration gradient in the axial direction will be occurred, resulting in diffusional mass flux along the direction, according to the Fick's second law which describes the change of the concentration as a function of time and as the equation

$$c(x,t) = \frac{Q_{\mathrm{inj}}}{(4\pi Dt)^{1/2}} \exp\left(-\frac{x^2}{4Dt}\right) \tag{2.28}$$

where $c_{(x,t)}$ is the concentration of analyte at given position x and time t, Q_{inj} the amount of analyte injected, and D the diffusion coefficient of the analyte. This is the formular for Gaussian-shaped with the variance $2Dt$, according to the random walk theory of the Einstein-Smoluchowski equation.

$$\sigma_{\text{diff}}^2 = 2Dt \qquad (2.29)$$

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

$$D = \frac{\mu^\circ k^* T}{ze} \qquad (2.30)$$

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

From Equations 2.10, 2.25 and 2.29, the plate height from longitudinal diffusion is derived as the equation

$$H_{\text{l}} = \frac{2D}{\mu_{\text{net}} E} \qquad (2.31)$$

According to Equations 2.31, H_{l} decreases with an increase in the migration velocity or applied voltage.

Since the media in MEEKC contains both aqueous and microemulsion phase, H_{l} in Equation 2.31 can be divided into two components and expressed by the following equation

$$H_{\text{l}} = \frac{2}{\mu_{\text{total}} E}\left(\frac{D_{\text{aq}} + kD_{\text{mc}}}{1+(t_{\text{eo}}/t_{\text{mc}})k}\right) \qquad (2.32)$$

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]. In the absence of EOF, it follows from Equation 2.32 that the plate height H_l is expressed by

$$H_{\text{l}} = \frac{2}{\mu_{\text{mc}} E}\left(\frac{D_{\text{aq}} + kD_{\text{mc}}}{k}\right) \qquad (2.33)$$

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

Thermal dispersion in MEKC and MEEKC is occurred when voltage is applied and heat is generated, called Joule heating, resulting in parabolic temperature gradient along the capillary in the radial direction. The parabolic temperature profile results in a parabolic velocity profile of the analyte because the mobility is a function of temperature. Analogous to the Einstein-Smoluchowski equation for one-dimensional diffusion in Equation 2.29, peak variance from thermal dispersion can be expressed by replacing D in Equation 2.29 by thermal dispersion coefficient, D_{th}, as the equation

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

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 the electric conductivity of the BGE, λ_s the thermal conductivity of the BGE, and r the capillary radius. According to Equations 2.18, 2.30 and 2.31, H_t can be 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 the electric current, and T the temperature [Terabe *et al*. 1989]. In MEEKC without EOF, H_t is rearranged to be the equation

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

From Equation 2.37, it should be note that H_t depends on κ, r, and E.

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

Analogous to the resistance to mass transfer in the mobile phase term for packed column LC, intermicelle mass transfer in MEKC and MEEKC is assumed to be the discrimination of analyte concentration distributing among the pseudo stationary phase. Therefore, H_{aq} can be derived from random walk theory [Terabe *et al*. 1989], as given by

$$H_{aq} = \left(\frac{k}{1+k}\right)^2 \left(\frac{\left(1-\frac{t_0}{t_{mc}}\right)^2}{1+\left(\frac{t_0}{t_{mc}}\right)k}\right)\left(\frac{d^2 v_{eo}}{4D_{aq}}\right) \qquad (2.38)$$

where t_0, t_{mc}, and d are the migration time of the insolubilised analyte, the migration time of micelle or microemulsion phase and the average between the pseudo stationary phase distance, respectively. From Equation 2.38, H_{aq} increases with increasing distances between the pseudo stationary phase ($H_{aq} \propto d^2$). Therefore, H_{aq} should decrease with increasing surfactant concentration. Moreover, H_{aq} also arises from the slow diffusion rate between the pseudo stationary phase.

2.5.4 Sorption-desorption kinetics in microemulsion solubilisation [Terabe *et al.* 1989, Khaledi 1998]

In MEEKC, sorption-desorption kinetics of the distribution of analyte between aqueous phase and pseudo stationary phase can be shown as the equation

$$A_{aq} \underset{k_d}{\overset{k_s}{\rightleftharpoons}} A_{mc} \qquad (2.39)$$

where A_{aq} and A_{mc} are the analytes in the aqueous and the microemulsion phase, respectively, and k_s and k_d the sorption and desorption rate constants, respectively.

The contribution of sorption-desorption kinetics results from the analytes migrating through the capillary with a distribution of velocities, as given by the equation

$$H_{mc} = \frac{2\left(1-\frac{t_0}{t_{mc}}\right)^2 k}{\left[1+\left(\frac{t_0}{t_{mc}}\right)k\right](1+k)^2}\left(\frac{v_{eo}}{k_d}\right) \qquad (2.40)$$

According to the non-equilibrium theory, H_{mc} from Equation 2.40 is small unless there are electrostatic forces or ionic interactions between the analytes and pseudo stationary phase [Terabe *et al*. 1989]. Therefore, H_{mc} may be negligible in this work.

2.5.5 Micellar polydispersity [Grossman and Colurn 1992, Khaledi 1998]

In MEKC, the difference in micelle aggregation number, the average number of surfactant monomers per micelle presented in a solution, 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 resulting in band broadening which is called micellar polydispersity. Plate height generated from micellar polydispersity can be expressed from the distribution of electrophoretic mobilities of micelles having different aggregation numbers. Therefore, H_{pd} in MEEKC, similar to MEKC, may be expressed by

$$H_{pd} = \left[\frac{\sigma_{\mu,mc}}{\mu_{mc}}\right]^2 \frac{\mu_{mc}^2(1-R)t_{mc}^* E}{\mu_{net}} \qquad (2.41)$$

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

From Section 2.5, it can be conclude that H_{mc} is negligible for analytes having strong partitioning into the pseudo stationary phase. 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.

2.6 Resolution in MEEKC

In CE, a resolution (R_s) of two analytes is defined as the ratio of the difference in their migration times to their peak width at base as the equation

$$R_s = \frac{t_{m2} - t_{m1}}{0.5(w_{b1} + w_{b2})} \qquad (2.42)$$

It follows from Equations 2.42 and 2.12 that, R_s can be related to the mobility and the average number of theoretical plates, $\overline{N}$, as the equation

$$R_s = \frac{1}{4}\left(\frac{\Delta\mu}{\overline{\mu} + \mu_{eo}}\right)\sqrt{\overline{N}} \qquad (2.43)$$

where $\Delta\mu$ is the difference in the mobility, and $\overline{\mu}$ the average electrophoretic mobility of the analytes. In MEKC and MEEKC with high EOF, it follows from Equations 2.16 and 2.43 that R_s of two analytes is expressed by [Terabe *et al.* 1985]

$$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)\left(\frac{1 - \frac{t_0}{t_{mc}}}{1 + \left(\frac{t_0}{t_{mc}}\right)k_1}\right) \qquad (2.44)$$

where α is the selectivity which is defined as the ratio of the retention factor, e.g. k_2/k_1 and $k_2 \geq k_1 > 0$, t_0 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 2.43 that the resolution of two analytes is given by

$$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\Delta\mu}{\overline{\mu}}\right) \qquad (2.45)$$

In the case where $|\mu_2| > |\mu_1|$ ($t_{m2} < t_{m1}$), the resolution in Equation 2.45 may relate to α, k and $\overline{N}$ as the equation

$$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\alpha - 1}{\frac{\alpha + 1}{2} + k_2}\right) \qquad (2.46)$$

In the case of $1 \le \alpha \le 1.3$, R_s may be assumed to be equal the simple equation [Nhujak *et al.* 2006]

$$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\alpha - 1}{1 + k_2}\right) \qquad (2.47)$$

Table 2.1 shows a comparison of equations expressed for retention and resolution in MEKC, MEEKC and HPLC. More details of a plot of R_s as a function of k, α and N are discussed in Section 4.3.1.

Table 2.1 Comparison of retention and resolution

	MEEKC or MEKC		HPLC
	high EOF	low EOF	
Definition of k	$k = \frac{n_{mc}}{n_{aq}}$	$k = \frac{n_{mc}}{n_{aq}}$	$k = \frac{n_{sp}}{n_{mp}}$
Measurement of k	$k = \frac{t_m - t_{eo}}{t_{eo}\left(1 - \frac{t_m}{t_{mc}}\right)}$	$k = \frac{t_{mc}}{t_m - t_{mc}}$	$k = \frac{t_R - t_{sp}}{t_{sp}}$
k order	$k_1 < k_2 < k_3$	$k_1 < k_2 < k_3$	$k_1 < k_2 < k_3$
t_R order	$t_{m,1} < t_{m,2} < t_{m,3}$	$t_{m,1} > t_{m,2} > t_{m,3}$	$t_{R,1} < t_{R,2} < t_{R,3}$
R_s	$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)\left(\frac{1 - \frac{t_0}{t_{mc}}}{1 + \left(\frac{t_0}{t_{mc}}\right)k_1}\right)$	$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\alpha - 1}{1 + k_2}\right)$	$R_s = \frac{\sqrt{\overline{N}}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)$

n_{sp} and n_{mp} are total moles of analyte in the stationary phase and those in the mobile phase, respectively, and t_R the retention time

CHAPTER III

PRELIMINARY STUDY OF RETENTION SCALES IN MEEKC EMPLOYING SUPPRESSED ELECTROOSMOSIS

3.1 Introduction and Theory

As described in Section 2.5, retention factor (*k*) is one of the characteristics that indicates retention behavior of analytes in chromatography and electrokinetic chromatography such as MEEKC and MEKC. Much of previous MEEKC work involved separation of either charged or neutral compounds using the basic buffer, such as phosphate or borate, to obtain high EOF [Hansen 2003, McEvoy *et al.* 2007]. In MEEKC with high EOF and anionic surfactant, as shown in Figures 3.1a and 3.2a, the neutral analyte with higher hydrophobicity or the greater number of carbons (*z*) has greater retention factor or favors partitioning into the pseudo stationary phase, resulting in higher electrophoretic mobility with the direction to the anode. Due to the electroosmotic mobility higher than the electrophoretic mobility of charged oil droplets and analytes, all species migrate toward the cathode. Therefore, the migration time of species in Figures 3.2a are in order an EOF marker < the analyte with smaller k < the analyte with higher k < a microemulsion marker. In contrast to MEEKC without EOF and reversed polarity as shown Figures 3.1b and 3.2b, all species migrate only due to electrophoretic mobility, and therefore, the reversal of migration time order is observed. On the other hand, the greater the retention factor, the slower the migration time of analytes in MEEKC with high EOF, but the faster the migration time of analytes in MEEKC without EOF. In order to obtain short analysis time, MEEKC with high EOF is suitable for hydrophilic analytes with small *k* [Altria *et al.* 2000a and 2000b], while MEEKC without EOF is suitable for hydrophobic analytes with high *k* [Altria *et al.* 2003, Nhujak *et al.* 2006, Seelanan *et al.* 2006, Pomponio *et al.* 2003].

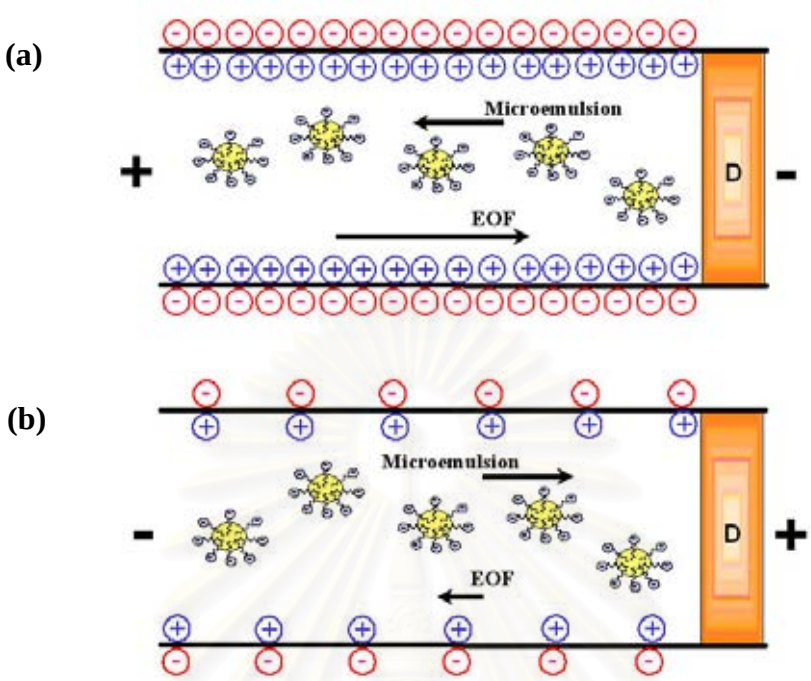


Figure 3.1 Schematic of the separation principles of MEEKC using anionic surfactant: (a) high EOF and (b) low EOF. Adapted from Altria [2000a].

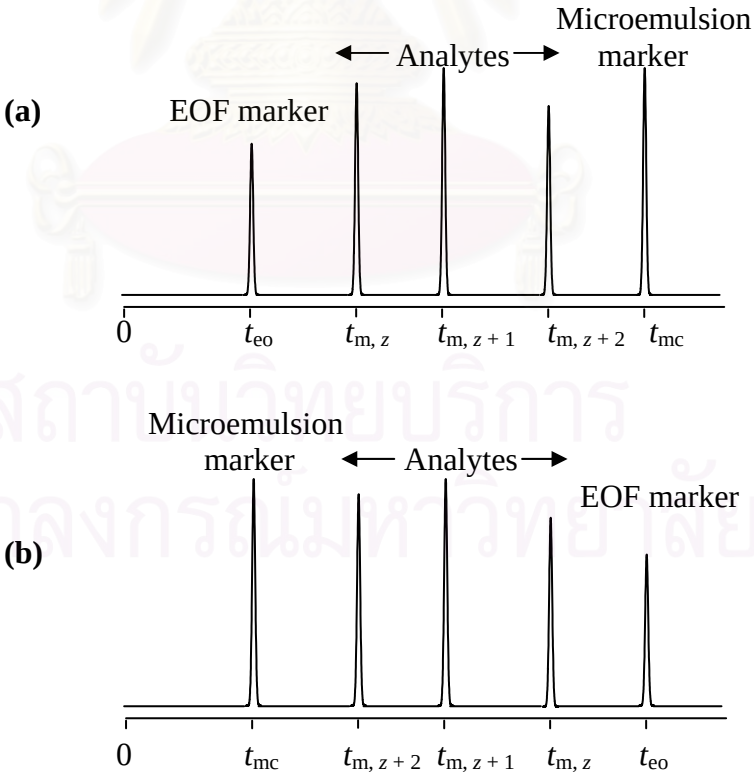


Figure 3.2 Typical elution order for analytes in MEEKC: (a) high EOF and (b) low EOF, where $k_{z+2} > k_{z+1} > k_z$ and z refer to the number of carbons of analytes. Adapted from Miola *et al*. [1998].

The retention factor of the neutral analyte in MEEKC without EOF can be calculated from electropherograms using the Equation 2.18. The higher the retention factor, the stronger the retention or the partitioning of analytes in the pseudo stationary phase. An increase in the concentration of surfactant results in an increase in *k* in MEEKC [Altria *et al.* 2000b, Nhujak *et al.* 2006, Seelanan *et al.* 2006, Ishihama *et al.* 1996, Song *et al.* 1995, Harang *et al.* 2004], while a decrease in *k* is affected by an increase in temperature [Nhujak *et al.* 2006, Seelanan *et al.* 2006, Harang *et al.* 2004], Joule heating caused by high voltage [Nhujak *et al.* 2006, Song *et al.* 1995] and the concentration of organic co-solvent [Nhujak *et al.* 2006, Seelanan *et al.* 2006, Harang *et al.* 2004, Gong *et al.* 2004]. Types of surfactant [Altria *et al.* 2000b], co-surfactant [Altria *et al.* 2000b] and organic co-solvent [Altria *et al.* 2000b, Nhujak *et al.* 2006, Ishihama *et al.* 1996] also influence a change in *k*. The relationship between log *k* of analytes in MEEKC and log K_{ow} was found to give a linear equation, where K_{ow} is the octanol-water distribution constant of the analyte [Gong *et al.* 2004, Klotz *et al.* 2001]. Using standards with known log K_{ow}, log K_{ow} of the analyte may be determined using log *k* of the analyte obtained from MEEKC. Most of previous MEEKC work above-mentioned was carried out using high EOF [Altria *et al.* 2000b, Hansen 2003, Ishihama *et al.* 1996, Song *et al.* 1995, Harang *et al.* 2004], except for a few work involving MEEKC with suppressed EOF [Nhujak *et al.* 2006, Seelanan *et al.* 2006].

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 [Muijselaar *et al.* 1994]. 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 [Muijselaar *et al.* 1994]

$$I = 100z + 100\frac{\log k_A - \log k_z}{\log k_{z+1} - \log k_z} \qquad (3.1)$$

where k_z and k_{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 2.8b and Equations 2.18 and 3.1, the retention index can be expressed as a function of the migration time as the equation

$$I = 100z + 100\frac{\log\left(\frac{t_{mc}}{t_{m,A} - t_{mc}}\right) - \log\left(\frac{t_{mc}}{t_z - t_{mc}}\right)}{\log\left(\frac{t_{mc}}{t_{z+1} - t_{mc}}\right) - \log\left(\frac{t_{mc}}{t_z - t_{mc}}\right)} \qquad (3.2)$$

According to the Martin's equation [Muijselaar *et al.* 1994], 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

$$\log k = az + b \qquad (3.3)$$

where a and b are the slope and the intercept, respectively. It follows from Equations 3.1 and 3.3 that the retention index may be calculated by interpolation and for the analyte with higher k than the first homologue, by extrapolation of the equation [Muijselaar *et al.* 1994]

$$I = \frac{100(\log k - b)}{a} \qquad (3.4)$$

In MEKC with EOF and using homologous series of alkylbenzenes (BZ) or alkylarylketones as retention index standards, the retention indices of analytes were obtained to be independent of the surfactant concentration, whereas slightly dependent on the temperature [Muijselaar *et al.* 1994]. However, in MEKC with addition of organic solvents in the buffer, some variations (RSD < 3%) of retention index were found at different SDS concentrations [Ahuja *et al.* 1994]. The retention index can be used for identification of neutral analytes [Muijselaar *et al.* 1994]. In addition, a good linear relationship between I and log K_{ow} was obtained [Muijselaar *et*

al. 1994]. Using the different surfactants as micellar phase in MEKC, the ΔI values, obtained from different micellar systems, can be used to elucidate the functional group selectivity of these specific micellar systems and to classify pseudo stationary phases in MEKC [Muijselaar *et al.* 1994]. Up-to-date, factors affecting retention index of analytes in MEEKC have not been reported previously.

The aim of this section is to investigate the retention behaviors, such as retention factor and retention index, of homologous series of hydrophobic neutral compounds in MEEKC with suppressed EOF using an acidic buffer. The following parameters affecting these retention scales were determined: the SDS concentration, temperature, voltage and types and concentrations of organic co-solvents.

Homologous series of BZ were chosen as standards because they are widely used as standards for studying retention factor and log K_{ow} in MEKC and MEEKC. In addition, BZ were found to be suitable retention index standards, in comparison with alkylaryl ketones (PN) in MEKC with anionic surfactant SDS [Muijselaar *et al.* 1994]. Other homologous compounds, such as PN, alkylbenzoates (BA) and alkylparabens (PB), were used as test analytes in order to study their retention factor and retention index. The structures of homologous series of BZ, PN, BA and PB are shown in Figure 3.3.

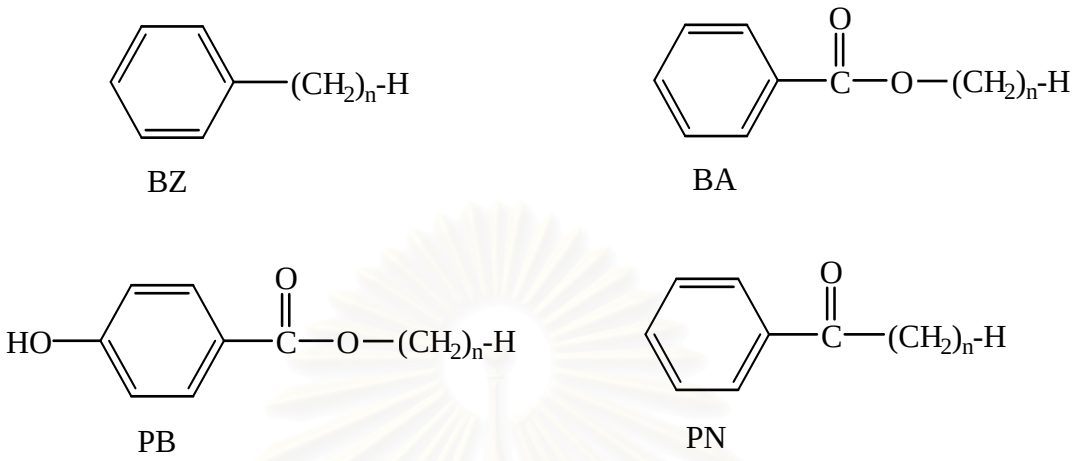


Figure 3.3 Chemical structures of homologous series. *n* is the number of methylene groups.

3.2 Experimental

3.2.1 Chemicals

Homologous series of BZ and BA were purchased from Fluka (Buchs, Switzerland): benzene (BZ6), methylbenzene (BZ7), ethylbenzene (BZ8), propylbenzene (BZ9), methylbenzoate (BA8), ethylbenzoate (BA9), propylbenzoate (BA10), and butylbenzoate (BA11). PN and PB were purchased from Sigma-Aldrich (Steinheim, Germany): acetophenone (PN8), propiophenone (PN9), butyrophenone (PN10), valerophenone (PN11), methylparaben (PB8), ethylparaben (PB9), propylparaben (PB10), butylparaben (PB11). *n*-Octane, 1-butanol and sodium hydroxide were obtained from Fluka, SDS from Sigma (MO, USA), phosphoric acid and all organic solvents from Merck (Darmstadt, Germany), and dodecylbenzene (DB)/microemulsion marker (M) from Sigma-Aldrich.

3.2.2 Preparation of buffers and analytes

3.2.2.1 Preparation of microemulsion buffers

The microemulsion buffer was prepared by pipeting the appropriate amounts of stock aqueous solutions of 500 mM SDS and 500 mM phosphate buffer at pH 2.5 into a 10 mL volumetric flask, followed by adding appropriate volumes of 1-butanol and *n*-octane. The final solution was made up to 10 mL with Milli-Q water. In the case of preparation of the microemulsion buffer containing organic co-solvent such as methanol (MeOH), ethanol (EtOH), acetonitrile (ACN) or 2- propanol (2-PrOH), the appropriate volume of organic co-solvent was added before addition of Milli-Q water. All microemulsion buffers were sonicated for 30 min to obtain a clear and highly stable microemulsion. The microemulsion buffers were filtered through 0.45 μm PTFE membrane filters prior to MEEKC analysis.

3.2.2.2 Preparation of analytes

Each homologous series of BZ, PN, BA and PB as analytes, and DB as a microemulsion marker were separately dissolved at a concentration of 10.0 mM in EtOH. The mixture of each homologous series at the concentration of 0.2 mM each was obtained by diluting 10.0 mM of each series of homologous and DB, and then diluting the mixture with microemulsion containing 180 mM SDS, 890 mM 1-butanol and 1.0% v/v *n*-octane. It should be noted that the mixture of each homologous series was separately prepared to avoid overlapping or co-migration of some analytes, leading to difficulty of identification of analytes and determination of migration times. All the sample solutions were filtered through 0.45 μm PTFE membrane filters prior to MEEKC analysis

3.2.3 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 nm.

An uncoated fused-silica capillary used was 40.2 cm in length (30 cm to detector) × 50 μm i.d., 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 duplicate, unless otherwise stated. Prior to MEEKC analysis each day, the capillary was rinsed with EtOH, 0.1 M NaOH, water, and the microemulsion buffer for 15 min each. Between consecutive runs, the capillary was rinsed with water, EtOH, 0.1 M NaOH, and water for 1 min each and then the microemulsion buffer for 2 min. After analysis, each day, the capillary was rinsed with water and EtOH for 5 min each, and then 0.1 M NaOH and water for 10 min each.

3.3 Results and Discussion

3.3.1 Choice of CE conditions

3.3.1.1 pH and concentration of buffer

Since homologous series of BZ, PN, BA and PB are highly hydrophobic compounds. MEEKC employing suppressed EOF should be carried to obtain fast analysis time as mentioned previously in Section 3.1. In this work, the phosphate buffer at pH 2.5 was used to eliminate EOF. The phosphate buffer contains weak acid (H_3PO_4 or HA) and conjugated base ($H_2PO_4^-$ or A^-) which can be expressed by a dissociation equilibrium as the equation

$$H_3PO_4\,(aq) \rightleftharpoons H_2PO_4^-\,(aq) + H^+\,(aq) \quad : pK_{a1} = 2.12 \qquad (3.5)$$

The pH of the solution is given by the Henderson-Hasselbalch's equation [Chang 2005]

$$pH = pK_a + \log\frac{[A^-]}{[HA]} \qquad (3.6)$$

Since pK_{a1} of H_3PO_4 is equal to 2.12, the phosphate buffer at pH 2.5 in a range of pK_{a1} ± 1.0 was chosen in order to provide high buffering capacity, resulting in high precision in the suppressed EOF and migration times of analytes.

A concentration of the phosphate buffer at 50 mM was used in this work, which is widely used in MEEKC at low pH [Altria *et al*. 2000b, Nhujak *et al*. 2006]. This is because the lower concentration of the buffer results in poor precision in migration time, while the higher concentration of the buffer generates high current and Joule heating which may limit the use of high voltage and temperature [Altria *et al*. 2000b, Nhujak *et al*. 2006].

3.3.1.2 Diameter and length of capillary

In MEEKC, the most commonly used capillaries are 50 and 75 mm μm i.d. [Chankvetadze 1997]. 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, 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, the 50 μm i.d. capillary was selected. For the length of the capillary, the 40.2 cm (30 cm to detector) capillary was used to give fast analysis time. In MEEKC, the mostly used internal diameter and length of capillary are 50 μm and 40.2 cm, respectively [Altria 2000].

3.3.1.3 Detection wavelength

The homologous series of BZ, PN, BA and PB and DB were found to absorb UV light with the maximum absorbance near 200 nm. However, the buffer produces high UV

background at 200 nm. Therefore, UV detection of the homologous series of BZ, PN, BA and PB was scanned from 190 to 300 nm, and electropherograms were monitored at 214 nm in this work.

3.3.1.4 Types of oil droplet

In MEEKC, *n*-octane or *n*-heptane is commonly used to form the oil droplet. In comparison with *n*-octane, *n*-heptane has lower toxicity, but faster vaporize [Ishihama *et al*. 1995] and gives poorer stability of the microemulsion [Fu *et al*. 1996]. *n*-Octane, used as the oil droplet, was reported to provide more repeatable microemulsions [Altria *et al*. 2000].

Others 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 a range of neutral compound [Altria *et al*. 2000]. Therefore, *n*-octane was chosen as oil droplet in this work, and the concentration of *n*-octane at 1.0% v/v was used, which is widely used in MEEKC.

3.3.1.5 Types of surfactant

In MEEKC, sodium dodecyl sulphate (SDS) is the most widely used as anionic surfactant because it is cheap and available in highly purified form. The C_{12} alkyl chain of the SDS surfactant penetrates into the oil droplet, while the negatively charged hydrophilic sulphate groups reside in the surrounding aqueous phase [Altria 2000a]. Other anionic surfactant such as sodium cholate has also been used to generate negatively charged droplets [Miksik *et al.* 1998, Altria 2000a]. However, it gave different selectivity compared to a SDS buffer and more expensive. Cationic surfactant such as cetyltrimethylammonium bromide (CTAB) is used in MEEKC [Bosco *et al.* 1995, Altria 2000a]. It produces positively charged oil droplets and also generates a positively charged surface bi-layer on the capillary wall, which reverses

the EOF direction. However, CTAB produces high UV background at the wavelength in a range of 190 to 230 nm due to UV absorption of Br^-. Therefore, SDS was chosen as the surfactant in this work, and the effect of SDS concentration on *k* and *I* will be described in Section 3.3.3.

3.3.1.6 Types of co-surfactant

Various types of alcohols, C_3 to C_6, were used as the co-surfactant in MEEKC. The greater *k* of analyte was found with increasing *z* of the alcohol, possible due to the larger microemulsion diameter with the larger size of the alcohol [Pomponio *et al*. 2003].

Previous work on separation of curcuminoids [Nhujak *et al*. 2006] showed that broad peaks and poor resolution were obtained for the co-surfactant as 1-propanol, 1-pentanol or 1-hexanol in MEEKC due to unstable microemulsion. In comparison with butanols, cyclohexanol gave the greater *k* of curcuminoids, but longer migration time due to higher viscosity. Similar selectivity and resolution of analytes such as curcuminoids were obtained with butanols and cyclohexanol used as the co-surfactant. However, previous work on separation of catechins [Pomponio *et al*. 2003] showed the significant difference in selectivity, resolution and migration time order using different C_3 to C_6 alcohols in MEEKC, especially butanols, in the MEEKC buffer for saparation of catechins. This is possibly due to the different structures of analytes or different concentration of alcohol used in their work. In this work, 1-butanol was chosen as the co-surfactant, and the concentration of 1-butanol at 890 mM was used, which is widely used in MEEKC [Hansen 2003, McEvoy *et al*. 2007].

3.3.2 Precision in retention factor and retention index

Intraday and interday precisions in *k* and *I* of homologous series were compared using one batch and batch-to-batch of the MEEKC buffer containing a 50 mM phosphate buffer at pH 2.5, 180 mM SDS, 1.0% v/v *n*-octane, and 890 mM 1-butanol. An example of electropherograms is shown in Figure 3.4. DB, a highly hydrophobic compound, spiked into a sample solution is used as a marker for t_{mc} [Altria *et al.* 2000a, Nhujak *et al.* 2006]. In comparison with the electrophoretic mobility of the analyte, negligible electroosmotic mobility was obtained in this work, measured by short-end injection using thiourea [Nhujak *et al.* 2006].

The values of *k* and *I* of standards (BZ series) and test analytes (PN, BA and PB series) as shown in Table 3.1 were determined from electropherograms using Equation 2.18 for *k* and Equation 3.4 for *I*. The quality of *k* and *I* for BZ standards is first considered. The slope (*a*) and the intercept (*b*) of the equation $\log k = az + b$ for a homologous series of BZ were also found to be fairly constant for one batch and batch-to-batch of intraday and interday runs, and a non-significant difference was obtained using paired *t*-test analysis at 95% confidence interval of the mean, indicating high precision in *k* of BZ standards used. Using the *t*-test comparing the calculated and nominal values of *I* for BZ, non-significant difference was obtained between one batch and batch-to-batch of intraday and interday runs, where the nominal values are 600, 700, 800 and 900 for BZ6, BZ7, BZ8 and BZ9, respectively. This indicates a high accuracy and precision in *I* of BZ standards used.

The quality of *k* and *I* for test analytes is now considered. In most of the case of this work, a slight difference in relative standard deviation (RSD) was obtained between one batch and batch-to-batch intraday precisions in *k* and *I* of test analytes, while higher RSD values of *k* and *I* for interday than those for intraday were found. Slightly poorer precision for interday than intraday is probably due to variation in chemistry properties of capillary wall surface each day, resulting in a change in suppressed EOF and the calculated retention factor [Nhujak *et al*. 2006]. However, using paired *t*-test analysis at 95% confidence interval of the mean, non-significant difference is found

for *k* and *I* obtained from intraday and interday or one batch and batch-to-batch. Precision in *k* and *I* in this work was found to be in a similar range, while significantly worse precision in *k* than *I* of analytes in MEEKC with high EOF was found in previous work [Ishihama *et al*. 1995]. This is probably because, in comparison with octane used as oil in this work, heptane used as oil in the previous work can vaporize more easily [Ishihama *et al*. 1995] and give poorer stability of the microemulsion [Fu *et al*. 1996].

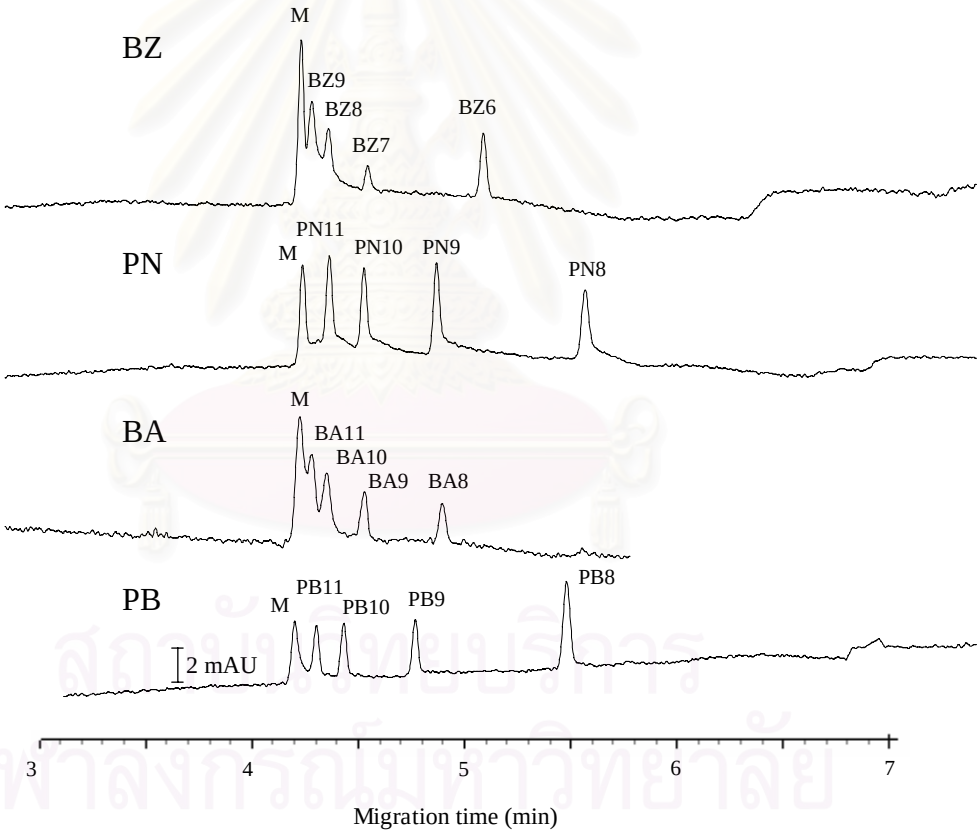


Figure 3.4 An example of electropherograms for homologous series in MEEKC using 180 mM SDS in a 50 mM phosphate buffer (pH 2.5) containing 1.0% v/v *n*-octane and 890 mM 1-butanol. CE conditions: uncoated fused silica capillary 50 μm i.d. × 40.2 cm (30 cm to detector), temperature 25°C, voltage -15 kV, 0.5 psi pressure injection for 3 s and UV detection at 214 nm.

Table 3.1 Intraday and interday precision of *k* and *I* of homologous series in MEEKC using 180 mM SDS and other conditions as shown in Figure 3.2

	RSD (%) and mean of *k*				RSD (%) and mean of *I*			
	One batch		Batch-to-batch		One batch		Batch-to-batch	
	Intraday[a)]	Interday[b)]	Intraday[c)]	Interday[b)]	Intraday[a)]	Interday[b)]	Intraday[c)]	Interday[b)]
Standards								
BZ6	0.5 (6.05)	2.0 (5.84)	0.8 (6.04)	1.6 (6.02)	0.1 (600)	0.1 (599)	0.1 (601)	0.3 (599)
BZ7	0.5 (15.66)	1.3 (15.59)	1.5 (15.32)	3.4 (15.91)	0.1 (702)	0.2 (703)	0.2 (701)	0.2 (703)
BZ8	0.9 (38.42)	1.6 (38.04)	1.7 (37.20)	3.8 (38.78)	0.1 (798)	0.2 (798)	0.1 (797)	0.2 (798)
BZ9	0.8 (100.08)	0.9 (99.05)	1.0 (99.07)	1.0 (99.90)	0.1 (901)	0.1 (900)	0.1 (902)	0.2 (900)
Analytes								
PN8	0.2 (3.35)	1.6 (3.30)	1.5 (3.36)	2.4 (3.37)	0.1 (536)	0.3 (538)	0.3 (537)	0.8 (536)
PN9	0.8 (7.18)	1.9 (7.19)	1.1 (7.18)	1.3 (7.14)	0.2 (618)	0.5 (621)	0.2 (619)	0.6 (617)
PN10	0.9 (15.31)	2.4 (15.58)	1.3 (15.42)	1.1 (15.29)	0.2 (699)	0.4 (703)	0.2 (702)	0.4 (699)
PN11	0.7 (34.79)	1.4 (34.84)	1.3 (34.87)	0.8 (34.54)	0.1 (787)	0.2 (789)	0.2 (790)	0.3 (786)
BA8	0.3 (7.97)	2.9 (7.82)	0.7 (7.93)	2.0 (7.83)	0.1 (629)	0.6 (630)	0.1 (630)	0.7 (627)
BA9	0.2 (17.93)	2.0 (17.61)	1.0 (17.31)	1.7 (17.48)	0.1 (716)	0.3 (716)	0.1 (714)	0.5 (713)
BA10	0.8 (40.58)	1.5 (40.68)	1.3 (39.99)	1.8 (40.26)	0.1 (804)	0.3 (805)	0.2 (804)	0.4 (803)
BA11	0.3 (88.55)	0.5 (88.61)	0.8 (88.93)	0.7 (89.06)	0.1 (888)	0.1 (888)	0.2 (890)	0.2 (888)
PB8	0.5 (3.97)	1.5 (3.90)	1.4 (3.94)	2.2 (3.93)	0.1 (554)	0.4 (556)	0.3 (555)	0.8 (553)
PB9	0.6 (8.85)	2.7 (8.67)	1.4 (8.86)	2.3 (8.75)	0.1 (640)	0.5 (641)	0.2 (642)	0.7 (639)
PB10	0.5 (21.46)	2.3 (21.70)	1.5 (21.69)	1.5 (21.53)	0.1 (736)	0.4 (738)	0.2 (738)	0.4 (735)
PB11	0.4 (51.42)	1.7 (52.08)	0.8 (51.31)	0.7 (51.45)	0.1 (829)	0.3 (832)	0.1 (831)	0.4 (829)

The values of mean are in parentheses. Homologous series of BZ (BZ6, BZ7, BZ8 and BZ9) were used as retention index standards.

[a)] n = 5 runs.

[b)] Five days and each day for 2 runs (n = 10 runs).

[c)] Five batches and each batch for 2 runs (n = 10 run).

3.3.3 Effect of SDS concentration on retention factor and retention index

To investigate the influence of SDS concentration ([SDS]) on *k* and *I* of analytes in MEEKC with suppressed EOF, experiments were carried out by varying [SDS] in a range of 100 to 200 mM. An example of electropherograms is shown in Figure 3.5. At SDS below 100 mM, broad peaks and poor precision in the migration time were obtained possibly due to low stability of the microemulsion [Nhujak *et al.* 2006].

From Figure 3.6a, the linear relationship between *k* and [SDS] was obtained, with high values of the coefficient of linear regression (r^2) > 0.99, except for BZ8, BZ9, BA11 and PB8 with r^2 ~ 0.98. In MEKC [Muijselaar *et al.* 1994, Quirino *et al.* 1999], *k* linearly increases with [SDS] as the equation $k = K\phi = K\bar{v}$ ([SDS]-CMC), where *K* is the distribution constant of the analyte between the micellar phase and aqueous phase, ϕ is the phase ratio of the volume of micellar phase to aqueous phase, $\bar{v}$ is the partial molar volume of the micelles, and CMC is the critical micelle concentration, respectively. However, this equation has not proven to be valid for MEEKC since the values of $\bar{v}$ and CMC are difficult to determine. The values $\bar{v}$ and CMC of in MEEKC may be assumed to be equal to those in MEKC with same concentration of [SDS] and 1-butanol [Fu *et al*. 1996]. However, the relationship between *k* and [SDS] was also seemed to be linear in previous work on MEEKC [Nhujak *et al.* 2006, Ishihama *et al.* 1996, Song *et al.* 1995].

At the equal *z*, the retention factors were found to be in the order BZ > BA > PB > PN, indicating the hydrophobicity for BZ > BA > PB > PN. From Figure 3.7 and Table 3.2, the excellent linear relationship between log *k* and *z* was obtained for each series at a given [SDS] in a range of 100 to 200 mM, with r^2 > 0.999, which is consistent with the Martin's equation in Equation 3.3. Over a wide range of [SDS], the values of slope, *a*, for each homologous series were found to be insignificantly different, with RSD < 1.5%. This indicates that [SDS] does not affect a change in methylene selectivity, α_{CH2}, of two adjacent compound of homologous series, where $\log \alpha_{CH2} = a$ [Mertzman *et al*. 2005]. For analytes with equal *z*, the values of $\log \alpha_{CH2}$

obtained are in order BZ > PB > BA > PN. A decrease in α_{CH2} is possibly due to the hydrophilic functional group of analytes. Using the average value of the slope ($\bar{a}$) obtained from a plot of log *k* versus *z*, the value of the adjusted intercept (b') is seen to increase linearly with an increase in [SDS] as given in Table 3.2. In previous work on MEKC with high EOF [Muijselaar *et al.* 1994], the slope of the linear equation $\log k = az + b$ for homologous series of BZ was also found to be fairly constant, whereas the intercept increased with an increase in [SDS], which is consistent with this work.

Using BZ as standards and Equation 3.4 with the values of *a* and *b* in Table 3.2, the calculated values (symbol) of *I* for analytes as a function of [SDS] in a range of 100 to 200 mM are shown in Figure 3.6b. The calculated values of *I* for BZ were found to be in excellent agreement with the nominal values, with $|\Delta I| <$ 0.7%. By plotting *I* against [SDS] and using *t*-test comparing the observed slope of the linear fit with zero for each analyte, non-significant difference was obtained, indicating that *I* for analytes in Figure 3.6b are independent of [SDS]. Therefore, the average values of *I* (solid line) for each analyte over a wide range of [SDS] from 100 to 200 mM are shown in Figure 3.6b, with RSD < 0.5%. The independent of *I* from [SDS] is because *I* of analyte is obtained from *k* of the analyte compared to *k* of standards, and [SDS] affects a similar trend of a change in *k* of the analyte and the standards. In previous work on MEKC with high EOF, the independence of [SDS] from *I* in neutral analytes was also reported [Muijselaar *et al.* 1994].

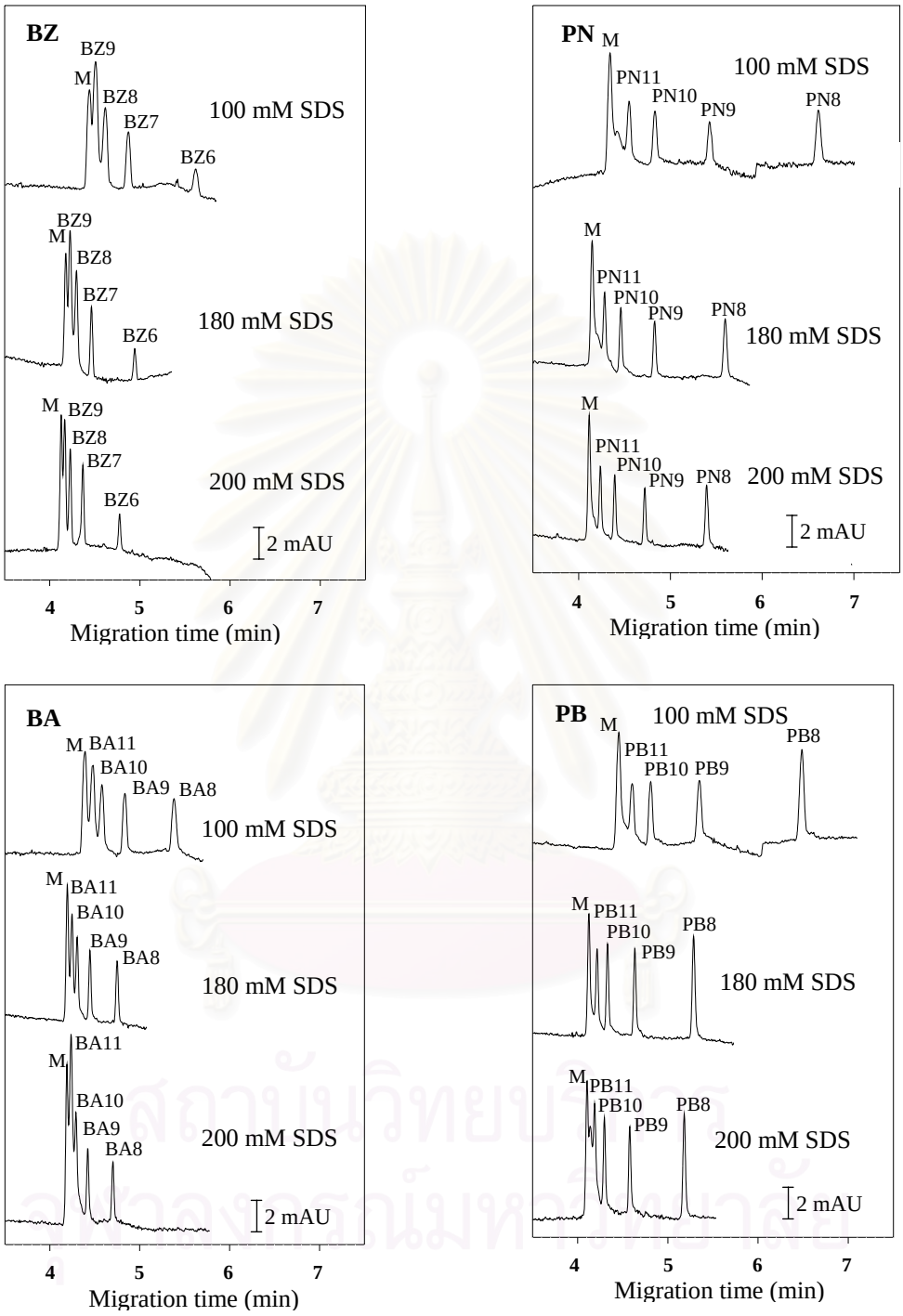


Figure 3.5 An example of electropherograms for homologous series in MEEKC using various concentrations of SDS. Other MEEKC conditions as shown in Figure 3.4.

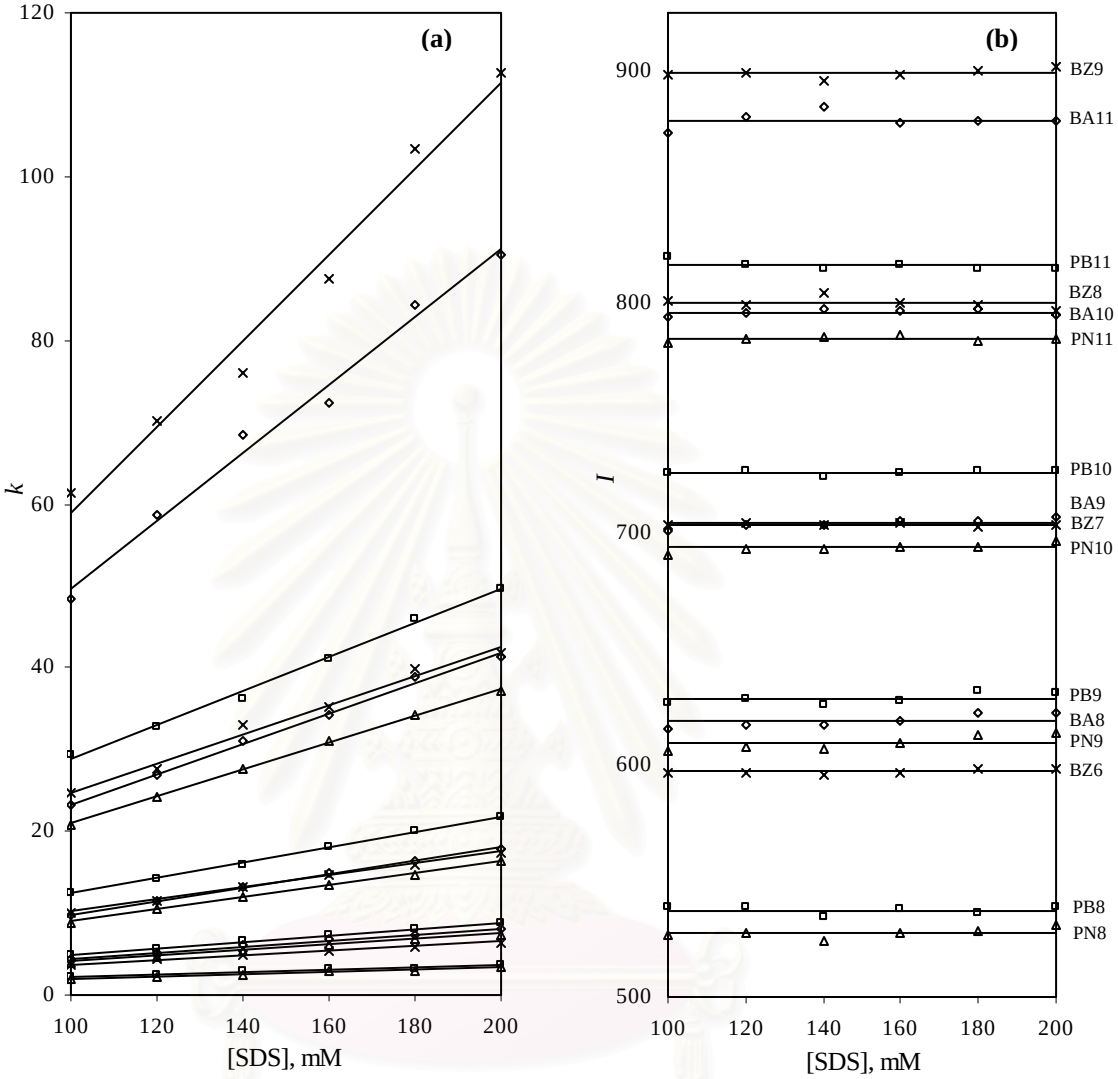


Figure 3.6 Retention factor (a) and retention index (b) of analytes as a function of SDS concentration. Conditions as shown in Figure 3.4. Symbols ×, △, ◇ and □ refer to the average observed value from two runs for homologous series of BZ, PN, BA and PB, respectively. Lines for *k* are linear fit, and lines for *I* are the average value obtained from 100 to 200 mM SDS.

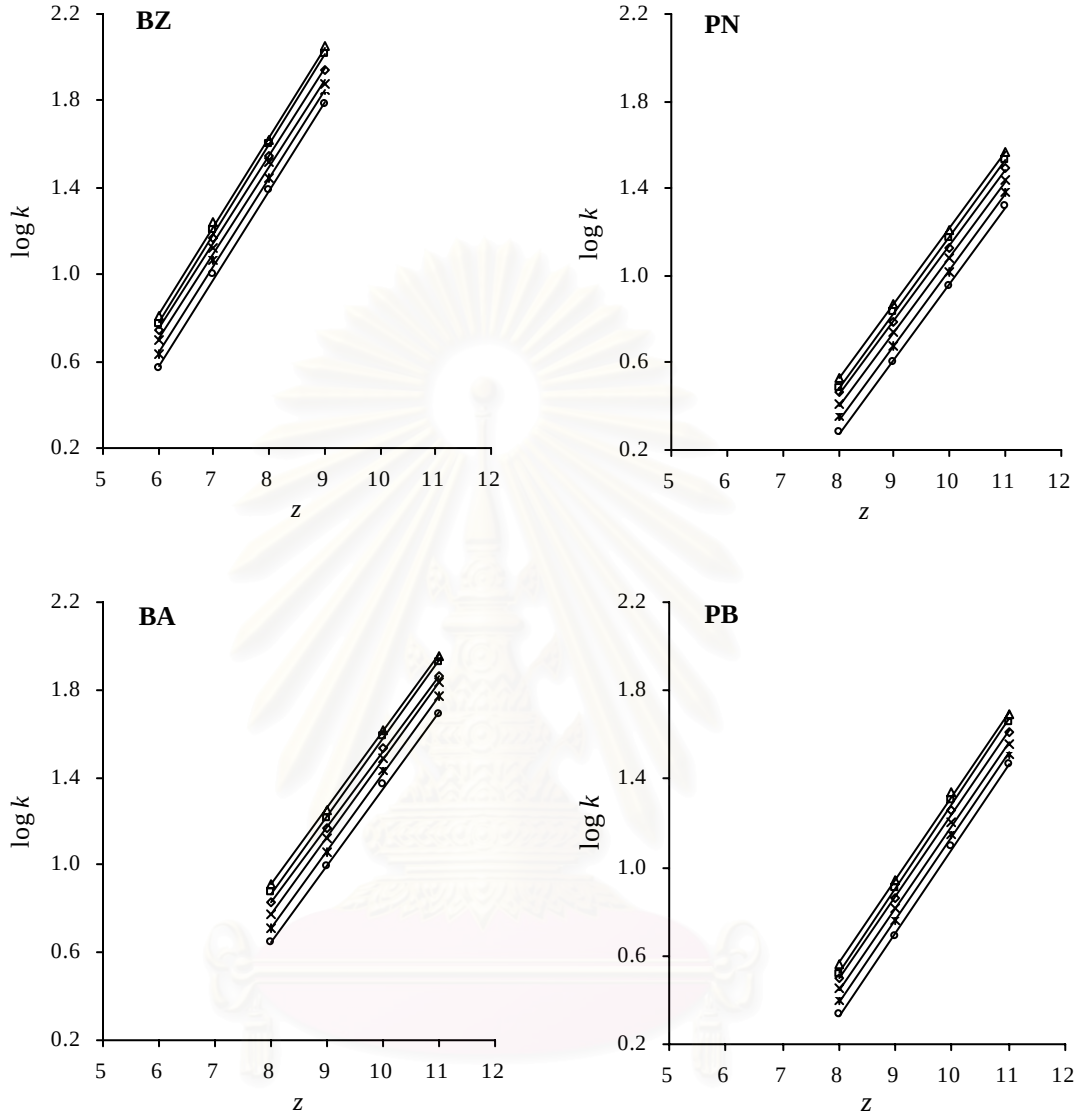


Figure 3.7 log *k* and *z* of analytes as a function of SDS concentrations of 100 (○), 120 (∗), 140 (×), 160 (◇), 180 (□) and 200 mM (△). Other MEEKC conditions as shown in Figure 3.4. Values of slope and *y*-intercept are listed in Table 3.2.

Table 3.2 Linear relationship between log *k* and *z* of homologous series compounds in MEEKC

[SDS], mM	log *k* = *az*+*b*	BZ	PN	BA	PB
100	*a*	0.405	0.345	0.350	0.381
	b	-1.847	-2.491	-2.151	-2.728
	r^2	0.9994	0.9989	0.9995	0.9995
	b'	-1.843	-2.488	-2.164	-2.686
120	*a*	0.402	0.344	0.353	0.375
	b	-1.768	-2.406	-2.112	-2.604
	r^2	0.9993	0.9993	0.9997	0.9996
	b'	-1.784	-2.420	-2.092	-2.626
140	*a*	0.396	0.344	0.354	0.372
	b	-1.664	-2.352	-2.059	-2.527
	r^2	0.9987	0.9996	0.9998	0.9997
	b'	-1.726	-2.361	-2.028	-2.575
160	*a*	0.399	0.343	0.346	0.373
	b	-1.642	-2.292	-1.935	-2.482
	r^2	0.9994	0.9994	0.9996	0.9996
	b'	-1.681	-2.309	-1.987	-2.523
180	*a*	0.412	0.348	0.354	0.383
	b	-1.696	-2.304	-1.961	-2.542
	r^2	0.9998	0.9997	0.9995	0.9995
	b'	-1.633	-2.272	-1.934	-2.485
200	*a*	0.410	0.346	0.351	0.379
	b	-1.647	-2.241	-1.896	-2.464
	r^2	0.9993	0.9998	0.9999	0.9997
	b'	-1.599	-2.231	-1.901	-2.447
$\bar{a}$		0.404±0.006	0.345±0.002	0.351±0.003	0.377±0.004
b'		0.00245[SDS]-2.079 r^2 = 0.9920	0.00254[SDS]-2.728 r^2 = 0.9866	0.00261[SDS]-2.410 r^2 = 0.9845	0.00239[SDS]-2.915 r^2 = 0.9920

$\bar{a}$ is the average value of the slope obtained from a plot of log *k* and *z* over 100 to 200 mM SDS. b' is the adjusted intercept obtained from a linear plot between log *k* and *z* with a constant slope of $\bar{a}$.

3.3.4 Effect of temperature on retention factor and retention index

The influence of temperatures, 15 to 40°C (298 to 313 K), on *k* and *I* in MEEKC was investigated using the microemulsion buffer as given in Figure 3.4. An example of electropherograms is shown in Figure 3.8. At each temperature, the constant current of 95 μA was used to exclude difference in Joule heating [Muijselaar *et al.* 1994], with a power < 1.5 W at the temperature above 25°C. As seen in Figure 3.9a, *k* linearly decreases with increasing temperature, with $r^2 > 0.96$. An increase in separation temperature results in a decrease in *k* due to an increase in the solubility of insoluble compounds in the organic-water phase [Altria *et al.* 2000b]. According to the van't Hoff equation [Muijselaar *et al.* 1994], ln *K* and ln *k*, where $k = K\phi$, are proportional to $1/T$ as the Equations

$$\ln K = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} \qquad (3.7)$$

$$\ln k = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \qquad (3.8)$$

where ΔH^o and ΔS^o are the standard enthalpy and standard entropy, respectively, and *R* is the gas constant. The phase ratio in MEECK is difficult to determine as previously mentioned. In previous work on MEEKC with high EOF using dodecoxycarbonylvaline (DDCV) as surfactant , ethyl acetate as oil and 1-butanol as co-surfactant, a linear increase in *K* was found with an increase in $1/T$ at 1% DDCV, while non-linear relationship was obtained at 4% DDCV [Mertzman *et al*. 2005]. Since 4% DDCV gave a higher concentration of surfactant to phase ratio, a temperature change had a much more dramatic effect with respect to either the phase ratio or the respective microemulsion conformation [Mertzman *et al*. 2005]. The linear relationship between the plot of ln *k* versus $1/T$ in Figure 3.9b may be due to a slight or no change in the phase ratio in MEEKC used in this work. The similar result was found in our previous work also on curcuminoids [Nhujak *et al*. 2006] and avermectins [Seelanan *et al*. 2006].

The ln *k* values of BZ in MEEKC in the temperature range of 15 to 40°C were also determined using constant power of 1.5 W. Above 25°C, results showed that the constant power of 1.5 W gave ln *k* slightly higher than did the constant current of 95 μA, with $|\Delta k| < 1.5\%$, while below 25°C, the slightly lower ln *k* was obtained using constant power of 1.5 W, with $|\Delta k| < 2$ %. At a given temperature, non-significant difference was found for ln *k* of BZ obtained from the constant current and the constant power, using paired *t*-test analysis at 95% confidence interval of the mean. This indicates insignificant deviation in ln *k* caused by Joule heating from use of the constant current and the constant power. In addition, plots of ln *k* and 1/*T* using the constant power give linear equations with $r^2 > 0.97$. However, a current lower than 95 μA or the power lower than 1.5 W may be used to keep Joule heating to the minimum, but a long analysis time will be obtained.

From Figure 3.10, the excellent linear relationship between log *k* and *z* was obtained for each series at a given temperature in a range of 15 to 40°C, with $r^2 > 0.999$, which is consistent with the Martin's equation in Equation 3.3. This result is similar to that obtained at a given [SDS] as described previously in Section 3.3.3.

According to Equation 3.4 and using BZ as retention index standards, the retention indices of analytes obtained are shown in Figure 3.9c. Using *t*-test comparing the observed slope of the linear fit with zero for each analyte over a temperature range of 15 to 40°C, non-significant difference was obtained, indicating that *I* for analytes in Figure 3.9c are independent of temperature. Therefore, the average values *I* (solid line) for each analyte over a wide range of temperature from 15 to 40°C are shown in Figure 3.9c, with RSD < 1.2%. The independent of *I* from temperature is because temperature affects a similar trend of a change in *k* of the analyte and the standards. In previous work on MEKC [Muijselaar *et al.* 1994], the rather small dependence of temperature on *I* was reported, using BZ as the retention index standards and constant current.

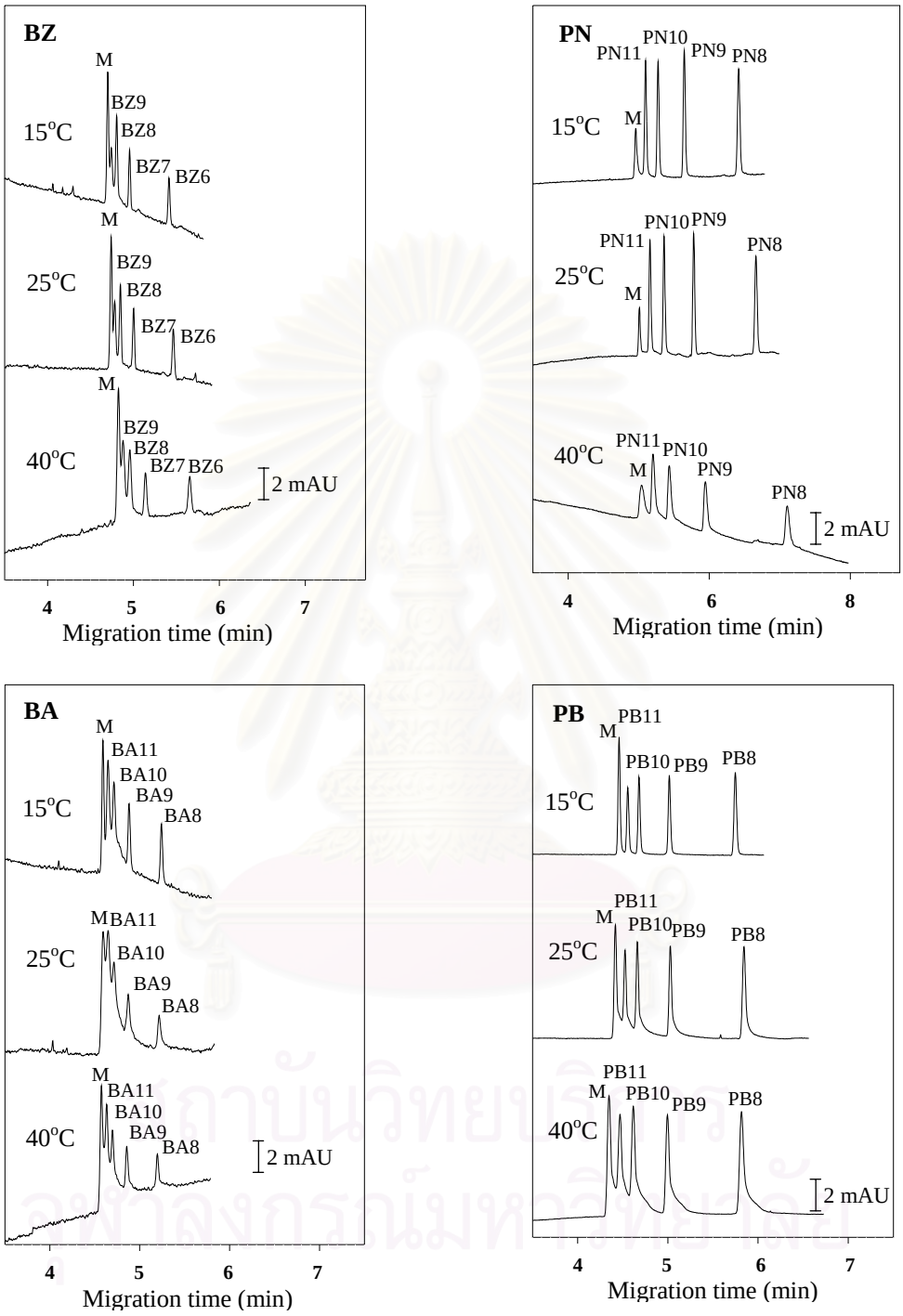


Figure 3.8 An example of electropherograms for homologous series in MEEKC using various temperatures. Other MEEKC conditions as shown in Figure 3.4.

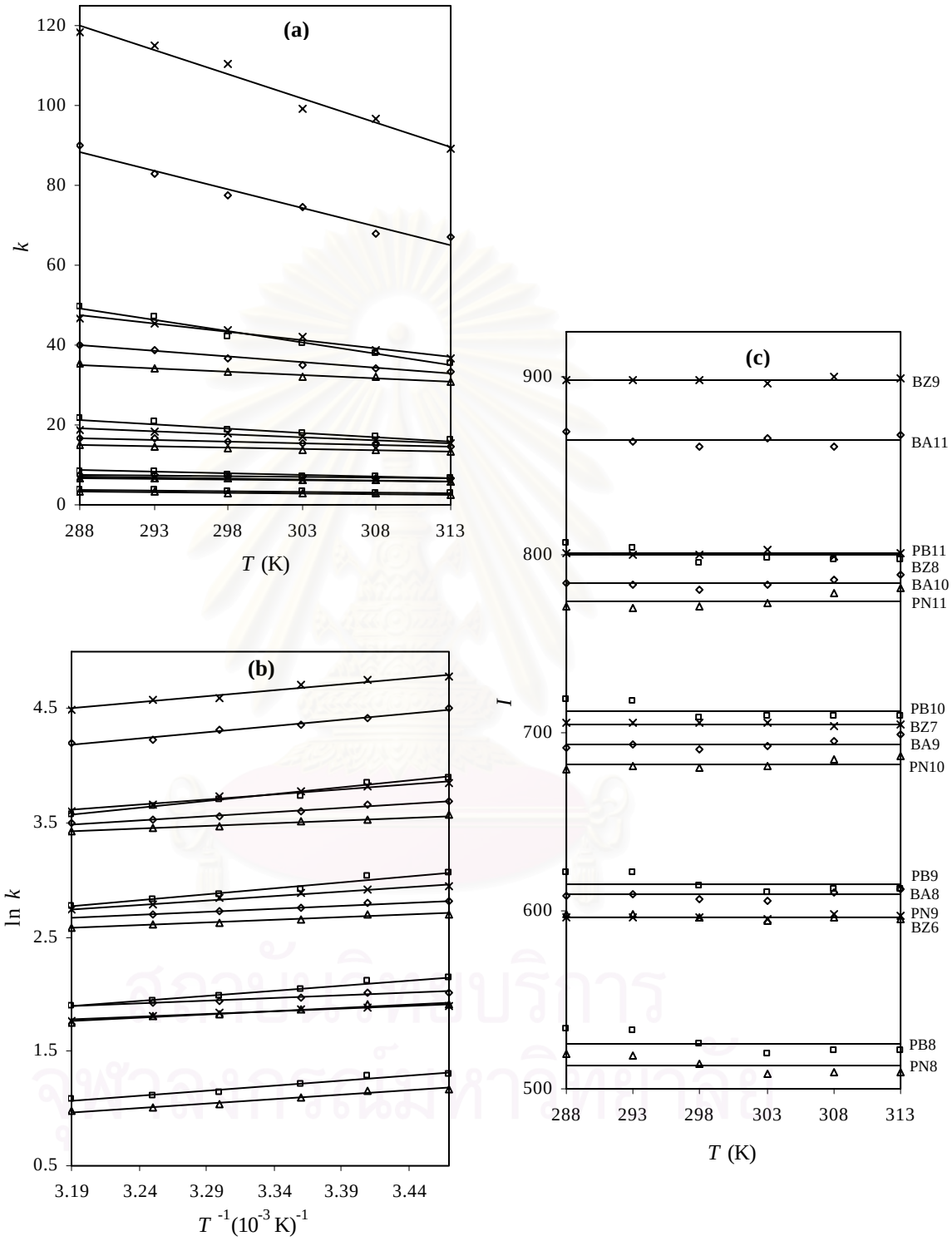


Figure 3.9 Retention factor (a), natural logarithm of retention factor (b), and retention index (c) of analytes as a function of temperature. Other MEEKC conditions as shown in Figure 3.4. Symbols refer to the average observed value from two runs for homologous series as given in Figure 3.6. Lines for *k* and ln *k* are linear fit, and lines for *I* are the average value obtained from 15 to 40°C.

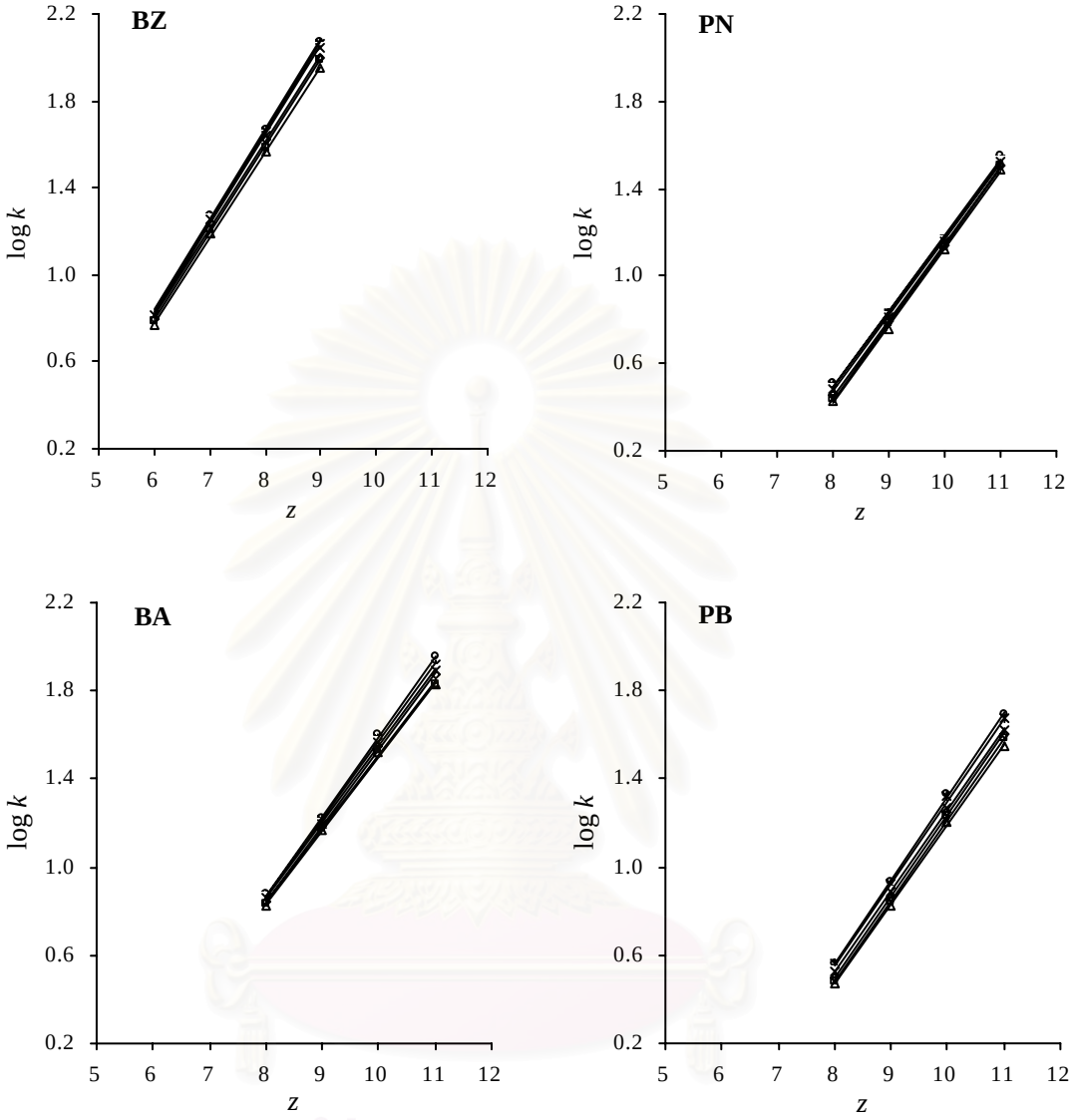


Figure 3.10 log *k* and *z* of analytes as a function of temperature of 15 (○), 20 (∗), 25 (×), 30 (◇), 35 (□) and 40°C (△). Other MEEKC conditions as shown in Figure 3.4.

3.3.5 Effect of voltage on retention factor and retention index

The influence of applied voltage, -10 to -20 kV, on *k* and *I* in MEEKC was carried out using the microemulsion buffer as given in Figure 3.4. An example of electropherograms is shown in Figure 3.11. At applied voltage over -20 kV, higher noise and broad peaks were obtained possibility due to excess Joule heating. Theoretically, the retention factor is independent of the voltage. However, as seen in Figure 3.12a, a decrease in *k* with increasing applied voltage in a range of -10 to -20 kV, was obtained possibility due to excess an increase in temperature caused by Joule heating.

From Figure 3.13, the excellent linear relationship between log *k* and *z* was obtained for each series at a given applied voltage in a range of -10 to -20 kV, with $r^2 > 0.999$, which is consistent with the Martin's equation in Equation 3.3. This result is similar to that obtained at a given [SDS] as described previously in Section 3.3.3.

According to Equation 3.4 and using BZ as retention index standards, the retention indices of analytes obtained are shown in Figure 3.12b. Using *t*-test comparing the observed slope of the linear fit with zero for each analyte over an applied voltage range of -10 to -20 kV, non-significant difference was obtained, indicating that *I* for analytes in Figure 3.12b are independent of applied voltage. Therefore, the average values *I* (solid line) for each analyte over a wide range of applied voltage from -10 to -20 kV are shown in Figure 3.12b, with RSD < 0.6%. The independent of *I* from applied voltage is because applied voltage affects a similar trend of a change in *k* of the analyte and the standards.

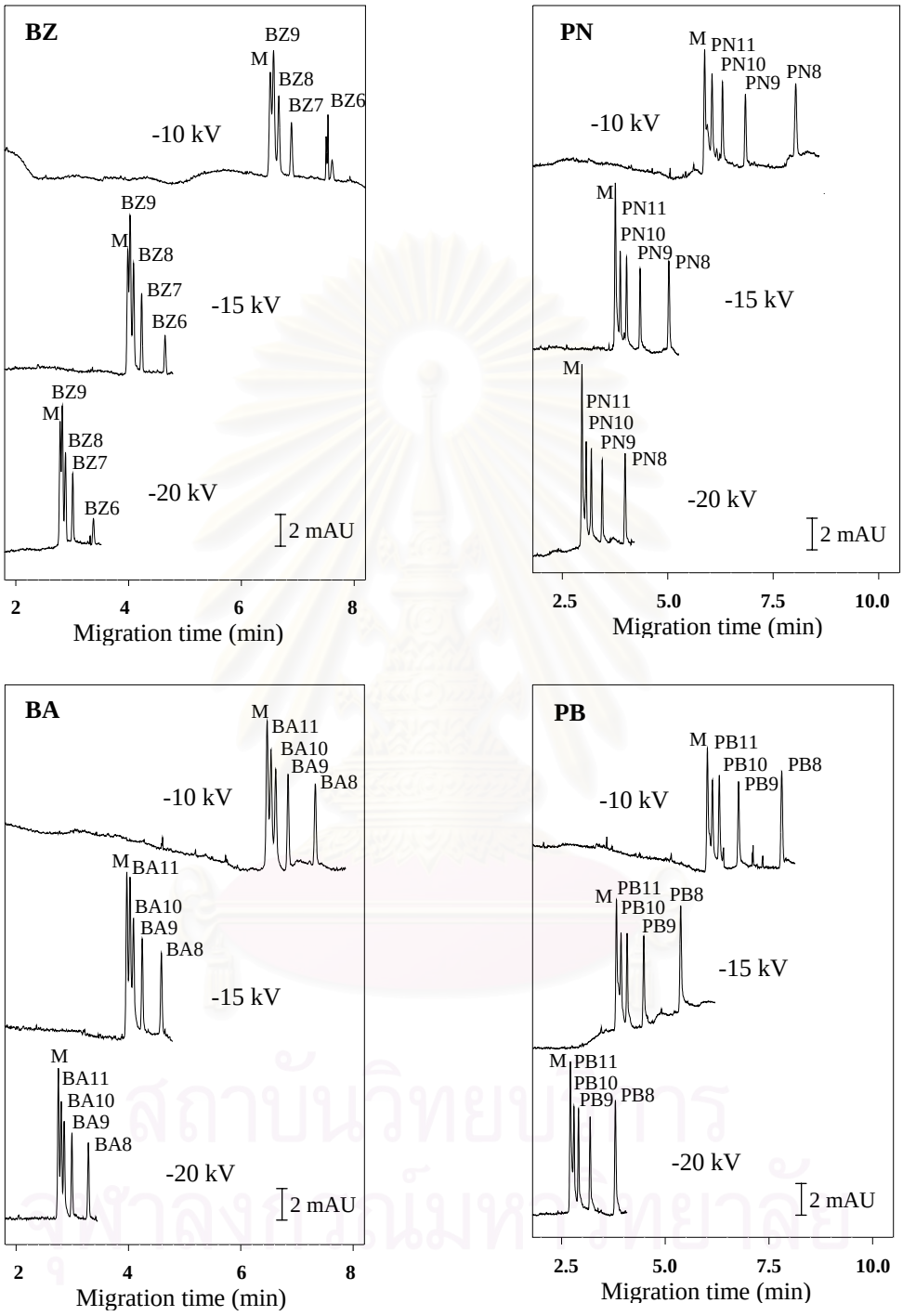


Figure 3.11 An example of electropherograms for homologous series in MEEKC using various applied voltage. Other MEEKC conditions as shown in Figure 3.4.

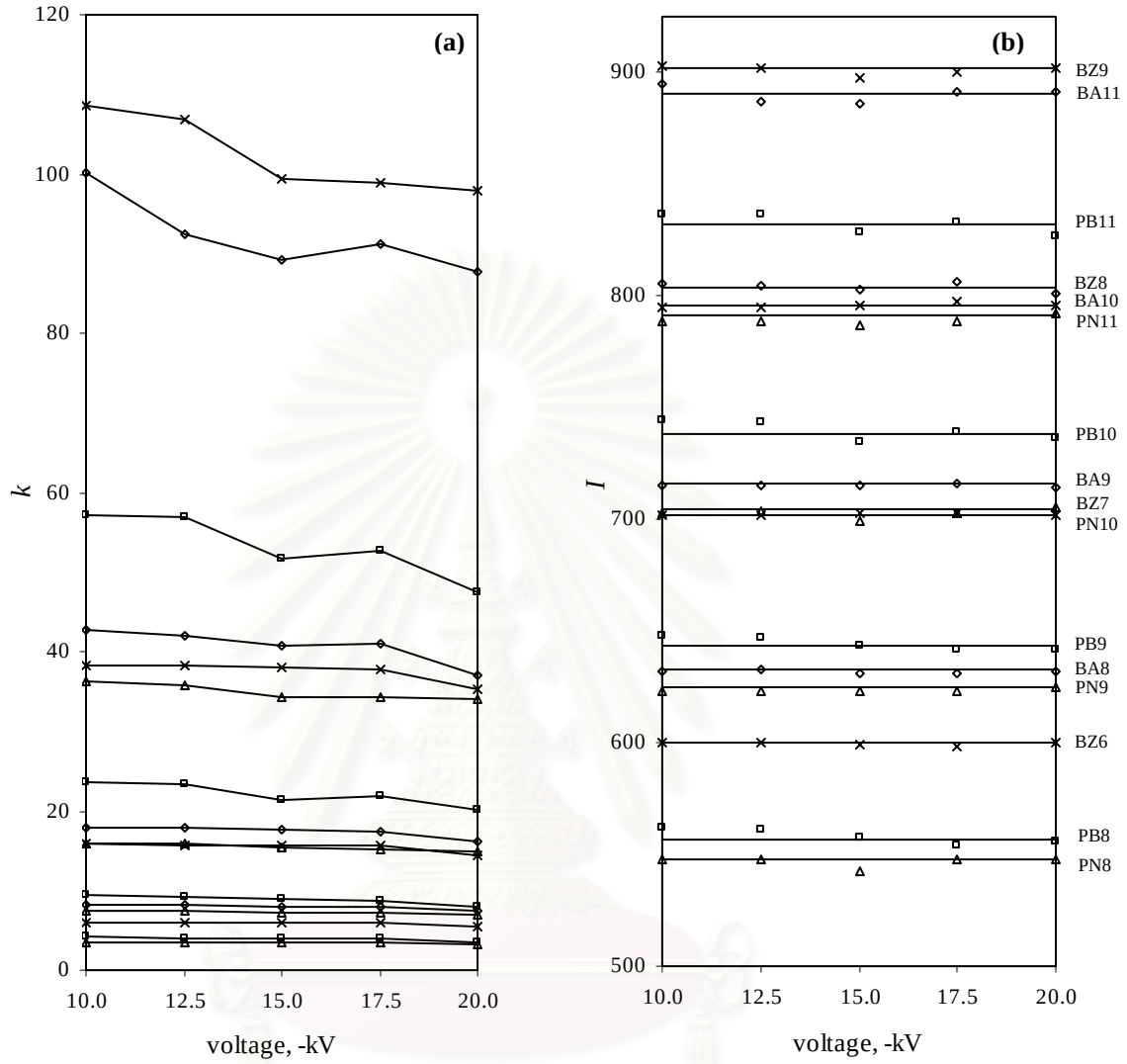


Figure 3.12 Retention factor (a), and retention index (b) of analytes as a function of applied voltage. Other MEEKC conditions as shown in Figure 3.4. Symbols refer to the average observed value from two runs for homologous series as given in Figure 3.6. Lines for *I* are the average value obtained from -10 to -20 kV.

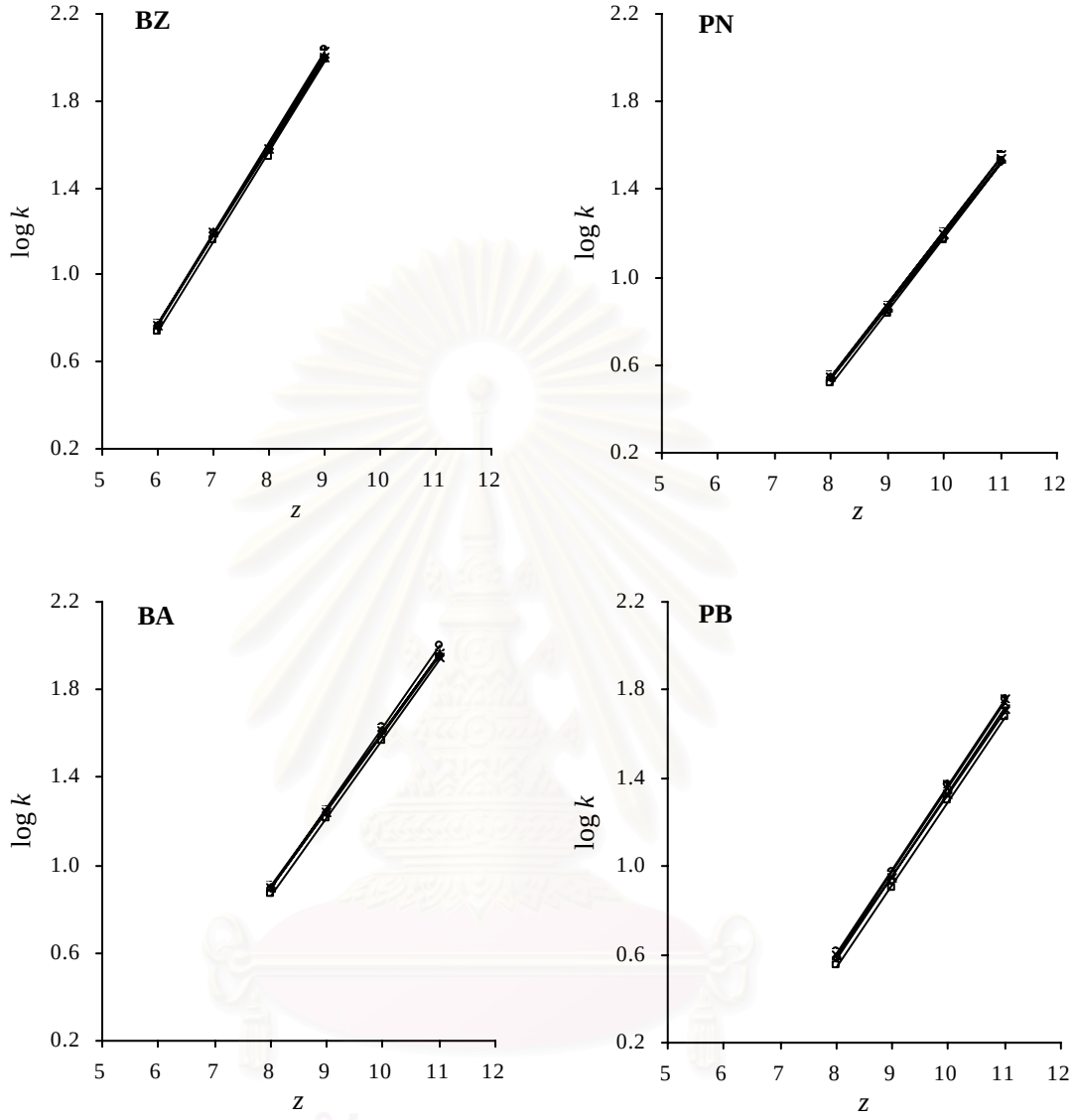


Figure 3.13 log *k* and *z* of analytes as a function of applied voltage of -10.0 (○), -12.5 (∗), -15.0 (×), -17.5 (◇) and -20.0 kV (□). Other MEEKC conditions as shown in Figure 3.4.

3.3.6 Effect of organic co-solvents on retention factor and retention index

To investigate the influence of organic co-solvent on *k* and *I*, the organic co-solvent, such as ACN, MeOH, EtOH and 2-PrOH, were separately added at the level of 0 to 30% v/v in the microemulsion buffer using 180 mM SDS and other components as given in Figure 3.4. An example of electropherograms is shown in Figures 3.14 to 3.17. From Figure 3.18, the retention factor was found to decrease with an increase in the concentration of organic co-solvent (φ) due to an increase in the solubility of analyte in organic-aqueous phase [Altria 2000a, Nhujak *et al.* 2006]. When ACN, MeOH and EtOH was used as organic co-solvent, the relationship between log *k* and φ gave good linear equations with r^2 ranging from 0.93 to 0.99, while the second-degree polynomial equations were obtained for 2-PrOH used as organic co-solvent with $r^2 > 0.99$. In reversed-phase liquid chromatography, a decrease in log *k* as a function of an increase in φ is given by the simple linear equation [Jandera *et al.* 1996, Muijselaar *et al.* 1995, Ahuja *et al.* 1995]

$$\log k = \log k_w - m\varphi \qquad (3.9)$$

where k_w is the retention factor extrapolated to pure water as the mobile phase, and *m* is the slope from the linearity of the plot of log *k* versus φ. However, the deviation from the linear plot of log *k* versus φ may be obtained, and a second-degree polynomial equation can be used to describe the curvature of the plot of log *k* versus φ as the equation [Jandera *et al.* 1996, Muijselaar *et al.* 1995]

$$\log k = \log k_w - p\varphi + q\varphi^2 \qquad (3.10)$$

where *p* and *q* are the constant values from the curvature of the plot of log *k* versus φ.

The different effect between 2-PrOH and other solvents may be caused by the different chromatographic behavior with more hydrophobic organic co-solvent. MeOH, EtOH and ACN are more polar organic co-solvent and have little or no interaction with the charged oil droplets, while 2-PrOH is more hydrophobic than

other organic co-solvents and can act as co-surfactant [Altria *et al.* 2006b]. Therefore, the less amount of free 2-PrOH in the aqueous phase than the total 2-PrOH added leads to higher *k* than expected (see Equation 3.10 and Figure 3.18), and the deviation of log *k* from linear relationship between log *k* and φ. In MEKC with high EOF and 0 to 20% v/v organic solvent, a linear relationship of ln *k* versus φ was obtained for MeOH [Muijselaar *et al.* 1995] and ACN [Ahuja *et al.* 1995], while the deviation of log *k* from linear relationship was observed at the high concentrations of ACN (20 to 30%) [Muijselaar *et al.*1995]. In MEEKC with high EOF, addition of organic co-solvents, such as ACN [Harang *et al.* 2004] and 2-PrOH [Gong *et al.* 2004], affected a decrease in *k*, but the equation for relationship between log *k* and φ was not reported.

From Figures 3.19 to 3.22, the excellent linear relationship between log *k* and *z* was obtained for each series at a given φ in a range of 0 to 30% v/v, with $r^2 > 0.999$, which is consistent with the Martin's equation in Equation 3.3. This result is similar to that obtained at a given [SDS] as described previously in Section 3.3.3.

According to Equation 3.4 and using BZ as retention index standards, the calculated values of *I* for analytes as a function of φ in a range of 0 to 30 %v/v are shown in Figure 3.23. The calculated values of *I* for all analytes were found to decrease with an increase in φ. This indicates that *I* is dependent on φ and types of organic co-solvents because the different solubility of analyte and standards in the organic co-solvents result in the different trend of a change in *k* of the analyte and the standards.

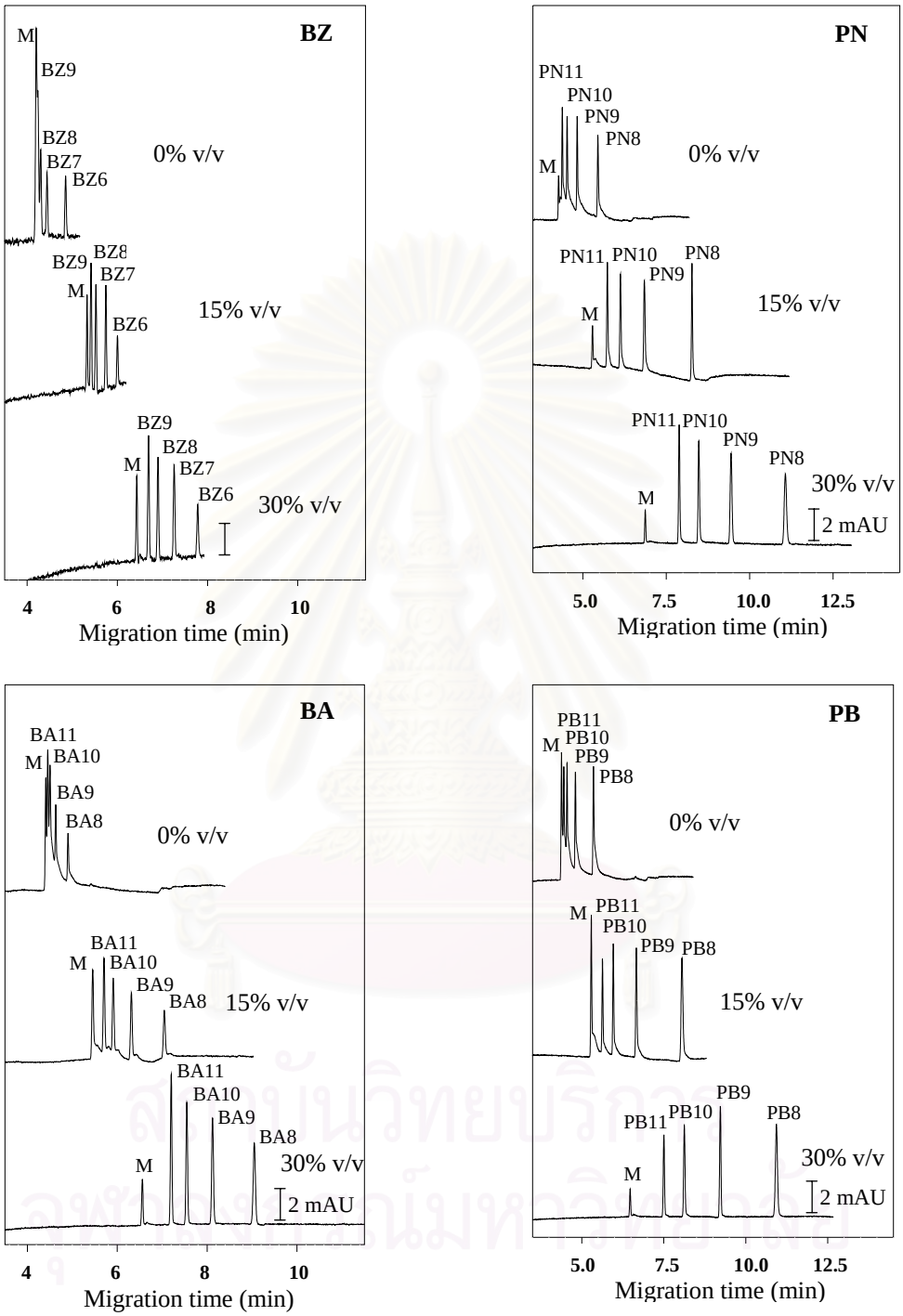


Figure 3.14 An example of electropherograms for homologous series in MEEKC using various concentrations of ACN. Other MEEKC conditions as shown in Figure 3.4.

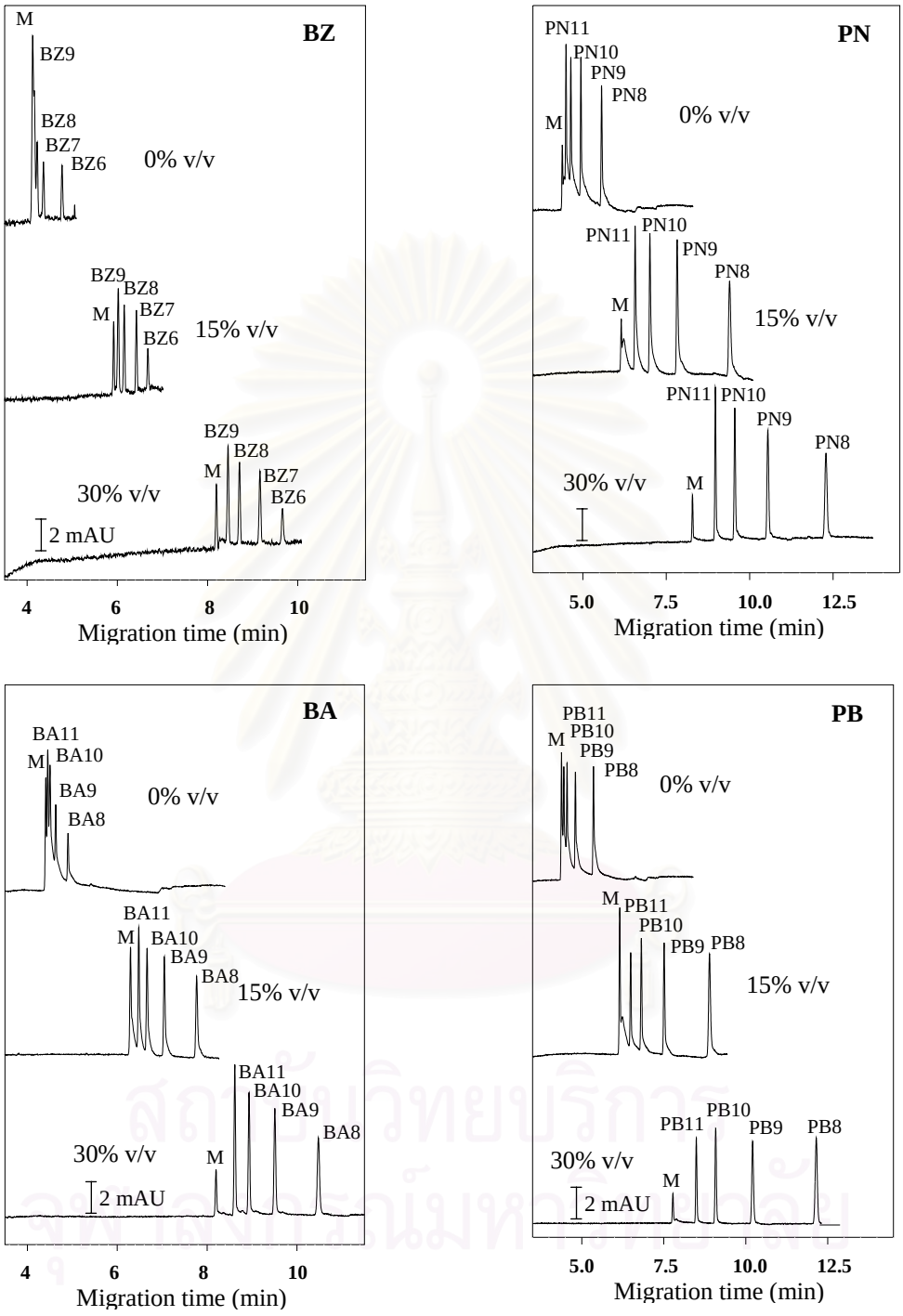


Figure 3.15 An example of electropherograms for homologous series in MEEKC using various concentrations of MeOH. Other MEEKC conditions as shown in Figure 3.4.

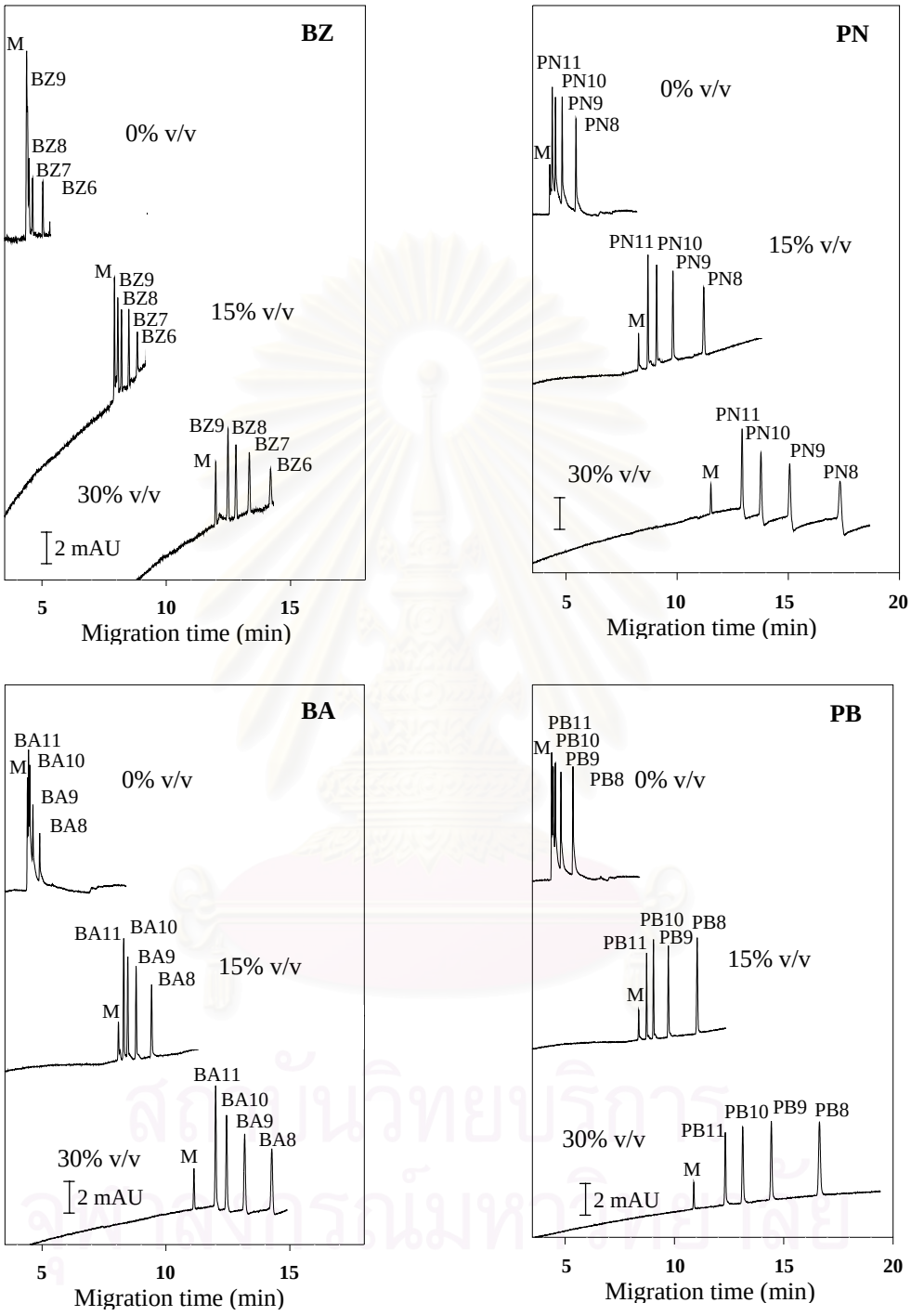


Figure 3.16 An example of electropherograms for homologous series in MEEKC using various concentrations of EtOH. Other MEEKC conditions as shown in Figure 3.4.

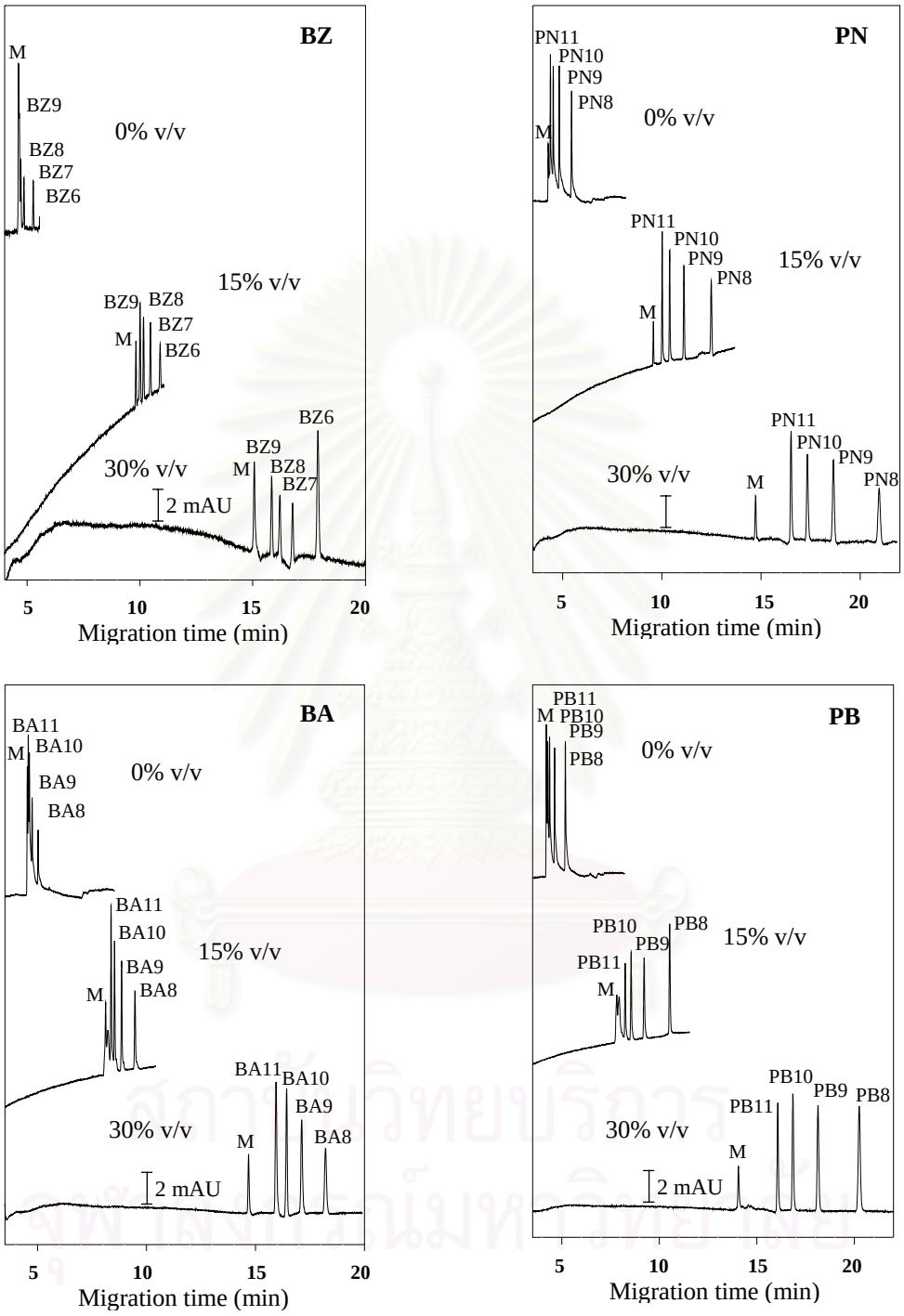


Figure 3.17 An example of electropherograms for homologous series in MEEKC using various concentrations of 2-PrOH. Other MEEKC conditions as shown in Figure 3.4.

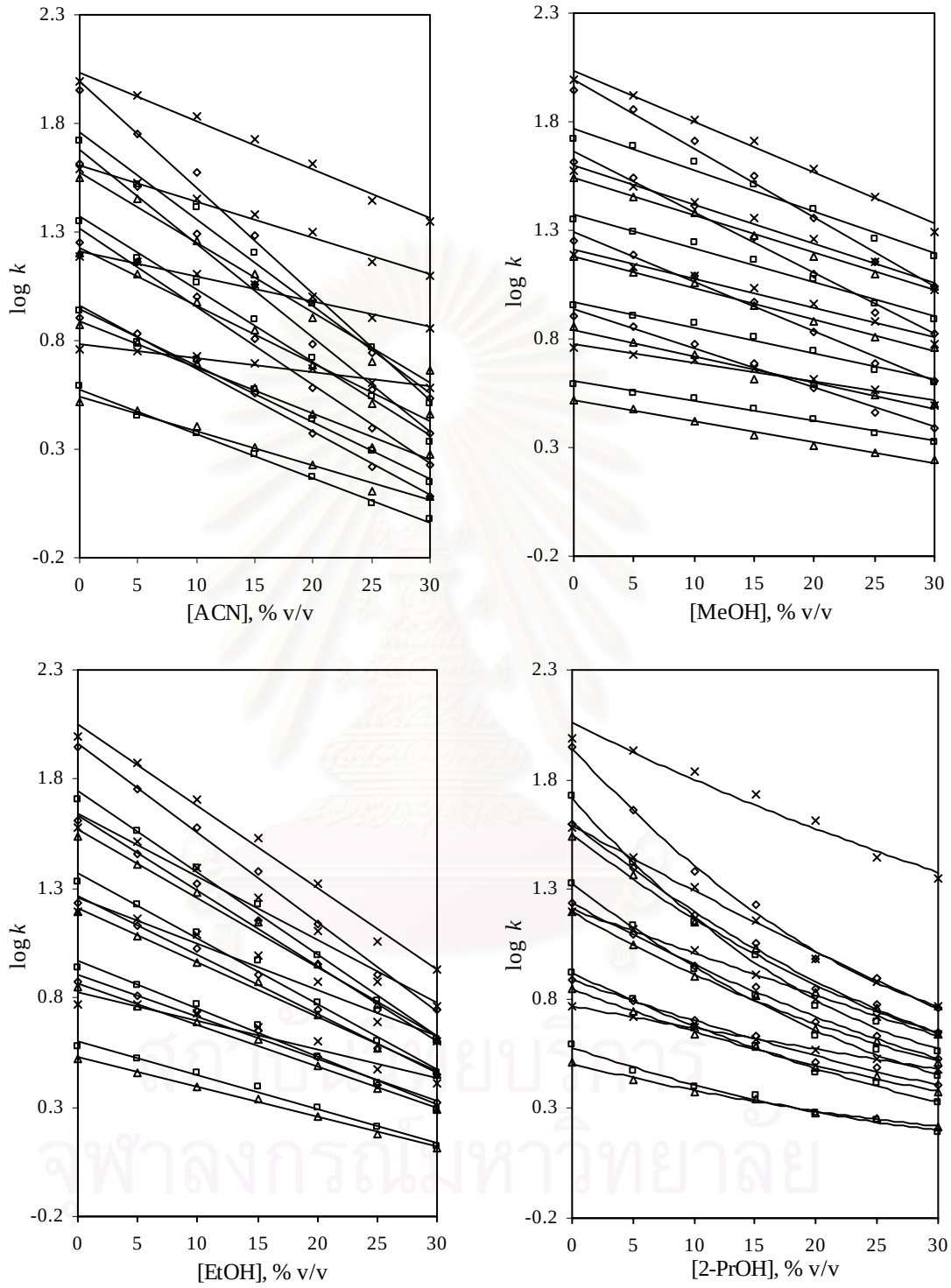


Figure 3.18 Retention factor of analytes as a function of the concentration of organic co-solvents using 180 mM SDS and other MEEKC conditions as shown in Figure 3.4. Symbols refer to the average observed value from two runs for homologous series as given in Figure 3.6. At 0% v/v organic co-solvent, observed log *k* is in order BZ9 > BA11 > PB11 > BZ8 > BA10 > PN11 > PB10 > BA9 > BZ7 > PN10 > PB9 > BA8 > PN9 > BZ6 > PB8 > PN8.

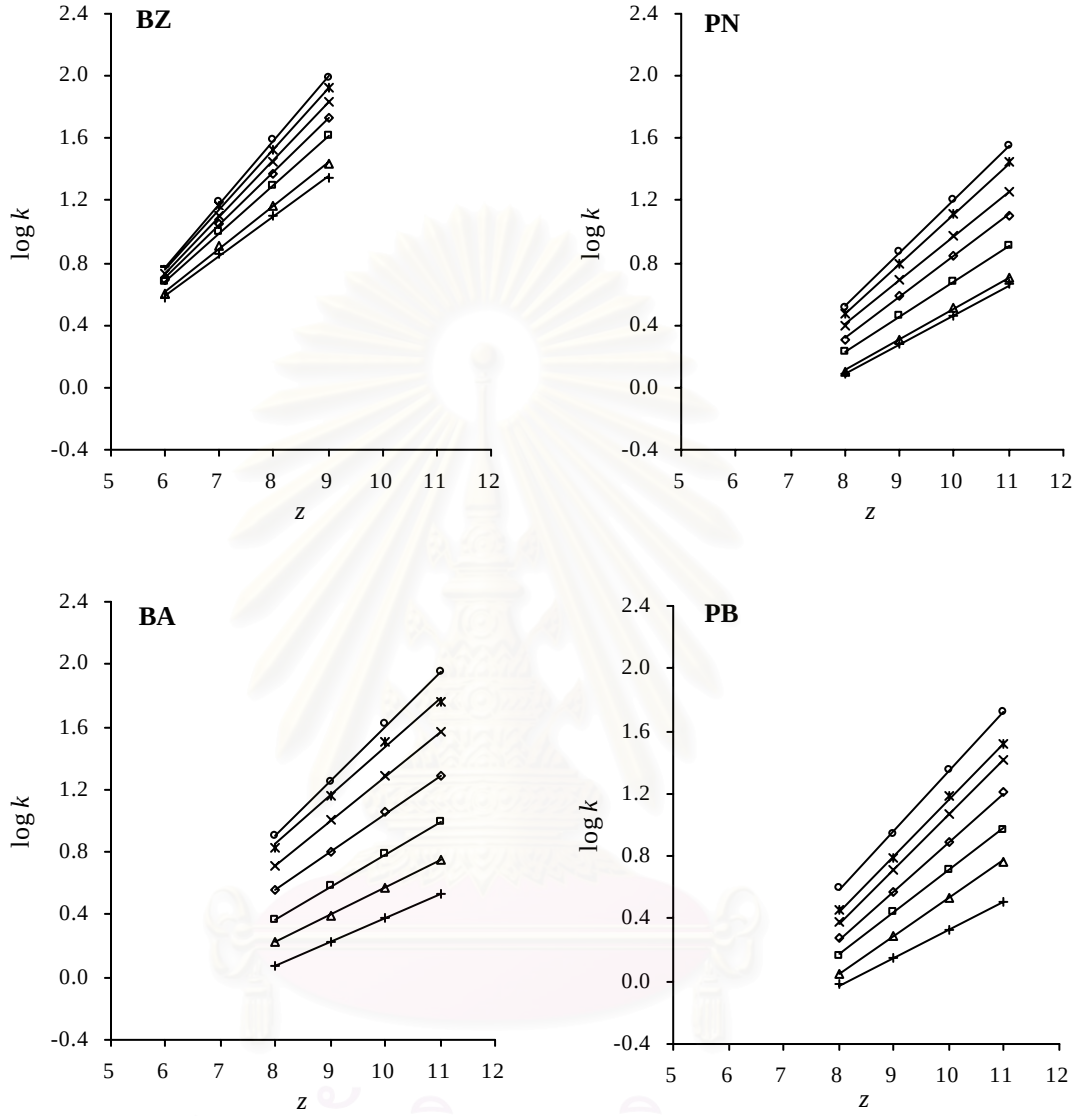


Figure 3.19 log *k* and *z* of analytes as a function of ACN concentrations of 0 (○), 5 (∗), 10 (×), 15 (◇), 20 (□), 25 (△) and 30% v/v (+). Other MEEKC conditions as shown in Figure 3.4.

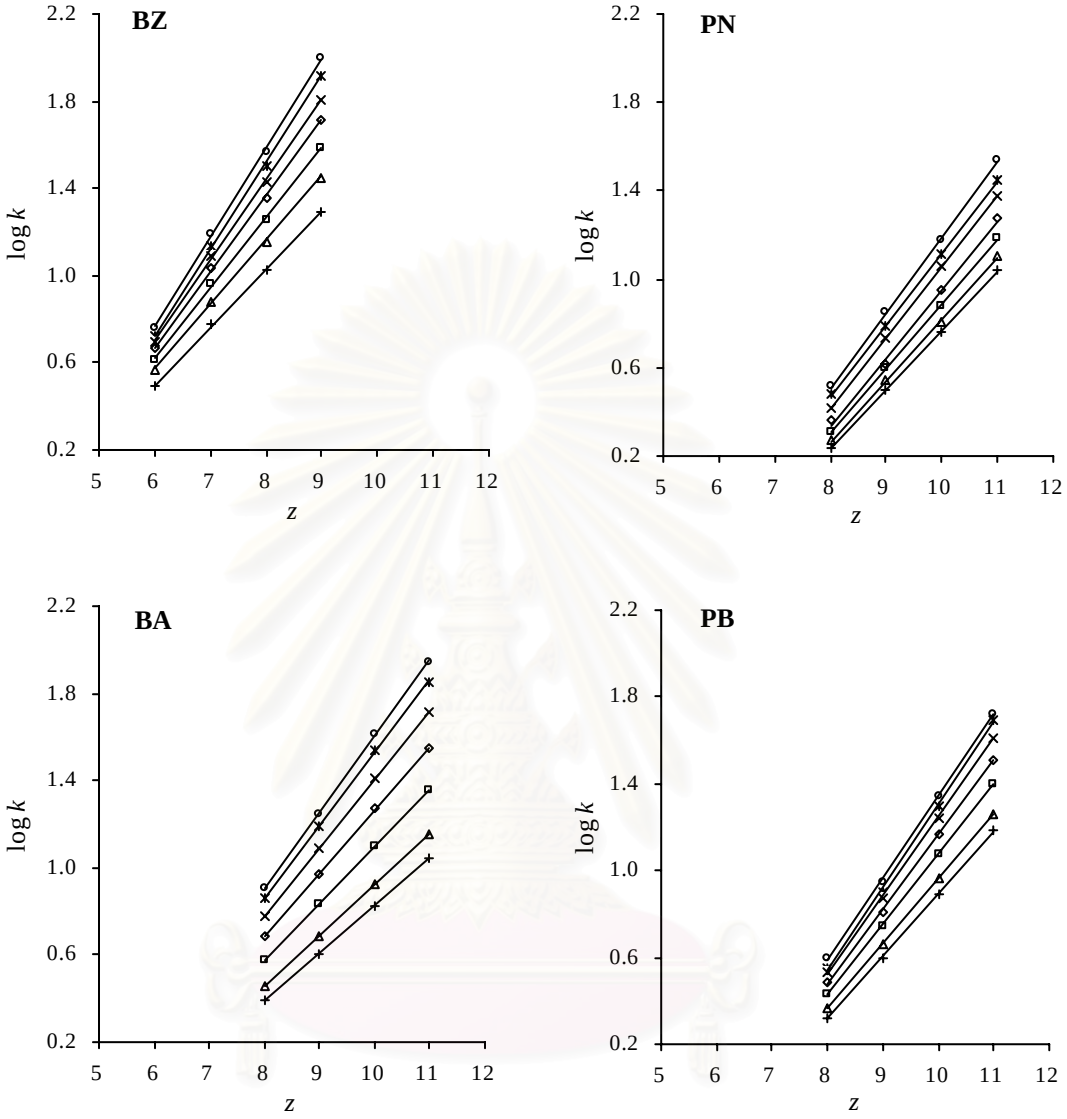


Figure 3.20 log *k* and *z* of analytes as a function of MeOH concentrations of 0 (○), 5 (∗), 10 (×), 15 (◇), 20 (□), 25 (△) and 30% v/v (+). Other MEEKC conditions as shown in Figure 3.4.

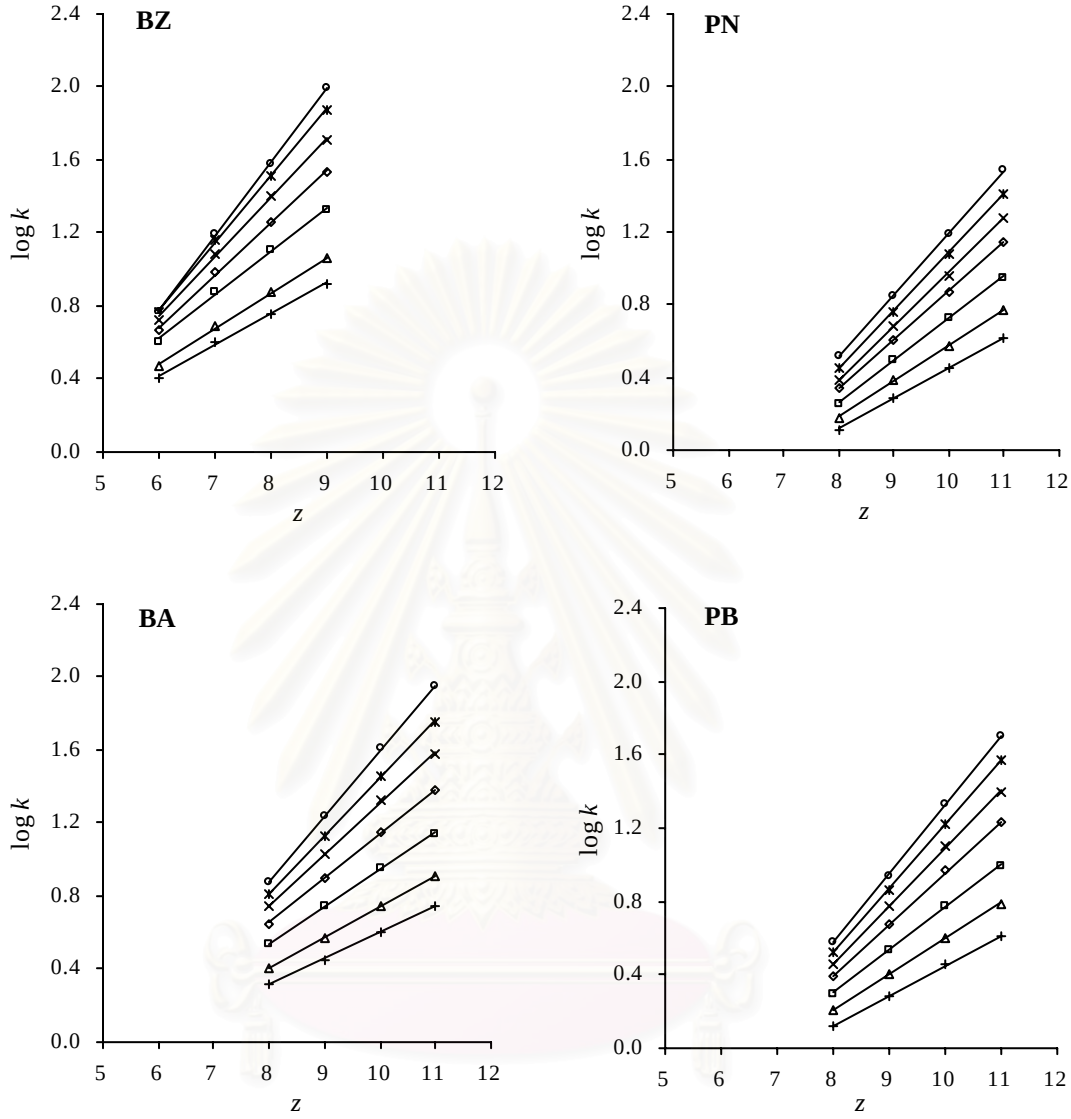


Figure 3.21 log *k* and *z* of analytes as a function of EtOH concentrations of 0 (○), 5 (∗), 10 (×), 15 (◇), 20 (□), 25 (△) and 30% v/v (+). Other MEEKC conditions as shown in Figure 3.4.

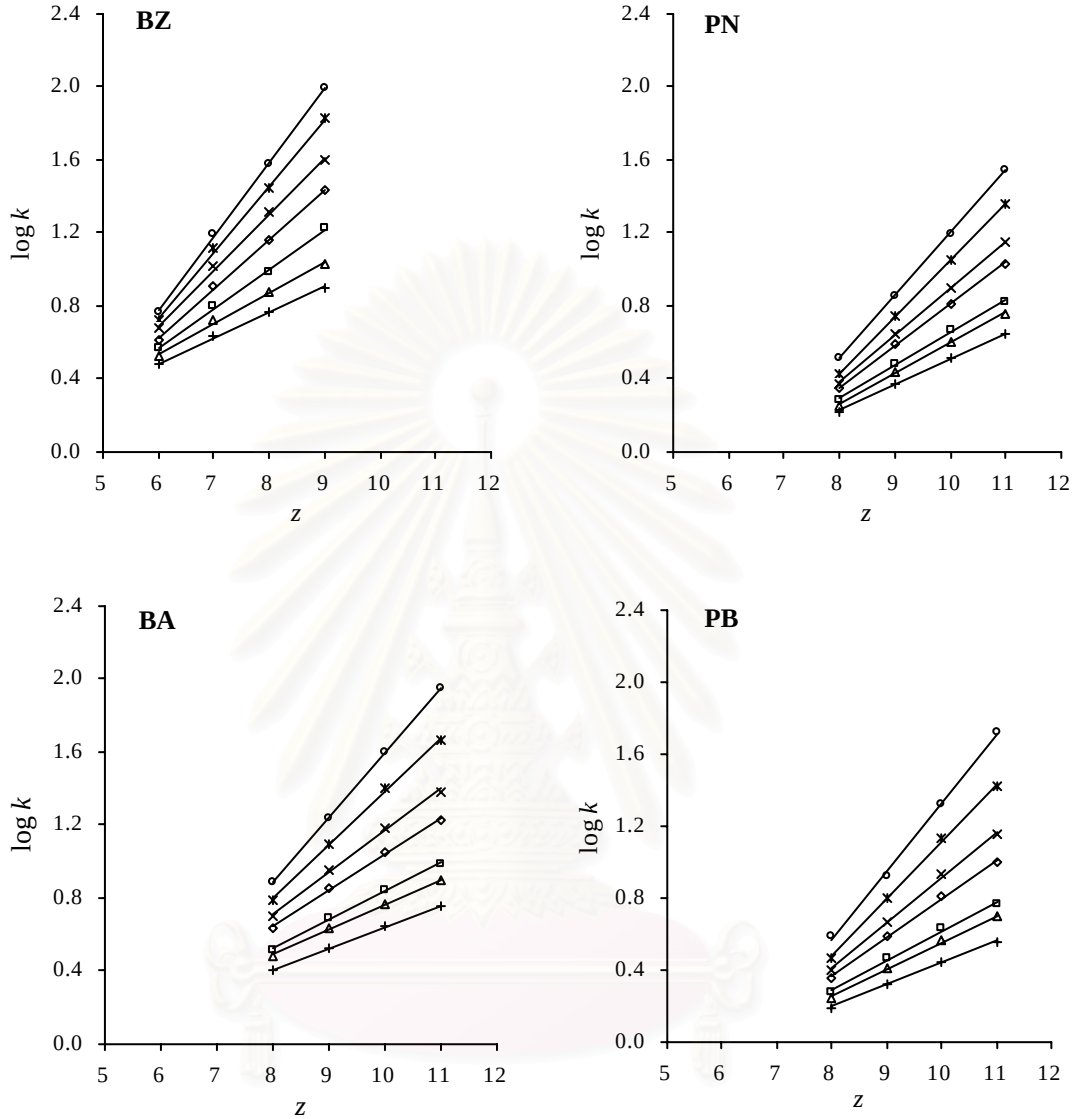


Figure 3.22 log *k* and *z* of analytes as a function of 2-PrOH concentrations of 0 (○), 5 (∗), 10 (×), 15 (◇), 20 (□), 25 (△) and 30% v/v (+). Other MEEKC conditions as shown in Figure 3.4.

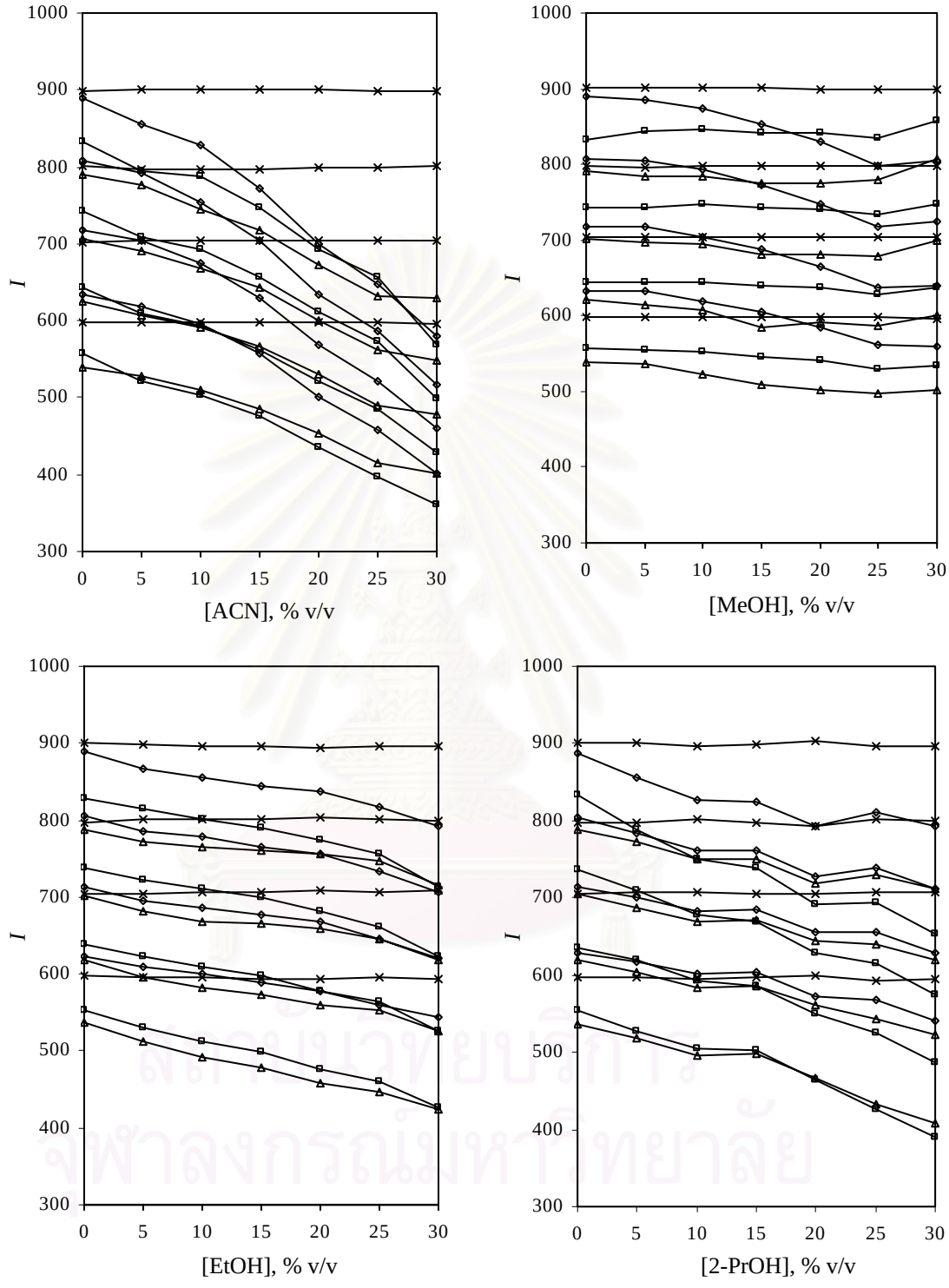


Figure 3.23 Retention index of analytes as a function of the concentration of organic co-solvents using 180 mM SDS and other MEEKC conditions as shown in Figure 3.4. Symbols refer to the average observed value from two runs for homologous series as given in Figure 3.6. At 0% v/v organic co-solvent, observed log *k* is in order BZ9 > BA11 > PB11 > BZ8 > BA10 > PN11 > PB10 > BA9 > BZ7 > PN10 > PB9 > BA8 > PN9 > BZ6 > PB8 > PN8.

3.4 Conclusion

In MEEKC with suppressed EOF, both retention factor and retention index can be used for peak identification of homologous series compounds, such as BZ, PN, BA and PB. However, the retention index shows better precision. The retention index is independent of [SDS] in a range of 100 to 200 mM, temperature in a range of 15 to 40^{o}C and applied voltage in a range of -10 to -20 kV, while is dependent on φ. The retention factor linearly increases with increasing [SDS], while linearly decreases with increasing temperature. The value of log *k* linearly decreases with increasing φ used as MeOH, EtOH or ACN, while a second-degree polynomial decrease with increasing concentration of 2-PrOH was obtained. In addition, the retention factor decrease with increasing applied voltage. Excellent linear relationship is obtained between log *k* and *z* of homologous series compounds over a wide range of [SDS], φ, temperature and applied voltage. Therefore, factors affecting retention behaviors in MEEKC can be explained using theory of HPLC and MEKC.

CHAPTER IV

COMPARISON OF RESOLUTION IN MEEKC AND MEKC EMPLOYING SUPPRESSED ELECTROOSMOSIS: APPLICATION TO BISPHENOL-A-DIGLYCIDYL ETHER AND ITS DERIVATIVES

4.1 Introduction

In MEKC and MEEKC, separation of two analytes can be described by selectivity and resolution. To obtain high separation, the high difference in *k* or high selectivity is required. In addition, the higher the resolution, the better the separation. In MEEKC, both microemulsion buffer components and parameters of CE instrument have been shown to affect separation of a wide range of compounds [Altria *et al.* 2000a, McEvoy *et al.* 2007].

Typically, MEEKC separations are performed using phosphate or borate buffers at the concentration of 50 or 10 mM for phosphate or borate buffer, respectively. Using a low buffer concentration results in poor precision in migration time, while the higher concentration of the buffer generates high current and Joule heating, which may limit the use of high voltage and temperature [Altria *et al*. 2000a]. Zwitterionic buffers such as 2-[2-amino-2-oxoethyl)amino]ethanesulfonic acid (ACES) have been used in MEEKC to reduce the amount of current produced, which allows higher voltages to be applied and faster separation [Mertzman *et al*. 2004].

In MEEKC, hexane, heptane or octane are commonly used to form microemulsions in MEEKC. Although all three oils have been shown to give similar selectivity, octane has been reported to give more repeatable microemulsions with better peak resolution, efficiency and precision [Sánchez *et al*. 2002, Fogarty *et al*. 2003]. Other oils used include medium chain alcohols such as 1-pentanol, 1-hexanol and 1-octanol have been shown to be not immiscible with water [Vomastová *et al*. 1996, Mahuzier *et al*.

2001]. Low interfacial oils as ethyl acetate has also been used as oil in MEEKC, which requires a lower concentration of SDS to form the microemulsion, allowing high voltages to be applied [Mahuzier *et al*. 2001]. Increasing the concentration of oil has been shown to insignificantly affect the separation in MEEKC [Cherkaoui *et al*. 2002].

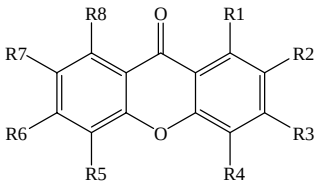
Typically, SDS is the most widely used as anionic surfactant in MEEKC because it is cheap and available in highly purified form. Increasing [SDS] results in a more stable and reproducible microemulsion system but higher current and Joule heating which may limit the use of high voltage and temperature [Fogarty *et al*. 2003]. Selectivity can be greatly altered by mixing SDS with another surfactant or by replacing it completely with another surfactant. A microemulsion containing 100 mM SDS and 80 mM bile salt sodium chlorate (SC) has been shown to provide optimum selectivity for separation of six biphenyl nitrile compounds and three related substances with high hydrophobicity and similar structure [Gong *et al*. 2004]. Chiral surfactants such as dodecoxycarbonylvaline (DDCV) have been used in MEEKC to improve enantiomeric separations [Mertzman *et al*. 2004, Kahle *et al*. 2006] and resolutions were found to increase with increasing [DDCV] [Mertzman *et al*. 2004].

In MEEKC, 1-butanol is the most widely used as a co-surfactant in order to reduce interfacial tension and to enhance stability of a microemulsion system [Altria *et al*. 2000a and 2000b, Hansen *et al*. 2001]. Other alcohols used such as 1-propanol, 1-pentanol, or 1-haxanol has been shown to give broad peaks and poor resolution due to unstable microemulsion [Nhujak *et al.* 2006]. Although baseline resolution was obtained with the co-surfactant as 1-butanol and cyclohexanol, cyclohexanol gave longer migration time due to higher viscosity [Nhujak *et al.* 2006]. Branched-chain alcohols such as 2-butanol do not use to form microemulsion because they can not bridge the oil-water interface effectively [Altria *et al*. 2000a]. Increasing the concentration of co-surfactant has been shown to alter the selectivity and improve resolution in MEEKC [Altria *et al*. 2000a].

Organic co-solvents such as ACN, MeOH, EtOH or 2-PrOH can be added to the microemulsion buffer in order to improve separations of highly hydrophobic compounds in MEEKC [Altria *et al.* 2000a, Nhujak *et al.* 2006, Seelanan *et al*. 2006]. The amount of organic co-solvent that can be added before microemulsion distruption varies for each organic co-solvent [Altria *et al.* 2000a]. An increase in the concentration of organic co-solvents such as ACN, MeOH, EtOH or 2-PrOH has been shown to improve the resolution in MEEKC [Gong *et al.* 2004, Huang *et al.* 2005a and 2005b, Sánchez *et al.* 2002, Fogarty *et al.* 2003].

In addition, parameters of CE instrument such as temperature and applied voltage have been shown to affect separation in MEEKC. Increasing temperature can either decrease or increase resolutions in MEEKC [Huang *et al.* 2003, Mertzman *et al*. 2005, Gong *et al.* 2004]. Faster analysis time was obtained using higher temperature and/or voltage due to a decrease in the viscosity of the microemulsion buffer, but poorer resolution of analytes was found due to Joule heating [Altria *et al.* 2000a, Nhujak *et al*. 2006].

In previous work, the better separation in MEEKC than MEKC was reported for curcuminoids [Nhujak *et al.* 2006], biphenyl nitriles [Gong *et al.* 2004], catechins [Pomponio *et al.* 2003], benzophenones [Huang *et al.* 2005b], vitamins [Sánchez *et al.* 2002], and xanthones [Bo *et al.* 2003]. Figures 4.1 and 4.2 show comparison of MEEKC and MEKC separations of xanthones [Bo *et al.* 2003] and catechins [Pomponio *et al.* 2003], respectively. However, separation scales of α, k and N have not been compared.



Structure of xanthones

No	R1	R2	R3	R4	R5	R6	R7	R8
1	HO	MeO	HO	H	H	H	HO	H
2	OH	H	HO	H	H	H	HO	H
3	OH	H	HO	MeO	H	H	HO	H
4	HO	MeO	MeO	H	H	HO	HO	H
5	MeO	H	H	H	H	H	HO	H
6	HO	MeO	MeO	H	H	H	HO	H
7	HO	H	H	MeO	H	H	HO	H
8	H	H	MeO	HO	H	H	MeO	H
9	MeO	H	H	MeO	H	H	HO	H

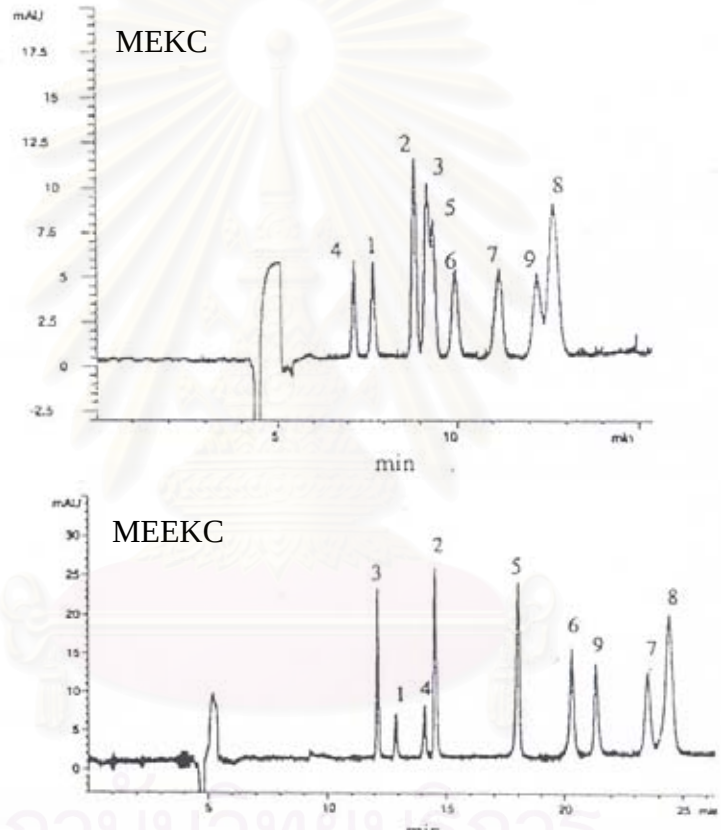


Figure 4.1 Comparison between MEEKC and MEKC for separation of xanthones. MEEKC buffer: 80 mM *n*-heptane, 10% v/v *n*-butanol, 120 mM SDS, 50 mM borate buffer at pH 9.5 and 5 mM sulfated β-CD; MEKC buffer: 100 mM borate buffer at pH 10.5, 60 mM SDS and 5 mM sulfated β-CD; fused-silica capillary, 48.5 cm in length (40 cm to detector) × 50 μm i.d. for MEEKC analysis and 58.5 cm in length (50 cm to detector) × 50 μm i.d. for MEKC analysis; injection of 5 kPa for 10 s; temperature, 35°C; applied voltage, 20 kV; UV detection at 265 nm. Reproduced from [Bo *et al.* 2003].

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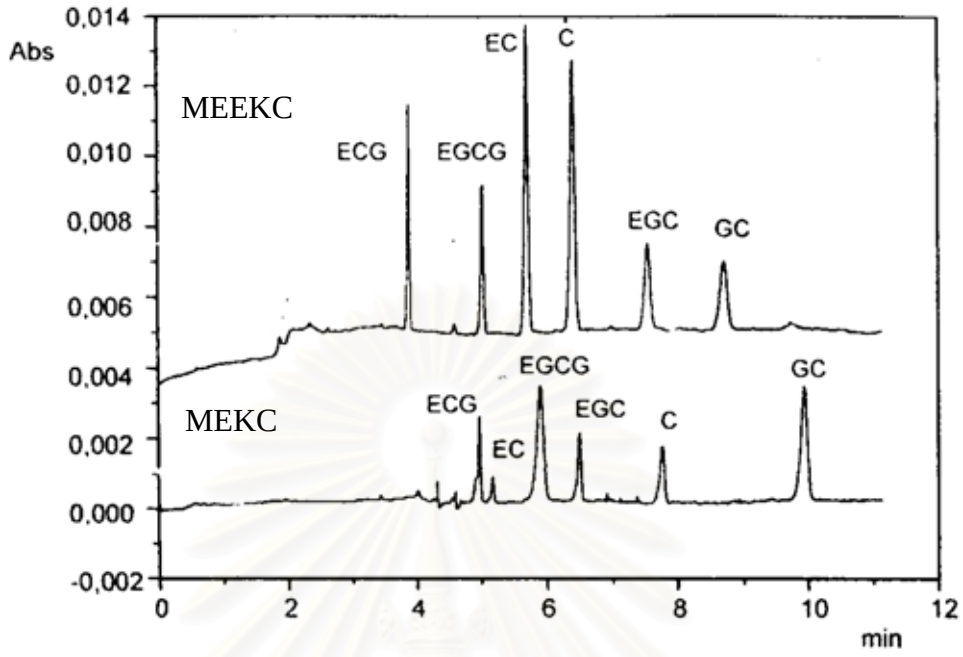
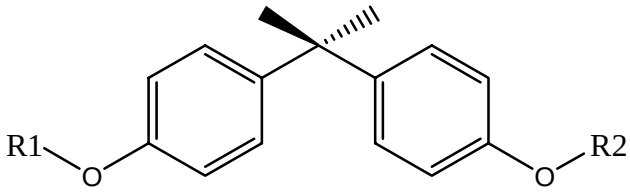


Figure 4.2 Comparison between MEEKC and MEKC for separation of catechins. MEEKC buffer: 1.36% w/v heptane, 9.72% w/v 1-butanol, 2.31% w/v SDS and 50 mM sodium phosphate buffer at pH 2.5; MEKC buffer: 50 mM sodium phosphate buffer at pH 2.5 and 2.31% w/v SDS; fused-silica capillary, 24 cm in length (19.5 cm to detector) × 50 μm i.d.; injection of 5 psi for 1 s; temperature, 40°C; applied voltage, -25 kV; UV detection at 200 nm. Abbreviations C, EC, EGC, GC, EGCG and ECG refer to (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-gallocatechin, (-)-epigallocatechin gallate and (-)-epicatechin gallate, respectively. Reproduced from [Pomponio *et al.* 2003].

The aims of this section is to compare the quantity of resolution of hydrophobic analytes in MEEKC and MEKC with suppressed electroosmosis, and to explain the quantity of separation scales of α, k and N in MEEKC and MEKC using the resolution equation recently reported in a previous work [Nhujak *et al.* 2006]. MEEKC and MEKC will be carried out using a 50 mM phosphate buffer at pH 2.5 to suppress electroosmosis, and the resolution of test analytes will be compared at given MEEKC and MEKC conditions used: the SDS concentrations of 100 to 180 mM, temperatures of 15 to 40°C, and organic co-solvents as ACN, MeOH, EtOH and 2-PrOH at levels of 20 to 30% v/v. The test analytes used are bisphenol-A-diglycidyl ether (BADGE) and its derivatives. BADGE is starting material for preparation of epoxy resins commonly used as protective coating for interior of metal food cans [Leepipatpiboon *et al.* 2005]. Residual BADGE and it derivatives in the coatings may migrate into the food upon contact.



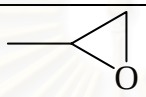
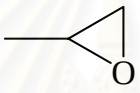
BADGEs	R1	R2
BADGE (B)		R2 = R1
BADGE·H_2O (D)		-$CH_2CH(OH)CH_2OH$
BADGE·$2H_2O$ (E)	-$CH_2CH(OH)CH_2OH$	R2 = R1
BADGE·2HCl (A)	-$CH_2CHClCH_2OH$	R2 = R1
BADGE·HCl·H_2O (C)	-$CH_2CHClCH_2OH$	-$CH_2CH(OH)CH_2OH$

Figure 4.3 Structures of bisphenol-A-diglycidyl ether and its derivatives.

4.2 Experimental

4.2.1 Chemicals

The following test analytes were purchased from Fluka (Buchs, Switzerland): bisphenol-A-diglycidyl ether (BADGE), bisphenol-A-(2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O), bisphenol-A-bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$), bisphenol-A-bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl), bisphenol-A-(3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BADGE·HCl·H_2O). *n*-Octane, 1-butanol and sodium hydroxide were obtained from Fluka, SDS from Sigma (MO, USA), phosphoric acid and all organic solvents from Merck (Darmstadt, Germany), and dodecylbenzene (DB)/microemulsion marker (M) from Sigma-Aldrich.

4.2.2 Preparation of buffers and analytes

4.2.2.1 Preparation of buffers

The buffer used for MEKC was prepared by pipeting the appropriate amounts of stock aqueous solutions of 500 mM SDS and 500 mM phosphate buffer at pH 2.5 into a 10 mL volumetric flask, followed by adding appropriate volumes of organic co-solvent such as MeOH, EtOH, 2-PrOH or ACN, while the buffer used for MEEKC was prepared by pipeting the appropriate amounts of stock aqueous solutions of 500 mM SDS and 500 mM phosphate buffer at pH 2.5 into a 10 mL volumetric flask, followed by adding appropriate volumes of 1-butanol, *n*-octane and organic co-solvent. The final solution was made up to 10 mL with Milli-Q water. All buffers were sonicated for 30 min to obtain the clear solutions and to degas in the solutions. The buffers were filtered through 0.45 μm PTFE membrane filters prior to CE analysis.

4.2.2.2 Preparation of analytes

Each test analytes and DB were separately dissolved at a concentration of 1000 ppm in EtOH. In MEKC, the mixture of the test analytes and DB at the concentration of 25 ppm each was obtained by diluting 1000 ppm of the test analytes and DB and then diluting the mixture with buffer containing 180 mM SDS, while in MEEKC, the mixture of the test analytes and DB at the concentration of 25 ppm each was obtained by diluting 1000 ppm of test analytes and DB and then diluting the mixture with buffer containing 180 mM SDS, 890 mM 1-butanol and 1.0% v/v *n*-octane. All the test analytes solutions were filtered through 0.45 μm PTFE membrane filters prior to CE analysis.

4.2.3 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 nm. An uncoated fused-silica capillary used was 40.2 cm in length (30 cm to detector) ×

50 μm i.d., 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 duplicate. Prior to CE analysis each day, the capillary was rinsed with ethanol, 0.1 M NaOH, water, and the buffer for 15 min each. Between consecutive runs, the capillary was rinsed with water, ethanol, 0.1 M NaOH, and water for 1 min each and then with buffer for 2 min. After analysis, each day, the capillary was rinsed with water and ethanol for 5 min each, and then 0.1 M NaOH and water for 10 min each.

4.3 Results and Discussion

4.3.1 Principle of resolution in MEEKC and MEKC without EOF

In MEKC or MEEKC without EOF, the resolution relates to k, N and α as described in Section 2.6. Figure 4.4 shows plots of the values of resolution as a function of k at given values of N and α. The values of resolution can be determined using Equation 2.47. From Figure 4.4a, in order to obtain the high resolution, the high values of α and N and small k are needed. However, a small change in the resolution with decreasing k_2 from 0.10 to 0.01 (Figure 4.4b) due to a slight change in $1+k_2$ or $1+k_2 \approx 1.0$. For analytes with $k_2 > 10$, a slight decrease in the resolution is due to $1+k_2 \approx k_2$. Therefore, the values of $0.1 < k_2 < 10$ are suitable for separation in MEEKC or MEKC without EOF. The smaller the value of k, the greater the resolution, but the longer the migration time. It should be noted that Equation 2.47 is not defined for $k_2 = 0$, and therefore, it does not mean that the analytes without interaction with the emulsion/micelles is always the best conditions for electrophoretic separation. An improved resolution may be obtained for analytes with higher α and/or higher N. For the analytes with high k, the high α of analytes is needed to achieve resolution at given N. For the analytes with small α and high k, the high N is needed for the analytes to achieve resolution. It should be noted that for $\alpha > 1.3$ and small k_2, i.e. $k_2 < 1$, Equation 2.46 should be used in stead of Equation 2.47. However, in most case, the optimization of analytes with $1 \leq \alpha \leq 1.3$ is theoretically discussed, and therefore, Equation 2.47 is used in this work.

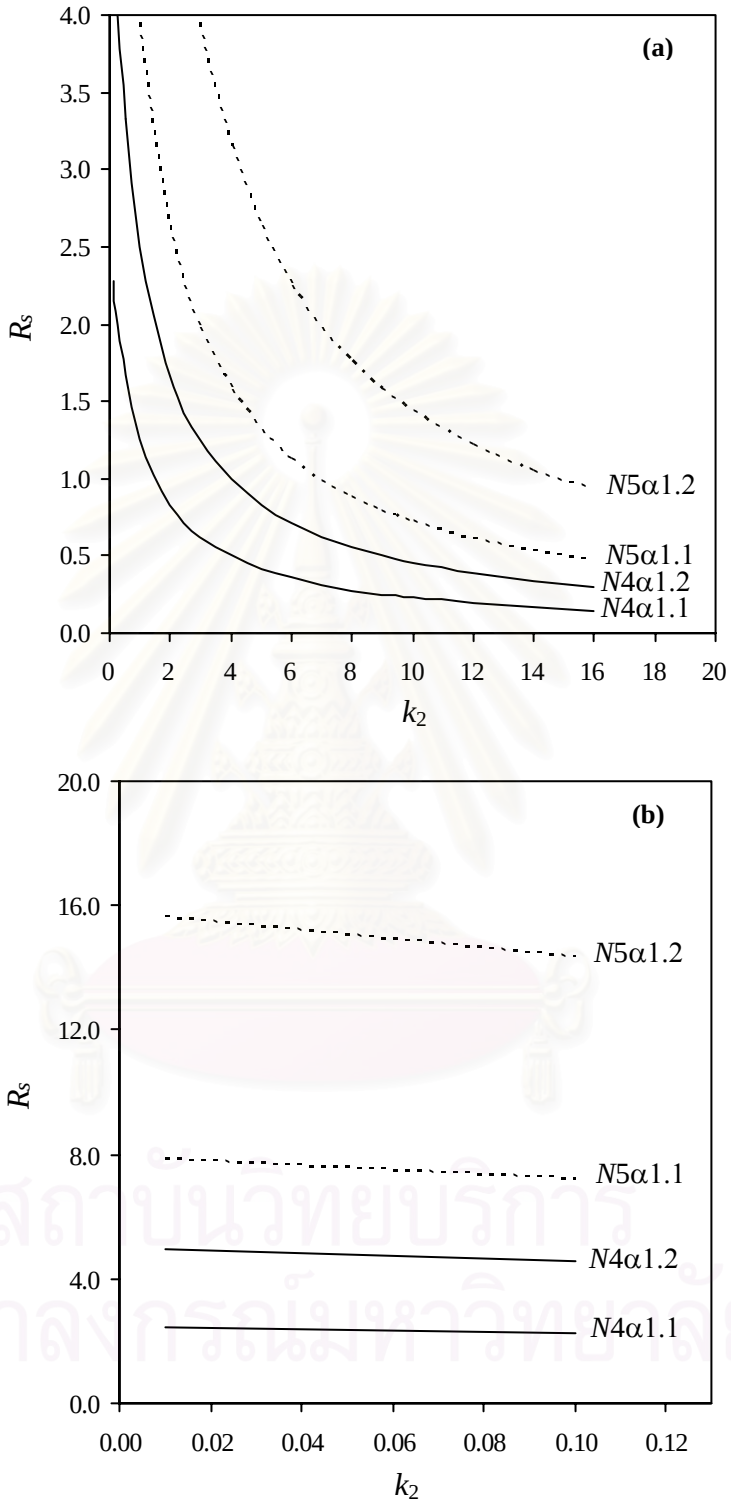


Figure 4.4 Resolution as a function of k_2 from 0.10 to 16 (a) and 0.01 to 0.10 (b), at given values of N and α. $N4$ and $N5$ refer to N of 10^4 and 10^5, and $\alpha 1.1$ and $\alpha 1.2$ refer to α of 1.1 and 1.2, respectively.

4.3.2 Comparison of hydrophobicity of pseudo stationary phase in MEEKC and MEKC

The hydrophobicity of pseudo stationary phase may be compared using the logarithm of methylene selectivity, log α_{CH2}, that is obtained from a slope of a linear plot between log *k* of the homologous series in MEEKC or MEKC versus the number of carbons [Ahuja *et al.* 1995]. The greater the value of log α_{CH2}, the higher the hydrophobicity of the pseudo stationary phase [Ahuja *et al.* 1995]. Figure 4.5 shows a comparison of log α_{CH2} for C_6 to C_9 alkylbenzene series in MEEKC and MEKC containing various types of organic co-solvents as ACN, MeOH, EtOH or 2-PrOH at a level of 0 to 30% v/v. At a given concentration of each organic co-solvent, log α_{CH2} obtained from MEEKC is smaller than that from MEKC, indicating the lower hydrophobicity of pseudo stationary phase in MEEKC than that in MEKC due to 1-butanol penetrating the oil droplets in MEEKC. From Figure 4.5, a decrease in the values of log α_{CH2} with increasing the concentration of each organic co-solvent in MEEKC and MEKC was obtained in this work, indicating that the difference in the polarity of the aqueous phase and pseudo stationary phase is decreased with an increase in organic co-solvent concentration [Ahuja *et al.* 1995].

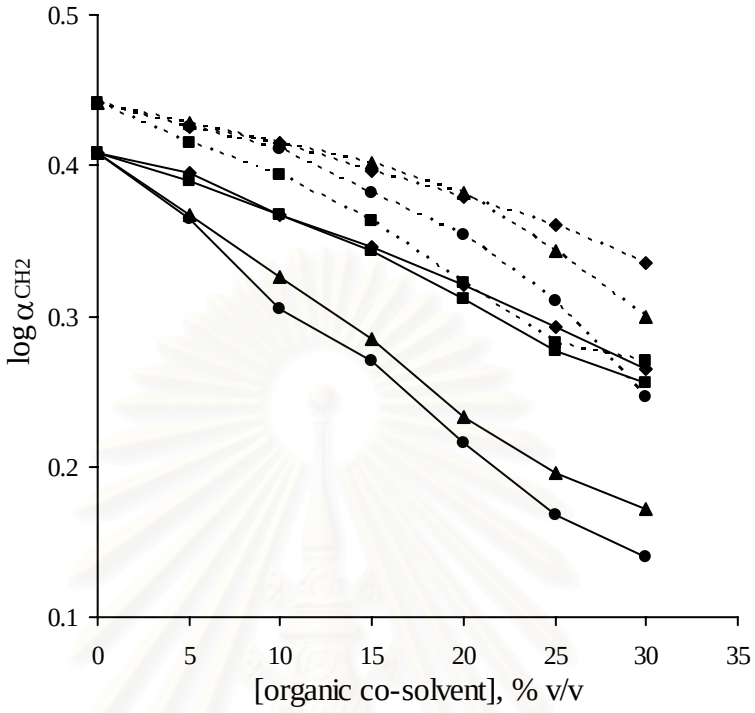


Figure 4.5 Methylene selectivity, log α_{CH2}, for MEEKC (solid lines) and MEKC (dash lines) at various types and concentrations of organic co-solvents, where log α $\log_{CH2}$ was obtained from the slope of a linear plot between log *k* of alkylbenzene series versus the number of carbons as described in Section 3.3.3. Symbols ■, ◆, ▲ and ● refer to ACN, MeOH, EtOH and 2-PrOH, respectively

4.3.3 Effect of types and concentrations of organic co-solvents

In initial work, MEKC analysis was carried out using a pH 2.5 50 mM phosphate buffer containing SDS as surfactant, while MEEKC analysis was carried out using a pH 2.5 50 mM phosphate buffer containing 1.0% v/v *n*-octane as oil, SDS as emulsifier surfactant and 890 mM 1-butanol as co-surfactant. DB, a highly hydrophobic compound, spiked into a sample solution is used as a marker for t_{mc} [Altria *et al.* 2000a, Nhujak *et al.* 2006]. In comparison with the electrophoretic mobility of the anlayte, negligible electroosmotic mobility was obtained in this work, measured by short-end injection using thiourea [Altria *et al.* 2000a, Nhujak *et al.* 2006]. No resolution of BADGEs was obtained in MEEKC or MEKC, and co-retention of these compounds and DB was observed using the buffer without organic co-solvent. This indicates that these compounds strongly and completely partition into a pseudo stationary phase of micelle in MEKC or charged oil droplets in MEEKC.

Organic solvents at 20 to 30% v/v, such as ACN, MeOH, EtOH and 2-PrOH, were separately added in the MEEKC and MEKC buffer in order to obtain the difference in partitioning of these analytes into the aqueous phase. An example of electropherograms of BADGEs for MEEKC and MEKC separations is shown in Figure 4.6. Table 4.1 shows the effects of types and concentrations of organic co-solvents on separation scales of test analytes in MEEKC and MEKC. From Equation 2.18 and Figure 4.6, k_2 in Table 4.1 refers to the retention factor of the test analytes with the higher k, i.e. $k_2 = k_A$ for a pair of A:B, and therefore the value of α refers to k_A/k_B for a pair of A:B. The values of R_s shown in Table 4.1 are calculated from electropherograms using Equation 2.47. The value of $\overline{N}$ in Table 4.1 refers to the average efficiency for two analytes, i.e. $\overline{N} = (N_A + N_B)/2$, for a pair of A:B, and N_A and N_B are calculated from electropherogram using Equation 2.24. It should be noted that, using pared *t*-test analysis at 95% confidential of the mean, non-significant difference is found between the calculated R_s using Equation 2.47 and measured R_s obtained from $R_s = 1.18\Delta t_m / \overline{w}_h$, $\overline{w}_h$ is the average peak width at half-height.

From Table 4.1, an increase in the concentration of organic co-solvent in the MEEKC or MEKC buffer resulted in a decrease in *k*, due to an increase in the partitioning of the test analytes in the organic-aqueous phase [Altria *et al.* 2000a, Nhujak *et al.* 2006]. Theoretically, *k* of analytes in each MEKC or MEEKC system increases with the larger size and higher hydrophobicity of analytes [Yang *et al.* 1995a and 1995b]. From Table 4.1 and Figures 4.3, 4.6 and 4.7, the *k* order BADGE·2HCl (A) > BADGE (B) > BADGE·$2H_2O$ (E) was obtained to be in consistent with the hydrophobicity and size order of two identical substituting groups of BADGEs, 3-chloro-2-hydroxypropyl > glycidyl ether > 2-hydroxypropyl of BADGE·2HCl, BADGE and BADGE·$2H_2O$, respectively. In addition, *k* of BADGEs containing two different substituting groups was found to be between that of BADGEs containing two identical substituting groups, BADGE·2HCl > BADGE·HCl·H_2O (C) > BADGE·$2H_2O$, and BADGE > BADGE·H_2O (D) > BADGE·$2H_2O$.

In MEEKC and MEKC without EOF, the test analytes having greater *k* or stronger partitioning of the test analytes into the pseudo stationary phase migrate with a smaller migration time as discussed previously in Section 3.1 and shown in Figure 3.2b. From Figures 4.6 and 4.7 and Table 4.1, in MEEKC and MEKC buffer containing 20 to 30% v/v organic co-solvent, the migration time order for BADGEs in ACN < MeOH < EtOH < 2-PrOH was not consistent with the retention factor order for BADGEs in MeOH > ACN > EtOH > 2-PrOH. This is because an increase in the migration time is also due to two additional effects caused by an increase in the viscosity, 2-PrOH > EtOH > MeOH > ACN, and the smaller charge-to-size ratio caused by solvation of organic solvent [Altria *et al.* 2000a, Nhujak *et al.* 2006].

In MEEKC and MEKC, separation of two analytes can be described by α or R_s. α is used to indicate the difference in the retention of analytes, while R_s is used to measure the quantity of separation of analytes. Over a wide range of 20 to 30% v/v organic co-solvent in the MEEKC and MEKC system, MeOH did not give the separation of BADGEs in the MEKC system, α of 1.0 and R_s of 0, which are not shown in Table 4.1. It should be noted that Equation 2.47 for resolution is used with reasons as

previously discussed in Section 4.3.1. Although $\alpha > 1.3$ for some analytes as shown in Table 4.1, the different values of R_s less than 5% are obtained from calculation using Equations 2.46 and 2.47, due to $k_2 > 1$. It should be also noted that no resolution of the test analytes in MEKC containing 20 to 30% v/v MeOH is due to very high k of the test analytes, ($k > 60$), indicating that the test analytes completely partitioning into the micellar phase and migrate together with the micellar phase. Therefore, it can be followed from Equation 2.47 and Figure 4.4 that, to obtain $R_s = 1.5$, α should be greater than 2.2 for the test analytes with N of 10^5.

In the presence of 25 to 30% v/v organic co-solvents in the MEEKC and MEKC buffers, a slight change of α was obtained in MEEKC and MEKC. Although ACN, EtOH and 2-PrOH gave a similar range of α for the test analytes, EtOH provided significantly greater R_s of the test analytes. This is due to the greater $\overline{N}$ and smaller k_2 of the test analytes in the buffer containing EtOH, $R_s \propto \overline{N}^{1/2}$, as shown in Equation 2.47. In previous work on MEKC and MEEKC, the organic modifiers can either enhance or reduce efficiency due to several effects such as an increase in viscosity and a change in micellar structure and different diffusion in organic solvent [Altria *et al.* 2000a, Nhujak *et al.* 2006]. In most cases, simple explanation for the change in N is not clear [Khaledi 1998, Nhujak *et al.* 2006].

From Table 4.1, although MEKC gave higher selectivity of the test analytes, α or α-1, than did MEEKC, poorer resolution in MEKC was obtained. This is because R_s is also proportional to the efficiency scale, $\sqrt{\overline{N}}$, and the retention scale, $1/(1+k_2)$, as given in Equation 2.47. Table 4.2 shows a comparison of the separation scales of test analytes in MEEKC and MEKC, expressed as the ratios of R_s, α-1, $\sqrt{\overline{N}}$ and $1/(1+k_2)$ of the test analytes in MEEKC to MEKC. For example, in the presence of 25% v/v EtOH in the MEEKC or MEKC buffer, the ratios of α-1, $\sqrt{\overline{N}}$ and $1/(1+k_2)$ for C:D in MEEKC to MEKC were obtained to be 0.38, 1.14 and 6.41, respectively, giving the product of the ratios or the R_s ratio of 2.72 for MEEKC to MEKC. This indicates that, in spite of smaller α of analytes in MEEKC than MEKC, higher resolution of the test

analytes in MEEKC is due to significantly smaller *k* in MEEKC than MEKC. It should be noted from Equation 2.47 and Figure 4.4 that, for two pairs of the test analytes with the similar range of α and $\overline{N}$, the smaller the value of *k*, the higher the resolution.

At a given concentration of each organic co-solvent, a smaller α of the test analytes in MEEKC than MEKC was obtained in this work. In previous work on MEKC and MEEKC with high EOF [Song *et al.* 1995], although the same SDS surfactant was used, MEKC and MEEKC gave a different separation selectivity due to the differences in interaction mechanism among MEKC and MEEKC possibly caused by the differences in the structures of micelles and microemulsions as discussed in their work [Song *et al.* 1995].

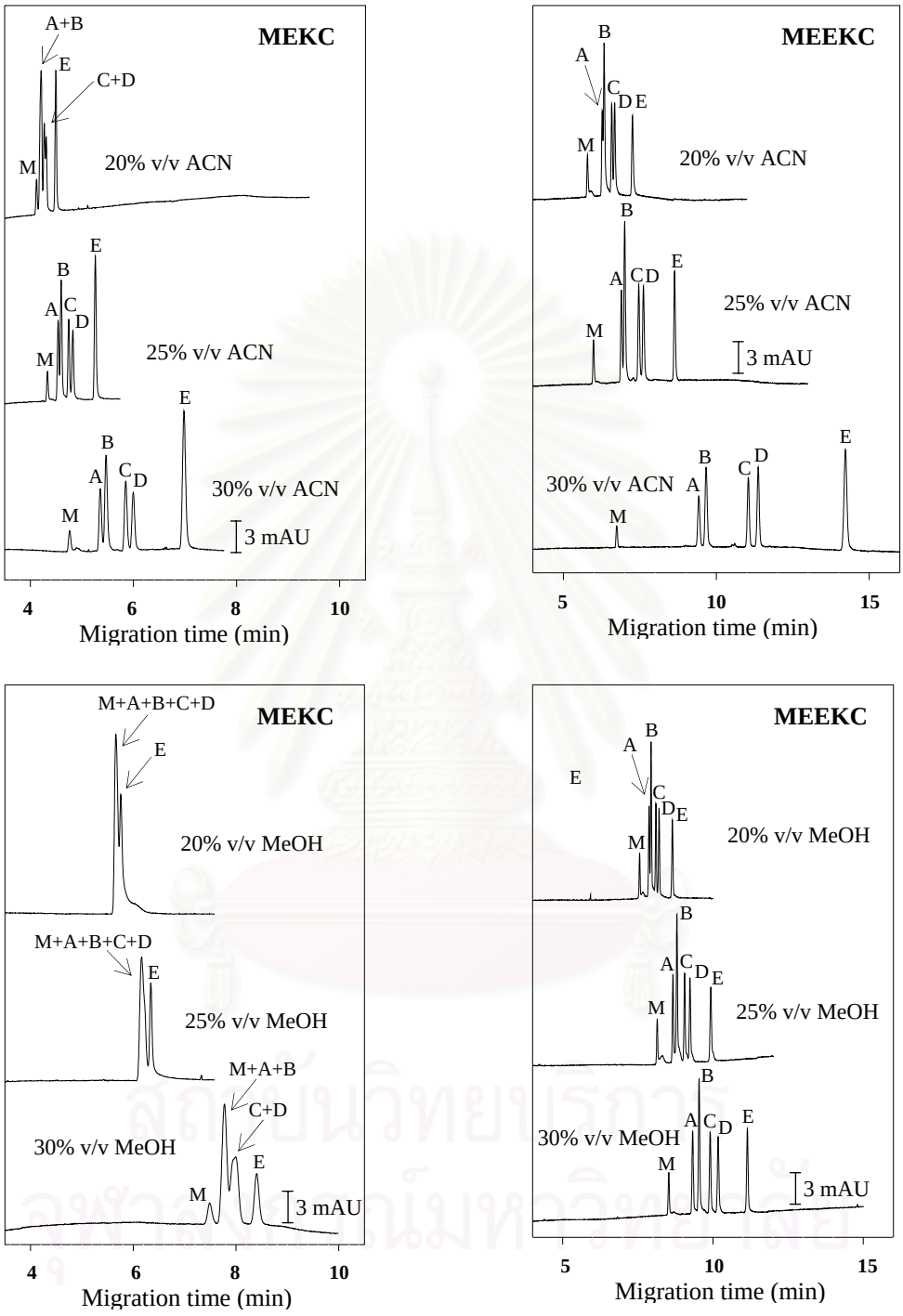


Figure 4.6 An example of electropherograms of test analytes in MEKC and MEEKC in the presence of organic co-solvents as ACN and MeOH. The MEKC buffer contained a 50 mM phosphate buffer at pH 2.5, 180 mM SDS and organic co-solvents, while the MEEKC buffer contained a 50 mM phosphate buffer at pH 2.5, 1.0% v/v *n*-octane, 180 mM SDS, 890 mM 1-butanol and organic co-solvents. CE instrument conditions: temperature of 25°C, applied voltage of -15 kV, UV detection at 214 nm, 0.5 psi pressure injection for 3 s. Abbreviations M, A, B, C, D and E refer to pseudo stationary phase marker, BADGE·2HCl, BADGE, BADGE·HCl·H_2O, BADGE·H_2O and BADGE·$2H_2O$, respectively.

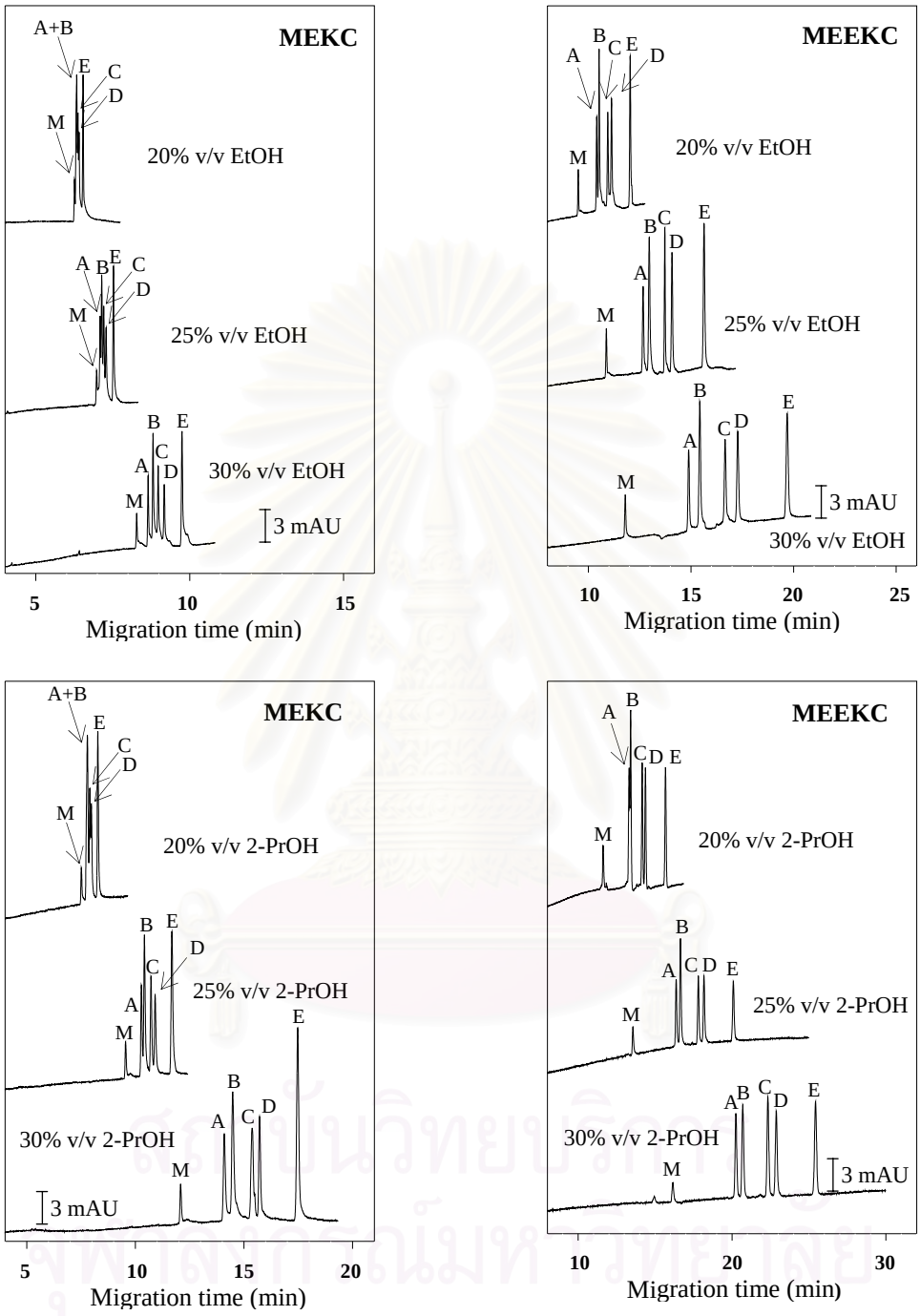


Figure 4.7 An example of electropherograms of test analytes in MEKC and MEEKC in the presence of organic co-solvents as EtOH and PrOH. Other CE conditions as shown in Figure 4.6. Abbreviations refer to pseudo stationary phase marker and test analytes as shown in Figure 4.6.

Table 4.1 Effect of types and concentrations of organic co-solvents on separation scales of test analytes in MEEKC and MEKC in the presence of 180 mM SDS in the buffers. Each value is the average from two runs

Organic solvent	A pair of analytes	MEEKC				MEKC			
		k_2	α	$\overline{N}$ (10^5)	R_s	k_2	α	$\overline{N}$ (10^5)	R_s
20% v/v ACN	A:B	12.1	1.13	nd	nd	nd	nd	nd	nd
	B:C	10.7	1.45	0.90	2.88	nd	nd	nd	nd
	C:D	7.38	1.12	0.98	1.12	27.7	1.24	nd	nd
	D:E	6.57	1.65	0.89	6.40	22.4	2.01	nd	nd
25% v/v ACN	A:B	6.65	1.12	0.81	1.12	21.1	1.28	0.74	0.86
	B:C	5.95	1.45	0.81	4.61	16.4	1.55	0.81	2.25
	C:D	4.10	1.11	0.84	1.56	10.6	1.19	0.84	1.19
	D:E	3.71	1.62	1.16	11.2	8.91	1.89	0.84	6.51
30% v/v ACN	A:B	2.54	1.09	0.77	1.76	8.08	1.19	0.54	1.22
	B:C	2.33	1.47	0.93	10.8	6.79	1.54	0.54	4.03
	C:D	1.59	1.08	1.22	2.70	4.41	1.14	0.57	1.54
	D:E	1.47	1.62	1.12	21.0	3.88	1.80	0.60	10.0
20% v/v MeOH	A:B	24.2	1.22	2.34	1.06	nd	nd	nd	nd
	B:C	19.9	1.43	2.33	2.48	nd	nd	nd	nd
	C:D	13.9	1.19	2.40	1.56	nd	nd	nd	nd
	D:E	11.7	1.68	2.29	6.41	nd	nd	nd	nd
25% v/v MeOH	A:B	15.7	1.26	2.13	1.80	nd	nd	nd	nd
	B:C	12.5	1.39	2.13	3.33	nd	nd	nd	nd
	C:D	8.95	1.19	2.07	2.17	nd	nd	nd	nd
	D:E	7.49	1.64	1.81	8.02	nd	nd	nd	nd
30% v/v MeOH	A:B	10.8	1.27	1.87	2.47	nd	nd	nd	nd
	B:C	8.52	1.37	1.81	4.13	nd	nd	nd	nd
	C:D	6.23	1.19	1.61	2.64	nd	nd	nd	nd
	D:E	5.22	1.59	1.83	10.1	nd	nd	nd	nd
20% v/v EtOH	A:B	10.5	1.13	2.07	1.29	nd	nd	nd	nd
	B:C	9.31	1.41	1.97	4.41	nd	nd	nd	nd
	C:D	6.58	1.13	1.99	1.91	56.3	1.33	nd	nd
	D:E	5.84	1.56	2.20	9.60	42.2	1.87	nd	nd
25% v/v EtOH	A:B	6.06	1.17	1.93	2.64	60.2	1.44	2.12	0.83
	B:C	5.20	1.36	2.36	7.05	41.8	1.40	2.04	1.06
	C:D	3.82	1.12	2.53	3.13	29.9	1.32	1.96	1.15
	D:E	3.40	1.49	2.15	12.9	22.6	1.78	2.28	3.95
30% v/v EtOH	A:B	4.00	1.17	2.18	3.97	22.3	1.43	3.16	2.59
	B:C	3.41	1.34	2.08	8.79	15.6	1.31	2.97	2.54
	C:D	2.54	1.12	1.74	3.54	11.9	1.29	2.91	3.03
	D:E	2.26	1.44	1.59	13.5	9.26	1.64	2.79	8.24
20% v/v 2-PrOH	A:B	6.90	1.06	nd	nd	nd	nd	nd	nd
	B:C	6.49	1.42	1.59	5.59	nd	nd	nd	nd
	C:D	4.57	1.08	1.76	1.51	19.4	1.19	nd	nd
	D:E	4.22	1.47	1.88	9.76	16.4	1.64	nd	nd
25% v/v 2-PrOH	A:B	4.84	1.10	1.52	1.67	13.3	1.20	1.28	1.25
	B:C	4.40	1.38	1.57	6.97	11.1	1.35	1.25	2.56
	C:D	3.20	1.09	1.70	2.21	8.21	1.16	1.23	1.52
	D:E	2.95	1.41	1.67	10.6	7.07	1.57	1.08	5.80
30% v/v 2-PrOH	A:B	3.93	1.11	1.55	2.20	6.06	1.19	0.85	1.96
	B:C	3.55	1.36	1.43	7.48	5.07	1.37	1.00	4.82
	C:D	2.61	1.09	1.43	2.36	3.70	1.10	1.30	1.92
	D:E	2.40	1.38	1.55	11.0	3.35	1.49	1.32	10.2

nd = not determined due to peak overlapping

Table 4.2 Effect of types and concentration of organic co-solvents on ratio of separation scales of test analytes in MEEKC to MEKC in the presence of 180 mM SDS in the buffers. Each value is the average from two runs

Organic solvent	A pair of analytes	Ratio of separation scales in MEEKC to MEKC			
		$1/(1+k_2)$	α-1	$\sqrt{N}$	R_s
20% v/v ACN	A:B	nd	nd	nd	nd
	B:C	nd	nd	nd	nd
	C:D	3.42	0.50	nd	nd
	D:E	3.09	0.64	nd	nd
25% v/v ACN	A:B	2.89	0.43	1.05	1.30
	B:C	2.50	0.82	1.00	2.05
	C:D	2.27	0.58	1.00	1.31
	D:E	2.10	0.70	1.18	1.72
30% v/v ACN	A:B	2.56	0.47	1.19	1.44
	B:C	2.34	0.87	1.31	2.68
	C:D	2.09	0.57	1.46	1.75
	D:E	1.98	0.78	1.37	2.09
20% v/v EtOH	A:B	nd	nd	nd	nd
	B:C	nd	nd	nd	nd
	C:D	7.56	0.39	nd	nd
	D:E	6.32	0.64	nd	nd
25% v/v EtOH	A:B	8.67	0.39	0.95	3.18
	B:C	6.90	0.90	1.08	6.65
	C:D	6.41	0.38	1.14	2.72
	D:E	5.36	0.63	0.97	3.27
30% v/v EtOH	A:B	4.66	0.40	0.83	1.53
	B:C	3.76	1.10	0.84	3.46
	C:D	3.64	0.41	0.77	1.17
	D:E	3.15	0.69	0.75	1.64
20% v/v 2-PrOH	A:B	nd	nd	nd	nd
	B:C	nd	nd	nd	nd
	C:D	3.66	0.42	nd	nd
	D:E	3.33	0.73	nd	nd
25% v/v 2-PrOH	A:B	2.45	0.50	1.09	1.34
	B:C	2.24	1.09	1.12	2.72
	C:D	2.19	0.56	1.18	1.45
	D:E	2.04	0.72	1.24	1.83
30% v/v 2-PrOH	A:B	1.43	0.58	1.35	1.12
	B:C	1.33	0.97	1.20	1.55
	C:D	1.30	0.90	1.05	1.23
	D:E	1.28	0.78	1.08	1.08

nd = not determined due to peak overlapping

4.3.4 Effect of SDS concentration

As described in Section 4.3.3, in the presence of 25 to 30% v/v organic co-solvents in the MEEKC and MEKC buffers, EtOH provided significantly greater R_s of the test analytes than other organic co-solvents. 25 and 30% v/v EtOH gave slight difference in the resolution of the test analytes, but long analysis time was obtained at the higher concentration of EtOH. Therefore, 25% v/v EtOH was chosen as organic co-solvent in this section. The influence of [SDS] in a range of 100 to 180 mM on resolution of test analytes was investigated in MEEKC and MEKC using 25% EtOH in the buffer and other CE conditions as shown in Figure 4.6. An example of electropherograms of BADGEs for MEEKC and MEKC separations is shown in Figure 4.8. Below 100 mM SDS in MEEKC or 60 mM SDS in MEKC, broad peaks and poor peak shapes of the test analytes were observed, possibly due to unstable of oil droplets in MEEKC and micelle phase in MEKC. In addition, over 180 mM SDS in MEEKC or MEKC may cause higher Joule heating, resulting in poorer resolution of analytes. From Figure 4.8, the slower migration time of pseudo stationary phase marker and the test analytes were observed with decreasing [SDS] due to a decrease in surface charge density of the pseudo stationary phase [Altria *et al*. 2000b].

Table 4.3 shows the effect of [SDS] on the separation scales of the test analytes in MEEKC and MEKC. From Table 4.3, the retention factor in MEEKC and MEKC increased with an increase in [SDS] due to an increase in the phase ratio as described previously in Section 3.3.3. A decrease of 180 to 100 mM [SDS] in the buffer resulted in a decrease in k, but small change in R_s was found due to a decrease in $\overline{N}$ and no change in α.

The selectivity insignificantly changed with an increase in [SDS] due to the independent of K with [SDS] ($\alpha = k_2/k_1 = K_2/K_1$). The efficiency increased with an increase in [SDS] possibly caused by the reduction of H_{aq} and H_{pd}, similarly explained in MEKC and MEEKC at high EOF [Terabe *et al*. 1989 and 1992]. In addition, an increase in μ_{sp} with an increase in [SDS] results in a decrease in H_l (Equation 2.26). However, an increase in [SDS] may cause higher Joule heating, leading to an increase

in H_t and a decrease in N. A decrease of 180 to 100 mM [SDS] in the buffer resulted in long analysis time and lowers stability of the pseudo stationary phase.

Table 4.4 shows a comparison of the separation scales of test analytes in MEEKC and MEKC, expressed as the ratios of R_s, α-1, $\sqrt{N}$ and $1/(1+k_2)$ of the test analytes in MEEKC to MEKC. At the given [SDS], the better resolution of the test analytes in MEEKC than MEKC was also found, which is can be explained using the ratio of separation scales similar to Table 4.2.

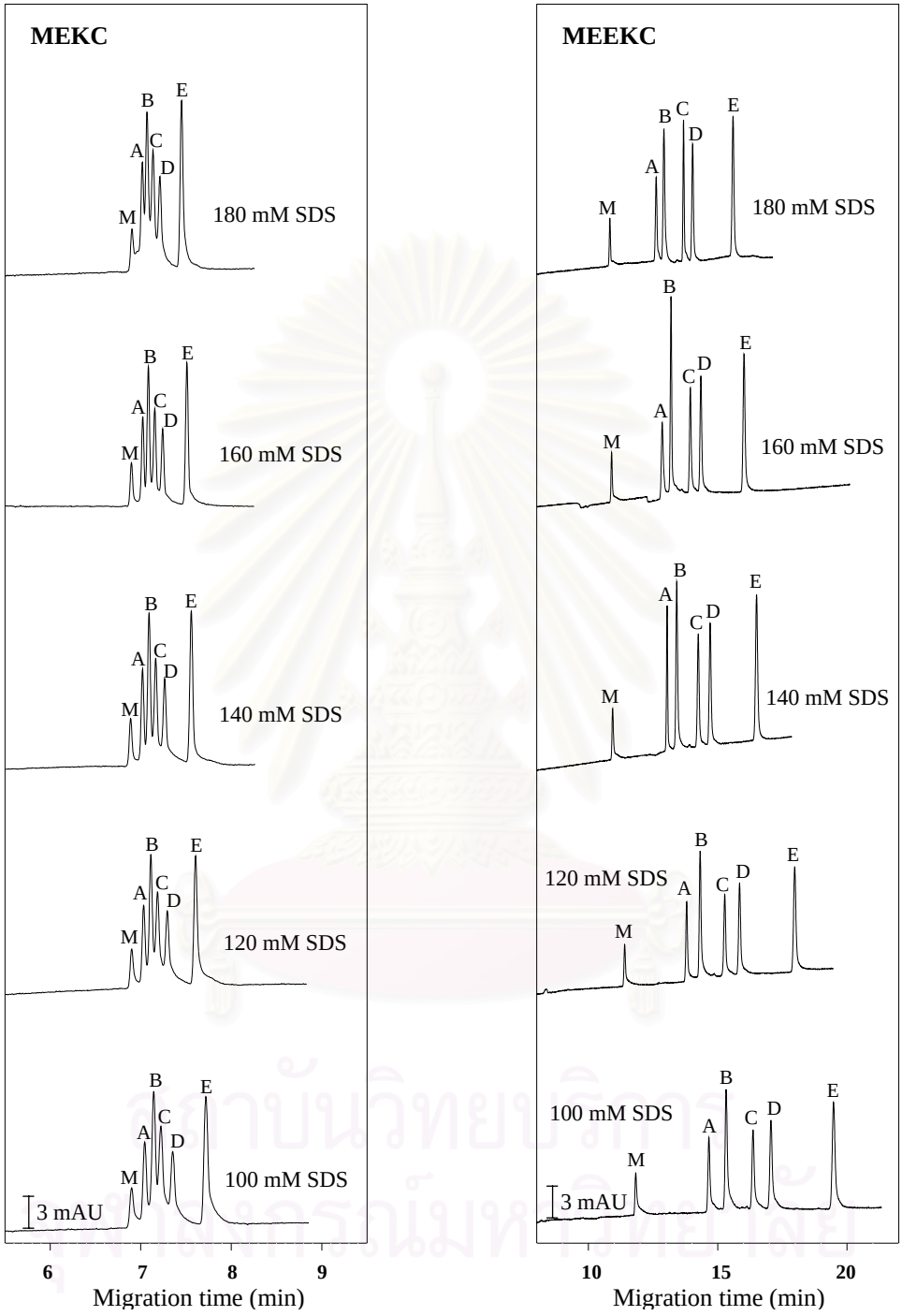


Figure 4.8 An example of electropherograms of test analytes in MEKC and MEEKC in the presence of 25% v/v EtOH and using various concentrations of SDS. Other CE conditions as shown in Figure 4.6. Abbreviations refer to pseudo stationary phase marker and test analytes as shown in Figure 4.6.

Table 4.3 Effect of SDS concentration on separation scales of test analytes in MEEKC and MEKC in the presence of 25% v/v EtOH in the buffers. Each value is the average from two runs

[SDS], mM	A pair of analytes	MEEKC				MEKC			
		k_2	α	$\overline{N}$ (10^5)	R_s	k_2	α	$\overline{N}$ (10^5)	R_s
180	A:B	6.06	1.17	1.93	2.64	60.2	1.44	2.12	0.83
	B:C	5.20	1.36	2.36	7.05	41.8	1.40	2.04	1.06
	C:D	3.82	1.12	2.53	3.13	29.9	1.32	1.96	1.15
	D:E	3.40	1.49	2.15	12.9	22.6	1.78	2.28	3.95
160	A:B	5.57	1.16	2.23	2.88	56.4	1.49	2.46	1.06
	B:C	4.79	1.33	2.45	7.05	37.9	1.38	2.43	1.20
	C:D	3.59	1.13	1.99	3.16	27.5	1.34	2.40	1.46
	D:E	3.17	1.48	2.03	13.0	20.5	1.77	2.60	4.57
140	A:B	5.28	1.18	2.22	3.38	54.0	1.55	1.80	1.06
	B:C	4.46	1.33	1.80	6.41	34.8	1.37	1.63	1.04
	C:D	3.34	1.14	1.85	3.47	25.5	1.36	1.54	1.33
	D:E	2.93	1.47	1.76	12.5	18.8	1.76	1.77	4.04
120	A:B	4.78	1.22	1.72	3.95	51.9	1.57	1.72	1.12
	B:C	3.93	1.32	1.91	7.09	33.0	1.36	1.48	1.02
	C:D	2.97	1.15	1.82	4.03	24.4	1.38	1.23	1.31
	D:E	2.59	1.48	1.59	13.3	17.7	1.79	1.55	4.16
100	A:B	4.25	1.24	1.44	4.34	47.8	1.67	1.12	1.15
	B:C	3.44	1.30	1.60	6.76	28.6	1.32	0.93	0.82
	C:D	2.65	1.15	1.66	4.19	21.6	1.40	0.80	1.25
	D:E	2.30	1.46	1.48	13.4	15.4	1.81	1.00	3.90

Table 4.4 Effect of SDS concentration on ratio of separation scales of test analytes in MEEKC to MEKC in the presence of 25% v/v EtOH in the buffers. Each value is the average from two runs

[SDS], mM	A pair of analytes	Ratio of separation scales in MEEKC to MEKC			
		$1/(1+k_2)$	α-1	$\sqrt{\overline{N}}$	R_s
180	A:B	8.67	0.39	0.95	3.18
	B:C	6.90	0.90	1.08	6.65
	C:D	6.41	0.38	1.14	2.72
	D:E	5.36	0.63	0.97	3.27
160	A:B	8.74	0.33	0.95	2.72
	B:C	6.72	0.87	1.00	5.88
	C:D	6.21	0.38	0.91	2.16
	D:E	5.16	0.62	0.89	2.84
140	A:B	8.76	0.33	1.11	3.19
	B:C	6.56	0.89	1.05	6.16
	C:D	6.11	0.39	1.10	2.61
	D:E	5.04	0.62	1.00	3.09
120	A:B	9.15	0.39	1.00	3.53
	B:C	6.90	0.89	1.14	6.95
	C:D	6.40	0.39	1.22	3.08
	D:E	5.21	0.61	1.01	3.20
100	A:B	9.30	0.36	1.13	3.77
	B:C	6.67	0.94	1.31	8.24
	C:D	6.19	0.38	1.44	3.35
	D:E	4.97	0.57	1.22	3.44

4.3.5 Effect of temperature

The influence of temperature in a range of 15 to 40°C at the constant of applied voltage of -15 kV was investigated in MEEKC and MEKC in the presence 25% v/v EtOH in the buffers and other CE conditions as shown in Figure 4.6. An example of electropherograms of BADGEs for MEEKC and MEKC separations is shown in Figure 4.9. From Figure 4.9, the faster migration time of pseudo stationary phase marker and the test analytes were observed with increasing temperature due to a decrease in the viscosity of the buffer in MEEKC and MEKC system.

Table 4.5 shows the effect of temperature on the separation scales of the test analytes in MEEKC and MEKC. From Table 4.5, a decrease in *k* of the test analytes in MEEKC and MEKC was found with an increase in separation temperature. This can be explained by the fact that an increase in the temperature results in an increase in the solubility of hydrophobic compounds in the organic-water phase [Altria *et al.* 2000a, Nhujak *et al.* 2006]. Theoretically, the smaller *k* gives the slower migration time of the analyte in MEEKC or MEKC without EOF. However, experimental resulted showed the faster migration time of the analyte with an increase in temperature, mainly due to a decrease in the viscosity of the buffer in MEEKC and MEKC system. Although *k* decreased at higher temperature, small change in R_s of the test analytes was obtained in MEKC and MEEKC because a decrease in *N* due to an increase in H_t caused by Joule heating but small change in α.

Table 4.6 shows a comparison of the separation scales of test analytes in MEEKC and MEKC, expressed as the ratios of R_s, α-1, $\sqrt{N}$ and $1/(1+k_2)$ of the test analytes in MEEKC to MEKC. At the given temperature, the better resolution of the test analytes in MEEKC than MEKC was also found, which is can be explained using the ratio of separation scales similar to Table 4.2.

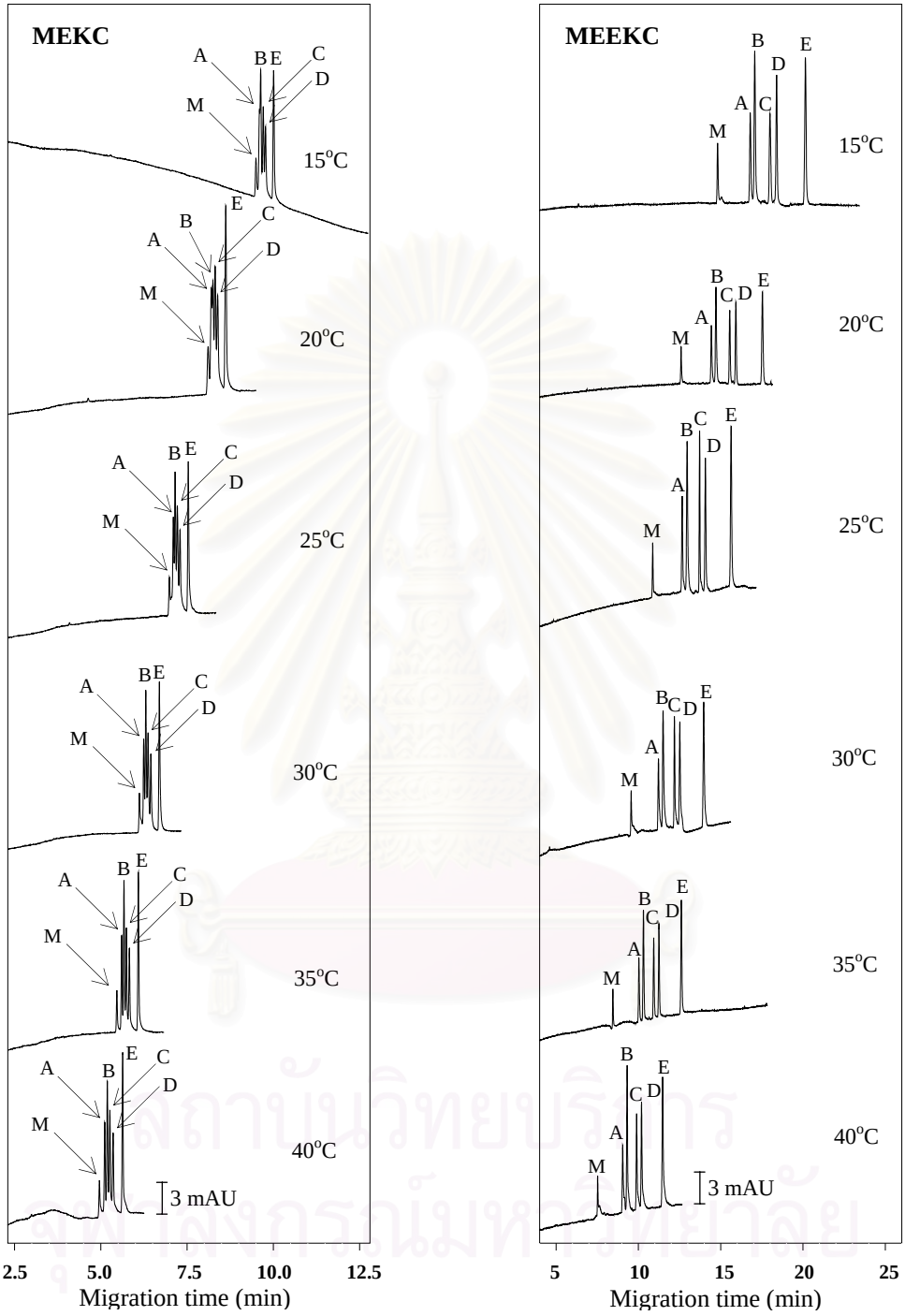


Figure 4.9 An example of electropherograms of test analytes in MEKC and MEEKC in the presence of 25% v/v EtOH and using various temperatures. Other CE conditions as shown in Figure 4.6. Abbreviations refer to pseudo stationary phase marker and test analytes as shown in Figure 4.6.

Table 4.5 Effect of temperature on separation scales of test analytes in MEEKC and MEKC in the presence of 180 mM SDS and 25% v/v EtOH in the buffers. Each value is the average from two runs

Temperature, °C	A pair of analytes	MEEKC				MEKC			
		k_2	α	$\overline{N}$ (10^5)	R_s	k_2	α	$\overline{N}$ (10^5)	R_s
15	A:B	7.50	1.14	2.34	1.99	91.8	1.26	nd	nd
	B:C	6.59	1.41	2.16	6.28	73.0	1.56	nd	nd
	C:D	4.68	1.12	2.34	2.55	46.6	1.33	2.13	0.80
	D:E	4.16	1.49	2.43	11.7	35.1	1.85	2.59	3.00
20	A:B	6.88	1.15	2.15	2.21	80.4	1.38	nd	nd
	B:C	5.98	1.40	2.33	6.92	58.3	1.48	nd	nd
	C:D	4.27	1.12	2.55	2.87	39.4	1.34	1.36	0.78
	D:E	3.80	1.48	2.43	12.3	29.4	1.84	1.72	2.86
25	A:B	6.06	1.17	1.93	2.64	60.2	1.44	2.12	0.83
	B:C	5.20	1.36	2.36	7.05	41.8	1.40	2.04	1.06
	C:D	3.82	1.12	2.53	3.13	29.9	1.32	1.96	1.15
	D:E	3.40	1.49	2.15	12.9	22.6	1.78	2.28	3.95
30	A:B	5.76	1.17	1.46	2.40	49.0	1.48	2.12	1.11
	B:C	4.93	1.36	1.85	6.53	33.2	1.36	1.89	1.14
	C:D	3.63	1.12	2.07	2.95	24.5	1.32	1.86	1.35
	D:E	3.24	1.49	1.71	11.9	18.5	1.75	2.09	4.40
35	A:B	5.37	1.17	1.32	2.42	39.4	1.49	1.94	1.34
	B:C	4.57	1.34	1.37	5.65	26.5	1.34	1.81	1.31
	C:D	3.42	1.13	1.66	3.00	19.9	1.31	1.72	1.54
	D:E	3.03	1.49	1.81	12.9	15.2	1.73	1.95	4.97
40	A:B	4.94	1.18	1.12	2.54	32.2	1.48	1.46	1.38
	B:C	4.19	1.32	1.41	5.79	21.7	1.32	1.35	1.29
	C:D	3.17	1.13	1.54	3.06	16.5	1.31	1.26	1.57
	D:E	2.82	1.48	1.51	12.2	12.6	1.70	1.41	4.83

nd = not determined due to peak overlapping

Table 4.6 Effect of temperature on ratio of separation scales of test analytes in MEEKC to MEKC in the presence of 180 mM SDS and 25% v/v EtOH in the buffers. Each value is the average from two runs

Temperature, °C	A pair of analytes	Ratio of separation scales in MEEKC to MEKC			
		$1/(1+k_2)$	α-1	$\sqrt{N}$	R_s
15	A:B	10.9	0.54	nd	nd
	B:C	9.75	0.73	nd	nd
	C:D	8.38	0.36	1.05	3.19
	D:E	7.00	0.58	0.97	3.90
20	A:B	10.3	0.39	nd	nd
	B:C	8.50	0.83	nd	nd
	C:D	7.67	0.35	1.37	3.68
	D:E	6.33	0.57	1.19	4.30
25	A:B	8.67	0.39	0.95	3.18
	B:C	6.90	0.90	1.08	6.65
	C:D	6.41	0.38	1.14	2.72
	D:E	5.36	0.63	0.97	3.27
30	A:B	7.40	0.35	0.83	2.16
	B:C	5.77	1.00	0.99	5.73
	C:D	5.51	0.38	1.05	2.19
	D:E	4.60	0.65	0.90	2.70
35	A:B	6.34	0.35	0.82	1.81
	B:C	4.94	1.00	0.87	4.31
	C:D	4.73	0.42	0.98	1.95
	D:E	4.02	0.67	0.96	2.60
40	A:B	5.59	0.38	0.88	1.84
	B:C	4.37	1.00	1.02	4.49
	C:D	4.20	0.42	1.11	1.95
	D:E	3.56	0.69	1.03	2.53

nd = not determined due to peak overlapping

4.5 Conclusion

The quantity of resolution of test analytes, BADGEs, in MEEKC and MEKC with suppressed electroosmosis was compared, using an equation for the resolution relating to separation scales of α, k and N, $R_s = (\sqrt{\overline{N}}/4)\ (\alpha\text{-}1)/(1+k_2)$ [Nhujak *et al*. 2006]. At a given CE condition, MEEKC was found to provide better resolution of the test analytes than did MEKC. This can be explained from experimental results and the resolution equation that MEEKC gave significantly greater $1/1+k_2$ or smaller k of the test analytes, while smaller α-1 in MEEKC and a comparable range of $\overline{N}$. This indicates that the better resolution of BADGEs in MEEKC than that in MEKC is mainly due to the significantly smaller retention factor in MEEKC, but not the greater selectivity in MEEKC. Improved resolution of BADGEs was obtained with increasing the concentration of organic co-solvents, such as ACN, MeOH, EtOH or 2-PrOH, from 20 to 30% v/v in MEEKC and MEKC, while small change in resolution with the SDS concentration in a range of 100 to 180 mM. In addition, a decrease in temperature from 40 to 15^{o}C results in slightly better R_s. However, long analysis time was obtained with low concentration of SDS and high concentration of organic co-solvent in the buffer, and low temperature used.

CHAPTER V

PREDICTION AND APPLICATION OF RETENTION IN MEEKC

5.1 Introduction

The value of *k* is one of the characteristics that indicates hydrophobicity of compounds in chromatography and electrokinetic chromatography such as MEEKC and MEKC [Muijselaar *et al.* 1995 and 1997, Gong *et al.* 2004, Klotz *et al.* 2001, Ishihama *et al.* 1995, Kelly *et al.* 2001]. The higher the value of *k*, the higher the hydrophobicity or the partitioning of the analytes in the pseudo stationary phase. As described in Sections 3.3.3 and 3.3.6, excellent linear relationship is obtained between log *k* and the number of carbons of analyte (*z*) of homologous series compounds in a wide range of the concentration of SDS ([SDS]) and organic co-solvents (φ). The equations may be modified for prediction of retention factor of homologous series compounds in MEEKC in a wide range of [SDS] and φ in this section.

The value of log K_{ow} is also one of the most commonly used as an indicator of hydrophobicity of compounds that can be used to predict biomembrane transport, bioaccumulation in plants and animals, and soil adsorption [Klotz *et al.* 2001, Poole *et al.* 2003, Jia *et al*. 2003]. A number of methods have been applied for log K_{ow} measurements. Direct measurement of log K_{ow} using the conventional shake flask method is time consuming (often in excess of 1 day/solute), tedious and required large amounts of pure compounds [Poole *et al*. 2003, Jia *et al*. 2003, Herbert *et al*. 1995].

Reversed-phase liquid chromatography (RPLC) has been used as a measurement of log K_{ow} through a linear relationship of log K_{ow} and log *k* of compounds [Dorsey *et al*. 1993, Klotz *et al.* 2001]. This method is advantageous over conventional shake flask method including providing faster analysis times, improved reproducibility, and requiring less amounts of sample for testing [Klotz *et al.* 2001, Poole *et al*. 2003].

Electrokinetic chromatography such as MEKC and MEEKC has been recently used for log K_{ow} measurement [Poole *et al*. 2003]. Using standards with known log K_{ow}, the linear plot between log K_{ow} versus log k or log K_{ow} versus I were obtained in MEKC [Herbert *et al.* 1995, Ishihama *et al.* 1995] or MEEKC [Klotz *et al.* 2001, Gong *et al.* 2004], and the values of log K_{ow} of anlytes were predicted from log k or I. Excellent agreement was found between literature and predicted values of log K_{ow} estimated from log k or I [Herbert *et al.* 1995, Ishihama *et al.* 1995, Klotz *et al.* 2001, Gong *et al.* 2004]. Therefore, the retention factor and retention index are used to determine log K_{ow} of analytes in this section.

In previous work on MEKC with the SDS surfactant and high EOF [Kelly *et al.* 2001], the retention factor of compounds can be predicted from the structure-retention relationship. Excellent agreement was found between observed and predicted values of retention factor [Kelly *et al.* 2001]. Therefore, the structure-retention relationship is also used to explain and predict the retention factor of compounds in this section.

The aim of this section is to predict the retention factor and log K_{ow} of analytes. The linear equation is developed for prediction of retention factor of homologous series compounds in MEEKC in a wide range of [SDS] and φ, using the simple linear relationship between log k and z, log k and [SDS] and log k and φ. The structure-retention relationship is also used to explain and predict the retention factor of compounds containing two different substituting groups. In addition, the retention factor and retention index are used to determine log K_{ow} of analytes.

5.2 Experimental

All chemicals used are previously given in Sections 3.2.1 and 4.2.1. Preparation of buffers and analytes used are previously described in Sections 3.2.2 and 4.2.2. In addition, all CE conditions used are previously mentioned in Sections 3.2.3 and 4.2.3.

5.3 Results and Discussion

5.3.1 Prediction of retention factor of homologous series compounds

As previously described in Section 3.3.3, the excellent linear relationship between log *k* and *z* as shown in Figure 3.7 and Table 3.2 was obtained for each homologous series at a given [SDS] in a range of 100 to 200 mM, with $r^2 > 0.999$, which is consistent with the Martin's equation in Equation 3.3. Over a wide range of [SDS], the values of slope, *a*, for each homologous series were found to be insignificantly different, while the value of intercept, *b*, for each homologous series increased with an increase in [SDS]. Using the average value of the slope, $\bar{a}$, obtained from a plot of log *k* versus *z*, the value of the adjusted intercept, b' , is seen to increase linearly with an increase in [SDS] as given in Table 3.2. In order to predict the retention factor of homologous series compounds in MEEKC in a wide range of [SDS], the linear relationship between log *k* and *z* in Equation 3.3 may be modified into the equation

$$\log k = \bar{a}z + b' \qquad (5.1)$$

where $\bar{a}$ is dependent on homologous series, but independent of [SDS] for each homologous series as shown in Table 3.2. At a given [SDS], the value of b' may be predicted using the linear relationship between b' and [SDS] as given in Table 3.2. The predicted and observed values of log *k* of homologous series are shown in Table 5.1, using 130 and 190 mM SDS in MEEKC and predicted log *k* from Equation 5.1 and Table 3.2. The linear plots in Figure 5.1 indicate excellent agreement between observed and predicted log *k* of homologous series with the slope values of 1.001 (r^2 = 0.995) and 0.994 (r^2 = 0.996) for MEEKC at 130 and 190 mM SDS, respectively.

Table 5.1 Observed and predicted log *k* of analytes in MEEKC using 130 and 190 mM SDS

Analytes	log *k* of analytes in MEEKC using [SDS] at			
	130 mM		190 mM	
	Predicted	Observed[a)]	Predicted	Observed[a)]
BZ6	0.664	0.628 ± 0.006	0.811	0.788 ± 0.003
BZ7	1.07	1.06 ± 0.01	1.22	1.22 ± 0.01
BZ8	1.47	1.44 ± 0.01	1.62	1.60 ± 0.01
BZ9	1.88	1.87 ± 0.01	2.02	2.01 ± 0.01
PN8	0.362	0.401 ± 0.001	0.515	0.496 ± 0.004
PN9	0.707	0.745 ± 0.003	0.860	0.831 ± 0.003
PN10	1.05	1.09 ± 0.01	1.21	1.18 ± 0.01
PN11	1.40	1.45 ± 0.01	1.55	1.54 ± 0.01
BA8	0.737	0.753± 0.001	0.894	0.915 ± 0.001
BA9	1.09	1.09 ± 0.01	1.25	1.26 ± 0.01
BA10	1.44	1.46 ± 0.01	1.60	1.62 ± 0.01
BA11	1.79	1.79 ± 0.02	1.95	1.94 ± 0.01
PB8	0.460	0.410 ± 0.004	0.507	0.551 ± 0.023
PB9	0.837	0.782 ± 0.011	0.884	0.929 ± 0.043
PB10	1.21	1.19 ± 0.02	1.26	1.32 ± 0.04
PB11	1.59	1.57 ± 0.04	1.64	1.68 ± 0.04

[a)] the average and SD values obtained from two runs.

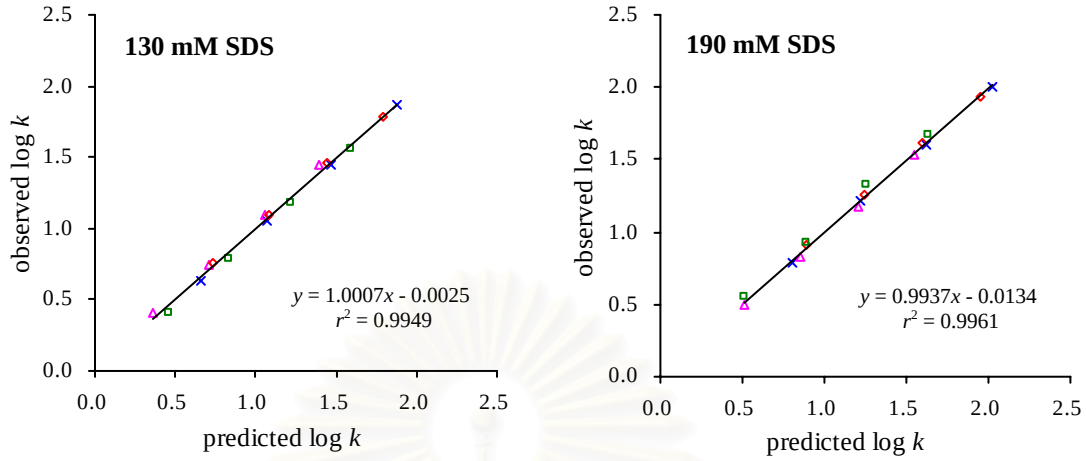


Figure 5.1 Linear relationship between observed and predicted log *k* of analytes in MEEKC using various concentrations of SDS. Other MEEKC conditions as shown in Figure 3.2. Symbols refer to the average observed value from two runs for homologous series as given in Figure 3.3, and predicted values are obtained using Equation 5.1 and Table 3.2.

In the case of MEEKC with an organic co-solvent, it follows from Equations 3.9 and 3.10 and Figure 3.9 that good agreement was found for log k_w obtained from the experiments without any organic co-solvent and from the Y-intercept of plots in Figure 3.9. The values of *m* for each homologues series were also found to linearly increase with *z* ($r^2 > 0.99$). In addition, at a given φ for each organic co-solvent, linear relationship between log *k* and *z* was observed, log $k = az + b$ with $r^2 > 0.995$ for each homologues series. With an increase in φ, a linear decrease in *a* and a linear increase in *b* were obtained for each homologous series.

Using MeOH, EtOH or ACN as the organic co-solvent in MEEKC, it follows from Equation 3.3 and 3.9 that the relationship between log *k* as a function of φ may be given by the equation

$$\log k = (\bar{a}\, z + b') - (rz + s)\, \varphi \qquad (5.2)$$

where *r* and *s* are the values of slope and intercept, respectively, for a plot of *m* (in Equation 3.9) and *z*, for each homologous series.

The predicted and observed values of log *k* of homologous series are shown in Table 5.2, using 12 or 22%v/v organic co-solvents in MEEKC and other CE conditions in Figure 3.4, and predicted log *k* from Equation 5.2 and Table 3.2. The linear plots in Figure 5.2 indicate excellent agreement between observed and predicted log *k* of homologous series with the values of slope near 1.00 and $r^2 > 0.991$. Therefore, analytes with similar *k* or *I*, such as BA9 and BZ7, BZ8 and BA10 or PB8 and PN8, in MEEKC without any organic co-solvent may be identified using their *k* obtained from MEEKC with appropriate types and concentrations of organic co-solvents.

Table 5.2 Observed and predicted log *k* of analytes in MEEKC using 12 and 22% v/v organic co-solvents

Analytes	log *k* of analytes in MEEKC using											
	12% v/v MeOH		22% v/v MeOH		12% v/v EtOH		22% v/v EtOH		12% v/v ACN		22% v/v ACN	
	Predicted	Observed[a)]	Predicted	Observed[a)]	Predicted	Observed[a)]	Predicted	Observed[a)]	Predicted	Observed[a)]	Predicted	Observed[a)]
BZ6	0.684	0.679 ± 0.001	0.599	0.581 ± 0.002	0.632	0.635 ± 0.001	0.503	0.533 ± 0.001	0.711	0.705 ± 0.001	0.648	0.644 ± 0.001
BZ7	1.03	1.06 ± 0.01	0.896	0.906 ± 0.006	0.939	0.973 ± 0.001	0.729	0.777 ± 0.001	1.05	1.07 ± 0.01	0.935	0.956 ± 0.001
BZ8	1.38	1.39 ± 0.01	1.19	1.19 ± 0.01	1.25	1.28 ± 0.01	0.955	0.982 ± 0.001	1.39	1.40 ± 0.01	1.22	1.24 ± 0.01
BZ9	1.72	1.75 ± 0.01	1.49	1.50 ± 0.01	1.55	1.60 ± 0.01	1.18	1.20 ± 0.01	1.73	1.75 ± 0.01	1.51	1.54 ± 0.01
PN8	0.376	0.373 ± 0.003	0.282	0.279 ± 0.009	0.332	0.329 ± 0.001	0.201	0.216 ± 0.001	0.300	0.264 ± 0.002	0.142	0.102 ± 0.003
PN9	0.691	0.678 ± 0.001	0.571	0.551 ± 0.012	0.604	0.612 ± 0.001	0.412	0.431 ± 0.001	0.579	0.538 ± 0.001	0.367	0.316 ± 0.003
PN10	1.01	0.984 ± 0.001	0.860	0.823 ± 0.019	0.875	0.890 ± 0.001	0.622	0.628 ± 0.002	0.859	0.807 ± 0.001	0.592	0.516 ± 0.005
PN11	1.32	1.31 ± 0.01	1.15	1.12 ± 0.03	1.15	1.17 ± 0.01	0.832	0.835 ± 0.002	1.14	1.08 ± 0.01	0.817	0.710 ± 0.007
BA8	0.649	0.664 ± 0.001	0.466	0.444 ± 0.004	0.636	0.660 ± 0.001	0.443	0.481 ± 0.001	0.519	0.523 ± 0.003	0.229	0.228 ± 0.015
BA9	0.943	0.955 ± 0.001	0.713	0.682 ± 0.006	0.901	0.934 ± 0.001	0.636	0.667 ± 0.001	0.789	0.790 ± 0.004	0.430	0.404 ± 0.023
BA10	1.24	1.26± 0.01	0.960	0.931 ± 0.008	1.17	1.21 ± 0.01	0.829	0.859 ± 0.002	1.06	1.07 ± 0.01	0.632	0.578 ± 0.031
BA11	1.53	1.57 ± 0.01	1.21	1.18 ± 0.01	1.43	1.45 ± 0.01	1.02	1.04 ± 0.01	1.33	1.33 ± 0.01	0.833	0.747 ± 0.040
PB8	0.424	0.411 ± 0.001	0.335	0.325 ± 0.001	0.349	0.335 ± 0.010	0.196	0.217 ± 0.001	0.291	0.282 ± 0.002	0.090	0.088 ± 0.007
PB9	0.761	0.731 ± 0.001	0.638	0.611 ± 0.002	0.637	0.623 ± 0.012	0.411	0.428 ± 0.001	0.589	0.583 ± 0.003	0.322	0.289 ± 0.011
PB10	1.10	1.07 ± 0.01	0.940	0.913 ± 0.002	0.926	0.916 ± 0.016	0.626	0.627 ± 0.002	0.887	0.896 ± 0.005	0.554	0.513 ± 0.006
PB11	1.43	1.39 ± 0.01	1.24	1.21 ± 0.01	1.21	1.18 ± 0.02	0.841	0.819 ± 0.002	1.18	1.19 ± 0.01	0.786	0.741 ± 0.004

[a)] the average and SD values obtained from two runs.

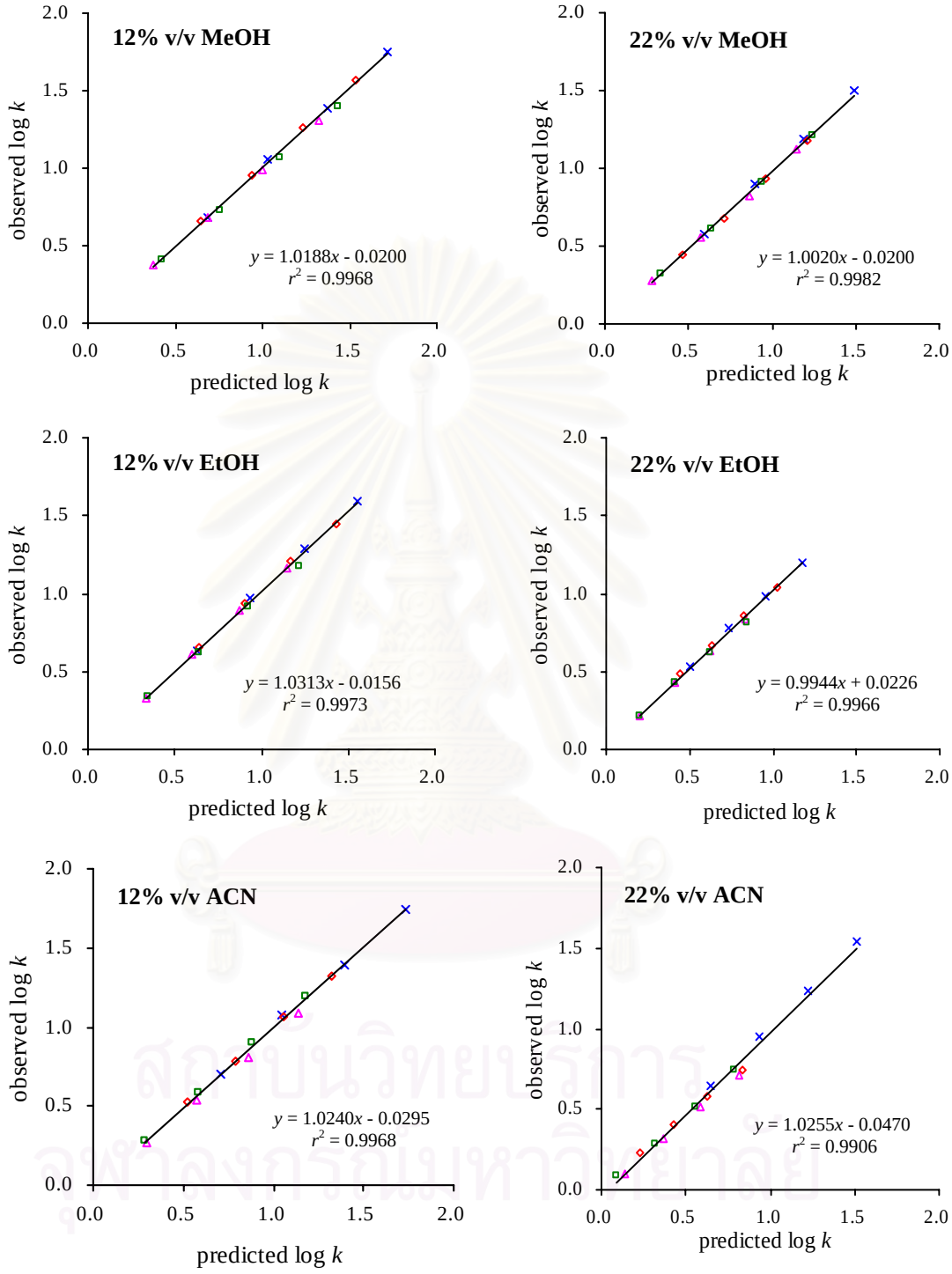


Figure 5.2 Linear relationship between observed and predicted log *k* of analytes in MEEKC containing organic co-solvents. Other MEEKC conditions as shown in Figure 3.2. Symbols refer to the average observed value from two runs for homologous series as given in Figure 3.3, and predicted values are obtained using Equation 5.2 and Table 3.2.

5.3.2 Prediction of octanol-water distribution constant of homologous series compounds using MEEKC

The value of log K_{ow} is one of the most commonly used as an indicator of solubility or hydrophobicity of compounds, and may be determined from the relationship between log K_{ow} and log k or log K_{ow} and I as the equations [Muijselaar *et al.* 1995 and 1997, Gong *et al.* 2004, Klotz *et al.* 2001, Muijsellar *et al.* 1997]

$$\log K_{ow} = c \log k + d \qquad (5.3)$$

$$\log K_{ow} = eI + f \qquad (5.4)$$

where c and d are the slope and intercept values, respectively, obtained from the linear plot between log K_{ow} and log k for BZ as standards at each [SDS] as shown in Figure 5.3a and Table 5.3; e and f are the slope and intercept values, respectively, obtained from the linear plot between log K_{ow} and nominal I of BZ as standards as shown in Figure 5.3b and Table 5.3.

The average values of predicted log K_{ow} as shown in Table 5.4 were obtained from 100 to 200 mM SDS and BZ as standards. The predicted values of log K_{ow} using Equation 5.4 were obtained from the linear relationship between log K_{ow} and $\bar{I}$, where $\bar{I}$ is the average value of the retention index of the analyte over a wide range of SDS concentrations from 100 to 200 mM in Figure 3.4b. From Table 5.4, using paired *t*-test analysis at 95% confidence interval of the mean, non-significant difference was obtained for the predicted log K_{ow} estimated from log k and I. Good agreement was also found for the predicted and literature values of log K_{ow} for each analyte, except for PN8 and PB8. The slight difference is probably because the predicted values of log K_{ow} for PN8 and PB8 are not in the range of a calibration plot using BZ as standards. However, using paired *t*-test analysis at 95% confidence interval of the mean, non-significant difference was obtained between the predicted and literature values for all analytes.

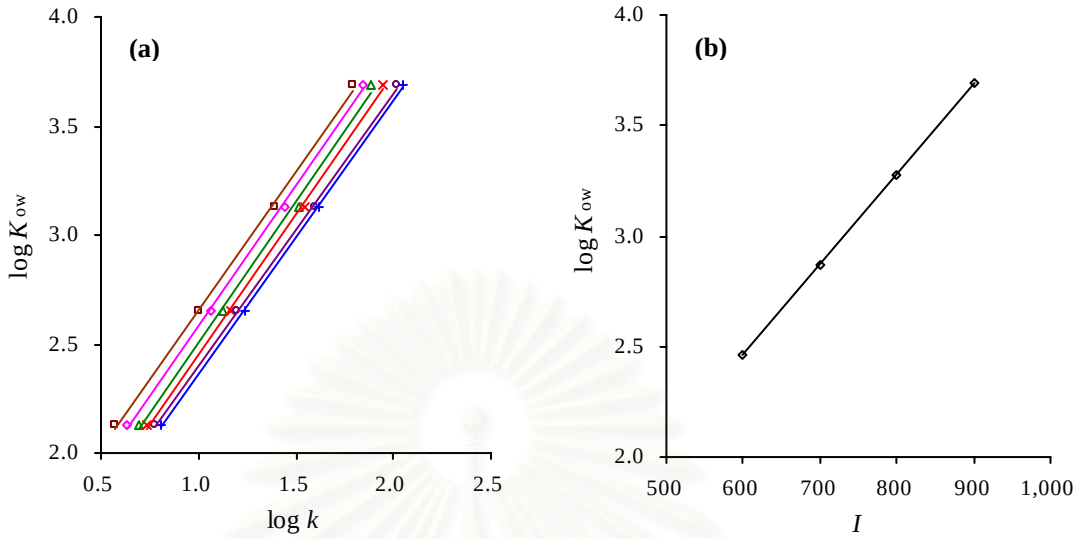


Figure 5.3 Relationship between (a) log K_{ow} and log k and (b) log K_{ow} and I of homologous series in MEEKC using BZ as standards. The values of I in (b) refer to nominal I of BZ (600, 700, 800 and 900 for BZ6, BZ7, BZ8 and BZ9, respectively). Symbols □, ◇, △, ×, o and + in (a) refer to the average observed value from two runs for BZ at 100, 120, 140, 160, 180 and 200 mM SDS, respectively. Other MEEKC conditions as shown in Figure 3.2.

Table 5.3 Linear equations of calibration plots of log K_{ow} and log k and log K_{ow} and I of homologous series in MEEKC using BZ as standards

[SDS], mM	$\log K_{ow} = c \log k + d$			$\log K_{ow} = eI + f$		
	c	d	r^2	e	f	r^2
100	1.275	1.387	0.9985			
120	1.284	1.301	0.9990			
140	1.301	1.203	0.9965			
160	1.293	1.156	0.9986	0.00516	-0.970	0.9992
180	1.251	1.152	0.9992			
200	1.257	1.101	0.9997			

Table 5.4 Predicted and literature values of log K_{ow} of analytes in MEEKC using BZ as standards

analyte	log K_{ow}		
	predicted values		literature values
	$\log K_{ow} = c \log k + d$	$\log K_{ow} = e\bar{I} + f$	
PN8	[a)]1.72 ± 0.05	1.72 ± 0.05	1.58 [Ishihama *et al.* 1995]
PN9	[a)]2.13 ± 0.06	2.13 ± 0.07	2.20 [Ishihama *et al.* 1995]
PN10	[a)]2.56 ± 0.05	2.56 ± 0.07	2.66 [Ishihama *et al.* 1995]
PN11	[a)]3.02 ± 0.05	3.02 ± 0.08	3.11 [Poole *et al.* 2000]
BA8	[a)]2.19 ± 0.07	2.18 ± 0.06	2.12 [Poole *et al.* 2000]
BA9	[a)]2.62 ± 0.04	2.63 ± 0.06	2.64 [Poole *et al.* 2000]
BA10	[a)]3.11 ± 0.06	3.11 ± 0.04	NA
BA11	[a)]3.56 ± 0.05	3.56 ± 0.01	NA
PB8	[a)]1.78 ± 0.04	1.78 ± 0.04	1.96 [Hansen *et al.* 2000]
PB9	[a)]2.24 ± 0.05	2.24 ± 0.05	NA
PB10	[a)]2.75 ± 0.04	2.75 ± 0.04	NA
PB11	[a)]3.22 ± 0.03	3.23 ± 0.02	NA

c and *d* are the slope and intercept values, respectively, obtained from the linear calibration plot between log K_{ow} and log *k* for BZ at each SDS concentration. *e* and *f* are the slope and intercept values, respectively, obtained from the linear calibration plot between log K_{ow} and nominal *I* of BZ as standards. $\bar{I}$ is the average value of retention index from 100 to 200 mM SDS as shown Figure 3.4b. log K_{ow} = 2.13, 2.65, 3.13 and 3.69 for BZ6, BZ7, BZ8 and BZ9, respectively [Muijselaar *et al.* 1994]. NA = not available. [a)]the average value obtained from 100 to 200 mM SDS.

5.3.3 Relationship of BADGEs structure-retention in MEEKC

It should be noted from Section 4.3.3 that the retention factor of BADGEs containing two different substituting groups was found to be between that of BADGEs containing two identical substituting groups, BADGE.2HCl (A) > BADGE.HCl.H_2O (C) > BADGE.2H_2O (E) and BADGE (B) > BADGE.H_2O (D) > BADGE.2H_2O (E). The retention factor is seem to be log k_C = (log k_A + log k_E)/2 and log k_D = (log k_B + log k_E)/2, and not k_C = (k_A + k_E)/2 and k_D = (k_B + k_E)/2. This may be explained using the additivity approach for estimation of log P_{OW} [Hansch *et al.* 1979, Kelly *et al.* 2001] and log K_{MW} [Smith *et al.* 1987, Kelly *et al.* 2001], where log P_{OW} is the octanol-water partition coefficient and log K_{MW} is the micelle-water partition coefficient. In our case, log K_{PW} is used instead of log K_{MW}, where log K_{PW} is the pseudo stationary phase-organic solvent/water partition coefficient. The pseudo stationary phase in MEEKC refers to the charged oil droplets surrounding with charged surfactant and co-surfactant, while the pseudo stationary phase in MEKC refers to the micellar phase.

The group additive approach is based on the assumption that free energy of transfer from a bulk organic/aqueous phase into a pseudo stationary phase has additive-constitutive properties as [Kelly *et al.* 2001]

$$\Delta G(\mathrm{PR}) = \Delta\Delta G(\mathrm{P}) + \sum \Delta\Delta G(\mathrm{R}) \qquad (5.5)$$

where $\Delta G(\mathrm{P})$ is the free energy of partitioning of a parent moiety and $\sum \Delta\Delta G(\mathrm{R})$ the sum of the free energies of the substituents. Since ΔG is also proportional to log K_{PW}, it follows that log K_{PW} of the test analytes (PR) is related to log K_{PW} of the parent P and substituent R, as the equation

$$\log K_{PW}(\mathrm{PR}) = \log K_{PW}(\mathrm{P}) + \log K_{PW}(\mathrm{R}) \qquad (5.6)$$

For the analyte containing two identical substituting groups, such as X and X or Y and Y, it follows from Equation 5.6 that

$$\log K_{PW}(PXX) = \log K_{PW}(P) + 2\log K_{PW}(X) \qquad (5.7)$$

$$\log K_{PW}(PYY) = \log K_{PW}(P) + 2\log K_{PW}(Y) \qquad (5.8)$$

In the case of the analyte containing two different substituting groups, such as X and Y, it follows from Equation 5.6 that

$$\log K_{PW}(PXY) = \log K_{PW}(P) + \log K_{PW}(X) + \log K_{PW}(Y) \qquad (5.9)$$

From Equations 5.7 to 5.9, the relationship of log K_{PW} of these analytes can be written by

$$\log K_{PW}(PXY) = \frac{\log K_{PW}(PXX) + \log K_{PW}(PYY)}{2} \qquad (5.10)$$

From Equation 2.12, substituting $K = k/\phi$ in Equation 5.11 gives the relationship of log k of these analytes as the equation

$$\log k(PXY) = \frac{\log k(PXX) + \log k(PYY)}{2} \qquad (5.11)$$

In order to check Equation 5.11, the predicted and observed values of log k of BADGEs are shown in Table 5.5, in a wide range of MEEKC and MEKC conditions used in our experiment, and predicted log k from Equation 5.11 and Tables 4.1, 4.3 and 4.5. The linear plots in Figure 5.4 indicate excellent agreement between observed and predicted log k of BADGEs with the slope values of 1.02 (r^2 = 0.996) and 1.01 (r^2 = 0.996) for MEEKC and MEKC, respectively.

Table 5.5 Observed and predicted values of log *k* for BADGEs containing two different substituting groups

CE conditions	MEEKC				MEKC			
	log k_C		log k_D		log k_C		log k_D	
	predicted	observed	predicted	observed	predicted	observed	predicted	observed
(a) Various types and concentrations of organic co-solvents								
20% v/v ACN	0.841 ± 0.001	0.868 ± 0.007	0.814 ± 0.006	0.818 ± 0.006	nd	nd	nd	nd
25% v/v ACN	0.591 ± 0.008	0.613 ± 0.009	0.567 ± 0.008	0.569 ± 0.008	1.00 ± 0.01	1.03 ± 0.01	0.945 ± 0.008	0.950 ± 0.006
30% v/v ACN	0.182 ± 0.008	0.200 ± 0.010	0.164 ± 0.008	0.167 ± 0.008	0.621 ± 0.006	0.644 ± 0.007	0.583 ± 0.006	0.589 ± 0.006
20% v/v MeOH	1.11 ± 0.01	1.14 ± 0.01	1.07 ± 0.01	1.07 ± 0.01	nd	nd	nd	nd
25% v/v MeOH	0.928 ± 0.003	0.952 ± 0.002	0.878 ± 0.003	0.875 ± 0.002	nd	nd	nd	nd
30% v/v MeOH	0.775 ± 0.005	0.795 ± 0.004	0.723 ± 0.004	0.718 ± 0.005	nd	nd	nd	nd
20% v/v EtOH	0.798 ± 0.001	0.819 ± 0.002	0.772 ± 0.001	0.767 ± 0.001	nd	nd	nd	nd
25% v/v EtOH	0.571 ± 0.001	0.582 ± 0.001	0.538 ± 0.001	0.531 ± 0.001	1.44 ± 0.01	1.48 ± 0.01	1.36 ± 0.01	1.36 ± 0.01
30% v/v EtOH	0.399 ± 0.001	0.405 ± 0.001	0.365 ± 0.001	0.354 ± 0.001	1.05 ± 0.01	1.08 ± 0.01	0.971 ± 0.001	0.967 ± 0.001
20% v/v 2-PrOH	0.648 ± 0.001	0.660 ± 0.001	0.635 ± 0.001	0.625 ± 0.001	nd	nd	nd	nd
25% v/v 2-PrOH	0.502 ± 0.001	0.506 ± 0.002	0.481 ± 0.001	0.469 ± 0.001	0.887 ± 0.001	0.914 ± 0.003	0.850 ± 0.005	0.849 ± 0.003
30% v/v 2-PrOH	0.418 ± 0.001	0.417 ± 0.001	0.396 ± 0.001	0.380 ± 0.001	0.568 ± 0.004	0.568 ± 0.004	0.529 ± 0.004	0.525 ± 0.004
(b) Various [SDS]								
180 mM	0.571 ± 0.001	0.582 ± 0.001	0.538 ± 0.001	0.531 ± 0.001	1.44 ± 0.01	1.48 ± 0.01	1.36 ± 0.01	1.36 ± 0.01
160 mM	0.538 ± 0.001	0.555 ± 0.001	0.505 ± 0.001	0.501 ± 0.001	1.41 ± 0.01	1.44 ± 0.01	1.32 ± 0.01	1.31 ± 0.01
140 mM	0.511 ± 0.001	0.524 ± 0.001	0.474 ± 0.001	0.467 ± 0.001	1.38 ± 0.01	1.41 ± 0.01	1.29 ± 0.01	1.28 ± 0.01
120 mM	0.462 ± 0.002	0.473 ± 0.002	0.419 ± 0.002	0.413 ± 0.001	1.36 ± 0.01	1.39 ± 0.01	1.26 ± 0.01	1.25 ± 0.01
100 mM	0.413 ± 0.001	0.424 ± 0.002	0.368 ± 0.002	0.362 ± 0.003	1.31 ± 0.01	1.34 ± 0.01	1.20 ± 0.01	1.19 ± 0.01
(c) Various temperature								
15°C	0.661 ± 0.002	0.670 ± 0.002	0.663 ± 0.002	0.619 ± 0.004	1.63 ± 0.01	1.67 ± 0.02	1.58 ± 0.02	1.55 ± 0.02
20°C	0.623 ± 0.001	0.630 ± 0.001	0.593 ± 0.001	0.580 ± 0.001	1.56 ± 0.01	1.60 ± 0.01	1.49 ± 0.01	1.47 ± 0.01
25°C	0.571 ± 0.001	0.582 ± 0.001	0.538 ± 0.001	0.531 ± 0.001	1.44 ± 0.01	1.48 ± 0.01	1.36 ± 0.01	1.36 ± 0.01
30°C	0.548 ± 0.001	0.560 ± 0.001	0.514 ± 0.001	0.510 ± 0.001	1.36 ± 0.01	1.39 ± 0.01	1.28 ± 0.01	1.27 ± 0.01
35°C	0.520 ± 0.001	0.534 ± 0.001	0.485 ± 0.001	0.481 ± 0.001	1.27 ± 0.01	1.30 ± 0.01	1.19 ± 0.01	1.18 ± 0.01
40°C	0.487 ± 0.001	0.501 ± 0.001	0.451 ± 0.001	0.450 ± 0.001	1.19 ± 0.01	1.22 ± 0.01	1.11 ± 0.01	1.10 ± 0.01

nd = not determined due to peak overlapping, (a), (b) and (c) other CE conditions in Figure 4.6, 4.8 and 4.9, respectively.

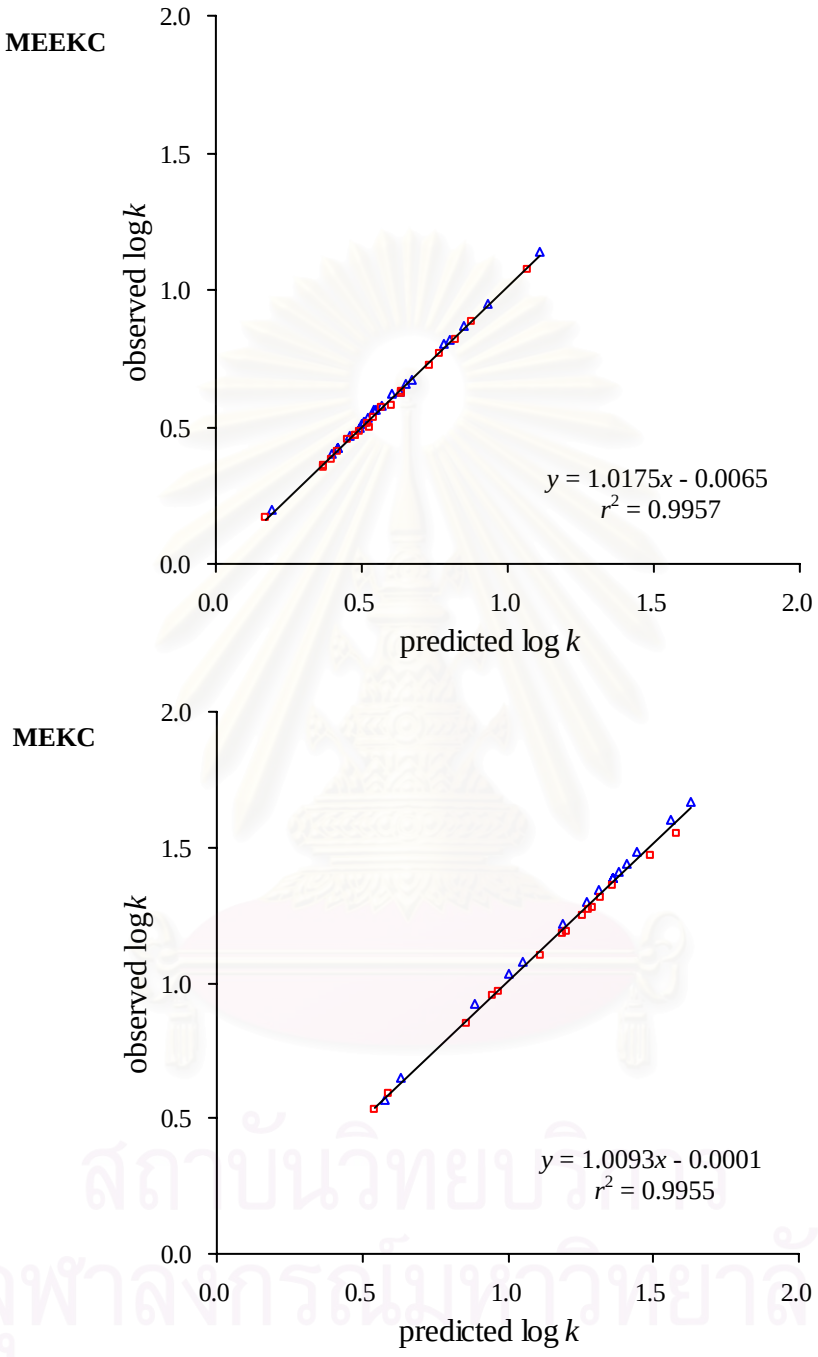
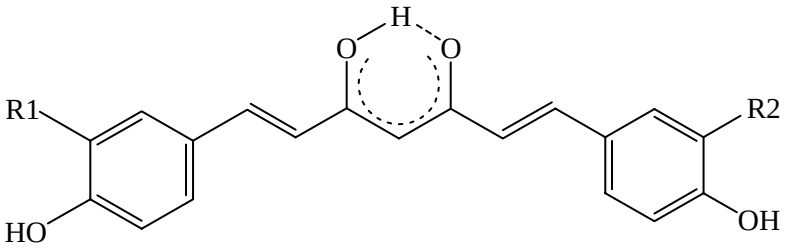


Figure 5.4 Linear relationship between observed and predicted log *k* of analytes in a wide range of MEEKC and MEKC conditions as shown in Table 5.5. Other CE conditions as shown in Figure 4.6. Symbols △ and □ refer to the average observed value from two runs for C and D respectively, and predicted values are obtained using Equation 5.11 and Tables 4.1, 4.3 and 4.5.

5.3.4 Relationship of structure-retention in previous work on MEEKC: Application to curcuminoids [Nhujak *et al.* 2006]

The objective of this section is to explain retention of other compounds containing two different substituting groups using the additive approach for estimation of log *k*. In recently previous work of our group, we have reported MEEKC for separation and analysis of curcuminoids, such as curcumin (C), demethoxycurcumin (D), and bis-demethoxycurcumin (B) [Nhujak *et al.* 2006]. As shown in Figure 5.5, bis-demethoxycurcumin and curcumin contain identical R1 and R2 groups, while demethoxycurcumin contains different R1 and R2 groups. Using an MEEKC buffer containing a pH 2.5 phosphate buffer, 1.1% v/v *n*-octane, 890 mM 1-butanol, 115 to 200 mM SDS, and organic co-solvents at leves of 20 to 30% v/v, the retention factor was reported to be in order B > D > C [Nhujak *et al.* 2006]. Using experimental results from previous work, [Nhujak *et al.* 2006], predicted and observed values of log *k* of D (log k_D) are compared as shown in Table 5.6 and Figure 5.6, where log k_D is the average value of log k_B and log k_C. The linear plot in Figure 5.6 indicates excellent agreement between observed and predicted values of log k_D in MEEKC with the slope values of 0.992 (r^2 = 0.9999).

Therefore, results from MEEKC on BADGEs and curcuminoids suggest that the retention factor of compounds containing two different substituting groups be predicted using the additive approach from log *k* of the compounds containing the identical substituting groups.



curcuminoids	R1	R2
curcumin (C)	$-OCH_3$	R2 = R1
demethoxycurcumin (D)	$-OCH_3$	H
bis-demethoxycurcumin (B)	H	R2 = R1

Figure 5.5 Structures of curcuminoids.

Table 5.6 Observed and predicted values of log *k* of demethoxycurcumin (log k_D) containing two different substituting groups

MEEKC conditions	log k_D Predicted[c)]	log k_D Observed[d)]
(a) Various types and concentrations of organic co-solvents		
20% v/v ACN	0.858 ± 0.001	0.856 ± 0.001
25% v/v ACN	0.651 ± 0.001	0.649 ± 0.001
30% v/v ACN	0.431 ± 0.001	0.430 ± 0.001
20% v/v EtOH	1.02 ± 0.01	1.01 ± 0.01
25% v/v EtOH	0.828 ± 0.001	0.825 ± 0.001
30% v/v EtOH	0.636 ± 0.001	0.636 ± 0.001
20% v/v 2-PrOH	0.839 ± 0.001	0.837 ± 0.001
25% v/v 2-PrOH	0.700 ± 0.001	0.698 ± 0.001
30% v/v 2-PrOH	0.602 ± 0.001	0.600 ± 0.001
(b)Various [SDS]		
115 mM	0.600 ± 0.005	0.598 ± 0.004
130 mM	0.646 ± 0.001	0.643 ± 0.001
150 mM	0.673 ± 0.002	0.670 ± 0.001
180 mM	0.700 ± 0.001	0.698 ± 0.001
200 mM	0.714 ± 0.002	0.712 ± 0.001

CE instrument conditions: uncoated fused silica capillary 50 μm i.d. × 40.2 cm (30 cm to detector), temperature 25°C, voltage –15kV, 0.5 psi pressure injection for 3 s and UV detection at 214 nm. MEEKC buffers used is a pH 2.5 phosphate buffer containing 1.1% *n*-octane, 890 mM 1-butanol and (a) various organic co-solvents and 180 mM SDS, and (b) 25% 2-PrOH and various concentration of SDS. [c)]obtained from the average value of log *k* of bis-demethoxycurcumin (B) and curcumin (C). [d)]data from previous work [Nhujak *et al.* 2006]

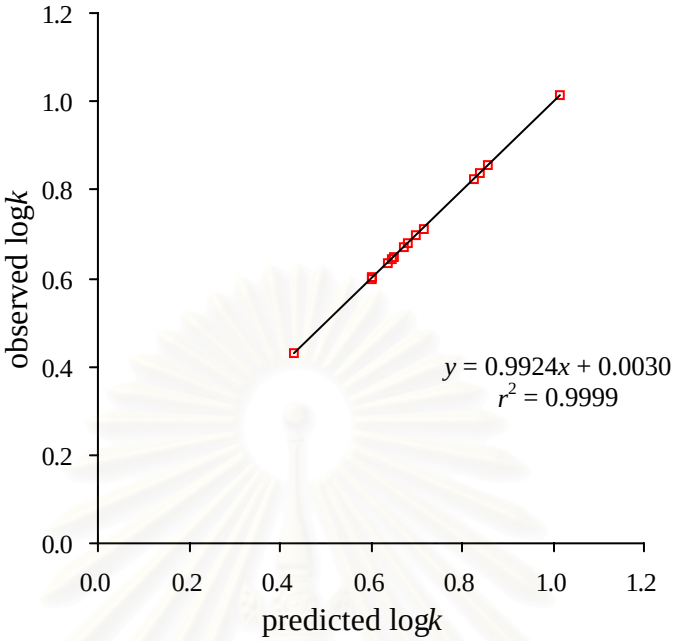


Figure 5.6 Linear relationship between observed and predicted log *k* of demethoxycurcumin in a wide range of MEEKC conditions as shown in Table 5.6. Symbol □ refers to the average observed value from two runs for analytes in previous work [Nhujak *et al.* 2006], and predicted values are obtained using Equation 5.11.

5.4 Conclusion

In MEEKC with suppressed electroosmosis, the equations were developed for prediction of *k* of homologous series compounds, such BZ, PN, BA and PB, in a wide range of [SDS] and φ. At a given [SDS] and φ in MEEKC, excellent agreement was found between observed and predicted values of log *k* of analytes, where the predicted values were obtained from our modified equations of the linear relationship of log *k* as functions of [SDS], *z*, and φ. Using BZ as standards, the linear calibration plot between log K_{ow} versus log *k* or log K_{ow} versus *I* were obtained over a wide range of [SDS] in MEEKC, and the values of log K_{ow} for the test analytes as PN, BA and PB were predicted from log *k* or *I*. Using paired *t*-test analysis at 95% confidence interval of the means, nonsignificant difference was obtained for the predicted log K_{ow} estimated from log *k* and *I*, and good agreement was also found for the predicted and

literature values of log K_{ow} for each analyte. In addition, in MEEKC and MEKC, the values of log k of compounds containing two different substituting groups can be predicted from the relationship of structure-retention of compounds containing two identical substituting groups. At a given [SDS], φ and temperature in MEEKC or MEKC, excellent agreement was found between observed and predicted values of log k of compounds containing two different substituting groups, where the predicted value was obtained from the average value of log k of compounds containing two identical substituting groups.

CHAPTER VI

SUMMARY AND FUTURE WORK

This work involves fundamental studies of retention, resolution and application of retention of hydrophobic aromatic compounds in microemulsion electrokinetic chromatography (MEEKC) with suppressed electroosmosis.

6.1 Preliminary Study of Retention Scales in MEEKC Employing Suppressed Electroosmosis

Retention behaviors, such as retention factor (*k*) and retention index (*I*), in MEEKC with suppressed electroosmosis were investigated. An MEEKC buffer used contains a pH 2.5 50 mM phosphate buffer to suppressed electroosmosis, 1.0% v/v *n*-octane as oil, sodium dodecyl sulphate (SDS) as surfactant, 890 mM 1-butanol as co-surfactant, and organic co-solvents. Following parameters affecting retention behaviors were determined: the SDS concentration ([SDS]), temperatures, voltage, and types and concentrations of organic co-solvents (φ). Test analytes used are homologous series compounds, such as alkylarylketones (PN), alkylbenzoates (BA) and alkylparabens (PB), while alkylbenzenes (BZ) is used as standards.

In MEEKC with suppressed electroosmosis, the retention factor linearly increases with increasing [SDS] in a range of 100 to 200 mM, due to an increase in the phase ratio. The retention factor linearly decreases with increasing temperature in a range of 15 to 40^{o}C, due to an increase in the solubility of insoluble compounds in the organic-aqueous phase. The retention factor was found to decrease with an increase in φ due to an increase in the solubility of analyte in organic-aqueous phase. The value of log *k* linearly decreases with increasing the concentration of organic co-solvents used as acetonitrile (ACN), methanol (MeOH) or ethanol (EtOH) in a range of 0 to 30% v/v, while a second-degree polynomial decrease with increasing concentration of or 2-

propanol (2-PrOH) was obtained. The different effect between 2-PrOH and other solvents may be caused by the different chromatographic behavior with more hydrophobic organic co-solvent. MeOH, EtOH and ACN are more polar organic co-solvent and have little or no interaction with the charged oil droplets, while 2-PrOH is more hydrophobic than other organic co-solvents and can act as co-surfactant. Therefore, the less amount of free 2-PrOH in the aqueous phase than the total 2-PrOH added leads to higher k than expected, and the deviation of log k from linear relationship between log k and φ. In addition, the retention factor decreases with increasing applied voltage possibly due to excess an increase in temperature caused by Joule heating. Excellent linear relationship is obtained between log k and the number of carbons (z) of homologous series compounds over a wide range of [SDS], φ, temperature and applied voltage.

Using BZ as standards, the retention indices of other homologous series compounds were determined in MEEKC and they were found to be independent of [SDS] in a range of 100 to 200 mM, temperature in a range of 15 to 40^{o}C and applied voltage in a range of -10 to -20 kV. This is because the retention index of analyte is obtained from the retention factor of the analyte compared to the retention factor of standards, and these parameters affect a similar trend of change in the retention factors of the analyte and the standards. However, the retention indices are dependent on the types and concentrations of organic co-solvents as ACN, MeOH, EtOH or 2-PrOH in a range of 0 to 30% v/v due to the different solubility of analyte and standards in the organic co-solvents, resulting in the different trend of a change in the retention factor of the analyte and the standards.

Therefore, factors affecting retention factor and retention index can be explained using the theory of liquid chromatography and micellar electrokinetic chromatography (MEKC). Both retention factor and retention index can be used to identify the analyte obtained from MEEKC electropherograms.

6.2 Comparison of Resolution in MEEKC and MEKC Employing Suppressed Electroosmosis: Application to Bisphenol-A-Diglycidyl Ether and Its Derivatives

The resolution (R_s) of test analytes in MEEKC and MEKC with suppressed electroosmosis was compared, using an equation for the resolution relating to separation scales of selectivity (α), k and efficiency (N), $R_s = (\sqrt{\overline{N}}/4)\ (\alpha\text{-}1)/(1+k_2)$ [Nhujak *et al*. 2006]. MEKC was carried out using a pH 2.5 50 mM phosphate buffer containing SDS as surfactant and organic co-solvents, while MEEKC was carried out using a pH 2.5 50 mM phosphate buffer containing 1.0% v/v *n*-octane as oil, SDS as surfactant, 890 mM 1-butanol as co-surfactant, and organic co-solvents. The resolution of test analytes was compared at a given [SDS], φ and temperature used in MEEKC and MEKC. The test analytes used are bisphenol-A-diglycidyl ether (BADGE) and its derivatives.

At a given CE condition, MEEKC was found to provide better resolution of the test analytes than did MEKC. This can be explained from experimental results and the resolution equation that MEEKC gave significantly greater $1/1+k_2$ or smaller k of the test analytes, while smaller α-1 in MEEKC and a comparable range of $\overline{N}$. This indicates that the better resolution of BADGEs in MEEKC than that in MEKC is mainly due to the significantly smaller retention factor in MEEKC, but not the greater selectivity in MEEKC. The smaller k of the analytes in MEEKC than that in MEKC is due to the less hydrophobicity of the pseudo stationary phase in MEEKC than that in MEKC. It should be noted that the pseudo stantionary phase in MEEKC is the charged oil droplet surrounding with the surfactant (SDS) and co-surfactant (1-butanol), while the pseudo stationary phase in the SDS micellar phase.

Improved resolution of BADGEs was obtained with increasing the concentration of organic co-solvents, such as ACN, MeOH, EtOH or 2-PrOH, from 20 to 30% v/v in MEEKC and MEKC, while small change in resolution with the SDS concentration in a range of 100 to 180 mM. In addition, a decrease in temperature from 40 to 15^{o}C results in a slightly change in R_s. However, long analysis time was obtained with low

concentration of SDS and high concentration of organic co-solvent in the buffer, and low temperature used.

The results from this work may be used to explain why MEEKC provided better resolution than did MEKC in previous work [Sánchez *et al.* 2002, Bo *et al.* 2003, Pomponio *et al.* 2003, Gong *et al.* 2004, Huang *et al.* 2005, Nhujak *et al.* 2006].

6.3 Prediction and Application of Retention in MEEKC

From theory and experimental results in Chapter 3, the value of log *k* was obtained to be linearly increases with increasing the number of carbons (*z*) of the analyte and the concentration of SDS ([SDS]). Therefore, the linear equation of log *k* as functions of *z* and [SDS] was modified for prediction of log *k* of homologous series compounds, such BZ, PN, BA and PB. At 130 or 190 mM SDS in MEEKC with suppressed electroosmosis and without organic co-solvent, the observed and predicted values of log *k* of analytes were found to be in excellent agreement. In addition, the value of log *k* was obtained to be linearly decreases with increasing the concentration of organic co-solvents (φ) used as ACN, MeOH or EtOH, and therefore, the linear equation of log *k* as functions of *z* and φ was modified for prediction of log *k* of homologous series compounds. Using each organic co-solvent at 12 or 22% v/v in MEEKC with suppressed electroosmosis and 180 mM SDS, the excellent agreement was also obtained between observed and predicted values of log *k* of analytes. Therefore, the values of log *k* of analytes in a wide range of *z* may be predicted in a wide range of [SDS] and φ.

Using BZ as standards, the linear calibration plot between log K_{ow} versus log *k* or log K_{ow} versus *I* were obtained over a wide range of [SDS] in MEEKC. The values of log K_{ow} for the test analytes as PN, BA and PB were predicted from log *k* or *I*, where K_{ow} is the octanol-water distribution constant. Using paired *t*-test analysis at 95% confidence interval of the means, non-significant difference was obtained for the predicted log K_{ow} estimated from log *k* and *I*. In addition, using paired *t*-test analysis at 95% confidence interval of the means, good agreement was also found for the

predicted and literature values of log K_{ow} for each analyte. This indicates that MEEKC can be used to determined log K_{ow} of analytes using the determined value of log *k* or *I*.

In addition, in MEEKC and MEKC, the values of log *k* of compounds containing two different substituting groups (PXY) can be predicted from the relationship of structure-retention of compounds containing two identical substituting groups (PXX or PYY), where P is the parent moiety, and X and Y are the substituting groups. At a given [SDS], φ and temperature in MEEKC or MEKC, excellent agreement was found between observed and predicted values of log *k* of compounds containing two different substituting groups, where the predicted log *k* of PXY is obtained from the average value of log *k* of PXX and PYY. This is very useful for optimization of resolution in MEKC and MEEKC.

6.4 Future Work

In the future work, the investigation of retention behaviors, retention factor and retention index, may be extended to the non-homologous series compounds in MEEKC with or without electroosmosis. The comparison of resolution of other analytes in MEEKC and MEKC may be carried out. It is also interesting to study the relationship between the structure and retention of disubstituted aromatic compounds with different or identical substituting groups. This MEEKC approach can be used as an alternative method for prediction of retention and optimization resolution of compounds in MEEKC. In addition, MEEKC can be used to determine the octanol-water distribution constant in order to indicate the hydrophobicity of analytes.

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2) K. Poouthree, N. Leepipatpiboon, A. Petsom, and T. Nhujak “Comparison of resolution in microemulsion electrokinetic chromatography and micellar electrokinetic chromatography employing suppressed electroosmosis: Application to bisphenol-A-diglycidyl ether and its derivatives” revised for publication in *Electrophoresis*. Impact factor = 3.850.

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1) K. Poouthree, N. Leepipatpiboon, A. Petsom, and T. Nhujak “A comparison of resolution in microemulsion electrokinetic chromatography and micellar electrokinetic chromatography: Application to bisphenol-A-diglycidyl ether and its derivatives” Oral Presentation, The Second Mathematics and Physical Science Graduate Congress, 6-7 December, 2006, National University of Singapore, Singapore.
2) K. Poouthree, N. Leepipatpiboon, A. Petsom, and T. Nhujak “Retention factor and retention index of homologous series compounds in microemulsion electrokinetic chromatography employing suppressed electroosmosis” Poster Presentation, The First Mathematics and Physical Science Graduate Congress, 6-7 December, 2005, Chulalongkorn University, Bangkok, Thailand.

National Presentations

1) K. Poouthree, N. Leepipatpiboon, A. Petsom, and T. Nhujak “Comparison of resolution in microemulsion electrokinetic chromatography and micellar electrokinetic chromatography: Application to bisphenol-A-diglycidyl ether and its derivatives” Oral Presentation, 32nd Congress on Science and Technology of Thailand, 10-12 October, 2006, Bangkok.
2) K. Poouthree, N. Leepipatpiboon, A. Petsom, and T. Nhujak “Retention factor and retention index of homologous series compounds in microemulsion electrokinetic chromatography employing suppressed electroosmosis”, Poster Presentation, 31st Congress on Science and Technology of Thailand, 19-21 October, 2005, Suranaree University of Technology, Nakhon Ratchasima.

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