การแยกที่มีประสิทธิภาพสูงในคะพิลลารีอิเล็กโทรโครมาโทกราฟีและคะพิลลารีลิควิดโครมาโทกราฟีโดยใช้เฟสคงที่ประเภทมอนอลิต

นางสาววสุรา สุนทรตันติกุล

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HIGH EFFICIENCY SEPARATION IN CAPILLARY ELECTROCHROMATOGRAPHY AND CAPILLARY LIQUID CHROMATOGRAPHY USING MONOLITHIC STATIONARY PHASE

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Field of Study	Chemistry
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วสุรา สุนทรตันติกุล: การแยกที่มีประสิทธิภาพสูงในคะพิลลารีอิเล็กโทรโครมาโทกราฟีและคะพิลลารีลิควิดโครมาโทกราฟีโดยใช้เฟสคงที่ประเภทมอนอลิต. (HIGH EFFICIENCY SEPARATION IN CAPILLARY ELECTROCHROMATOGRAPHY AND CAPILLARY LIQUID CHROMATOGRAPHY USING MONOLITHIC STATIONARY PHASE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.ธรรมนูญ หนูจักร, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. ณัฐชนัญ ลีพิพัฒน์ไพบูลย์, 173 หน้า.

ได้เตรียมคอลัมน์ประเภท C18 ซิลิกามอนอลิตที่ปรับปรุงพื้นผิวด้วยหมู่ออกตะเดกซิล (ODS) และพอลิออกตะเดกซิลเมทะคริเลต (ODM) สำหรับรีเวิร์สเฟสคะพิลลารีลิควิดโครมาโทกราฟี (CLC) และได้คอลัมน์สมรรถนะสูงที่มีความสูงของเพลต (*H*) ใกล้เคียงหรือน้อยกว่า 10 ไมโครเมตร และสภาพให้เฟสเคลื่อนที่ไหลผ่านในคอลัมน์อยู่ในช่วง 7.3×10^{-14} ถึง 9.4×10^{-14} m^2 เมื่อเปรียบเทียบสภาพเลือกจำเพาะของการแยกของเฟสคงที่ ODM และ ODS สำหรับสารประกอบที่มีแฮโลเจน ได้แก่ แฮโลจีเนเตดเบนซีนและคลอโรฟีนอล พบว่าคอลัมน์ ODM มีความชอบรีเทนและสภาพเลือกจำเพาะของการแยกที่มากกว่าสำหรับสารกลุ่มนี้ เนื่องจากอันตรกิริยาดิสเพอซีพที่มากกว่าจากหมู่ C18 ที่หนาแน่นกว่าบนคอลัมน์ที่เคลือบด้วยพอลิเมอร์ ODM ร่วมกับผลของหมู่คาร์บอนิลในสายโซ่ของ ODM นอกจากนี้ได้เตรียมคอลัมน์ประเภทซิลิกามอนอลิตแบบ ODS ซึ่งมีกลไกการแยกแบบรีเวิร์สเฟส และแบบ ODS/AP_1 หรือ ODS/AP_2 ซึ่งมีกลไกการแยกแบบผสมสำหรับคะพิลลารีอิเล็กโทรโครมาโทกราฟี (CEC) ที่มี *H* อยู่ในช่วง 3.7-4.0 μm เมื่อ AP_1 และ AP_2 คือ หมู่อะมิโนโพรพิลและหมู่ 3-(2-อะมิโนเอทิลอะมิโน)โพรพิล ตามลำดับ คอลัมน์ที่มีกลไกแบบผสมเหล่านี้มีความชอบรีเทนสำหรับสารประกอบฟีนอล นอกจากนี้ได้เปรียบเทียบสภาพเลือกจำเพาะของการแยกในคะพิลลารีอิเล็กโทรฟอริซิสที่มีกลไกการแยกแตกต่างกัน คะพิลลารีโซนอิเล็กโทรฟอริซิสที่เติมไซโคลเด็กซ์ทริน (CD-CZE) มีสภาพเลือกจำเพาะของการแยกสำหรับคลอโร- และเมทิลเบนโซเอตที่ดีกว่า CZE ที่ไม่เติมไซโคลเด็กซ์ทรินและไมเซลลาร์อิเล็กโทรไคเนทิกโครมาโทกราฟี และได้จำลองรูปแบบของสภาพเลือกจำเพาะของการแยกสำหรับการแยกสารอะไครัลที่แตกตัวได้สมบูรณ์โดยใช้ไซโคลเด็กซ์ทรินที่ไม่มีประจุ

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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KEYWORDS: CAPILLARY ELECTROPHORESIS/ CAPILLARY ELECTROCHROMATOGRAPHY/ CAPILLARY LIQUID CHROMATOGRAPHY/ MONOLITHIC COLUMNS/ SEPARATION SELECTIVITY

WASURA SOONTHORNTANTIKUL: HIGH EFFICIENCY SEPARATION IN CAPILLAY ELECTROCHROMATOGRAPHY AND CAPILLARY LIQUID CHROMATOGRAPHY USING MONOLITHIC STATIONARY PHASE. ADVISOR: ASSOC. PROF. THUMNOON NHUJAK, Ph.D. CO-ADVISOR: ASST. PROF. NATCHANUN LEEPIPATPIBOON, Dr.rer.nat, 173 pp.

C18 monolithic silica capillary columns modified with octadecyl moieties (ODS) and poly(octadecyl methacrylate) (ODM) were prepared for reversed-phase capillary liquid chromatography (CLC). A high column performance was obtained with plate height (*H*) nearly or less than 10 μm and permeability in a range of 7.3×10^{-14}-9.4×10^{-14} m^2. In comparison of ODM and ODS stationary phase selectivities for halogenated compounds, such as halogenated benzenes and chlorophenols, the ODM columns have greater preference and greater selectivity for these compounds due to the greater dispersive interaction by more densely packed C18 groups on ODM polymer coated column together with the contribution of carbonyl groups in ODM side chain. In addition, the reversed-phase ODS and mixed-mode ODS/AP_1 or ODS/AP_2 monolithic silica capillary columns were prepared for capillary electrochromatography (CEC) with *H* in the range of 3.7-4.0 μm, where AP_1 and AP_2 refer to aminopropyl and 3-(2-aminoethylamino)propyl moieties, respectively. These mixed-mode columns have the preference for phenolic compounds. Moreover, the separation selectivity was compared in capillary electrophoresis with different modes. Capillary zone electrophoresis with the addition of cyclodextrin (CD-CZE) showed the better separation selectivity for chloro- and methylbenzoates than CZE without cyclodextrin and micellar electrokinetic chromatography. The separation selectivity patterns were also developed for the separation of fully charged achiral compounds using a neutral cyclodextrin.

Department : Chemistry Student's Signature .....
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LIST OF ABBREVIATIONS AND SYMBOLS

α	separation selectivity
α_0	separation selectivity of the analytes at zero cyclodextrin concentration
α_∞	separation selectivity of the analyte-cyclodextrin complex
α_{CD}	mobility selectivity in capillary zone electrophoresis with the addition of cyclodextrin
α_{CZE}	mobility selectivity in capillary zone electrophoresis
α_k	retention selectivity
α_{MEKC}	mobility selectivity in micellar electrokinetic chromatography
$\alpha(CH_2)$	methylene group selectivity
β	a change in electrophoretic mobility of the analytes in free and complex forms
ε	permittivity
ε_T	total column porosity
ζ	zeta potential
χ	product of binding constant and cyclodextrin concentration
η	viscosity
κ	binding selectivity
$\Delta\mu$	electrophoretic mobility difference
$\bar{\mu}$	average electrophoretic mobility
μ	electrophoretic mobility
μ_0	electrophoretic mobility at zero concentration of cyclodextrin
μ_∞	electrophoretic mobility at infinite concentration of cyclodextrin
μ_{eo}	electroosmotic mobility
μ_{net}	net electrophoretic mobility
σ	standard deviation
A	contribution to band broadening by eddy diffusion
B	contribution to band broadening from longitudinal diffusion
C	contribution to band broadening from resistance to mass transfer
C	free cyclodextrin concentration
C_i	total concentration of species *i*
C_m	molar concentration of the analyte in the mobile phase
C_s	molar concentration of the analyte in the stationary phase

E	electric field strength
e	electronic charge
F_e	electric force
F_F	frictional force
H	plate height
K	column permeability
K	binding constant
k	retention factor
k_c	retention factor in capillary electrochromatography
k_e	electromigration velocity factor
k''	chromatographic retention factor that measure under conditions used in CEC experiments
k''_{cc}	peak locator of chromatographic formalism
k''_{ce}	peak locator of electrophoretic formalism
k''_e	electromigration factor that measure under conditions used in CEC experiments
L	column length
l	effective length of the column
N	number of the theoretical plates
$\overline{N}$	average number of the theoretical plates
n_m	mole ratio of the analytes in the mobile phase
n_s	mole ratio of the analytes in the stationary phase
ΔP	pressure drop
q	charge of analytes
R_s	resolution
r_h	hydrodynamic radius of the ion
t_0	retention time of an unretained compound
t_{eo}	migration times of the EOF marker
t_R	retention time of the analyte
t_m	migration times of the analyte
u	linear velocity of mobile phase
V	applied voltage
V_m	volume of the mobile phase
V_s	volume of the stationary phase
v_{ep}	electrophoretic velocity
v_{eo}	electroosmotic velocity

v_{net}	net velocity of the analyte
w_b	peak width at base
w_h	peak width at half height
x_i	mole fraction of species *i*
z	charge of ions
z	number of carbon atom in aliphatic chain
2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DB	4-(2,4-dichlorophenoxy) butyric acid
2,4,5-T	(2,4,5-trichlorophenoxy) acetic acid
ACN	acetonitrile
AFF	acifluorfen
AIBN	α, α´-azobis-isobutyronitrile
AP_1	aminopropyltrimethoxysilane
AP_2	3-(2-aminoethylamino)propyl] trimethoxysilane
BADGE	bisphenol-A-diglycidyl ether
BADGE·$2H_2O$	bisphenol-A-bis(2,3-dihydroxypropyl) ether
BAGDE·2HCl	bisphenol-A-bis(3-chloro-2-hydroxypropyl) ether
BADGE·H_2O	bisphenol-A-(2,3-dihydroxypropyl)glycidyl ether
BADGE·HCl	bisphenol-A-(3-chloro-2-hydroxypropyl) glycidyl ether
BADGE·HCl·H_2O	bisphenol-A-(3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) ether
BFDGE	bisphenol-F-diglycidyl ether
BFDGE·$2H_2O$	bisphenol-F-bis(2,3-dihydroxypropyl) ether
BFDGE·2HCl	bisphenol-F-bis(3-chloro-2-hydroxypropyl) ether
BFDGE·H_2O	bisphenol-F-glycidyl-(2,3-dihydroxypropyl) ether
BFDGE·HCl	bisphenol-F-glycidyl-(3-chloro-2-hydroxypropyl) ether
BFDGE·HCl·H_2O	bisphenol-F-(3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) ether
BGE	background electrolyte
C^-	chlorobenzoate
CD	cyclodextrin
CD-CZE	capillary zone electrophoresis with the addition of cyclodextrin
CE	capillary electrophoresis
CEC	capillary electrochromatography
CLC	capillary liquid chromatography
CPR	clopyralid
CZE	capillary zone electrophoresis

DC	dichlorobenzoic acid
DCB	dicamba
DM	dimethylbenzoic acid
DM-β-CD	heptakis-(2,6-di-*O*-methyl)-β-CD
EOF	electroosmotic flow
FXP	fluroxypyr
GC	gas chromatography
GC/MS	gas chromatography coupled with mass spectrometry
HPLC	high performance liquid chromatography
i.d.	internal diameter
LC	liquid chromatography
LC/MS	liquid chromatography coupled with mass spectrometry
LC/MS/MS	liquid chromatography coupled with tandem mass spectrometry
M^-	methylbenzoate
MCPA	4-chloro-2-methylphenoxyacetic acid
MCPB	4-(2-methyl-4-chlorophenoxy) butyric acid
MCPP	mecoprop
MEKC	micellar electrokinetic chromatography
MeOH	methanol
MOP	3-methacryloxypropyltrimethoxysilane
MTMS	methyltrimethoxysilane
ODA	column modified with poly(octadecyl acrylate)
ODM	column modified with poly(octadecyl methacrylate)
ODS	column modified with octadecyl moieties
ODS/AP_1	column modified with octadecyl and aminopropyl moieties
ODS/AP_2	column modified with octadecyl and 3-(2-aminoethylamino)propyl moieties
ODS-DEA	octadecyldimethyl-*N*,*N*-diethylaminosilane
ODS-Cl	octadecyldimethylchlorosilane
o.d.	outer diameter
PCR	picloram
PEG	poly(ethylene glycol)
PSP	pseudo stationary phase
QCR	quinclorac
RP	reversed-phase
RSD	relative standard deviation
SEM	scanning electron microscope

SDS	sodium dodecyl sulfate
TCP	triclopyr
TEOS	tetraethoxysilane
TMOS	tetramethoxysilane
WAX	weak anion exchange
XDB	extra dense bonding

CHAPTER I

INTRODUCTION

1.1 Miniaturization of separation techniques [Hernández-Borges *et al*. 2007]

The miniaturization in chromatographic or electrophoretic separation techniques, such as capillary liquid chromatography (CLC) and capillary electrophoresis (CE) in various modes, has become very attractive in recent years. These systems are usually performed in capillary columns with the internal diameter (i.d.) in the range of 25-200 μm. In comparison with conventional systems, they provide a number of advantages, such as higher efficiency, lower solvent consumption, possibility to work with extremely small sample volumes, and better compatibility with mass spectrometry.

1.1.1 Introduction to capillary liquid chromatography (CLC) [Neue 1997, Hernández-Borges *et al*. 2007]

Liquid chromatography (LC) is one type of chromatographic techniques in which analytes are distributed between a liquid mobile phase and a solid or a liquid immobilized on solid stationary phase. High performance liquid chromatography (HPLC) is the term to define LC that employs high pressure to force the liquid through a column containing stationary phase materials.

Various i.d. columns (downscaling from millimeter to micrometer i.d. ranges) have been used in HPLC system. So far, definitions of microscale HPLC columns have been frequently suggested in the literatures [Chervet *et al*. 1996, Vissers *et al*. 1997, Saito *et al*. 2004, Noga *et al*. 2007], but have not yet found a widely acceptance. According to several literatures, Table 1.1 summarizes the most common names for different HPLC techniques based on the ranges of columns i.d. and flow rate of mobile phase. Generally speaking, the HPLC technique using capillary columns,

especially the i.d. less than 500 μm, is named capillary LC (CLC). However, 50-100 μm capillary columns in HPLC is preferred to classify as nano LC according to the flow rate of mobile phase in the levels of nL/min. In part of this work, HPLC separation is performed using 200 μm i.d. capillary columns, and therefore, the method should be classified as "CLC".

Table 1.1 Classification of LC techniques.

Proposed by [Chervet *et al*. 1996]

Column i.d.	Flow rate	Name
3.2-4.6 mm	0.5-2.0 mL/min	Conventional HPLC
1.5-3.2 mm	100-500 μL/min	Microbore HPLC
0.5-1.5 mm	10-100 μL/min	Micro LC
150-500 μm	1-10 μL/min	Capillary LC (CLC)
10-150 μm	10-1000 nL/min	Nano LC

Proposed by [Saito *et al.* 2004]

Column i.d.	Name
3-5 mm	Conventional HPLC
2 mm	Narrow-bore HPLC
0.5- 1 mm	Micro LC
100-500 μm	Capillary LC
10-100 μm	Nano LC

Proposed by [Noga *et al*. 2007]

Column i.d.	Flow rate	Name
8-20 mm	> 2 mL/min	Semi-preparative LC
4.6 mm	1-2.5 mL/min	Analytical LC
1.1 mm, 2.1 mm	100-500 (1000) μL/min	Microanalytical LC
0.75 mm, 0.8 mm	10-1000 μL/min	Narrowbore/microbore LC
500 μm	0.5-10 μL/min	Capillary/narrowbore LC
150 μm, 250 μm	300-500 nL/min	Capillary LC
75 μm	100-300 nL/min	Capillary LC

1.1.2 Introduction and modes of capillary electrophoresis (CE) [Li 1992]

Capillary Electrophoresis (CE) is a separation technique that the electromigration of the analytes is performed in a small internal diameter capillary (typically 25- 100 μm i.d.) containing a background electrolyte (BGE) under the effect of an electric field. The separation mechanism is based on the different electrophoretic mobilities (μ) of the analytes which depends on sizes and charges of the analytes.

Six modes in CE have been developed with different separation mechanisms. They include capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), capillary electrochromatography (CEC), capillary gel electrophoresis, capillary isoelectric focusing and capillary isotachophoresis. Three modes of CE, namely CZE, MEKC and CEC, are frequently used for the separation of a wide variety of compounds. Therefore, a brief description of these three modes is given in this section.

Capillary zone electrophoresis is the most widely used techniques in CE. The separation mechanism is based on the difference in electrophoretic mobilities of the analytes, mainly depends on the ratio of charge to hydrodynamic radius of the analytes at a given pH. In CZE, an uncoated fused silica capillary is typically used and filled with BGEs which are a common buffer such as phosphate, borate and acetate. According to the separation mechanism of CZE, cations and anions can be separated by the difference in charge-to-size ratio of the analytes but not for neutral compounds.

Micellar electrokinetic chromatography was developed by Terabe and co-workers in 1984. In MEKC, the surfactant is added to buffer solution in concentration above its critical micellar concentration to form micelles. The most commonly used surfactant is sodium dodecyl sulfate (SDS) which is an anionic surfactant. Micelles serve as pseudo stationary phases (PSP), similar to stationary phase in HPLC, but micelles can be moved through the column under an electric field. The separation mechanism in MEKC is based on the difference in partitioning of the analytes between the micelle

phase and the aqueous phase and also the difference in electrophoretic mobility of the analytes. Therefore, this mode is suitable for separating both charged and neutral compounds.

Capillary electrochromatography is a hybrid technique between CZE and HPLC. It combines the selectivity of HPLC and high efficiency of CZE. Typically, the capillary used in CEC is a column containing stationary phases similar to that in HPLC and the analytes in CEC is moved to the detector by electro-driven flow, instead of pressure-driven flow as in HPLC. The differential migration of the analytes in CEC is due to interactions of the analytes between stationary and mobile phases plus electrophoretic mobility of the charged analytes.

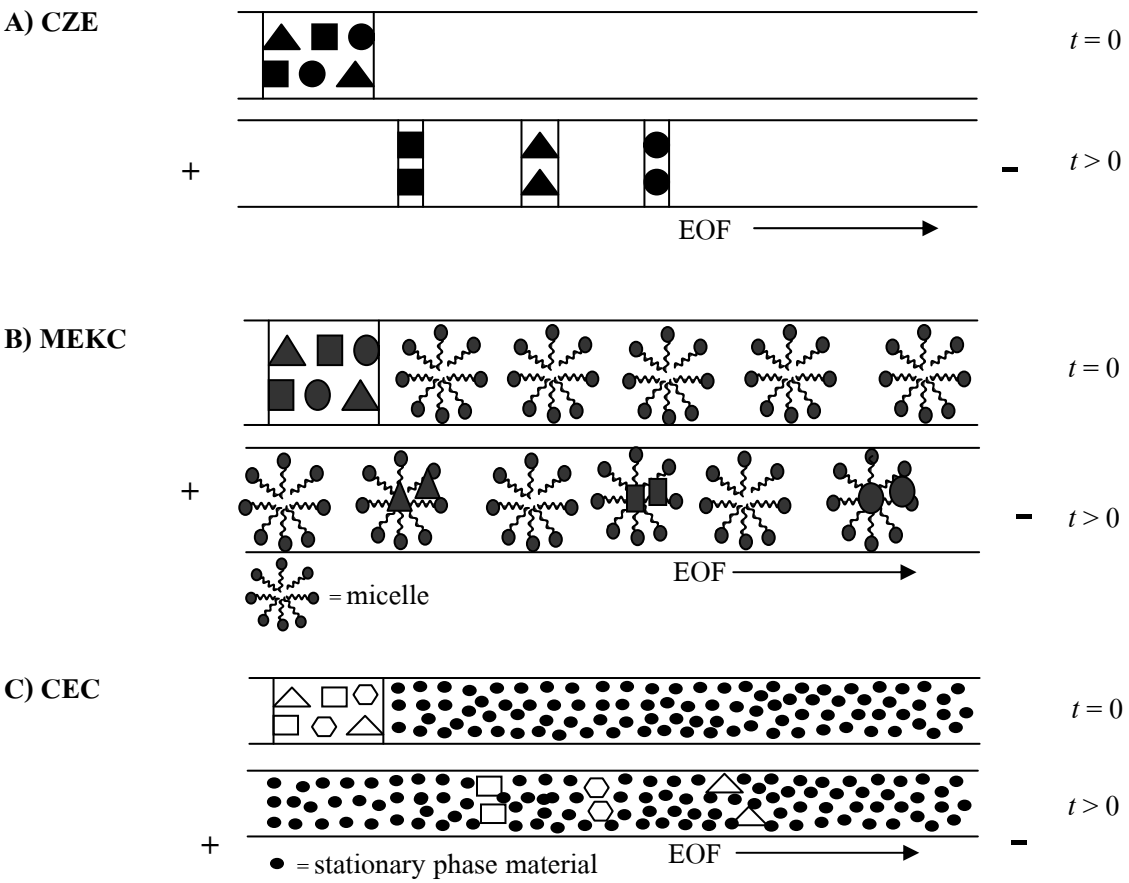


Figure 1.1 Separation mechanism of several modes in CE: (A) CZE, (B) MEKC and (C) CEC. Adapted from Weston and Brown [1997].

1.2 Monolithic columns [Núñez *et.al*. 2008, Legido-Quigley *et al*. 2003, Unger *et. al*. 2011, Szumski *et al*. 2002, Yan *et al.* 2004]

Classical columns in CLC and CEC are particle-packed capillary columns. Like most of LC columns, the packed capillary column consists of a capillary tube, a stationary phase and the frits to retain the bed of particles, as shown in Figure 1.2. In order to fill small particles into small diameter of capillary columns, several techniques have been proposed, such as dry packing, slurry packing, supercritical fluid packing, or electrokinetic packing. The selection of the method depends on particle size, column diameter, the mechanical strength of the material, and material type. The columns with smaller i.d. are usually equipped with in-column frits which can be obtained by preparing porous silica plug or sintering the packed particles. The main drawback for preparation of the packed capillary column is the difficulty to homogeneously pack the capillary columns with small-sized particles and also the tricky manufacture of frits.

The reduction of particle size can lead to better column efficiency because of smaller eddy diffusion and shorter diffusion path length. However, in CLC, an increase in backpressure has to be expected. The performance of a packed column is limited by the pressure limit of a solvent delivery system, up to 40 MPa, with current HPLC pumps. The 3-5 μm particles are currently used in packed column due to the compromise between the column efficiency and the backpressure. Otherwise, the ultrahigh-pressure pump is required for the smaller particles size. In CEC, the presence of frits in packed column can be responsible for bubble formation resulting in the current breakdown and the stop of the mobile phase flow. As they are made from sintered-packing material, they create zones of heterogeneities inside the packed column. Some compounds may interact with the frits in addition to the packed bed.

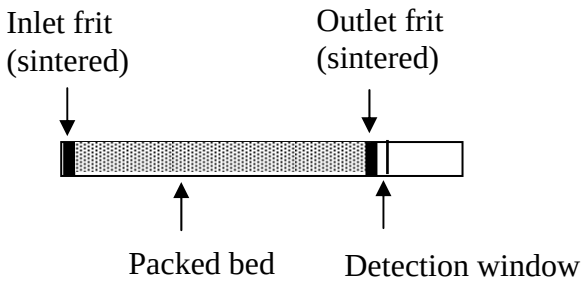


Figure 1.2 Particle-packed capillary column. Adapted from Szumski *et al*. [2002].

The use of monolithic stationary phase, instead of packing materials, is an alternative approach to avoid or to minimize the abovementioned problems in capillary columns. Monolithic columns are described as a single piece of porous material that fills entirely the column space without any interparticular voids typical of packed column. The monolith is created inside the capillary by *in situ* polymerization and covalently attached to the capillary wall. In comparison with a particle-packed column, the major advantages of monolithic columns include high efficiency separation, no need for a frit system, adjustable porosity, high permeability and low column pressure drop at higher mobile phase flow rates.

According to the nature of monolith network, the monolithic stationary phases can be divided into two groups: organic and inorganic ones. Organic-based monolith can be formed by polymerization of suitable organic monomers such as acrylamide, acrylate and methacrylate. Polymerization reaction mixtures usually consist of a combination of monomers and cross-linker, initiator and a porogenic mixture of solvents. Polymer-based monoliths have such advantages such as simple polymerization procedure and wide application range of pH values. However, they suffer from swelling and shrinking when exposes the organic solvent, leading to the change of pore structure and lack of mechanical stability. Moreover, the existence of micropores negatively affects the efficiency and peak symmetry for small molecules. Inorganic-based monolith is typically made of silica which can be prepared using sol-gel technology to create a continuous network throughout the column formed by the gelation of a sol solution within the column. Silica monoliths offer high mechanical strength, good solvent resistance and easy transfer methods from silica-particle packed columns.

However, silica monoliths present the same drawbacks as silica particles, such as a relatively narrow pH stability range. Figure 1.3 shows an example of scanning electron microscope (SEM) image of silica monolithic stationary phase. Continuous bed consists of skeleton and a bimodal pore size distribution: macropores and mesopores. Macropores (or through pores) form a continuous network and enables a convective flow through the continuous bed, while mesopores on skeletons provide a sufficiently large surface for the retention of analytes.

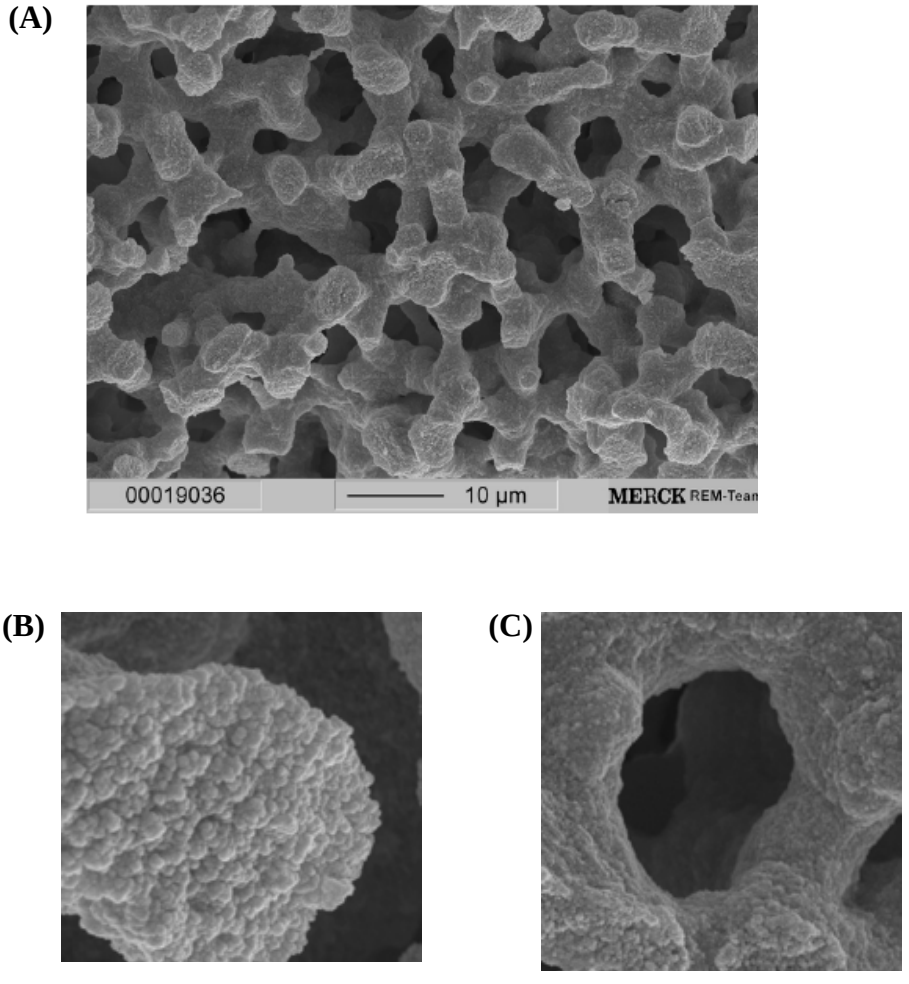


Figure 1.3 Scanning electron microscope (SEM) picture of the typical porous structure of monolithic silica columns (A), mesoporous structure of the silica skeleton (B), and macropores or through pores (C). Reproduced from Cabrera [2004].

Previously in Motokawa *et al.* [2002], various sizes of skeletons (≈ 1-2 μm) and through-pores (≈ 2-8 μm) has been prepared in fused-silica capillaries (50-200 μm i.d.). The through-pore sizes of monolithic silica capillary columns can be much larger than the skeleton size, resulting in a high through-pore size/skeleton size ratio of up to 1.5-4 which is greater than these ratios of 0.25-0.4 with particle-packed columns.

In general, the porosity of monolithic silica columns is much greater than that of a particle-packed column. The major difference is seen in interstitial porosities typically 90-95% for monolithic silica capillary columns, compared to 40% for a particle-packed column. Therefore, the permeability (*K*) of monolithic silica capillary columns is much higher, in comparison with particle-packed columns. A typical monolithic silica capillary column with 2.0 μm through pores and 1.5 μm skeleton shows the *K* values of 7-14×10^{-14} m^2 which is about 2-4 times higher than that of a column packed with 5 μm particles (*K* values of 3.5-4.0×10^{-14} m^2).

According to the greater porosity and the smaller-sized skeletons, the monolithic silica network provided the faster diffusion a solute in mobile phase and in the stationary phase, compared to a conventional packed column with 5 μm particles. The smaller sized skeletons should reduce band broadening caused by slow mass transfer in the stationary phase. Therefore, in comparison with particle-packed columns, monolithic silica columns showed high efficiency at similar pressure drop.

1.3 Aims, scopes and previous works

The aims of this thesis can be classified into four categories: (i) to use the reversed-phase monolithic silica capillary columns with high efficiency for separating selected compounds in CLC, (ii) to compare the retention and separation selectivity of halogenated compounds on two reversed-phase monolithic columns in CLC, (iii) to investigate the electrochromatographic separation of some polar compounds on mixed-mode monolithic silica capillary columns in CEC, and (iv) to compare the

separation selectivity of compounds with slightly different electrophoretic mobility in different CE modes and to predict the separation selectivity patterns for fully charged achiral compounds in CZE with the addition of cyclodextrin.

In preliminary work as in Chapter III, the monolithic silica capillary columns for reversed-phase stationary phases were prepared and evaluated for column performance in CLC, and then used for the separation of chlorophenoxy acids and bisphenol-A-diglycidyl ether (BADGE), bisphenol-F-diglycidyl ether (BFDGE) and theirs derivatives. As previously mentioned in Section 1.2, monolithic columns have proven to be a good alternative to particle-packed column in HPLC for high efficiency separation at similar pressure drop. The first objective of this part is to prepare and to evaluate the column performance of monolithic silica capillary columns for reversed-phase chromatography, including monolithic columns modified with octadecyl moieties and with poly(octadecyl methacrylate). Chlorophenoxy acid herbicides were chosen as polar test analytes and bisphenol-A-diglycidyl ether (BADGE), bisphenol-F-diglycidyl ether (BFDGE) and theirs derivatives as non-polar test analytes. Previously, HPLC separation usually employs a conventional reversed phase C18 columns with different mobile phase systems such as acetonitrile/water/trifluoroacetic acid [Cappiello *et al.* 1997], methanol/ammonium acetate [Wu *et al.* 2005], and methanol/acetic acid/water [Køppen and Spliid 1998]. However, simultaneous separation of these 13 chlorophenoxy acid herbicides has not been reported using monolithic silica capillary columns in CLC. For the separation of BADGE, BFDGE, and theirs derivatives, reversed-phase HPLC with fluorescence detection is the most widely used method [Leepipatpiboon *et al.* 2005, Lintschinger and Rauter 2000, Berger *et al.* 2001, Nerin *et al.* 2002]. HPLC separation was typically performed by C18 or C8 particle-packed columns with acetonitrile/water mobile phase. According to Leepipatpiboon *et al.* [2005], a 12-standard mixture of BADGE, BFDGE and theirs derivatives was separated using a column packed with 5 μm particles and a gradient elution of methanol/water mobile phase. However, isomers of some BFDGE derivatives cannot be separated. It is expected that the monolithic silica capillary columns will give the high efficiency for separating selected polar and nonpolar compounds, chlorophenoxy acids, and BADGE, BFDGE

and theirs derivatives, respectively. Therefore, the second objective of this part is the develop the separation for chlorophenoxy acids, and BADGE, BFDGE and their derivatives on reversed-phase monolithic silica capillary columns using either methanol or acetonitrile aqueous mobile phase in CLC, compared with the separation of these compounds on particle-packed column.

The second work as in Chapter VI involves the comparisons of separation selectivity on two reversed-phase monolithic silica capillary columns, namely the column modified with octadecyl moieties and poly(octadecyl methacrylate), for the separation of halogenated compounds in CLC. Separation selectivity (α) is one of the most important characteristics of a separation. This parameter expresses the ability of an analytical method to distinguish analytes from each other. In chromatography, the separation selectivity is defined as the ratio of retention factor (k) for two analytes. To obtain the better separation, the high difference in k or high separation selectivity is required. Typically, monolithic silica capillary columns for reversed-phase separation can be obtained by chemical modification with octadecyldimethyl-*N*,*N*-diethylaminosilane (ODS-DEA), so called ODS columns [Ishizuka *et al.* 2002, Motokawa *et al.* 2002, Hara *et al.* 2006, Rieux *et al.* 2006, Droste *et al.* 2005, Cabrera *et al.* 2000, Yang *et al.* 2006]. Since the monolithic silica capillary columns have high porosities or the presence of a small amount of silica in capillary column, the small retention factor will be obtained with a monolithic silica capillary column, compared to C18 particle packed column [Ishizuka *et al.* 2000, Núñez *et al*. 2008]. The smaller retention factors could lead to the poorer resolution on a monolithic silica column in comparison with a particle-packed column. Recently, high efficiency and high retentive monolithic silica capillary columns have been reported by polymerization of octadecyl methacrylate using α, α'-azobis-isobutyronitrile (AIBN) as a free radical initiator [Núñez *et al*. 2007a], so called ODM columns. The selectivity on the ODM and ODS stationary phase was investigated for some polar and non-polar compounds such as benzene and naphthalene derivatives, polycyclic aromatic hydrocarbons, steroids and alkyl phthalates, and tocopherol homologues [Núñez *et al*. 2007b]. The ODM columns were reported to have a preference for the compounds with aromatic characters, rigid and planar structures and lower length-to-breadth ratios. Moreover,

the separation selectivity in conventional HPLC was previously compared for common halogenated organic solvents between a typical C18 stationary phase and polymer-based packing materials including poly(methyl methacrylate-ethylene dimethacrylate) beads and poly(styrene-divinylbenzene) beads [Hosoya and Frechet 1993]. In general, these polymeric stationary phases demonstrate preferential retention of halogenated compounds than a conventional silica-based C18 stationary phase although the polymer packing materials have shown low column efficiency. Therefore, this work will aim to extend previous work [Hosoya and Frechet 1993, Núñez *et al*. 2007b] to investigate the retention of halogenated and alkylbenzenes and then to compare the selectivity of these analytes on ODS and ODM columns in CLC. The chromatographic performance of analytes was studied using either methanol or acetonitrile mobile phase in a range of 50-80% (v/v). In addition to halogenated benzenes, ODS and ODM columns were applied for the separation of 17 chlorophenols.

The third part of this thesis as in Chapter V is extended to CEC separation involving the preparation and evaluation of mixed-mode monolithic silica capillary columns and also the separation selectivity comparisons for some polar and nonpolar aromatic compounds. Recently, monolithic columns have also attracted much attention in CEC because of no need of the retaining frits. CEC columns play a dual role as a separation media and as a pump to push the mobile phase through the columns. Reversed-phase (RP) monolithic silica columns, such as C18, are widely used for the separation of a variety compounds in CEC. In order to obtain high selectivity for charged compounds, mixed-mode CEC columns or CEC column with two or more kinds of chromatographic retentions (*i.e.* RP/cation exchange [Chen *et al.* 2010], RP/anion exchange [Ye *et al.* 2009, Ding *et al.* 2006, Jemale *et al.* 2011]) have attracted more intention recently because the presence of charged groups can provide both stable EOF and also ion-exchange sites for charged compounds. Recently [Ye *et al.* 2009], mixed-mode of reversed-phase (RP) and weak anion exchange (WAX) for CEC columns has been reported by chemical modification of the silica monolith with hexadecyl and aminopropyl moieties. The electrochromatographic performance was evaluated using alkylbenzenes, benzoic acids and aniline as test analytes. Because of

the presence of aminopropyl groups in addition to alkyl chain on the monolithic silica surface, it is interesting to investigate the effect of aminopropyl groups on the retention behavior of various analytes. So far, the performance of the mixed-mode columns was mainly tested by using alkylbenzenes and a few other compounds as solutes in terms of electrochromatographic efficiency, ion-exchange mechanism and peak shape improvement for basic solutes. Therefore, the objective of this part is to investigate the electrochromatographic behavior and separation selectivity for some polar and nonpolar compounds, such as benzoates, phenols, halogenated benzenes and so on, on mixed-mode columns.

The last part of this thesis as in Chapter VI is moved to CZE separation with addition of a neutral cyclodextrin in the buffer to enhance the separation selectivity for achiral fully charged compounds. The CE separation selectivity can be expressed by the difference in electrophoretic mobilities (μ) of two analytes or the ratio of them. Typically, the higher separation selectivity, the better resolution. Since CZE separation is based on the difference in μ, a small different μ or a small separation selectivity will be obtained for the separation of structurally closely related compounds. In previous work [Hsieh and Huang 1996, Farran *et al.* 1999], only partial or no resolution was reported for some pairs of chlorophenoxy acids that differ in only one chloro and methyl substituent on a benzene ring, such as 2,4-dichlorophenoxyacetic acid (2,4-D)/4-chloro-2-methylphenoxyacetic acid (MCPA) and 4-(2,4-dichlorophenoxy) butyric acid (2,4-DB)/ 4-(2-methyl-4-chlorophenoxy) butyric acid (MCPB). The separation of these chlorophenoxy acid herbicides was enhanced using different CE approaches: CZE with the addition of an organic modifier for 2,4-D/MCPA [Farran *et al.* 1999], CZE with the addition of cyclodextrin (CD-CZE) for 2,4-D/MCPA, 2,4-DB/MCPB and other three chlorophenoxy acids [Hsieh and Huang 1996, Farran *et al.* 1999, Nielen 1993], MEKC with the addition of surfactants and an organic modifier for 2,4-D/MCPA [Farran *et al.* 1999], and CD-MEKC for 2,4-D/MCPA and other two phenoxy acids [Fung and Mak 2001]. Therefore, the first objective of this part is to investigate and to compare for a pair of benzoic acid derivatives that differ only in the chloro and methyl substituents. The separation of test analytes was employed using different additives in a borate buffer at

pH 9.2, as: i) CZE with different types and concentrations of organic solvents, ii) MEKC with different SDS concentrations, and different types and concentrations of organic modifiers, and iii) CD-CZE with different types and concentrations of cyclodextrin (CD). In addition, the simultaneous CE separation was also optimized for these benzoic acid derivatives and chlorophenoxy acid herbicides, including 2,4-D/MCPA, 2,4-DB/MCPB and other six chlorophenoxy acids. To predict the optimum condition for enantiomeric separation in CE, most of previous works deals with the development of theoretical models of separation selectivity [Wren and Rowe 1992, Rawjee *et al.* 1993a, Rawjee *et al.* 1993b, Williams and Vigh 1997, Yang *et al.* 2001]. The separation selectivity patterns for CE separation of chiral compounds were also predicted [Zhu and Vigh 2000a, Zhu and Vigh 2000b, Zhu *et al.* 2000]. However, these theoretical models for enantiomeric separation cannot be directly applied for achiral CE separation because of the typically different μ for achiral compounds but the same μ for enantiomers in the CZE buffer without CD. Therefore, the second objective of this part is to develop general separation selectivity equation and then to establish the possible separation selectivity patterns for fully charged achiral compounds with a neutral CD in order to explain a change in separation selectivity and $|\mu|$ order for fully charged achiral compounds over a wide range of CD concentration. In addition, the existence of these patterns was experimentally verified using positional isomers of chlorobenzoic acids and methylbenzoic acids, and phenoxy acids as test analyte and α-CD as a neutral CD.

CHAPTER II

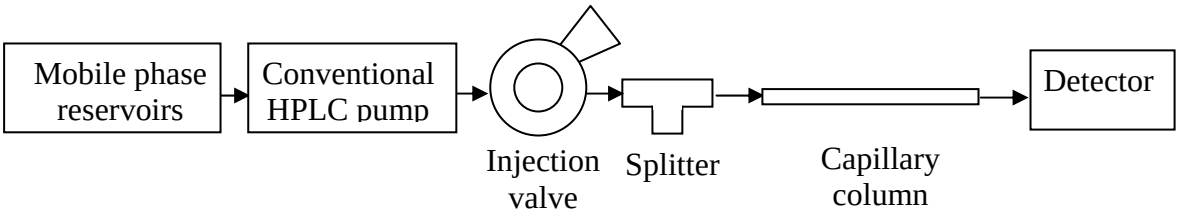
THEORY OF CLC, CE AND CEC

2.1 Instrumentation and fundamental of capillary liquid chromatography (CLC)

2.1.1 CLC instrumentation [Skoog and Leary 1992, Hernández-Borges *et al*. 2007, Vissers *et al*. 1997, Szumski *et al*. 2002, Noga *et al*. 2007]

Like in conventional HPLC, the major CLC equipment is composed of mobile phase reservoirs, pumping systems, sample introductions, columns and detectors, as illustrated in Figure 2.1.

(A) A split-flow/injection CLC system



(B) A splitless CLC system

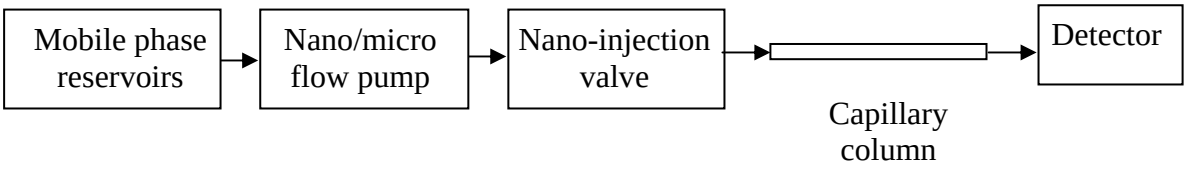


Figure 2.1 A schematic diagram of simple CLC instrument: (A) a split flow/injection system using conventional HPLC equipments and (B) a splitless system using a nano-liter injector and a nano/micro flow pump.

1) Mobile phase reservoirs

The reservoirs are made of glass or stainless steel and should contain at least 50 mL of solvents. A filter should be connected to the solvent feed line at the inlet end for removing dust and particulates from the solvent. Prior to feed mobile phases into the pump, solvent degassing is required to eliminate dissolved gases and to prevent the formation of bubbles in the check-valve pump or detector during the separation. Degassing can be performed by purging the mobile phase with inert gas, using a vacuum pump or heating and stirring the solvents.

2) Solvent delivery systems

The requirements for HPLC pumps are to deliver the solvents against the column back pressure with a steady and reproducible flow rates and a pulse-free output. Various types of pumps are available for HPLC systems. At the present time, reciprocating pumps are widely used in the commercial HPLC instruments. The pumps consist of the inlet and outlet check valves and a small chamber with a motor-driven piston. The flow of solvent into and out of a small chamber can be control by opening and closing alternately of two ball check valves. There are many advantages of reciprocating pumps, including their small internal volume (about 35-400 μL), their continuously solvent delivery, their high output pressures (up to 10,000 psi), their ready adaptability to gradient elution and their constant flow rates, which are independently of column backpressure and solvent viscosity. However, reciprocating pumps can produce a pulsed flow which can cause base line noise on the chromatogram.

There are two systems used in CLC operation: split and splitless flow systems. To create low flow rates delivery to a capillary column using conventional HPLC pumps, the method widely used is based on the split-flow technique. The device can be simply assembled by connecting the pump to a T-union with zero dead volume (served as a splitter). The other two ends are connected to the CLC capillary column

and to an empty tube of appropriate length and i.d. The major part of the flow is directed to the waste, and a small percent is introduced into a capillary column. The split ratio can be altered by changing the length and the i.d. of the empty tube. Another system used is to operate with a pump to create a direct nanoflow, called a splitless flow/injection system.

3) Sample introduction

The sample injection part is designed to introduce reproducibly a small aliquot of sample into the pressurized columns without stopping flow of mobile phases. The sample should be injected in a small amount as a narrow plug in order to minimize band broadening and to avoid column overloading. The most widely used device for injection in HPLC is the sampling valves. The function of a sample loop is to inject the sample into the mobile-phase steam prior to the column. The volume of sample injection into the column can be adjusted by changing sampling loop sizes (typically in the order of microliter in conventional HPLC system).

For CLC columns, injection volumes are in the order of a few nL up to approximately 1 μL. Various injection systems have been reported for introduction of a sample to capillary column. The internal loop of sampling valve can be replaced with commercial internal loops of 10-20 nL. However, the maximum injection volume should be less than one-third of the peak volume. The most popular injection technique is a split flow injection. It can be performed by connecting to a split vent between the micro-injection valve and the column. Alternatively, the stopped flow or heart-cut injection techniques can be used too.

4) Capillary

CLC columns are made of fused-silica, carbon, titanium, or stainless steel tubings. Fused-silica capillary is the most widely used tubing, while the last three materials are

used for the 150 μm or higher i.d. Capillary columns contain packing particles, monolith or wall coated with appropriate materials.

5) Detectors

The detectors used in HPLC can be classified into two types: bulk property and solute property detectors. The former is detectors that respond to some physical property of the mobile phase together with the solute such as refractive index. The latter detectors respond only to some property of the solute such as UV absorption, fluorescence or diffusion currents. UV-visible detectors are most currently used in commercial HPLC instrument.

As in conventional HPLC, UV-visible detectors are the most commonly used in CLC because of their simplicity relative low cost, and a broad range of applicability. In addition, they also allow on-column detection for accurate qualitative and quantitative analysis. However, they are not the most appropriate tools to be used due to the short pathlength of the capillaries resulting in the reduction of sensitivity. In order to overcome this limitation, the use of Z-shaped or U-shaped nano flow cells have been applied to reach an appropriate sensitivity.

2.1.2 Elution in LC [Neue 1997]

Elution of analytes from the column in LC can be divided into two types which are isocratic and gradient elution.

Isocratic elution: the compositions of mobile phase are held constant (without changing solvent strength during the separation).

Gradient elution: the compositions of mobile phases are continuously varied from low elution strength to high elution strength during the separation. The gradient elution is useful for separating a complex mixture of analytes having widely varying the

retention. In comparison with isocratic elution, the more strongly retained analytes not only eluted in a shorter time but the band shape is also improved in gradient elution.

There are two types of solvent mixing systems for gradient elution: low-pressure mixing and high-pressure mixing systems. In low-pressure mixing system, solvent mixtures are first formed by mixing two or more solvents at atmospheric pressure and then pumped using a single high pressure pump. While in high-pressure mixing system, the mixing of two solvents is generated by programming the delivery from the high pressure pumps and then mixed in the chamber before flowing into the column.

2.1.3 Reversed-phase chromatography [Weston and Brown 1997]

Reversed phase (RP) chromatography is the most widely used chromatographic mode. It is used to separate neutral molecules in solution based on their hydrophobicity. In this mode, the stationary phase used is non-polar bonded phases and the mobile phase used is polar solvent. As a result, a decrease in the polarity of the mobile phase results in a decrease in solute retention.

The separation mechanism in RP chromatography can be explained by two main theories: solvophobic and partitioning process, as shown in Figure 2.2. In solvophobic theory, the stationary phase is thought to behave more like a solid than a liquid, and retention is considered to be related primarily to hydrophobic interactions between solutes and the mobile phase (solvophobic effects). The solute binds to the surface of the stationary phase, thereby reducing the surface area of analyte exposed to the mobile phase. Adsorption increases as the surface tension of the mobile phase increases. Therefore, solutes are retained more as a result of solvophobic interactions with the mobile phase than through specific interactions with the stationary phase. In partitioning model of retention, the stationary phase plays a more important role in the retention process. The solute is thought to be fully embedded in the stationary phase chains, rather than adsorbed on the surface, and therefore is considered to be partitioned between the mobile phase and a “liquid like” stationary phase. Depending

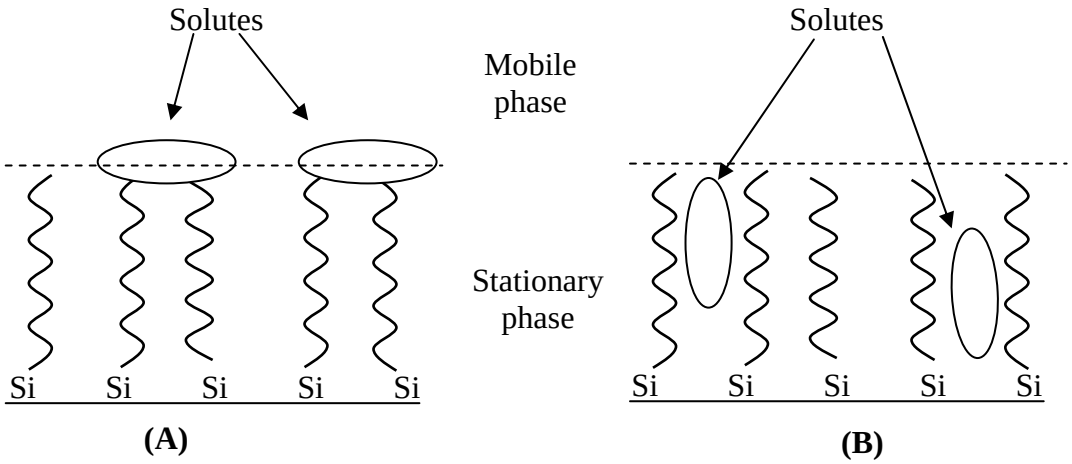


Figure 2.2 Models of solute retention: (A) solvophobic and (B) partitioning models. Adapted from Weston and Brown [1997].

on the nature of the bonded phase, the mechanism may be a combination of both those described above.

2.1.4 Molecular interactions [Simpson 1982]

There are three different types of molecular interaction that can take place between a solute and the stationary or mobile phases: ionic, polar and dispersive interactions.

Ionic forces result from the electrical interactions that can take place between ions. Therefore, they occur with the molecules having net positive or negative charges.

Polar interactions also result from electrical charges, but polar charges are either induced in the molecule or exist as permanent dipoles in the molecule (the molecules in this situation do not carry a net charge). Dipoles can also be induced in molecules by an electric field resulting from another molecule having permanent dipoles.

Dispersive interactions are also electric in nature, but do not result from a net charge on the molecule or a permanent dipole. They are typified by the interactions between hydrocarbon molecules or the hydrocarbon chains of polar molecules.

Therefore, to effect a separation in the chromatographic system one has to exploit the correct intermolecular forces between the solute and the mobile phase and the solute and the stationary phase.

2.1.5 Retention in LC [Skoog and Leary 1992, Synder and Kirkland 1979]

In LC, the analytes are distributed between the mobile phase and stationary phases at equilibrium. The distribution coefficient, *K*, is given as the equation,

$$A_m \rightleftharpoons A_s \qquad K = \frac{C_s}{C_m} \tag{2.2}$$

where C_s is the molar concentration of the analyte in the stationary phase and C_m is its molar concentration in the mobile phase.

The retention factor, *k*, is defined as the mole ratio of the analytes in the stationary phase (n_s) to the mobile phase (n_m).

$$k = \frac{n_s}{n_m} = K_A \frac{V_s}{V_m} \tag{2.3}$$

where V_s and V_m are the volume of the stationary phase and mobile phase, respectively.

From the chromatogram, the retention factor, *k*, can be calculated using Equation (2.4),

$$k = \frac{t_R - t_0}{t_0} \tag{2.4}$$

where t_R and t_0 are the retention time of the analytes and an unretained compound, respectively.

Selectivity, α, is a parameter that describes the relative migration rate of the analytes. It is defined as the ratio of retention factor for two analytes

$$\alpha = \frac{k_2}{k_1}; k_2 > k_1 \quad (2.5)$$

Since $k_2 > k_1$, the selectivity is always greater than 1. If α is approaching 1, there will be no separation.

2.1.6 Band broadening in LC [Synder and Kirkland 1979, Weston and Brown 1997]

Peaks in chromatography are assumed to have a Gaussian shape with standard deviation, σ, as shown in Figure 2.3. The width of the peak at base (w_b) is obtained by drawing lines at tangents to the points of inflection, and measuring the separation between the points of intersection with the baseline. The peak width at base is equal to 4σ, while the peak width at half height (w_h) is equal to 2.354σ.

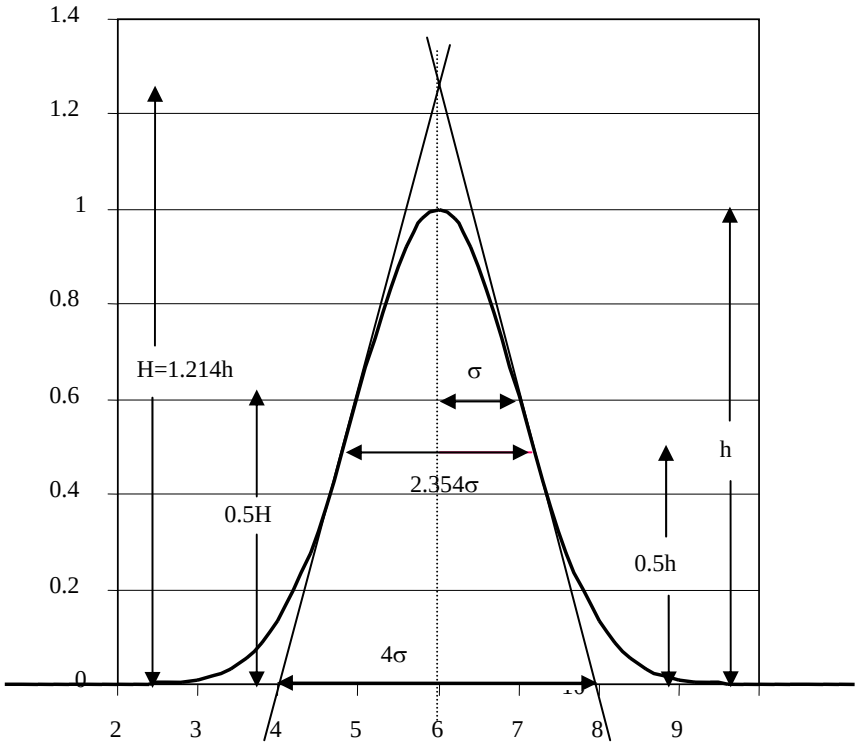


Figure 2.3 Gaussian peak, with parameters given in distance units. Adapted from Dyson [1990].

Column efficiency is described in terms of the number of the theoretical plates (*N*) which is a measure of the quality of the separation or the relative ability of a column to provide narrow bands. The number of the theoretical plates, *N*, is related to σ as the equation,

$$N = \left(\frac{t_R}{\sigma} \right)^2 \tag{2.6}$$

From a chromatogram, *N* can be calculated according to the equation,

$$N = 16\left(\frac{t_R}{w_b} \right)^2 = 5.54\left(\frac{t_R}{w_h} \right)^2 \tag{2.7}$$

An alternative measure of column efficiency is the plate height or height equivalent of a theoretical plate, *H*, which is related to *N* and *L* as the equation,

$$H = \frac{L}{N} \tag{2.8}$$

where *L* is the column length.

According to Equation 2.8, small *H* values mean more efficient columns and large *N* values.

The linear velocity, *u*, is based on the time for unretained compounds (t_0) as follows;

$$u = \frac{L}{t_0} \tag{2.9}$$

In practical, the dependence of H on u is the key to understanding and controlling separation after a column has been selected for a given LC separation. This relationship is so called the van Deemter plot as the following equation,

$$H = A + B/u + Cu \tag{2.10}$$

The A term represents the contribution to band broadening by eddy diffusion, the B term represents the contribution from longitudinal diffusion, and the C term represents the contribution from resistance to mass transfer.

Eddy diffusion arises from the presence of different flowpaths between different particles in the column, resulting in the different distance and velocity of the analyte molecules.

Longitudinal diffusion is a band broadening process in which analytes diffuse in the mobile phase randomly in all directions due to a concentration gradient.

Resistance to mass transfer is caused by the slow equilibration of the analytes between the mobile phase and stationary phase. There are two major contributions to the resistance to mass transfer: mass transfer in mobile phase and mass transfer in stationary phase. In the liquid mobile phase, the analytes move with different flow rates for different parts of a single flowstream. In addition, an increase in molecular spreading in mobile phase mass transfer results from the contribution of stagnant mobile-phase mass transfer. With porous particles, the mobile phase contained within the pores of the particle is stagnant. The analytes diffuse into and out of the porous particles in the stagnant mobile phase with different distance, and therefore, the analytes move out of the pores to the external mobile phase at different time. The effect of stationary phase mass transfer to band broadening arises from, after the analytes diffuse into a pore, the different penetration of analytes deep into the stationary phase.

In addition to the band broadening that takes place in the column, extracolumn effects also contribute to peak broadening. Extracolumn dispersion is the contribution to peak varaince that takes place outside the column. In CLC system, it is very important to minimize extracolumn contributions. There are four major sources of extracolumn dispersion: (i) dispersion due to the injection volume, (ii) dispersion due to the volume of detector cell, (iii) dispersion due to the detector response time, and (iv) dispersion resulting from the volume in the connecting tubing between the injector and the column and also between the column and the detector.

2.1.7 Resolution in LC [Synder and Kirkland 1979**]**

Resolution, R_s, is an important parameter that describes the degree of separation between two adjacent peaks. This parameter can be measured by the ratio of the distance between the two peaks to their peak width.

$$R_s = \frac{\Delta x}{0.5(w_1 + w_2)} = \frac{\Delta t_R}{0.5(w_1 + w_2)} \tag{2.11}$$

where Δx is the distance between the peak measured in length units, Δt_R is the distance between the peak in time units, and w is the width of the peaks at base (length unit for Δx and time unit for Δt_R).

Theoretically, resolution is related to retention factor (k), column efficiency (N) and selectivity (α) as the equation,

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{k}{k+1}\right)\left(\frac{\alpha - 1}{\alpha}\right) \tag{2.12}$$

This equation can be used as a guide for improving the separation by varying N, k, α. Efficiency is increased by increasing the column length (L) or decreasing mobile phase velocity (u) or by changing to a different column that provides lower H. Retention factor is varied by changing solvent strength to maintain k values in the

optimum range of $1 \leq k \leq 10$. Selectivity is varied by changing the composition of the mobile phase, stationary phase and/or temperature.

2.2 Instrumentation and fundamental in capillary electrophoresis (CE) and capillary electrochromatography (CEC)

2.2.1 CE instrumentation

Figure 2.4 shows a schematic of basic CE instrument. It consists of a capillary (typically fused-silica capillary) with the inner diameter of 10 to 200 μm, two BGE reservoirs at the inlet and the outlet ends of capillary (usually buffer solutions), two electrodes (typically platinum wire) which are immersed in BGE vials, a high voltage power supply (-30 kV to +30 kV), a detector generally used UV-visible detector, a cooling system for controlling temperature and minimizing the Joule heating effect, and electrokinetic or hydrodynamic injection systems.

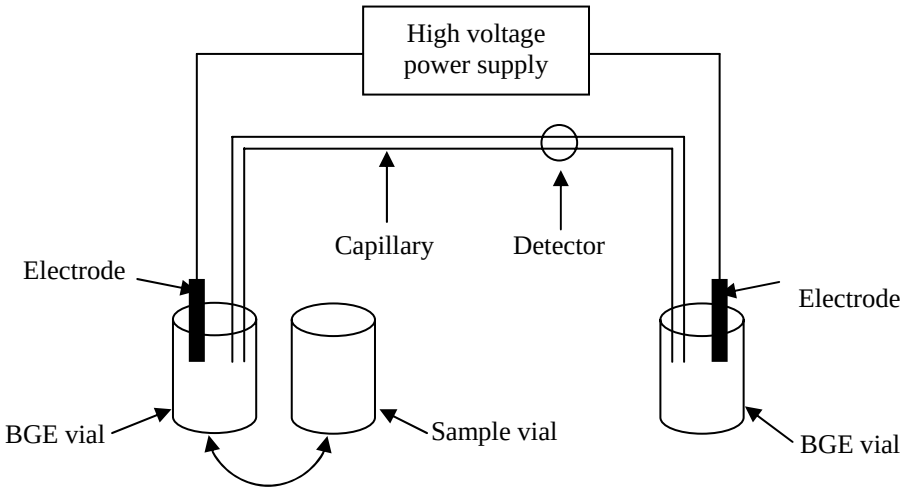


Figure 2.4 A basic schematic diagram of CE instrument. Adapted from Weinberger 1993].

2.2.2 Electrophoretic mobility [Camilleri 1993, Grossman and Colburn 1992]

When the external electric field is applied, the charged analytes can migrate by an electric force (F_e) which depends on the charge of analytes, q ($q = ze$, where z is the charge of ions and e the electronic charge) and the electric field strength, E,

$$F_e = zeE \tag{2.13}$$

Once the ions begin to move, the frictional force (F_F) takes place to resist the movement of ions. This force is proportional to the viscosity of buffer (η), electrophoretic velocity (v_{ep}), the hydrodynamic radius of the ion (r_h). For a spherical molecule, F_F is related to those parameters as,

$$F_F = 6\pi\eta\, r_h \tag{2.14}$$

At equilibrium, the electric force is counterbalanced by the frictional force, giving the equation

$$zeE = 6\pi\eta\, r_h \tag{2.15}$$

$$v_{ep} = \frac{ze}{6\pi\eta r_h} E = \mu E \tag{2.16}$$

The electrophoretic mobility (μ) is defined as an electrophoretic velocity (v_{ep}) of the analyte migrating along the capillary under the electric field strength (E) of 1 V m^{-1},

$$\mu = \frac{v_{ep}}{E} = \frac{ze}{6\pi\eta r_h} \tag{2.17}$$

2.2.3 Electroosmotic flow [Chankvetadze 1997a, Grossman and Colburn 1992]

Electroosmosis is an important driving force in CE in addition to the electrophoretic mobility for migrating the analytes along the capillary. The capillary commonly used in CE is an uncoated fused-silica capillary which contains the siloxane bonds (–Si–O–Si–) at the inner wall. When the capillary is in contact with an aqueous solution, the silanol groups (–Si–OH) can be formed by the hydrolysis of siloxane bonds, and then will ionize to be negatively charged at pH > 2

$$SiOH + H_2O \rightleftharpoons SiO^- + H_3O^+ \qquad (2.18)$$

The positive ions in an aqueous solution are attracted to balance the surface negative charges by the electrostatic force, resulting in the formation of the electrical double layer, as illustrated in Figure 2.5A. Some cations exist in a stagnant layer, called Helmholtz or Stern layer, and some of them are distributed in an outer diffuse layer. The rest of the excess cations is in the bulk solution.

When the electrical field is applied, the solvated cations in the diffuse layer migrate toward the cathode, leading to the movement of the solvent or water molecules in the same direction. This phenomenon is called electroosmosis, and the movement of the liquid is called electroosmotic flow (EOF) (Figure 2.6). The thickness of double layer is about only 100 Å, however, EOF is transmitted through the diameter of the capillary, presumably due to intermolecular interactions in the liquid phase.

As shown in Figure 2.5B, the electrical potential at the capillary wall/aqueous solution interface linearly decreases with increasing the distance from the surface in the Stern layer and exponentially decreases to zero in the diffuse layer. The electrical potential at shear plane, located just outside the imaginable boundary lines between the Stern and diffuse layer, is known as the zeta potential (ζ). The electroosmotic velocity (v_{eo}) is directly proportional to the zeta potential as,

$$v_{eo} = -\frac{\varepsilon\zeta}{4\pi\eta}E \tag{2.19}$$

where ε and η are the permittivity and the liquid viscosity in the double layer. From Equation 2.19, dividing v_{eo} by E gives electroosmotic mobility (μ_{eo}, $m^2\ V^{-1}\ s^{-1}$) as follows,

$$\mu_{eo} = \frac{v_{eo}}{E} = -\frac{\varepsilon\zeta}{4\pi\eta} \tag{2.20}$$

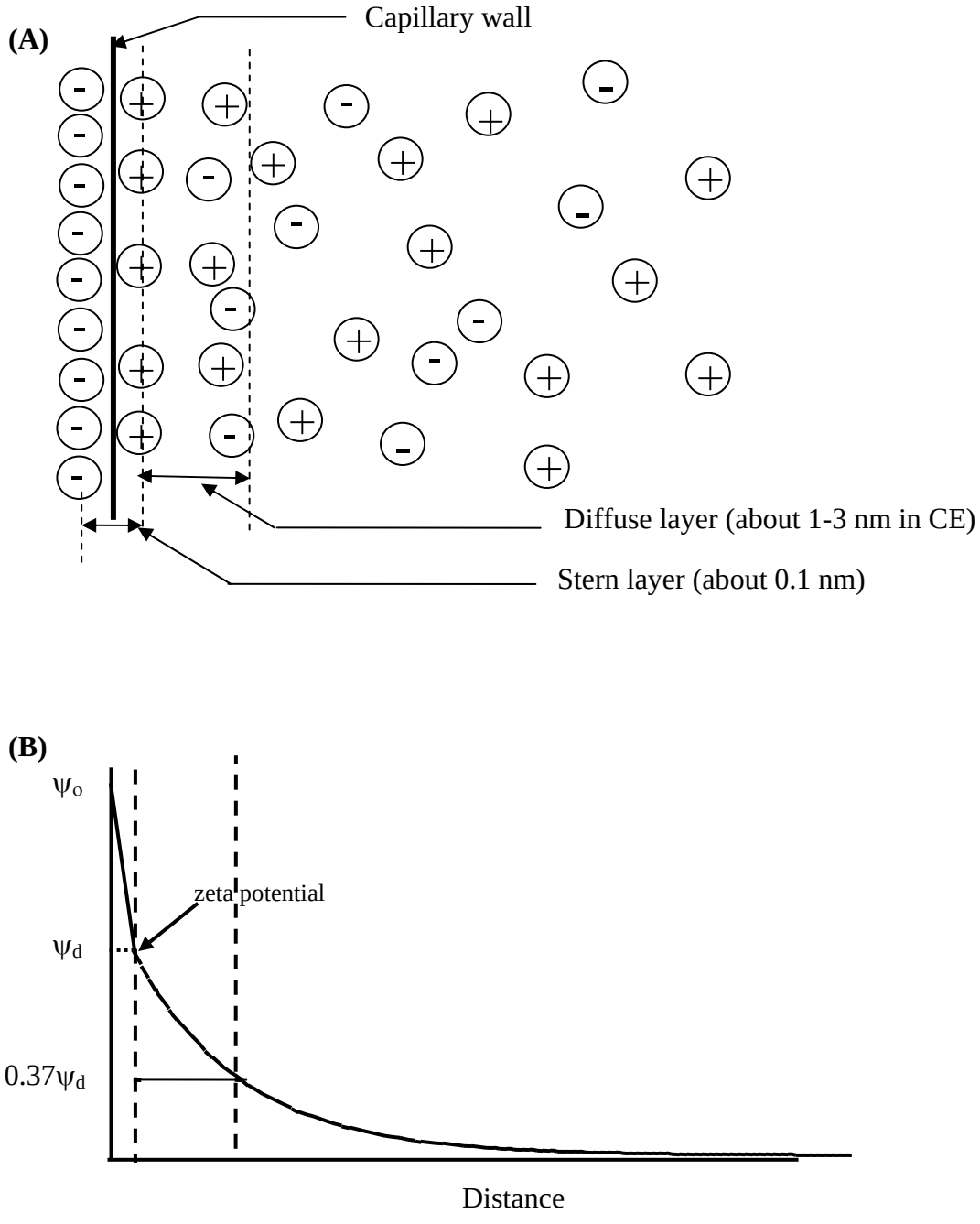


Figure 2.5 A model of a double electrical layer (A) and electrical potential (B). Adapted from Hiemenz and Rajagopalan [1997].

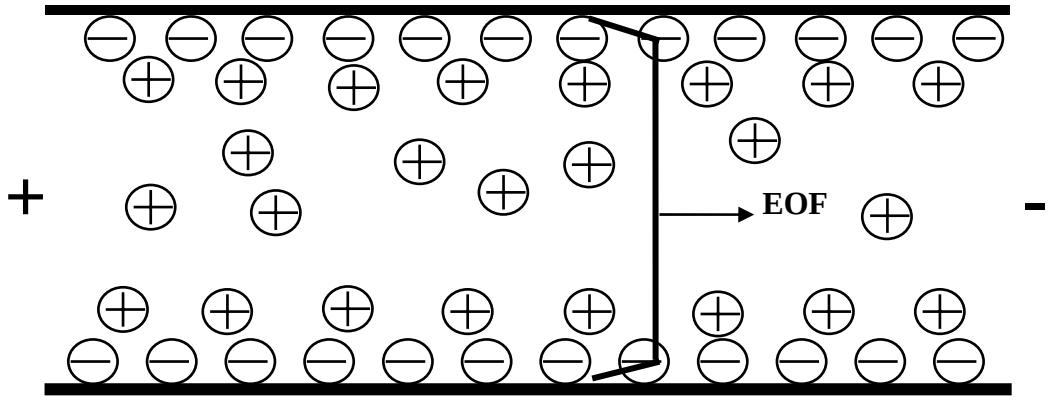


Figure 2.6 Electroosmotic flow (EOF). Adapted from Landers [1997].

The electroosmotic velocity increases with the distance far away from the capillary wall, and reaches a constant value at the distance of approximately 15 nm from the wall. Owing to a very thin double layer (up to several hundred nanometers) relative to the capillary diameter (typically 20 to 100 μm or 20,000 to 100,000 nm), it can be mentioned that the electroosmotic velocity is constant across the capillary diameter. Therefore, the EOF has a flat flow profile which gives lower band dispersion (higher efficiency), in comparison with a parabolic profile in pressure-driven systems such as HPLC (Figure 2.7).

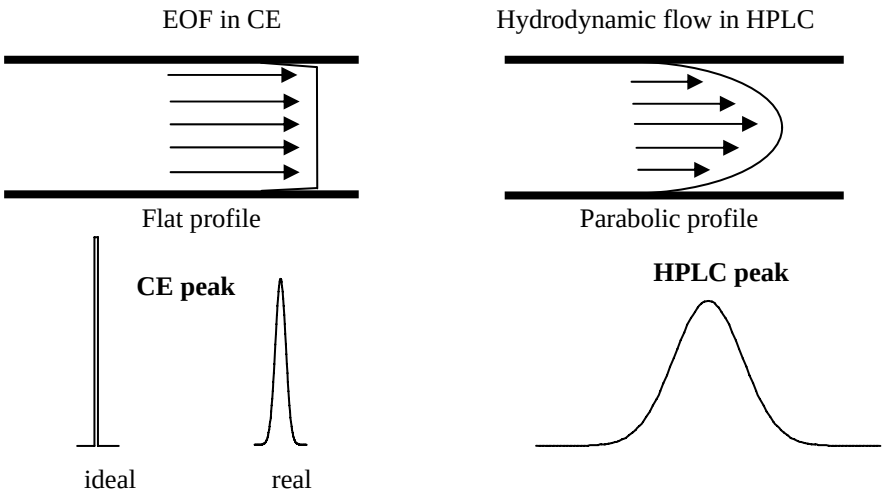


Figure 2.7 Flow profiles and peak in CE and HPLC. Adapted from Li [1992].

In CE, the analyte ions migrate towards to the detector due to the influence of both electrophoretic mobility of the analyte and electroosmotic flow. In the presence of EOF, the net velocity of the analyte (v_{net}) is the sum of the electrophoretic velocity of the analyte and the EOF as follows;

$$v_{net} = v_{ep} + v_{eo} \tag{2.21}$$

At high EOF, negative, positive and neutral analytes can migrate in the same direction as shown in Figure 2.8. In case of 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 gave the higher $v_{net,+}$. In contrast, $v_{ep,-}$ for anions has the direction toward the anode (opposite direction to EOF), and therefore, the higher ion charges and the smaller ion size gave the smaller $v_{net,-}$. Neutral molecules migrate toward the cathode only by the influence of EOF.

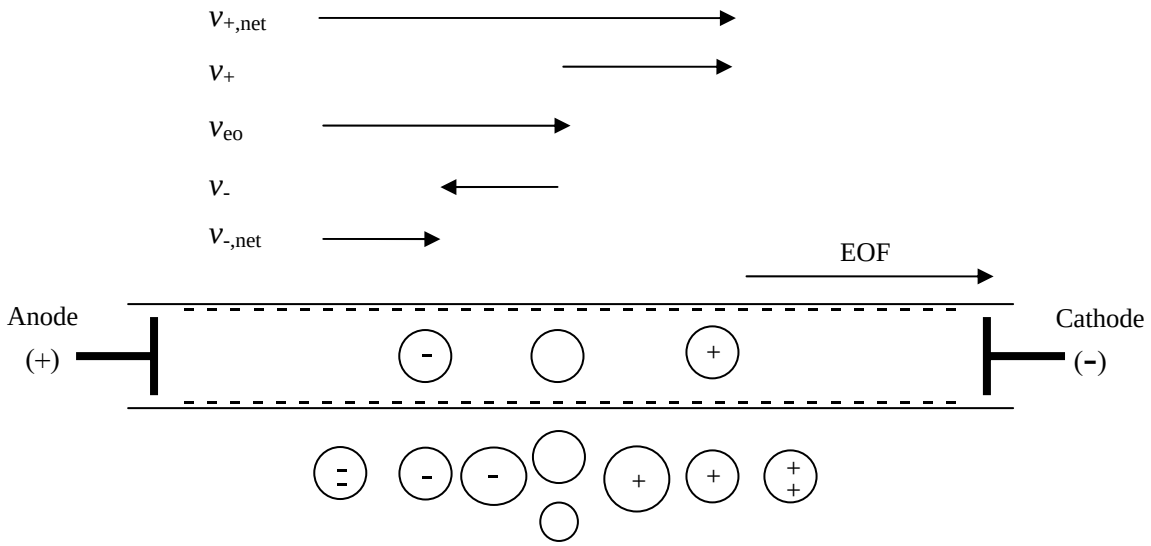


Figure 2.8 Migration behavior of cationic, anionic and neutral analytes. Adapted form Li [1992].

In practical, the net electrophoretic mobility ($\mu_{net} = \mu + \mu_{eo}$), μ_{eo} and μ can be calculated from an electropherogram as given in Equation 2.22-2.24;

$$\mu_{net} = \frac{\nu_{net}}{E} = \frac{lL}{Vt_m} \tag{2.22}$$

$$\mu_{eo} = \frac{\nu_{net}}{E} = \frac{lL}{Vt_{eo}} \tag{2.23}$$

$$\mu = \mu_{net} - \mu_{eo} = \left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right)\frac{lL}{V} \tag{2.24}$$

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

2.2.4 Resolution in CE [Khaledi 1998]

According to Equations 2.11 and 2.24, R_s in CE is related to the mobility and the average number of the theoretical plates, $\overline{N}$, as the equation

$$R_s = \frac{1}{4}\left(\frac{\Delta\mu}{\bar{\mu} + \mu_{eo}}\right)\sqrt{\overline{N}} \qquad (2.25)$$

where $\Delta\mu$ is the electrophoretic mobility difference and $\bar{\mu}$ is the average electrophoretic mobility.

Equation 2.25 shows that the improvement of separation can be achieved by increasing the mobility difference between the analytes and also the square root of the efficiency. The resolution in Equation 2.25 may be rearranged to relate to efficiency term, separation selectivity term and mobility term, like in chromatography as the equation;

$$R_s = \left(\frac{\sqrt{\overline{N}}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left|\frac{\mu_2}{\bar{\mu} + \mu_{eo}}\right| \qquad (2.26)$$

where α is the separation selectivity or mobility selectivity, defined as the ratio of μ for two analytes: $\alpha = \mu_2/\mu_1$, $\alpha > 1$.

2.2.5 Capillary electrochromatography (CEC)

As previously mentioned in Chapter I, CEC is carried out using capillary columns containing stationary phase used in CLC and the mobile phase is driven by EOF at high electric field strength in an instrument similar to that used in CZE. In comparison with CLC or CZE, CEC offers many advantages, such as higher efficiency than CLC, higher loading capacity than CZE, and possibility of different selectivity than CLC and CZE. The key components that impact the separation in a CEC column include

EOF, electrophoretic migration of the analytes, and also interactions between analytes and stationary phase.

2.2.5.1 Electroosmotic flow in CEC [Khaledi 1998, Colón *et al.* 1997, Végvári and Guttman 2006]

According to Section 2.2.3, the electrical double layer in CE only exists around the walls of the capillary. In CEC using packed and monolithic columns, each particle possesses its own electrical double layer. Therefore, it can generate EOF in the capillary with each particle, as shown in Figure 2.9. Since the surface areas of the beds are much greater than that of the capillary walls, most of EOF is generated from the surface of stationary phase.

As shown in Figure 2.10, the velocity of pressure driven flow in CLC varies with the diameter of the channels, while the velocity in an electrical driven system is independent on the channel width, resulting in the lower zone broadening in CEC than CLC. The particle size in packed columns or the macropores in monolithic columns should be 20 times larger than the thickness of double layer. Below this value, the electrical double layers that surround the particles will overlap, and then EOF will stop. The electrical double layers overlap within the channels, and therefore, there is no flow of the mobile phase within particle, only between particles.

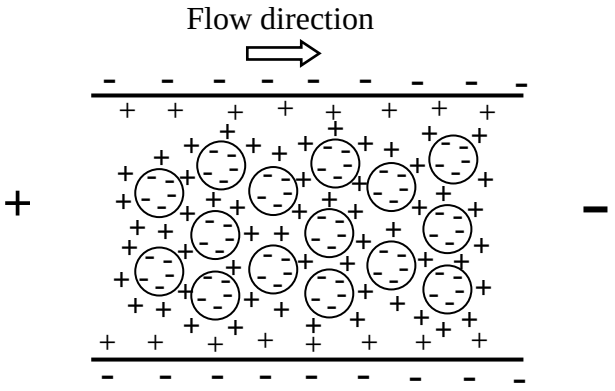


Figure 2.9 Electroosmotic flow in CEC. Adapted from Colón *et al.* [1997].

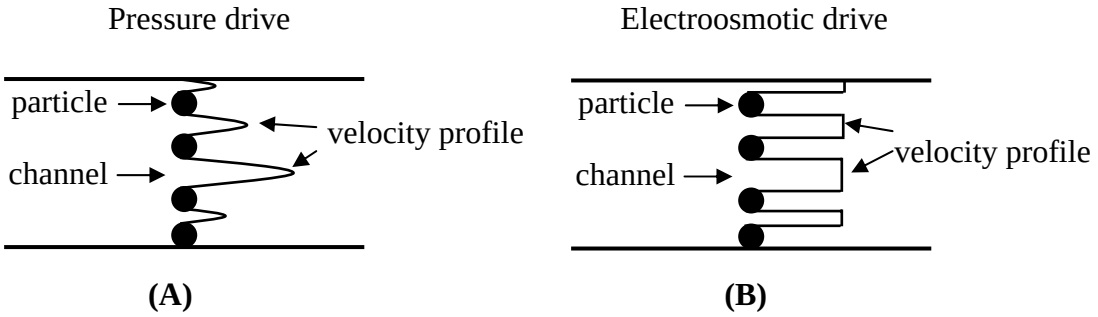


Figure 2.10 Flow profile in (A) CLC and (B) CEC. Adapted from Dittman and Rozing [1996].

2.2.5.2 Retention in CEC [Svec 2004, Bartle and Myers 2001, Rathore 2002, Rathore and Horváth 2002, Rathore and Horváth 1996]

In case of neutral analytes, the migration of analytes in CEC is equal to that in CLC, and then the retention factor of analytes in CEC can be described as given in Equation 2.4. However, the migration of charged compounds in CEC is more complex because of the interplay of electromigration and EOF. Since the elution of the analytes in CEC columns depends on the magnitudes of their different retardation and migration velocities, Rathore and Horváth [Rathore and Horváth 1996] defined the retention factor in CEC (k_c) as the ratio of the separative and non-separative virtual migration length. The separative components involve the selective interactions with the stationary phase or the electric field that is directly reponsible for the separation, while the non-separative components represents the migration that does not contribute directly to the separation. Therefore, the electrochromatographic retention factor, k_c, is given by the equation;

$$k_c = k + k\,k_e + k_e \qquad (2.27)$$

where k is the chromatographic retention factor in CLC (Equation 2.4), and k_e is the electromigration velocity factor in CZE, defined as the ratio of electromigration velocity of the analytes and that of EOF marker ($k_e = v_{ep}/v_{eo}$). The product of $k\,k_e$ is the consequence of simultaneous chromatography and electrophoresis. If $k_e = 0$ then only CLC operates and $k_c = k$, and if $k = 0$, then only process is CZE since $k_c = k_e$.

In CEC, the overall migration velocity of charged solutes, v_c, is related to the sum of its velocities due to EOF and electrophoretic migration in the mobile phase and the retardation factor $1/(1+k'')$ as;

$$\nu_c = \left(\frac{\nu_{ep} + \nu_{eo}}{1+k''} \right) = \frac{\nu_{eo}\left(1+k_e''\right)}{1+k''} \tag{2.28}$$

where k'' and k_e'' are chromatographic retention factor and the electromigration velocity factor, respectively, that measure under conditions used in CEC experiments. They are given by the equation;

$$k_e'' = \frac{\nu_{ep}}{\nu_{eo}} = \frac{\mu_{ep}}{\mu_{eo}} \tag{2.29}$$

$$k'' = \frac{t_m(1+k_e'') - t_0}{t_0} \tag{2.30}$$

According to Equations 2.29 and 2.30, the chromatographic and electrophoretic contributions to migration of charged analytes in CEC cannot be determined from CEC data alone and electrophoretic measurements must also be carried out. Yet, often a dimensionless peak locator would be sufficient to define the results of the separation. For such a case, Rathore and Horváth [Rathore and Horváth 2002] developed a simpler approach using a peak locator defined in terms of k'' and k_e'' by following a chromatographic or an electrophoretic formalism, respectively. The peak locator is devoid of any mechanistic insight offered by the more sophisticated factors, and therefore has only limited utility. However, the peak locator can be evaluated directly from the electrochromatograms without any additional measurements.

The simplest formalism of the peak locator, k_{cc}'', is expressed in a manner similar to Equation 2.4 for chromatography as follows,

$$k''_{cc} = \frac{t_m - t_{eo}}{t_{eo}} \quad (2.31)$$

where t_m and t_{eo} are the migration time of the analyte and an unretained neutral marker in CEC column, respectively. In contrast to CLC, since $t_m < t_{eo}$ may be possible for positively charged compounds migrating faster than the EOF, k''_{cc} would be negative for those cases. This equation has been used many times throughout the CEC literature [Xie *et al.* 2004].

Another way to define a peak locator is using the electrophoretic formalism, k''_{ce}, for analytes moving faster than the marker;

$$k''_{ce} = \frac{t_{eo} - t_m}{t_m} \quad (2.32)$$

where both t_m and t_{eo} defined with the same meaning as in Equation 2.31. In contrast to k''_{cc}, k''_{ce} may be negative for the analytes moving slower than EOF.

2.2.5.3 Band broadening in CEC [Khaledi 1998, Rathore 2002, Bartle and Myers 2001]

The mechanisms that contribute to band broadening in CEC can originate from both its electromigration and chromatographic characteristics. Similar to CLC, the chromatographic plate height, *H*, can be described by a van Deemter equation (Equation 2.10). The main difference in *H* between CLC and CEC is due to the different mobile phase flow profiles as previously mentioned in Section 2.2.5.1. The band broadening due to electromigration characteristic can be contributed by Joule heating and electrodispersion. In addition, it is also caused by the effect of sample plug size, detection window size and band broadening due to the presence of frits.

CHAPTER III

PREPARATION AND PERFORMANCE EVALUATION OF MONOLITHIC SILICA CAPILLARY COLUMNS FOR REVERSED-PHASE STATIONARY PHASE IN CLC, AND APPLICATION TO PHENOXY ACID HERBICIDES AND BISPHENOL-A-DIGLYCIDYL ETHER, BISPHENOL-F-DIGLYCIDYL ETHER AND THEIRS DERVATIVES

3.1 Introduction

3.1.1 Silica monolith preparation [Unger *et al*. 2011, Núñez *et al*. 2008, Nakanishi and Nobuo 2007, Tanaka *et al*. 2001, Hench and West 1990]

Silica-based monolithic columns can be prepared by a sol-gel process accompanied by phase separation mechanism. The sol-gel process involves the preparation of a sol solution and the gelation of the sol to form a network in a continuous liquid phase. Sols are dispersion of colloidal particles in a liquid. Colloids are solid particles with diameter of 1-100 nm. A gel is an interconnected, rigid network with pores of submicrometer dimensions and polymeric chains whose average length is greater than a micrometer. The gelation is defined as the loss of the bulk fluidity of the reaction mixture.

The sol-gel transition of a silica alkoxide solution is generally caused by two kinds of reactions: hydrolysis and polycondensation, which are known to proceed in parallel from the beginning of the reaction. The hydrolysis of Si-OR is initiated by mixing water with alkoxide to generate Si-OH (Equation 3.1). After that, Si-OH condenses with another Si-OH to produce polycondensed species containing a Si-O-Si linkage and water (Equation 3.2). A successive condensation leads to the growth of siloxane

oligomers that subsequently link together to form a gel network. In the case of a limited amount of water presents in the mixing solution, the alcohol producing condensation is also possible (Equation 3.3). The overall reactions for silica alkoxide, $Si(OR)_4$, are expressed as follows,

Hydrolysis: $Si(OR)_4 + nH_2O \longrightarrow Si(OH)_n(OR)_{4-n} + nROH$ (3.1)

Water condensation: $\equiv Si\text{-}OH + \equiv Si\text{-}OH \longrightarrow \equiv Si\text{-}O\text{-}Si\equiv + H_2O$ (3.2)

Alcohol condensation: $\equiv Si\text{-}OH + \equiv Si\text{-}OR \longrightarrow \equiv Si\text{-}O\text{-}Si\equiv + ROH$ (3.3)

The overall kinetics of Equations 3.1-3.3 strongly depend on the amount of water, the kind of alkoxy groups, and the catalyst and its concentration. The gelation time depends strongly on pH of the solution. Because the macropore formation of the silica sol-gel system is a competitive process involving phase separation and the sol-gel transition, its dynamic behavior is influenced by the molecular mass and its distribution among silica oligomers, the compatibility between the solvent mixture and the oligomers, and the rate of gel network development. Therefore, the composition and catalyst concentration of the starting solution and the reaction temperature are the key parameters that determine the macropore morphology.

Phase separation in a binary system is experimentally observed as a formation of two conjugate phase regions having, respectively, different chemical compositions generally called “phase domains”. With the system containing tetraalkoxysilane and appropriate additives as a starting point, the polymerization induced phase separation, especially spinodal decomposition, has been extensively utilized to generate well defined heterogeneous structures. The spinodal decomposition starts with an infinitesimal composition fluctuation with a characteristic wavelength and its amplitude continuously grows to make higher contrast between the phase domains: a silica-rich domain (to be silica skeleton) and a solvent-rich domain (to be macropores). When the mixture has the critical composition of the system as well as comparable volume fractions, a cocontinuous domain structure tends to develop and remains unbroken for a substantial period of time. The domains continuously grow in size (coarsening), and finally result in fragmented domains and continuous matrix (Figure

3.1). When phase separation and gel formation occur competitively, various transient co-continuous structures can be permanently frozen in the network, resulting in the macropore and silica skeleton formation.

Since the silica gel skeletons of a macroporous network structure formed actually contain micropores, the pore structures on the silica skeleton is required to enlarge the mesopore size suitable for the separation of analytes. The mechanism of pore coarsening by the aging under a basic condition is called Ostwald ripening. This process involves the dissolution and the reprecipitation of the silica. The tailor of mesopores skeleton is usually treated with aqueous ammonia or urea.

The starting solution for silica monolith preparation contains four major components, namely silica source, an additive to induce phase separation, acid catalyst and water. In the synthesis of silica monoliths, alkoxysilanes, especially tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS), are the most widely used precursors due to purity, availability and price. With capillaries less than 100 μm i.d., pure silica composition can be prepared without serious defects to influence the performance of the column. However, with thicker capillaries, the partial substitution of TMOS with methyltrimethoxysilane (MTMS) is effective to minimize the shrinkage of silica gel network within the capillaries. The phase-separation inducer includes water-soluble polymers and surfactants. For example, poly(ethylene glycol) (PEG) controls the size and macropore volume in gels because the glycol forms strong hydrogen bonds with the silanols of growing silicate polymers. Since the phase separation tendency is determined mainly by the PEG/silica ratio, the average macropore size and skeleton size can be controlled by adjusting PEG/silica ratio. The volume fraction of the fluid phase, which becomes the pore space after drying, mainly depends on the volume fraction of solvent phase in the starting solution. Consequently, the size and volume of macropores can be independently controlled by adopting PEG or its analogues as an additive (Figure 3.2).

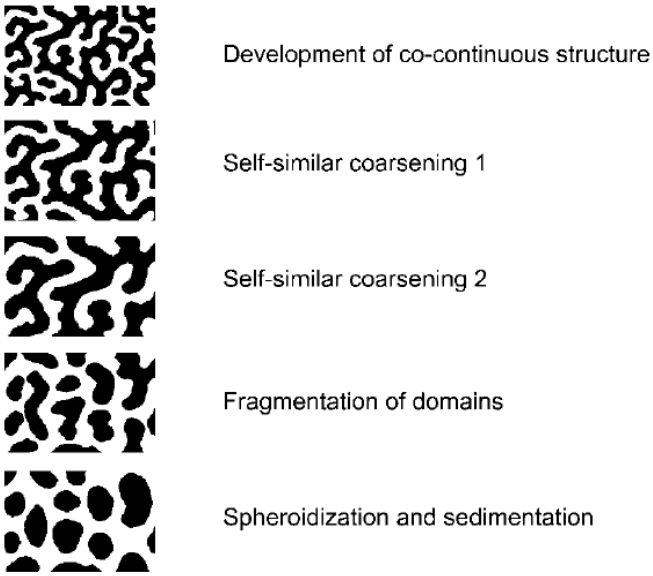


Figure 3.1 Time evolution of spinodally decomposed isotropic phase domains driven by surface energy. Reproduced from Nakanishi and Nobuo [2007].

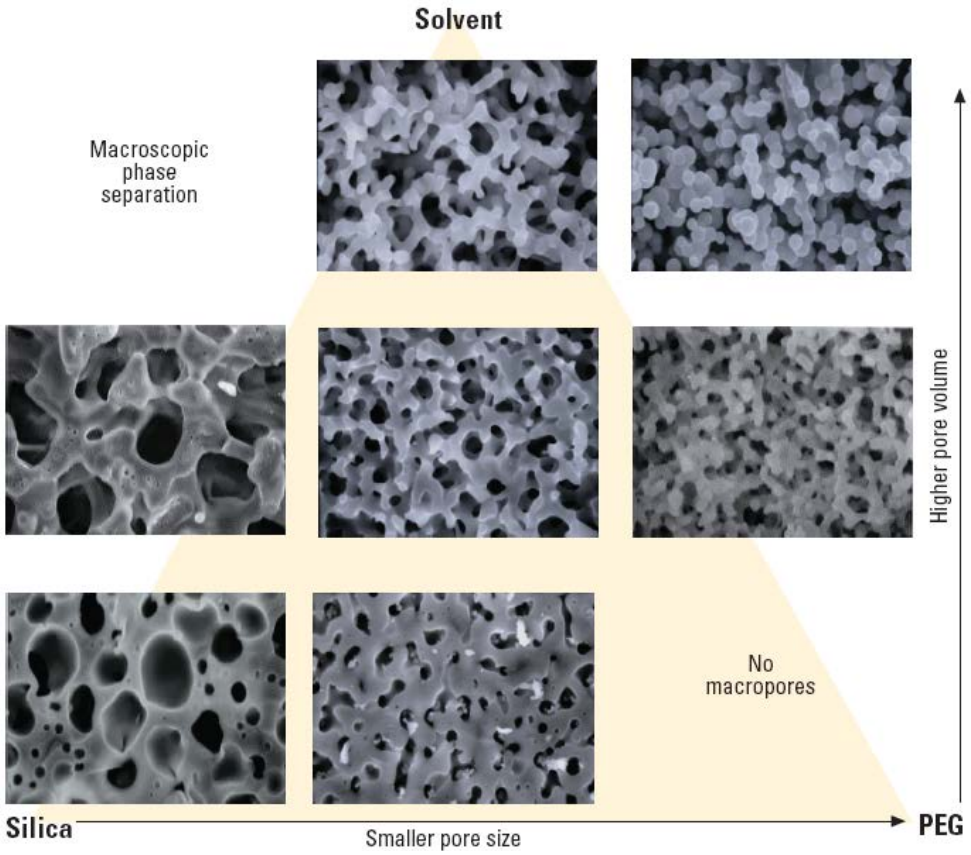


Figure 3.2 Relation between starting composition and resultant gel morphologies in a pseudoternary (silica/PEG/solvent) system. Pore size is controlled by PEG/Si ratio and pore volume by the fraction of solvent. Reproduced from Tanaka *et al.* [2001].

3.1.2 Chemical modification for reversed-phase stationary phase [Unger *et al*. 2011, Núñez *et al*. 2008]

In order to obtain the desired stationary phase, the surface of silica monolith can be modified by following either a silylation or a polymer-coating procedure, as shown in Figure 3.3. In most cases, the functionalization of monolithic silica columns was performed *in situ*. With a silylation reaction, the modification can be carried out by the direct reaction between the silica and a wide variety of silane reagents with the desired functional moieties, or by the introduction first of a spacer and then the chemical reaction with a ligand that will introduce the desired functional group. Polymerization methods can be performed by the introduction of a spacer or an anchor to be bonded with the silica surface before polymerization reaction.

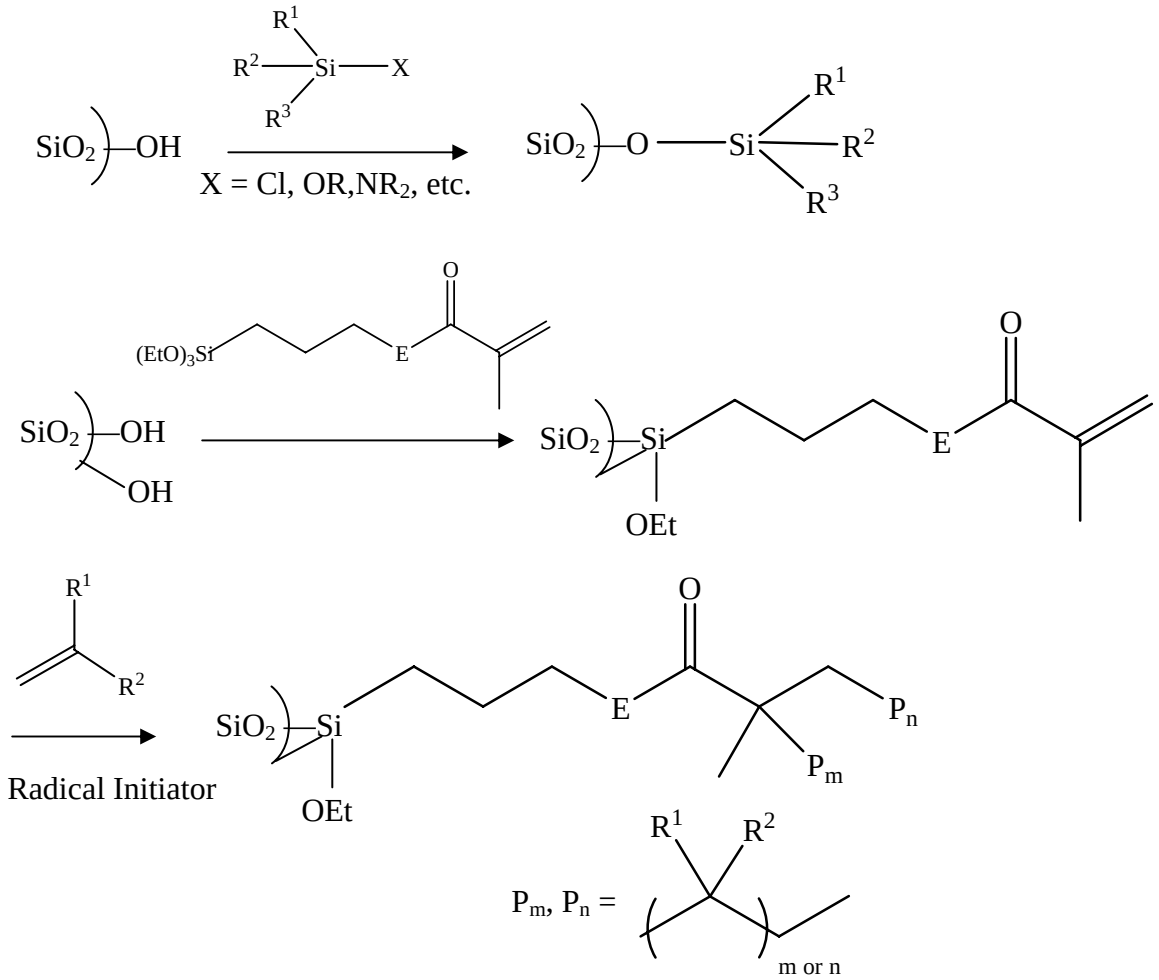
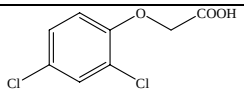
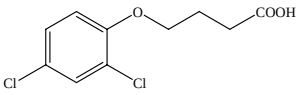
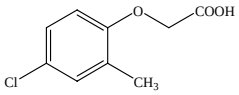
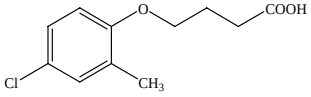
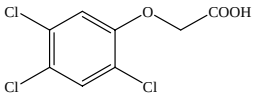
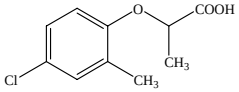
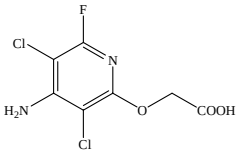
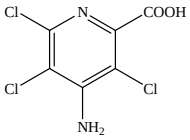
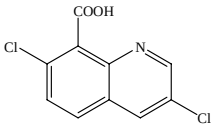
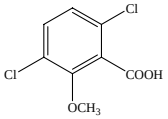
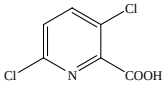
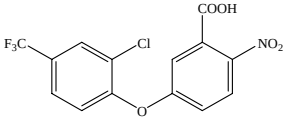
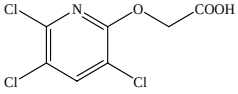


Figure 3.3 Chemical modification processes to functionalize monolithic silica columns. Adapted from Unger *et al*. [2011].

3.1.3 Previous works on separation of chlorophenoxy acid herbicides, and bisphenol-A-diglycidyl ether, bisphenol-F-diglycidyl ether and theirs derivatives

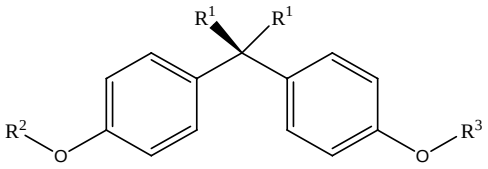
Chlorophenoxy acid herbicides are widely used in agriculture for weed and broadleaf plants control [Koesukwiwat *et al.* 2008]. Because of their high mobility in the soil, these compounds have been especially considered as a potential source of environmental contamination. The chemical structures of chlorophenoxy acids and their pK_a values are shown in Table 3.1. Several chromatographic methods have been reported for determination of chlorophenoxy acid herbides, such as gas chromatography (GC) [Santos-Delgado *et al.* 2000, Yu and Wells 2007, Kuang *et al.* 2006], GC coupled with mass spectrometry (GC/MS) [Bertrand *et al.* 1987, Pereiro *et al.* 2004, Catalina *et al.* 2000, Rodriguez *et al.* 2005, Nilsson *et al.* 1998, Ding *et al.* 2000], HPLC [Cappiello *et al.* 1997, Wu *et al.* 2005], LC coupled with mass spectrometry (LC/MS) [Geerdink *et al.* 1999, Takino *et al.* 2001, Garcia-Reyes *et al.* 2007], LC coupled with tandem mass spectrometry (LC/MS/MS) [Geerdink *et al.* 1999, Køppen and Spliid 1998, Lagana *et al.* 2002] and CE [Kruaysawat *et al.* 2001, Jung and Brumley 1995, Fung and Mak 2001, Starkey *et al.* 2003]. GC is a conventional chromatographic method for determination of these herbicides. Due to their low volatility and high polarity, a derivatization step (for example, using diamethane, alkyl and haloalkyl halides) is necessary prior to GC analysis. Since the derivatization was time-consuming and required for further clean-up step, HPLC was used as an alternative method for separating these compounds. The HPLC separation usually employs a conventional reversed phase C18 columns with different mobile phase systems such as acetonitrile/water/trifluoroacetic acid [Cappiello *et al.* 1997], methanol/ammonium acetate [Wu *et al.* 2005], and methanol/acetic acid/water [Køppen and Spliid 1998]. However, simultaneous separation of these 13 chlorophenoxy acid herbicides has not been reported in conventional HPLC and CLC using monolithic silica capillary columns.

Table 3.1 Chemical structures and pK_a of 13 chlorophenoxy acid herbicides.

Analyte	Structure	pK_a
2,4-D		2.7[a]
2,4-DB		4.58[b]
MCPA		3.1[a]
MCPB		4.86[b]
2,4,5-T		2.8[a]
Mecoprop		3.6[a]
Fluroxypyr		
Picloram		1.97[a]
Quinclorac		
Dicamba		1.9[a]
Clopyralid		2.0[a]
Acifluorfen		
Triclopyr		2.68[c]

[a]Values taken from Koesukwiwat *et al.* [2008].
[b]Values taken from Hsieh and Huang [1996].
[c]Value taken from Kimura *et al.* [2012].

Bisphenol-A-diglycidyl ether (BADGE) and bisphenol-F-diglycidyl ether (BFDGE) are commonly used as precursors for epoxy-resin based coating for food and beverage cans. In vinylic organosols (polyvinylchloride) based coating, BADGE and BFDGE are added mainly to remove hydrochloric acid that released during the heat treatment of the coating procedure. The epoxy groups on BADGE and BFDGE can react with hydrochloric acid to form many chlorinated compounds, such as BADGE·HCl, BAGDE·2HCl, BFDGE·HCl, and BFDGE·2HCl. Residual BADGE, BFDGE and theirs chlorohydroxy compounds can migrate from the inner coating into food upon contact. This can result in the formation of mono- and di-hydrolyzed products such as BADGE·H_2O, BADGE·$2H_2O$, BADGE·HCl·H_2O, BFDGE·H_2O, BFDGE·$2H_2O$, and BFDGE·HCl·H_2O. Figure 3.4 shows the structure of BADGE, BFDGE and their derivatives. The most frequently method used for the determination of BADGE, BFDGE and their derivatives is reversed-phase HPLC with fluorescence detection [Leepipatpiboon *et al.* 2005, Lintschinger and Rauter 2000, Berger *et al.* 2001, Nerin *et al.* 2002]. HPLC separation was typically performed by C18 or C8 particle-packed columns with mixture of acetonitrile/water mobile phase. In addition, mass spectrometry detection coupled with reversed-phase HPLC [Cottier *et al.* 1997, Garcier and Losada 2004] and with gas chromatography [Cottier *et al.* 1997, Salafranca *et al.* 1999] has been applied in order to confirm the presence of BADGE, BFDGE and theirs derivatives. According to Leepipatpiboon *et al.* [2005], a 12-standard mixture of BADGE, BFDGE and theirs derivatives was separated using a column packed with 5 μm particles and a gradient elution of methanol/water mobile phase.



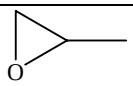
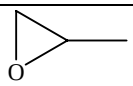
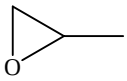
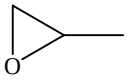
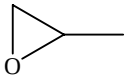
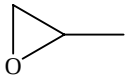
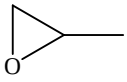
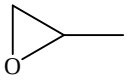
Analyte	R^1	R^2	R^3
BADGE	CH_3		
BADGE.H_2O	CH_3		$CH_2CH(OH)CH_2OH$
BADGE.$2H_2O$	CH_3	$CH_2CH(OH)CH_2OH$	$CH_2CH(OH)CH_2OH$
BADGE.HCl.H_2O	CH_3	$CH_2CH(Cl)CH_2OH$	$CH_2CH(OH)CH_2OH$
BADGE.2HCl	CH_3	$CH_2CH(Cl)CH_2OH$	$CH_2CH(Cl)CH_2OH$
BADGE.HCl	CH_3		$CH_2CH(Cl)CH_2OH$
BFDGE	H		
BFDGE.H_2O	H		$CH_2CH(OH)CH_2OH$
BFDGE.$2H_2O$	H	$CH_2CH(OH)CH_2OH$	$CH_2CH(OH)CH_2OH$
BFDGE.HCl.H_2O	H	$CH_2CH(Cl)CH_2OH$	$CH_2CH(OH)CH_2OH$
BFDGE.2HCl	H	$CH_2CH(Cl)CH_2OH$	$CH_2CH(Cl)CH_2OH$
BFDGE.HCl	H		$CH_2CH(Cl)CH_2OH$

Figure 3.4 Chemical structures of bisphenol-A-diglycidyl ether, bisphenol-F-diglycidyl ether and theirs derivatives.

In this chapter, the silica monolithic columns were prepared in 200 μm i.d. fused-silica capillary columns. Then, the columns were functionalized to be reversed-phase stationary phases by following a monomeric or a polymeric procedure. The performance of silica monolithic column was also evaluated. As previously mentioned in Chapter I, monolithic columns have shown to be higher efficiency and higher permeability than particle-packed columns. Therefore, in comparison with packed columns, the chromatographic separation of selected polar and nonpolar

compounds, chlorophenoxy acid herbicides and BADGE, BFDGE and their derivatives, respectively, was investigated on reversed-phase monolithic silica capillary columns in order to obtain the better separation and shorter analysis time.

3.2 Experimental

3.2.1 Chemicals and materials

All reagents were of analytical grade. Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMS), 3-methacryloxypropyltrimethoxysilane (MOP) and octadecyldimethylchlorosilane (ODS-Cl) were purchased from ShinEtsu Chemicals (Tokyo, Japan). Acetonitrile, urea, and pyridine were obtained from Wako Pure (Osaka, Japan); poly(ethylene glycol) (PEG, MW=10,000) and alkylbenzenes from Sigma-Aldrich (Steinheim, Germany); octadecyl methacrylate from TCI (Tokyo, Japan). Methanol, toluene, acetic acid (1 M), diethylamine and α,α′-azobis-isobutyronitrile (AIBN) were obtained from Nacalai Tesque (Kyoto, Japan). Methanol, acetonitrile and toluene were distilled before further use. Purified water (Arium 611 UV system, Sartorius, Goettingen, Germany) was used. Fused silica capillaries of 200 μm i.d. and 375 μm o.d. were purchased from Polymicro Technologies (Phoenix, AZ, USA).

All herbicide standards were obtained from Riedel de Haën (Seelze, Germany) and Dr. Ehrenstorfer GmbH (Augsburg, Germany): 2-methyl-4-chlorophenoxyacetic acid (MCPA), 4-(2-methyl-4-chlorphenoxy) butyric acid (MCPB), 2,4-dichlorophenoxyacetic acid (2,4-D), 4-(2,4-dichlorophenoxy) butyric acid (2,4-DB), fluroxypyr (FXP), quinclorac (QCR), triclopyr (TCP), (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T), acifluorfen (AFF), clopyralid (CPR), dicamba (DCB), mecoprop (MCPP) and picloram (PCR).

The following test analytes were purchased from Fluka (Buchs, Switzerland): bisphenol-A-diglycidyl ether (BAGDE), bisphenol-A-(3-chloro-2-hydroxypropyl)

glycidyl ether (BAGDE·HCl), bisphenol-A-bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl), bisphenol-A-(2,3-dihydroxypropyl)glycidyl ether (BAGDE·H_2O), bisphenol-A-bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$), bisphenol-A-(3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) ether (BADGE·H_2O·HCl), bisphenol-F-diglycidyl ether (BFDGE), bisphenol-F-bis(3-chloro-2-hydroxypropyl) ether (BFDGE·2HCl) and bisphenol-F-bis(2,3-dihydroxypropyl) ether (BFDGE·$2H_2O$). The other BFDGE derivatives, including bisphenol-F-glycidyl-(2,3-dihydroxypropyl) ether (BFDGE·H_2O), bisphenol-F-glycidyl-(3-chloro-2-hydroxypropyl) ether (BFDGE·HCl) and bisphenol-F-(3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) ether (BFDGE·HCl·H_2O), were gifted from Assistant Professor Dr. Natchanun Leepipatpiboon.

3.2.2 Preparation and chemical modification of monolithic silica capillary columns

3.2.2.1 Preparation of monolithic bare silica columns [Motokawa *et al.* 2002, Hara *et al*. 2010, Miyamoto *et al*. 2008]

Hybrid type monolithic silica capillary columns were prepared from a mixture of TMOS and MTMS (3:1 v/v) in 200 μm i.d. fused silica capillaries. The conditions employed for silica monolith preparation were similar to those previously reported. First, the fused silica capillary tube (about 3 meters length) was treated with 1 M NaOH at 40 °C for 3 h, followed by a flush with water, and then kept with 1 M HCl at 40 °C for 2 h. After a flush with water, and then with acetone, the capillary tube was air-dried at 40 °C.

A TMOS/MTMS mixture (9.0 mL) was added to a solution of PEG (0.90 g) and urea (2.03 g) in 0.01 M acetic acid (20.0 mL) at 0 °C and stirred for 30 min. The homogeneous solution was then stirred at 40 °C for 10 min, filtered through a 0.45 μm PTFE filter, filled into a pretreated fused-silica capillary tube, and allowed to react at 40 °C. The resultant gel was subsequently aged in the capillary overnight at the same temperature. Then, the temperature was increased slowly (over 10-20 h), and

the monolithic silica columns were treated at 120 °C for 4 h to form mesopores with the ammonia generated by the hydrolysis of urea, then cooled and washed with methanol. After that, the column was heat-treated at 330 °C for 24 h causing the decomposition of organic compounds in the column.

3.2.2.2 Preparation of monolithic silica columns modified with octadecyl moieties (ODS columns) [Tanaka *et al*. 1985]

ODS columns were obtained by chemical modification using octadecyldimethyl-*N*,*N*-diethylaminosilane (ODS-DEA prepared from ODS-Cl and diethylamine with a molar ratio 1:2.5 in toluene). The on-column bonding reaction was carried out as previously reported, by continuously feeding the columns with 20% (v/v) ODS-DEA solution in toluene at 0.8 MPa and allowing it to react for 3 h at 60 ºC, followed by washes with toluene, THF and methanol, respectively. Figure 3.5 shows the reaction pathway of ODS columns.

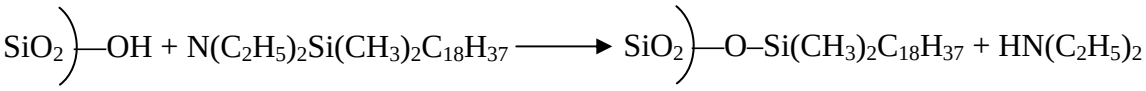


Figure 3.5 Surface modification of monolithic silica column with octadecylsilylation reaction.

3.2.2.3 Preparation of monolithic silica columns modified with poly(octadecyl methacrylate) (ODM columns) [Núñez *et al*. 2007a]

The surface modification of ODM columns was obtained similar to previously reported procedures by free radical polymerization of octadecyl methacrylate. The schematic reaction pathway of ODM column is shown in Figure 3.6. The first step in this chemical modification method consisted of a bonding reaction with MOP in order to anchor a monomer with a double bond that will undergo polymerization with octadecyl methacrylate using an AIBN initiator. After washing monolithic silica capillary columns with methanol and toluene for 24 h, MOP bonding was carried out

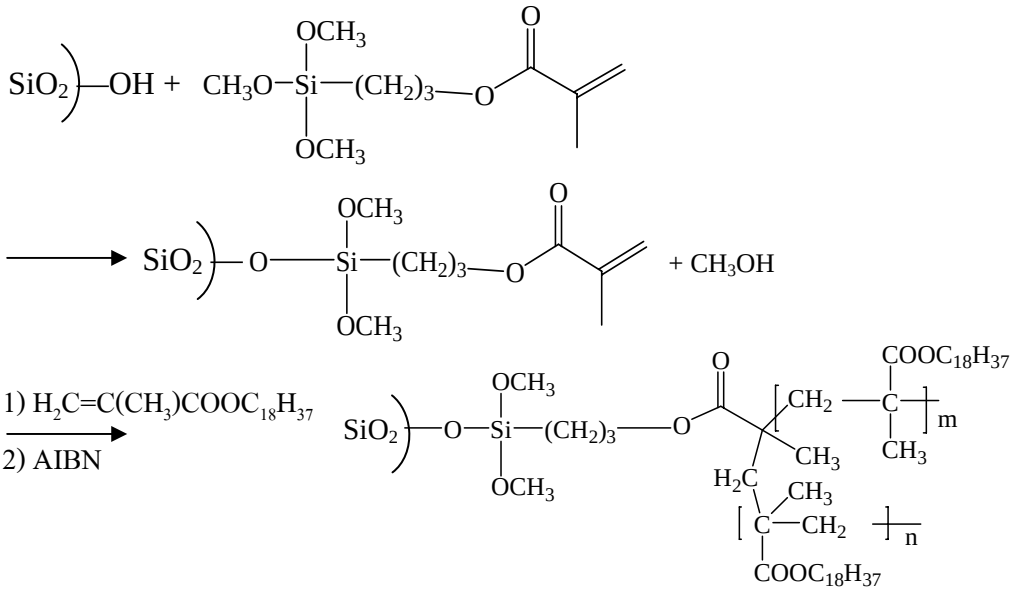


Figure 3.6 Surface modification of monolithic silica column with polymerization of octadecyl methacrylate.

by rinsing columns with a solution of MOP:pyridine 1:1 (v/v) for 48 h at 80 ºC, followed by a methanol wash. After that, $k_{hexylbenzene}$ of at least 0.3 was expected (using 80% methonol mobile phase), if not the procedure was repeated. To perform the polymerization reaction, MOP bonded columns were rinsed with toluene for 3 h and a polymerization reaction solution (250 μL of octadecyl methacrylate monomer and 250 μL of a 38 mg/mL AIBN solution in toluene) was flushed into columns and allowed to react at 80 ºC for 3 h. Then the columns were washed with toluene (24 h) followed by methanol.

3.2.3 HPLC and CLC instrumentations

Conventional HPLC analyses were performed on a Hewlett-Packard 1100 series equipped with an automatic degasser, binary pump, autosampler, column thermostat, and diode-array detector (Agilent Technologies, USA).

CLC measurements were performed by employing two sets of split-flow/injection HPLC system. More details about split- flow/injection system are previously discussed in Section 2.1 (Figure 2.1A). To evaluate the chromatographic performance

of prepared columns, the system consists of conventional pumps (Jasco 3085PU, Jasco, Tokyo, Japan), an injector valve (model 7725, Rheodyne, CA, USA) fitted with a T-union (as a splitter) with one end connected to the capillary column and another end to a flow restrictor (a stainless steel tubing 0.1 mm i.d., 1/16 in. o.d.), and a UV detector for on-column detection (Jasco CE-1575, Jasco, Tokyo, Japan). UV detection was carried out on-column when using monolithic bare silica columns. When chemical modification was performed by ODM polymerization and ODS-DEA silylation, detection was carried out off-column by connecting an uncoated fused-silica capillary (20 cm (12 cm effective length × 50 μm I.D.)) by means of a teflon tube. To optimize the separation condition for selected compounds, the system consists of pumps (Shimadzu LC-10AD), an injector valve (model 7725, Rheodyne, CA, USA) with a splitting T-joint, and a UV detector (GL Sciences MU701, GL Sciences Inc., Tokyo, Japan) equipped with capillary flow cell (GL Sciences, flow cell volumes 6 nL). The temperature of all systems was controlled in an air-circulating oven at 30 ºC. UV detection was monitored at 220, 254 and 280 nm. All chromatographic data were collected in duplicate runs using version 2.8.3 EZChrom Elite Client/Server software.

3.3 Results and discussion

3.3.1 Column performance of prepared monolithic silica capillary columns

In Section 3.2.2.1, the long monolithic silica capillary columns with 200 μm i.d. were prepared for several batches. Sections of 10-15 cm at both ends of the column were cut off, and then, the long monolithic columns were cut into 30 or 40 cm to obtain ≈ 40 columns. Size of skeleton and through-pore can be estimated for the scanning electron microscope (SEM) photograph. Using the previous preparation condition in Motokawa *et al*. 2002, similar to condition used in this work, SEM image of hybrid silica monolithic capillary column in Motokawa *et al*. 2002 is shown in Figure 3.7. According to this condition, monolithic silica columns with skeleton size of 1.3-1.5 μm and through-pore size of 1.8-2.0 μm has been reported [Motokawa *et al*. 2002,

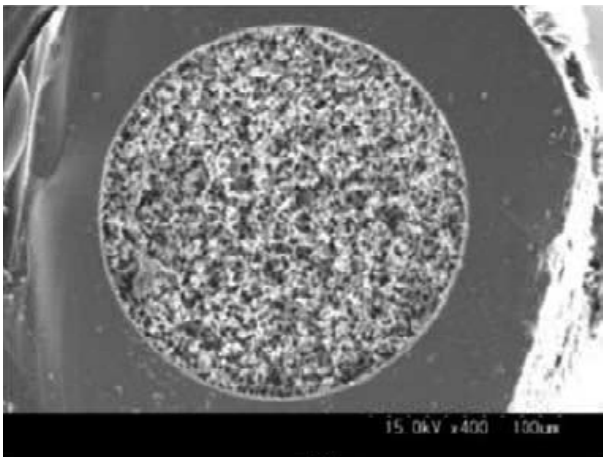


Figure 3.7 Scanning electron micrograph of a hybrid monolithic silica capillary column with 200 μm i.d., prepared in Motokawa *et al*. 2002 using condition as described in Section 3.2.2.1. Reproduced from Motokawa *et al*. [2002].

Hara *et al*. 2010]. In this work, the performance of monolithic silica capillary columns was evaluated in a split flow/injection HPLC system using 80% methanol mobile phase. A uracil (an unretained compound) was used as test analyte. The column with high separation efficiency was expected to produce H (H can be calculated using Equation 2.8) of uracil < 8.0 μm (or $N > 1.25\times10^5$ plates/meter) at u of 1.0 mm/s.

Since the column performance depends on the reaction batch used for preparation of the silica network, as well as the section of capillary used, only columns with high efficiency (H of uracil < 8.0 μm) were used for surface modification to be ODS (as described in Section 3.2.2.2) with 40 cm in length, and ODM columns (as described in Section 3.2.2.3) with 30 cm in length in next steps. It should be noted that the shorter ODM than ODS column was prepared due to high viscosity of polymerization mixtures. Plugged columns may be obtained when using smaller i.d. and longer silica monolithic column for ODM modification. In this work, two ODS and two ODM columns were chosen as representatives for discussion of their chromatographic performance. Small letters “a” or “b” refer to column number, for example, ODS-a and ODS-b are denoted as ODS column no. 1 and 2, respectively. The retention behavior of different compounds on these two reversed-phase columns was investigated using either 80% (v/v) methanol or 80% (v/v) acetonitrile mobile phase.

Test analytes were a mixtures of uracil and alkylbenzenes with C1 to C6 carbon atoms of alkyl chains for ODS columns, while a mixture of uracil and alkylbenzenes with C1 to C5 for ODM columns. Hexylbenzene highly retained on ODM column resulting in much longer analysis time than ODS. Linear velocity of the mobile phase (*u*) was varied from 0.2 mm/s to high *u* that is limited by the maximum flow rate of HPLC pumps (2.0 mL/min).

Figure 3.8 shows the plots of *k* of toluene and hexylbenzene over a wide range of *u* for ODS and ODM columns (*k* of test analytes can be calculated using Equation 2.4). A change in *u* does not affect *k* which is theoretically expected in reversed-phase chromatography [Synder *et al.* 1997]. The *k* values of alkylbenzenes can be used as a measure of the volume ratio of stationary phase to mobile phase or the amount of alkyl-bonded stationary phase bonded to the silica [Núñez *et al*. 2007a]. As can be seen, the retention of alkylbenzenes on ODM columns is greater than that on ODS columns under the same condition. However, in comparison with methanol mobile phase, the slightly higher retention on ODM was observed when using acetonitrile mobile phase due to the better solvation of acetonitrile on ODM stationary phase [Núñez *et al*. 2007a].

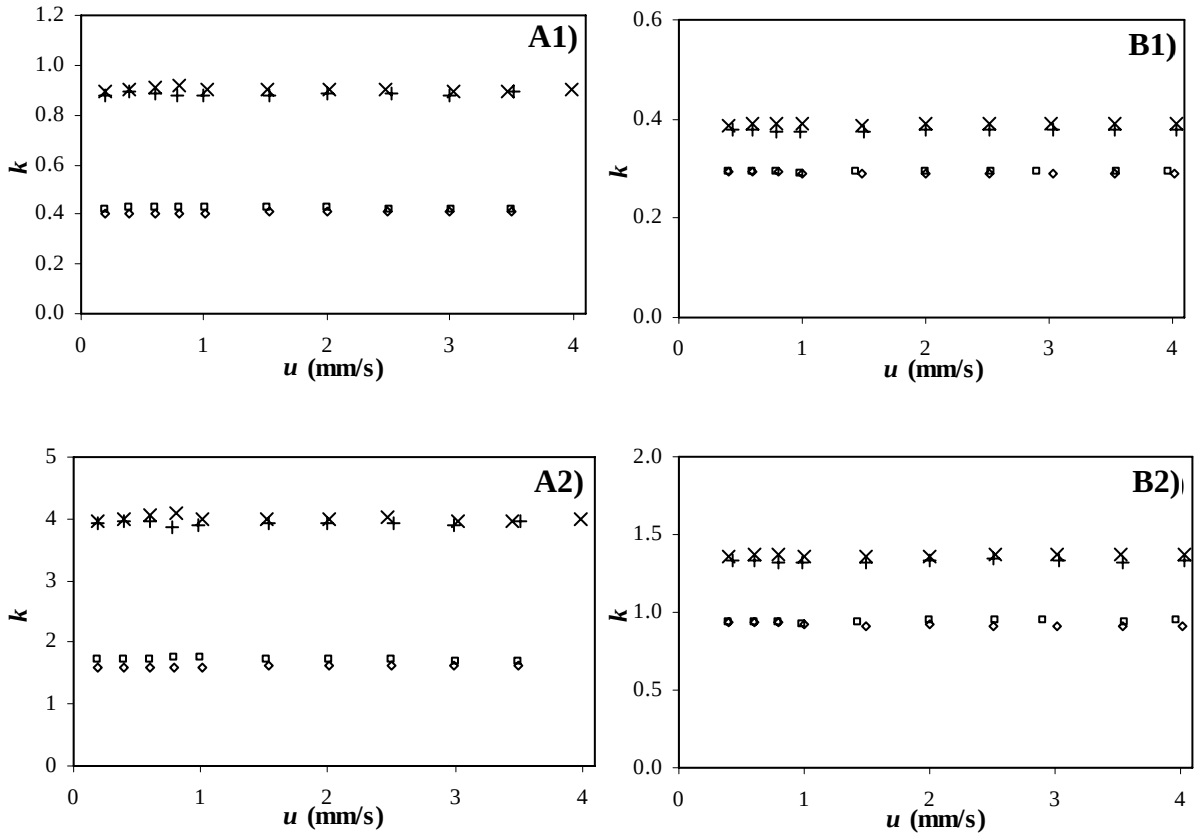


Figure 3.8 Plots of retention factors (*k*) of some alkylbenzenes over a wide range of linear velocity (*u*) on ODS (ODS-a (◇) and ODS-b (□)) and ODM (ODM-a (+) and ODM-b (×)) using mobile phase: (A) 80% (v/v) methanol and (B) 80% (v/v) acetonitrile. Toluene (A1, B1) and amyllbenzene (A2, B2) were used as test analytes.

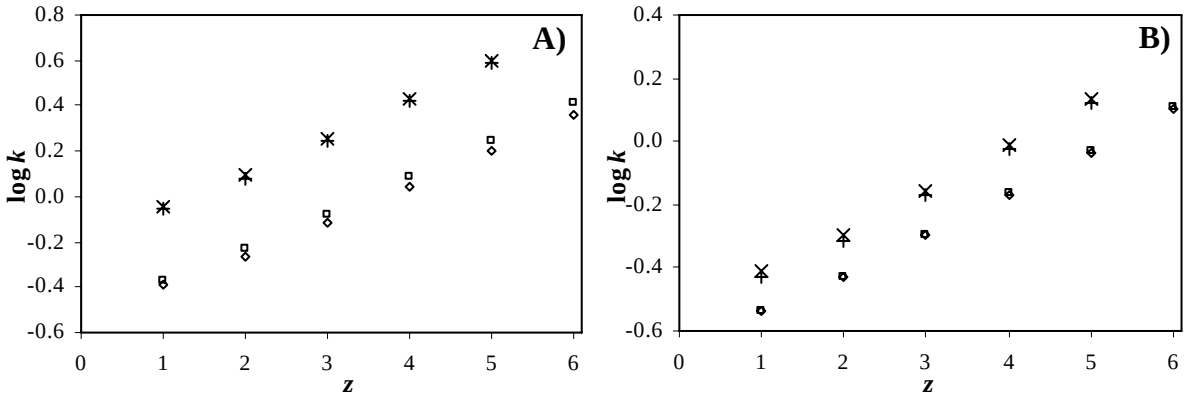


Figure 3.9 The plots of log *k* against *z* (*z* is the number of carbon atom in aliphatic chain), at *u* of 1.0 mm/s using mobile phase: (A) 80% (v/v) methanol and (B) 80% (v/v) acetonitrile, for ODS (ODS-a (◇) and ODS-b (□)) and ODM (ODM-a (+) and ODM-b (×)).

The retention of neutral solutes like alkylbenzenes can be used to evaluate the hydrophobicity of columns and is usually given by the selectivity of methylene increment ($\alpha(CH_2)$). Logarithm of methylene group selectivity (log $\alpha(CH_2)$) is determined from the slope of linear relationship between log *k* and *z* (*z* is the carbon number in aliphatic chain of alkylbenzenes), as shown in Figure 3.9. The higher the values of log $\alpha(CH_2)$ or $\alpha(CH_2)$, the greater the hydrophobicity of stationary phase [Núñez *et al*. 2007a]. Table 3.2 summarizes chromatographic properties of the columns: plate height (*H*), $\alpha(CH_2)$, pressure drop (ΔP), and column permeability (*K*), using either 80% methanol or 80% acetonitrile mobile phase at *u* of 1.0 mm/s. As can be seen, the $\alpha(CH_2)$ value on ODM column is slightly greater than that on ODS column.

Figure 3.10 shows the van Deemter plots of ODS and ODM columns obtained with uracil, toluene and amylbenzene as test analytes using either 80% (v/v) methanol or 80% (v/v) acetonitrile mobile phase. As can be seen that both columns provide similar plots with slightly smaller *H* on ODS than ODM columns. For example, *H* of 8.6-8.9 μm and 11.1 μm for amylbenzene on ODS and ODM columns, respectively, using 80% (v/v) methanol mobile phase at *u* of 1.0 mm/s (Table 3.2). In comparison with methanol mobile phase, acetonitrile provided better column efficiency (lower *H*) with a gradual increase in *H* at high *u*. This is because it has lower viscosity and it is also a better solvent for a poly(methacrylate) polymer [Núñez *et al*. 2007a].

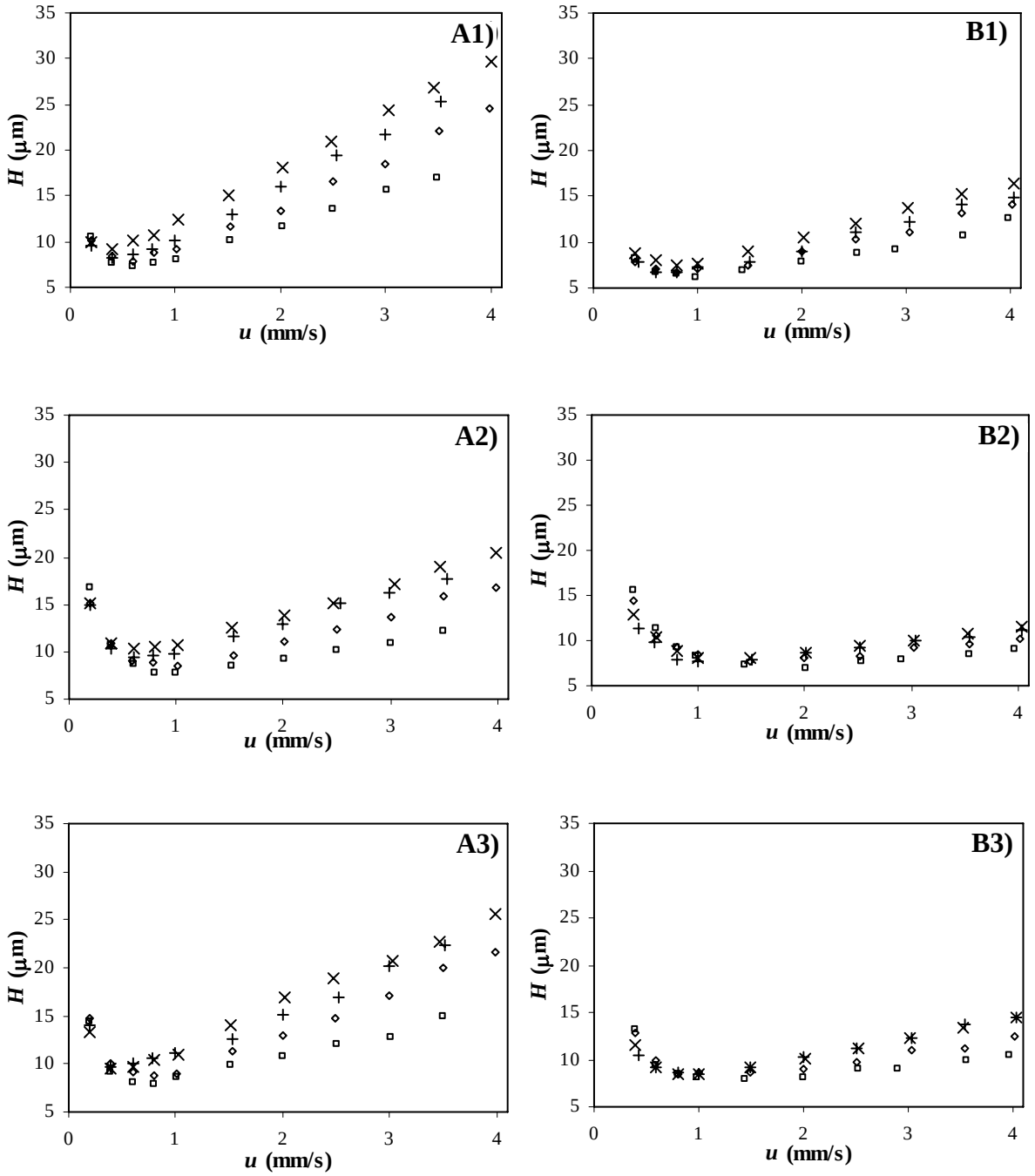


Figure 3.10 The van Deemter plots for ODS (ODS-a (◇) and ODS-b (□)) and ODM (ODM-a (+) and ODM-b (×)) using mobile phase: (A) 80% (v/v) methanol and (B) 80% (v/v) acetonitrile. Uracil (A1, B1), toluene (A2, B2) and amylbenzene (A3, B3) were used as test analytes.

Table 3.2 Plate Height (H), methylene selectivity ($\alpha(CH_2)$), pressure drop (ΔP) and column permeability (K) for ODS and ODM columns at u of 1.0 mm/s.

Mobile phase	column	H (μm)							$\alpha(CH_2)$	ΔP	$\Delta P/L$	K
		uracil	C1	C2	C3	C4	C5	C6		(MPa)	(MPa/m)	(10^{-14} m^2)
80% methanol	ODS-a	9.2	8.6	8.8	9.0	9.5	8.9	9.8	1.42	4.3	10.8	8.8
	ODS-b	8.0	7.7	7.8	8.1	8.6	8.6	8.5	1.43	4.7	11.8	9.4
	ODM-a	10.2	9.8	10.2	10.4	10.9	11.1	-	1.46	3.2	10.7	8.0
	ODM-b	12.4	10.7	11.1	11.1	11.1	11.1	-	1.46	3.9	13.0	7.3
80% acetonitrile	ODS-a	7.1	8.4	8.4	8.2	8.4	8.7	8.5	1.34	2.0	5.0	8.5
	ODS-b	6.1	8.4	8.1	7.8	8.0	8.1	8.1	1.35	2.2	5.5	7.2
	ODM-a	7.2	7.7	7.8	7.8	8.2	8.5	-	1.37	1.7	5.7	6.7
	ODM-b	7.8	8.1	8.2	8.1	8.1	8.4	-	1.37	1.8	6.0	6.7

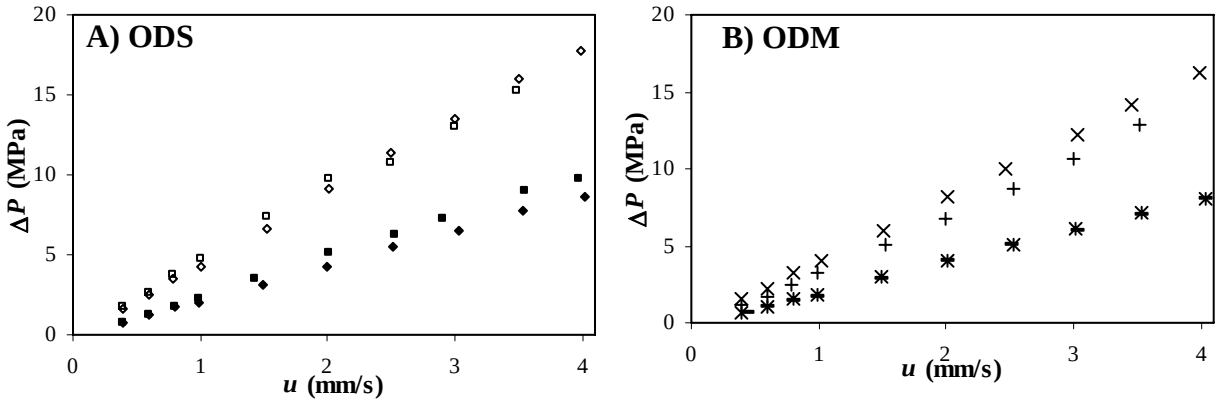


Figure 3.11 Plots of the column pressure drop against the linear velocity of mobile phase for (a) ODS 40 cm (ODS-a (◇, ◆) and ODS-b (□, ■)) and (b) ODM 30 cm (ODM-a (+, –) and ODM-b (×, ∗)). Mobile phase: 80% (v/v) methanol (◇, □, +, ×) and 80% (v/v) acetonitrile (◆, ■, –, ∗).

To drive the mobile phase at a certain velocity through a column, a certain pressure, Δ*P*, must be maintained at the column inlet. This pressure drop is related to the characteristics of the column and of the mobile phase, and to the flow velocity of this phase. Figure 3.11 shows the plots of column pressure drop against the linear velocity of a mobile phase, 80% (v/v) methanol or 80% (v/v) acetonitrile. Similar plot on ODM and ODS columns was observed. In order to compare the pressure drop on ODS and ODM with different length, the pressure drop per length (Δ*P*/*L*) was shown at *u* of 1.0 mm/s, as listed in Table 3.2. The Δ*P*/*L* for each column is in a range of 10.8-11.8 and 10.7-13.0 MPa/m for ODS and ODM columns, respectively, in 80% (v/v) methanol mobile phase, while 5.0-5.5 and 5.7-6.0 MPa/m for ODS and ODM columns, respectively, in 80% (v/v) acetonitrile mobile phase. The results are consistent with previous report in Núñez *et al*. [2007a]: 10.3 and 10.4 MPa/m for ODS and ODM columns, respectively, in 80% (v/v) methanol mobile phase, and 5.2 and 5.7 MPa/m for ODS and ODM columns, respectively, in 80% (v/v) acetonitrile mobile phase, at *u* of 1.0 mm/s.

The high permeability is an important advantage of monolithic silica capillary columns. Based on Darcy's Law, the permeability (*K*) is given by the equation [Guiochon *et al.* 2007, Hara *et al.* 2010]:

$$K = \frac{\varepsilon_T u \eta L}{\Delta P} \tag{3.4}$$

where u is the linear velocity, η is viscosity of a mobile phase, L is column length, and ε_T is total column porosity. The column porosity of monolithic silica capillary column after chemical modification is supposed to be 90% [Hara *et al*. 2010].

Using Equation 3.4 and slope of the ΔP vs u plots in Figure 3.11, the K values for ODS and ODM column are shown in Table 3.2. ODS columns gave a slight higher K than ODM columns, for example, 8.8×10^{-14}-9.4×10^{-14} and 7.3×10^{-14}-8.0×10^{-14} m^2 on ODS and ODM columns, respectively, in 80% methanol mobile phase. The K values on these ODS and ODM columns is consistent with that in previous works [Motokawa *et al.* 2002, Kobayashi *et al*. 2004, Núñez *et al*.: 2007, Miyamoto *et al*. 2008, Hara *et al*. 2010]: 6.2-17.1×10^{-14} m^2 in 80% methanol mobile phase. In addition, the K values of monolithic column in previous and present works are much higher than that of a column packed with 5 μm particles ($K \approx 4\times10^{-14}$ m^2) [Tanaka *et al.* 2001].

3.3.2 Separation of chlorophenoxy acid herbicides in conventional HPLC and CLC

3.3.2.1 Separation of chlorophenoxy acid herbicides in conventional HPLC

The HPLC method for the separation of 13 chlorophenoxy acid herbicides was developed using either methanol/acetic acid or acetonitrile/acetic acid mobile phase. The optimum condition is reported in Table 3.3. The chromatograms are illustrated in Figure 3.12A and 3.12B for acetonitrile and methanol, respectively.

Table 3.3 High performance liquid chromatographic conditions for the separation of chlorophenoxy acid herbicides.

HPLC parameters	HPLC conditions
Analytical column	4.6 ×150 mm , 5 μm Eclipse XDB-C8
Mobile phase	methanol/acetic acid pH 2.5 or acetonitrile/acetic acid pH 2.5
Flow rate	1 mL/min
Injection volume	10 μL
Gradient program	For acetonitrile/acetic acid pH 2.5 mobile phase
	Time (min) %Acetonitrile
	0 15
	1.5 15
	2 20
	8 30
	18 35
	22 38
	25 60
	30 60
	For methanol/acetic acid pH 2.5 mobile phase
	Time (min) %Methanol
	0 20
	2 20
	4 40
	12 50
	12.1 55
	35 55
Column temperature	30ºC
Detector	Photodiode array detector, 280 nm

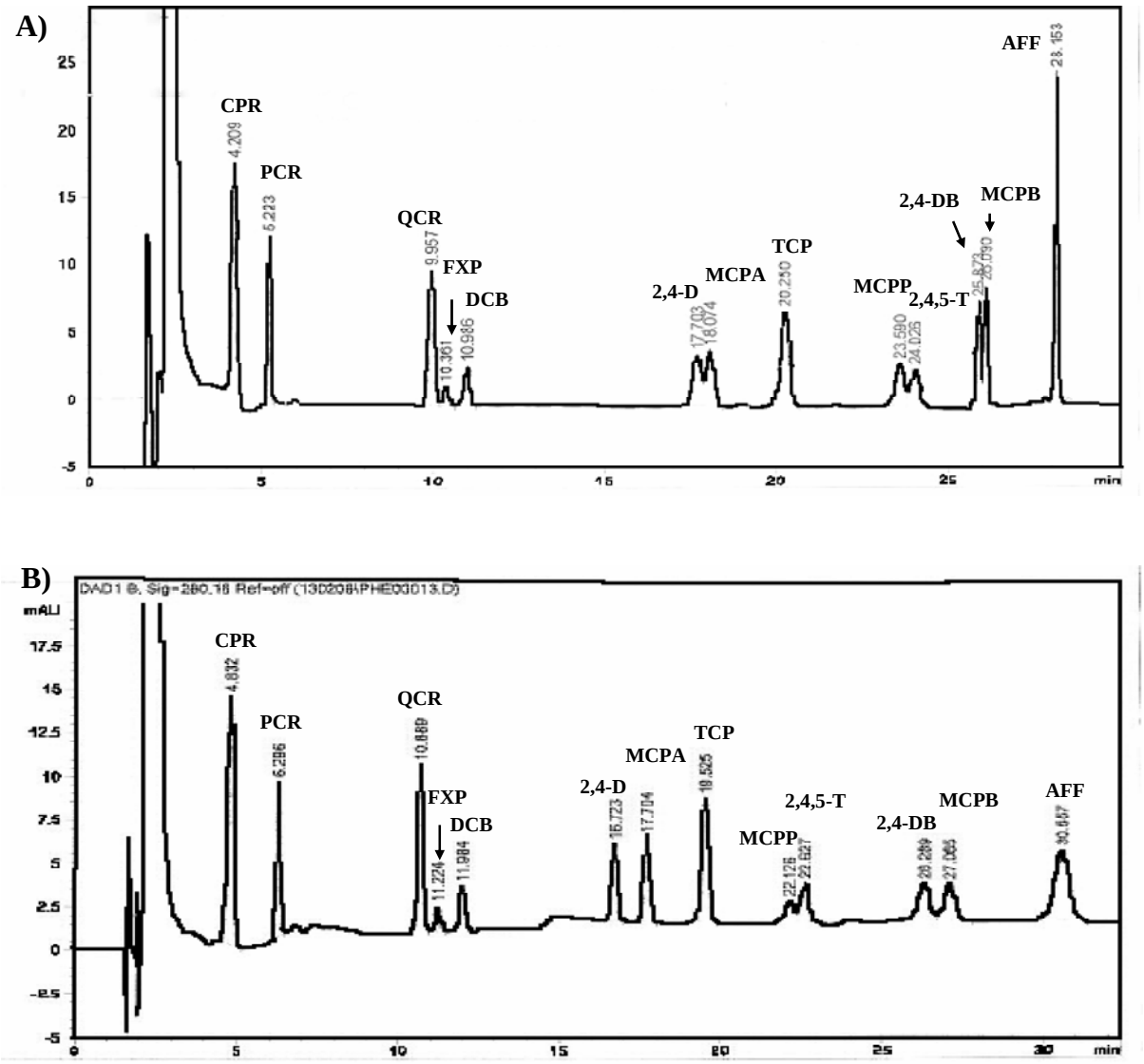


Figure 3.12 Chromatograms of a standard mixture of 13 chlorophenoxy acid herbicides (10 ppm each) using the HPLC conditions listed in Table 3.3. Mobile phase: (A) acetonitrile/acetic acid pH 2.5 and (B) methanol/acetic acid pH 2.5.

In initial work, HPLC separation of 13 chlorophenoxy acids was tested on Hypersil ODS 4.0×125 mm, 5 μm using acetonitrile/acetic acid pH 2.5 as mobile phase but could not achieved baseline separation. The distorted peak was also observed. Therefore, the analytical column was changed to a column packed with extra dense bonding (XDB) particles, Eclipse XDB-C8 4.6×150 mm with 5 μm particles column, to improve the peak shape when using low pH mobile phase. Adjustment of gradient elution using acetonitrile/acetic acid pH 2.5 mobile phase could not achieved baseline separation for some critical pairs such as MCPA/2,4-D, MCPP/2,4,5-T and

MCPB/2,4-DB (Figure 3.12A). Therefore, the organic solvent was changed from acetonitrile to methanol in order to alter the separation selectivity. In comparison with acetonitrile mobile phase, the better separation with methanol was obtained but a pair of MCPP/2,4,5-T was partially overlapped (Figure 3.12B).

3.3.2.2 Separation of chlorophenoxy acid herbicides in CLC

The CLC method was developed using ODS column with either methanol/0.1 % formic acid or acetonitrile/0.1 % formic acid mobile phase and the optimum condition is reported in Table 3.4. The chromatograms are illustrated in Figures 3.13A and 3.13B for acetonitrile and methanol, respectively.

Table 3.4 Capillary liquid chromatographic conditions for the separation of chlorophenoxy acid herbicides.

CLC parameters	CLC conditions
Analytical column	ODS-a monolithic silica column, 200 μm I.D.× 40 cm
Mobile phase	0.1 % formic acid in acetonitrile/0.1 % formic acid in water or 0.1 % formic acid in methanol/0.1 % formic acid in water
Flow rate	0.369 mL/min (*u* = 2.5 mm/s and split ratio *ca.* 78:1) for acetonitrile mobile phase 0.351 mL/min (*u* = 2.5 mm/s and split ratio *ca.* 75:1) for methanol mobile phase
Injection volume	1 μL
Gradient program	For 0.1 % formic acid in acetonitrile/0.1 % formic acid in water mobile phase Time (min) %Acetonitrile 0 32 3 40 18 40 19 42 25 42 30 60 40 60 For 0.1 % formic acid in methanol/0.1 % formic acid in in water mobile phase Time (min) %Methanol 0 45 3 45 25 55 26 60 30 60 35 80 40 80
Column temperature	30ºC
Detector	UV detector, 280 nm

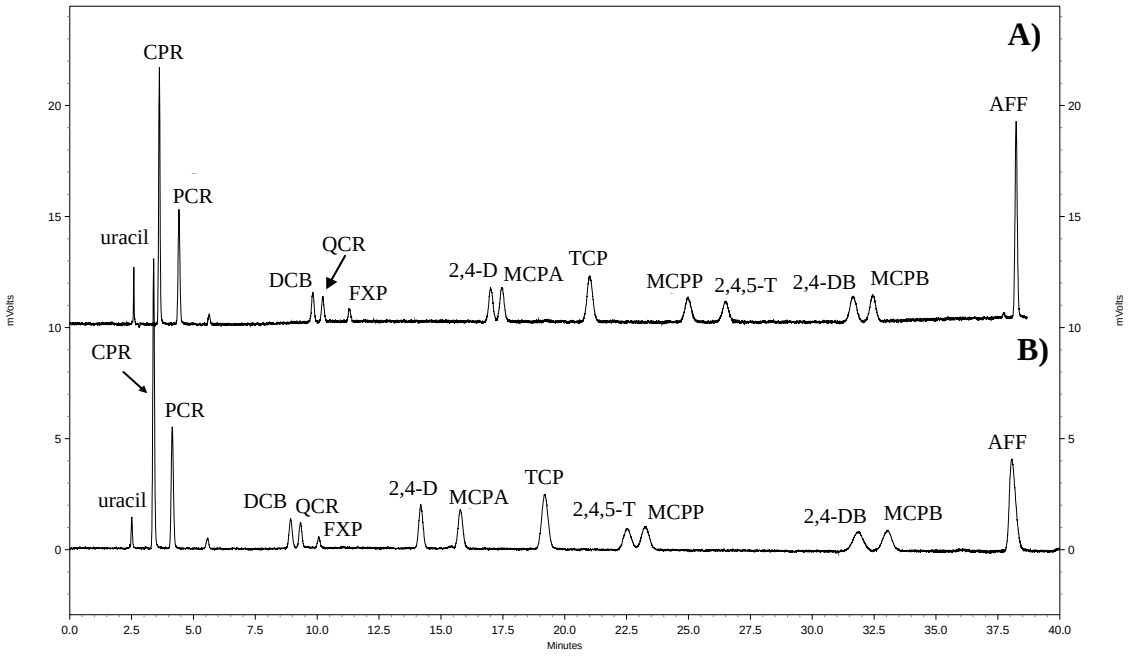


Figure 3.13 Chromatograms of a standard mixture of 13 chlorophenoxy acid herbicides (10 ppm each) using the CLC conditions listed in Table 3.4. Mobile phase: (A) 0.1 % formic acid in acetonitrile/0.1 % formic acid in water and (B) 0.1 % formic acid in methanol/0.1 % formic acid in water.

CLC separation of 13 chlorophenoxy acids was performed by using an ODS column (40 cm). Due to the better column efficiency, the baseline separation of all analytes was achieved with a gradient elution of either acetonitrile (Figure 3.13A) or methanol mobile phase (Figure 3.13B). The log *k* linearly decreases with increasing the volume percent organic solvent of mobile phase. In some cases, the elution order of peaks changes at different solvent composition. For example, in comparison with the gradient elution of acetonitrile and methanol mobile phase in HPLC (Figure 3.12) and the gradient elution of methanol mobile phase in CLC (Figure 3.13), the reversed elution order of MCPP/2,4,5-T was observed when using gradient elution of either acetonitrile in CLC.

Therefore, at the optimum condition on conventional (Figure 3.12) and monolithic column (Figure 3.13) is adequate for the determination of all 13 chlorophenoxy acid herbicides. The better separation on monolithic column was obtained on both acetonitrile and methanol mobile phase although the analysis time using monolithic column is longer (about 40 min) due to longer column length.

3.3.3 Separation of BADGE, BFDGE and theirs derivatives in CLC

The CLC method was developed using ODS column with either methanol/water or acetonitrile/water mobile phase and the optimum condition is reported in Table 3.5. The chromatograms are illustrated in Figures 3.14a and 3.14b for acetonitrile and methanol, respectively.

Table 3.5 Capillary liquid chromatographic conditions for the separation of BADGE, BFDGE and theirs derivatives.

CLC parameters	CLC conditions
Analytical column	ODS-b monolithic silica column, 200 μm I.D.× 40 cm
Mobile phase	acetonitrile/water or methanol/water
Flow rate	0.344 mL/min (*u* = 2.5 mm/s and split ratio *ca.* 73:1) for acetonitrile mobile phase 0.426 mL/min (*u* = 2.5 mm/s and split ratio *ca.* 90:1) for methanol mobile phase
Injection volume	1 μL
Gradient program	For acetonitrile/water mobile phase Time (min) %Acetonitrile 0 40 2 50 3 50 5 60 15 60 For methanol/water mobile phase Time (min) %Methanol 0 50 25 50 45 80
Column temperature	30ºC
Detector	UV detector, 220 nm

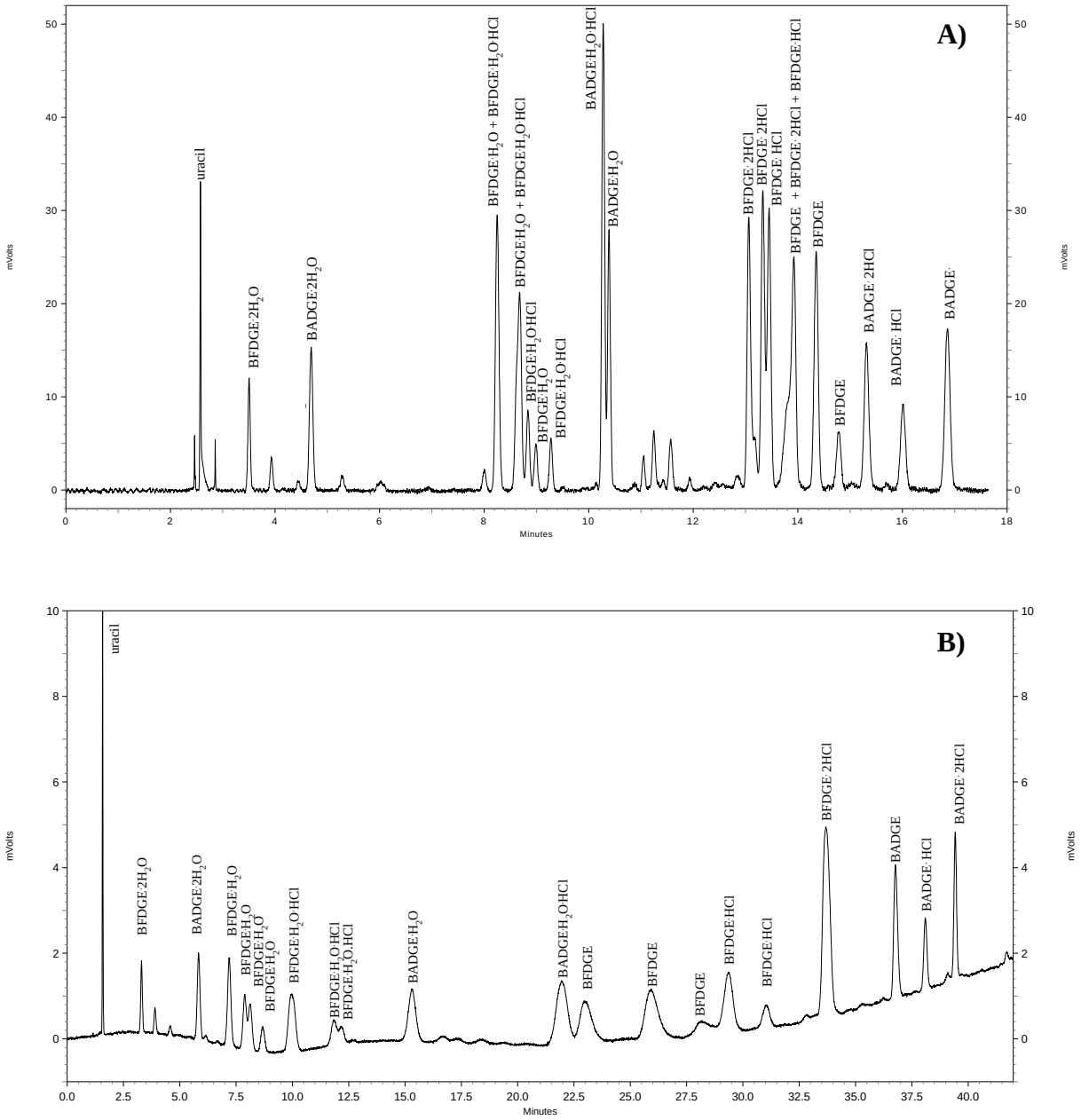


Figure 3.14 Chromatograms of a standard mixture of BADGE, BFDGE and their derivatives (using the CLC conditions listed in Table 3.5. Mobile phase: (A) acetonitrile/water and (B) methanol/water.

Separation of all 12 BADGE, BFDGE and their derivatives was optimized using ODS column and a gradient elution of acetonitrile/water, however, the co-elution of several analytes was observed (Figure 3.14A). In order to change the separation selectivity, a gradient elution of methanol/water was used for the separation of these analytes, and the baseline resolution was achieved (Figure 3.14B).

Since BFDGE exists in three isomer forms: *o,o*-, *p,p*-, and *o,p*-isomers, four possible isomers of BFDGE·H_2O, BFDGE·HCl·H_2O and BFDGE·HCl were obtained. In previous reported in Leepipatpiboon *et al*. [2005], HPLC separation condition can detect all four peaks of BFDGE·H_2O and BFDGE·HCl·H_2O (one peak of *o,o*-, *p,p*- , and two peaks of *o,p*-isomers), all three peak BFDGE (*o,o*-, *p,p*-, and *o,p*-isomers) but only two peaks of BFDGE·HCl (four isomers co-eluted), as shown in Figure 3.15. In this work, isomeric separation of all BFDGE derivatives could not be achieved due to the insufficient column efficiency. However, the separation may easily improve by using the longer monolithic column.

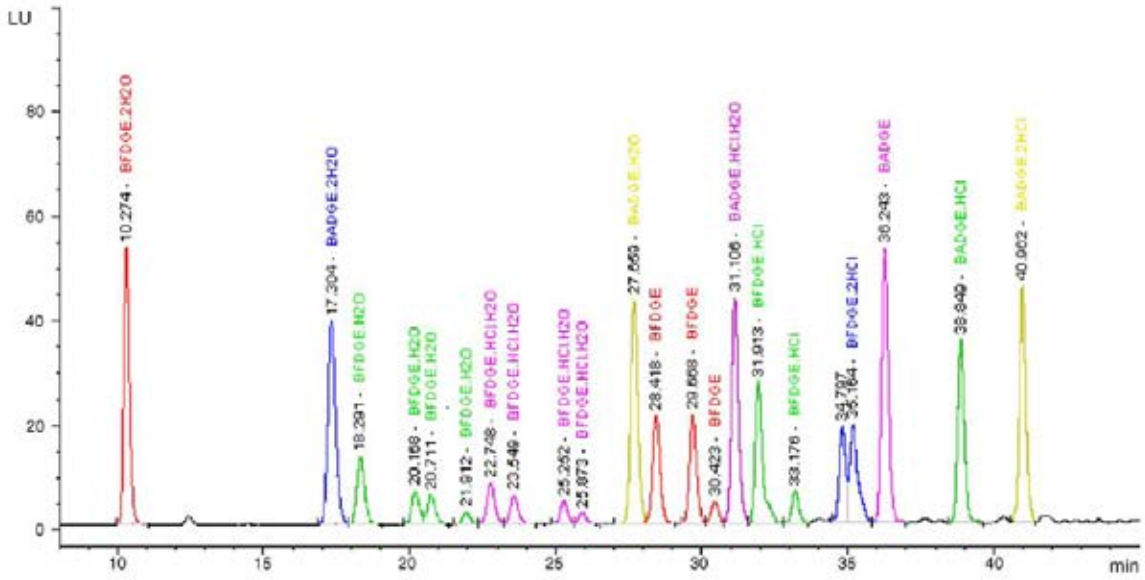


Figure 3.15 The chromatograms of a standard mixture of BADGE, BFDGE, and theirs derivatives using conventional HPLC. HPLC conditions: ODS Hypersil C18, 5 μm, 250 mm × 4.0 mm analytical column, a gradient elution of water (A) and methanol (B) mobile phase (at 0 min–40% B, 1 min–50% B, 15 min–55% B, 38 min–70% B, 45-55 min–90% B), fluorescence detection with excitation and emission wavelengths of 227 and 313 nm, respectively. Reproduced from Leepipatpiboon *et al*. [2005].

3.4 Conclusions

The monolithic silica capillary columns were prepared and chemically modified to be reversed-phase stationary phase: ODS and ODM columns. The performance of prepared columns was also evaluated in terms of column efficiency, retention properties and permeability. For example, the high efficiency monolithic silica columns was obtained with plate height of 8.6-8.9 μm and 11.1 μm on ODS and ODM columns, respectively, using amylbenzene as tested analyte and 80% (v/v) methanol as mobile phase at u of 1.0 mm/s. The slightly higher hydrophobicity on ODM than ODS column was obtained with methylene selectivity, $\alpha(CH_2)$, of 1.42-1.43 and 1.46 on ODS and ODM columns, respectively, using 80% (v/v) methanol mobile phase. In addition, ODS and ODM column shows the higher permeability with K of 8.8×10^{-14}-9.4×10^{-14} and 7.3×10^{-14}-8.0×10^{-14} m^2 on ODS and ODM columns, respectively, using methanol mobile phase. The performance of columns prepared in this work is consistent with that in previous report by Núñez *et al*. [2007a]. Using ODS column as an analytical column, the baseline separation of 13 chlorophenoxy acid herbicides was obtained in a gradient elution of either acetonitrile or methanol mobile phase. In addition, the CLC method for the separation of BADGE, BFDGE and theirs derivatives was developed on ODS column. All 12 BADGE, BFDGE and theirs derivatives can be achieved the baseline separation when using methanol mobile phase, while the co-elution of some analytes was observed in acetonitrile mobile phase. Although isomers of some BFDGE derivatives could not be separated, it can easily enhance the separation by using the longer ODS column.

CHAPTER IV

SELECTIVITY COMPARISONS OF MONOLITHIC SILICA CAPILLARY COLUMNS MODIFIED WITH POLY(OCTADECYL METHACRYLATE) AND OCTADECYL MOIETIES FOR HALOGENATED COMPOUNDS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

4.1 Introduction

Monolithic capillary columns are viable alternative to conventional particle packed columns for high efficiency HPLC separations at similar pressure drop because of small-sized skeletons and relatively large through pores [Motokawa *et al*. 2002, Eeltink *et al.* 2004, Cabrera 2004, Hara *et al.* 2006, Miyamoto *et al*. 2008]. In previous work, hybrid monolithic silica capillary columns were prepared from a mixture of tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMS) [Motokawa *et al*. 2002]. The surface of monolithic silica capillary column can be modified to obtain various stationary phases in different chromatographic modes such as reversed-phase [Motokawa *et al*. 2002, Cabrera 2004, Hara *et al.* 2006, Miyamoto *et al*. 2008], ion-exchange [Chambers *et al*. 2007] and hydrophilic interaction [Ikegami *et al*. 2008] by following a monomeric or polymeric procedure, for achieving desired solute selectivities in HPLC. Column characteristics and selectivities have been studied so that chromatographers can choose suitable stationary phases for particular separations, and these properties are very useful in method development [Stella *et al*. 2001a, Stella *et al*. 2001b, Turowski *et al.* 2001, Wilson *et al*. 2002, Neue *et al*. 2006].

C18 monolithic silica capillary columns for reversed-phase HPLC can be obtained by chemical modification with octadecyl moieties (ODS column) [Tanaka *et al*. 1985] or

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coating with poly(octadecyl methacrylate) (ODM column) [Núñez *et al*. 2007a]. Recently, the different performance of ODM and ODS monolithic silica capillary columns was reported for separating some polar and non-polar compounds such as benzene and naphthalene derivatives, polycyclic aromatic hydrocarbons, steroids and alkyl phthalates, and tocopherol homologues [Núñez *et al*. 2007b]. The ODM column had a preference for the compounds with aromatic characters, rigid and planar structures and lower length-to-breadth ratios. The polymeric ODM stationary phase also showed differences in selectivity against the ODS column. Better separations were observed for some compounds on ODM versus ODS columns under the same condition. In addition, poly(octadecyl acrylate) (ODA) grafted onto silica, which is similar structure of side chains with an ODM stationary phase, has been reported for conventional HPLC columns [Fukumoto *et al.* 1994, Chowdhury *et al*. 2000, Malik *et al.* 2008]. In comparison with an ODS phase, the comb-shaped polymer phase showed greater selectivity for polycyclic aromatic hydrocarbons. Selectivity in the ODA phase is enhanced by the molecular ordering of the long-chain octadecyl groups and interactions between the carbonyl groups of the polymer and π-containing compounds.

Halogenated compounds, a class of molecules of high environmental concern, can be generated in many industrial and natural processes [Liljelind *et al.* 2003, Boer and Law 2003]. These include a number of compound groups such as chlorophenols, polychlorinated biphenyls (PCBs), halogenated dibenzo-*p*-dioxins, which contain various homologues and isomers in each compound group. The development of methods to separate and analyze these compounds is needed. The separation selectivity of common halogenated organic solvents between a typical C18 stationary phase and polymer-based packing materials including poly(methyl methacrylate-ethylene dimethacrylate) beads and poly(styrene-divinylbenzene) beads was previously compared [Hosoya and Frechet 1993]. In general, these polymeric stationary phases demonstrate preferential retention of halogenated compounds than a conventional silica-based C18 stationary phase although the polymer packing materials have shown low column efficiency. Therefore, it is interesting to extend previous work [Hosoya and Frechet 1993, Núñez *et al*. 2007b] to study differences in

retention and selectivity of ODM and ODS stationary phases for halogenated compounds, using halogenated benzene derivatives as test analytes relative to alkylbenzenes.

This work investigates the retention of halogenated and alkyl substituted benzenes and compares the selectivity of these test analytes on ODM and ODS monolithic silica capillary columns in reversed-phase HPLC. In addition to halogenated benzenes, chlorophenols were separated on both ODM and ODS columns. The chromatographic performance of these compounds was studied using either methanol or acetonitrile mobile phase systems.

4.2 Experimental

4.2.1 Chemicals and materials

All chemicals and materials for preparation and modification of monolithic silica capillary columns are the same as listed in Section 3.2.1. Test analytes were purchased from Nacalai Tesque, TCI, Sigma-Aldrich and Wako Pure. The following solution mixtures were used in this study: *monoalkylbenzenes* (benzene (–H), toluene (–CH_3), ethylbenzene (–C_2H_5) and propylbenzene (–C_3H_7)); *monohalogenated benzenes* (fluorobenzene (–F), chlorobenzene (–Cl), bromobenzene (–Br) and iodobenzene (–I)); *dialkylbenzenes* (*o*-xylene (*o*-di–CH_3), *m*-xylene (*m*-di–CH_3), *p*-xylene (*p*-di–CH_3), *o*-diethylbenzene (*o*-di–C_2H_5), *m*-diethylbenzene (*m*-di–C_2H_5) and *p*-diethylbenzene (*p*-di–C_2H_5)); *dihalogenated benzenes* (*o*-difluorobenzene (*o*-di–F), *m*-difluorobenzene (*m*-di–F), *p*-difluorobenzene (*p*-di–F), *o*-dichlorobenzene (*o*-di–Cl), *m*-dichlorobenzene (*m*-di–Cl), *p*-dichlorobenzene (*p*-di–Cl), *o*-dibromobenzene (*o*-di–Br), *m*-dibromobenzene (*m*-di–Br) and *p*-dibromobenzene (*p*-di–Br)); *chlorophenols* ((1) 2-chlorophenol, (2) 4-chlorophenol, (3) 3-chlorophenol, (4) 2,6-dichlorophenol, (5) 2,3-dichlorophenol, (6) 2,5-dichlorophenol, (7) 2,4-dichlorophenol, (8) 3,4-dichlorophenol, (9) 3,5-dichlorophenol, (10) 2,3,6-trichlorophenol, (11) 2,3,4-trichlorophenol, (12) 2,4,5-trichlorophenol, (13) 3,4,5-

trichlorophenol, (14) 2,3,4,6-tetrachlorophenol, (15) 2,3,5,6-tetrachlorophenol, (16) 2,3,4,5-tetrachlorophenol and (17) pentachlorophenol).

4.2.2 Preparation and chemical modification of monolithic columns

ODS and ODM column can be prepared using the method as previously described in Section 3.2.2.2 and 3.2.2.3, respectively. Several ODS and ODM columns were obtained, but only two ODS and two ODM columns were used for investigation of separation selectivity in this chapter. The performance of ODM and ODS columns, such as plate height (*H*) and permeability (*K*), was evaluated using a mixture of uracil and alkylbenzenes in 80% methanol mobile phase at *u* of 1.0 mm/s. These two types of reversed-phase columns showed high column efficiencies and permeabilities, with H_{uracil} of 8.5 μm, $H_{\text{amylbenzene}}$ of 9.1 μm and *K* of 8.8×10^{-14} m^2 for ODS columns, while H_{uracil} of 10.9 μm, $H_{\text{amylbenzene}}$ of 11.6 μm and *K* of 8.7×10^{-14} m^2 for ODM columns. Retention factors of amylbenzene on ODM and ODS were obtained to be 3.35 and 1.10, respectively, with RSD of 1.2 and 1.0% (*n* = two columns in the same batch of preparation).

4.2.3 CLC instrumentation

Chromatographic measurements were performed using a split-injection/flow HPLC system which consisted of pumps (Jasco *X*-LC 3185PU, Jasco, Tokyo, Japan), an MU 701 UV-vis detector (GL Sciences Inc., Tokyo, Japan) and an injection valve (model 7725, Rheodyne, CA, USA) fitted with a T-union (as a splitter) with one end connected to the capillary column and another end to a flow restrictor (a stainless steel tubing 0.1 mm i.d., 1/16 in. o.d.). More details of a split/flow-injection system are described in Section 2.2.1. ODM and ODS columns used were 28.0 cm in length. The temperature of all systems was controlled in an air-circulating oven at 30 ºC. UV detection was monitoring at 214 nm. Linear velocity (*u*) was set at 1.0 mm/s. All chromatographic data were collected in duplicate runs using version 2.8.3 EZChrom Elite Client/Server software.

4.3 Results and discussion

In this study, test analytes including halogenated benzenes and alkylbenzenes were separated on ODM and ODS columns using mobile phases with various concentrations of methanol or acetonitrile in water. However, retention and selectivity for these compounds on both ODM and ODS columns were relatively compared with the same mobile phase composition, and therefore any difference in retention and selectivity reflects different properties of these two stationary phases for reversed-phase HPLC.

4.3.1 Monosubstituted benzenes

Figure 4.1 shows an example of chromatograms of monosubstituted benzenes in 70% methanol and 70% acetonitrile mobile phases on ODM and ODS columns. Monohalogenated benzenes were eluted on both ODM and ODS columns with the following retention order: –F < –Cl < –Br < –I and monoalkylbenzenes: –H < $–CH_3$ < $–C_2H_5$ < $–C_3H_7$. This retention order can be expected in the C18 reversed-phase system on the basis of the *n*-octanol/water partition process (log *P*). Higher retention of monosubstituted benzenes on ODM versus ODS columns was obtained in both acetonitrile and methanol mobile phases. It can be seen from Figure 4.1A that the separation of –Cl and $–CH_3$, and –I and $–C_2H_5$ is achieved using the methanol mobile phase for the ODM column, but co-elution of –Cl and $–CH_3$ and reversed order of –I and $–C_2H_5$ was obtained on the ODS column under the same condition. The separation of –F and –H, –Cl and $–CH_3$, and –I and $–C_2H_5$ was also obtained with the ODM column, while these pairs co-eluted on the ODS column in the acetonitrile mobile phase (Figure 4.1B). The difference in retention of halogenated compounds on ODM and ODS stationary phases was greater with respect to alkylbenzenes in either acetonitrile or methanol mobile phases.

Solute retention in reversed phase HPLC can be divided into two processes: mobile phase and stationary phase effects. The former commonly relates to hydrophobic interactions, while the latter includes dispersion interactions as universal interactions

along with possible contributions of dipolar interactions depending on a stationary phase structure and an organic solvent in the chromatographic system. Since the analytes were separated under the same mobile phase, the different elution behavior of halogenated and alkyl substituted benzene pairs was presumably due to the difference in the stationary phase effect. It should be noted that ODM at 70% methanol (Figure 4.2A) and ODS at 60% methanol (Figure 4.2B) gave comparable retention times for $–CH_3$. Baseline resolution was obtained for –Cl and $–CH_3$ on the ODM column, while partial separation of these two compounds was observed on the ODS column. However, this work compares the selectivity of a given stationary phase using the same mobile phase, but not separation selectivity at optimum conditions of mobile phases for each stationary phase.

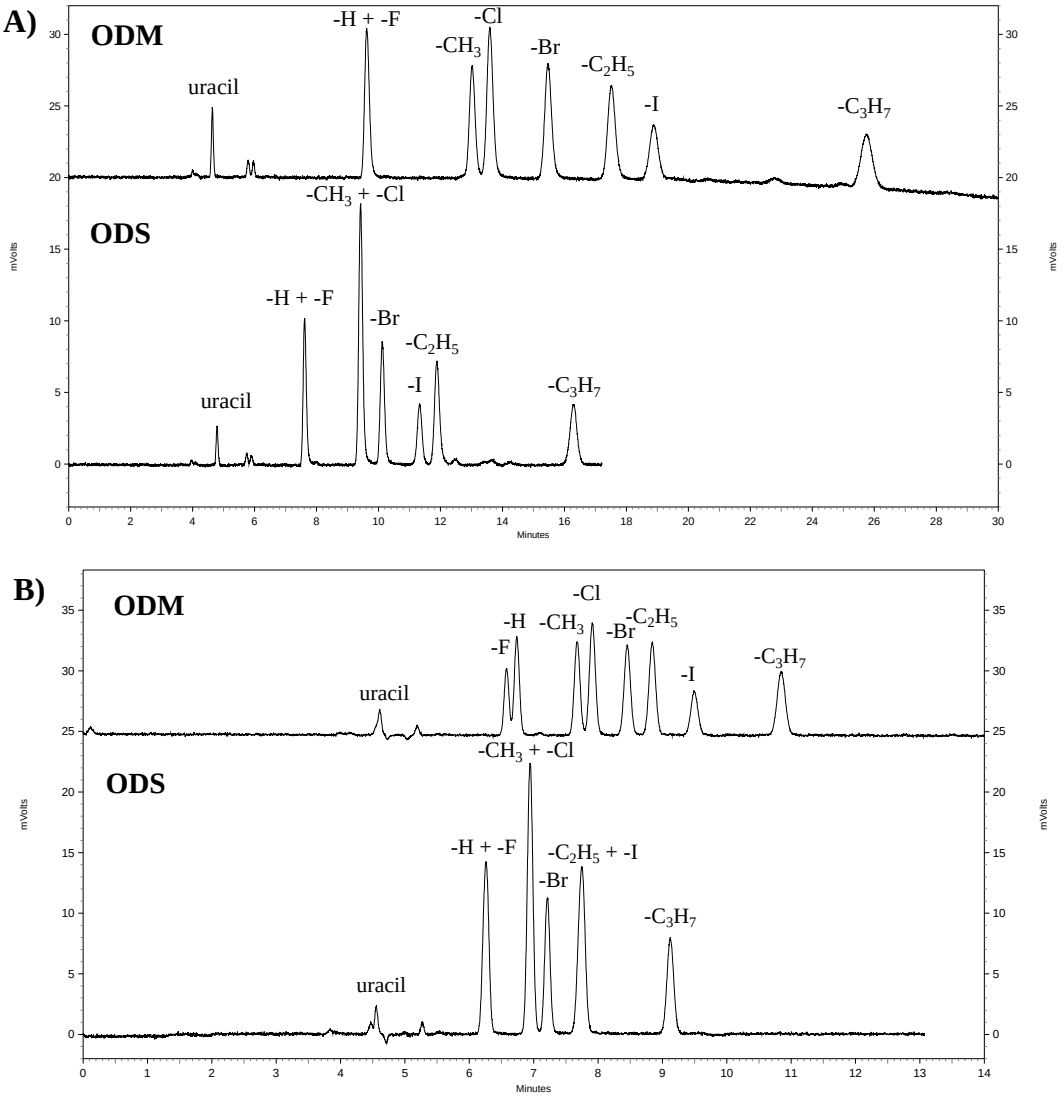


Figure 4.1 Chromatograms of mixtures of monohalogenated benzenes and monoalkylbenzenes on ODM and ODS columns using (A) 70% (v/v) methanol and (B) 70% (v/v) acetonitrile. Other CLC conditions were shown in Section 4.2.3. Uracil was used as an unretained marker.

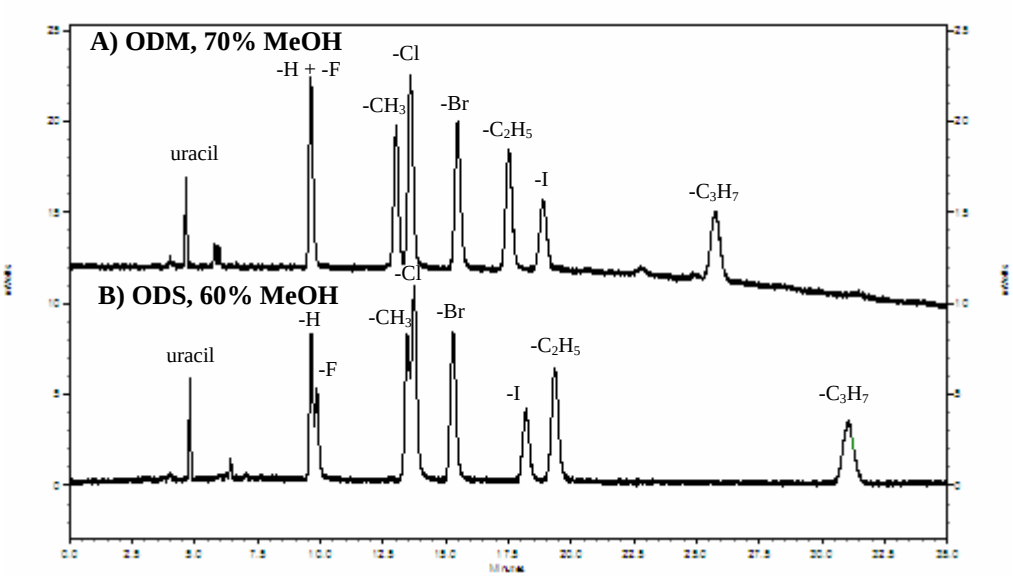


Figure 4.2 Chromatograms of mixtures of monohalogenated benzenes and monoalkylbenzenes: (A) 70% (v/v) methanol (MeOH) on ODM column and (B) 60% (v/v) MeOH on ODS column. Other CLC conditions were shown in Section 4.2.3. Uracil was used as an unretained marker.

In order to compare the preferential retention of alkylbenzenes and halogenated benzenes on ODM and ODS stationary phases over the amount of organic solvents in the mobile phase, examples of retention factors (*k*) for toluene and chlorobenzene are shown in Figure 4.3. Toluene and chlorobenzene showed higher retention on the ODM than ODS columns under the same condition. At the lower concentration of methanol, chlorobenzene, relative to toluene, gave stronger retention on both ODM and ODS stationary phases. The similar tendency was also observed using the acetonitrile mobile phase. Organic solvent effects on the retention of monohalogenated benzenes and monoalkylbenzenes were greater on the ODM column, especially for halogenated benzenes. As can be seen using the acetonitrile mobile phase, a slight decrease in retention of analytes was observed because of the loss of dispersion interactions in acetonitrile, in comparison with the methanol mobile phase [Núñez *et al*. 2007a].

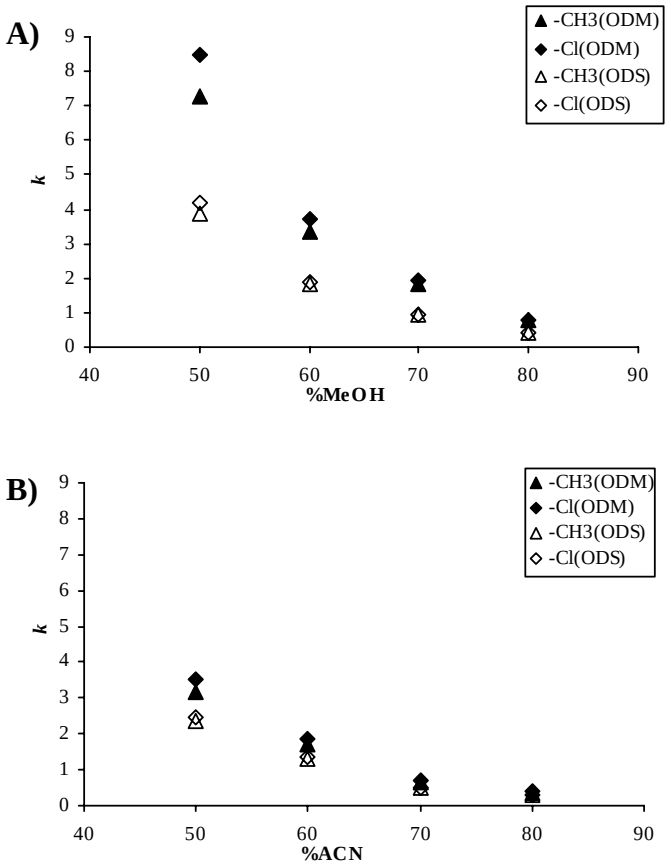


Figure 4.3 Retention factor (*k*) of toluene and chlorobenzenes on ODM and ODS columns in a wide range of organic modifier concentrations: (A) methanol and (B) acetonitrile.

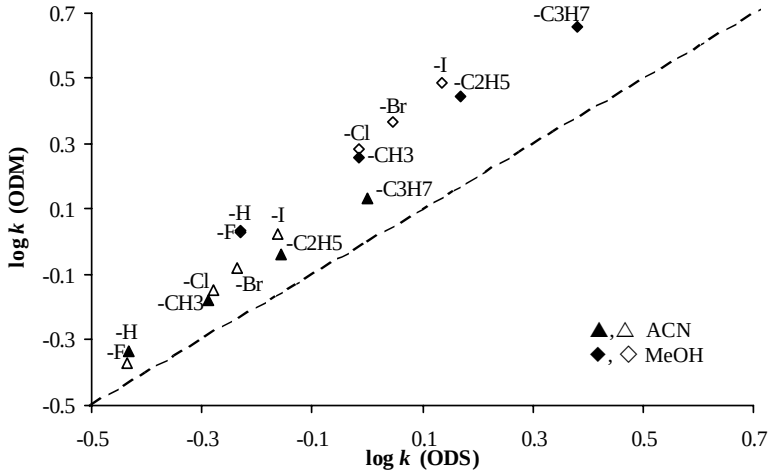


Figure 4.4 Plots of log *k* of monosubstituted benzenes on an ODM column against those on an ODS column using 70% (v/v) methanol (MeOH) and 70% (v/v) acetonitrile (ACN) as mobile phases.

Figure 4.4 demonstrates that, mobile phases with 70% methanol and 70% acetonitrile, log *k* values of monosubstituted benzenes on the ODM column are higher than those on the ODS column, indicating that both monohalogenated benzenes and monoalkylbenzenes have preferential retention for the ODM column in either acetonitrile or methanol mobile phase systems. From Figure 4.4, alkylbenzenes gave a linear increase with an increase in the number of carbon atoms in the aliphatic chain, which is common behavior for a reversed-phase system. Halogenated benzenes also demonstrated a linear tendency with increasing size and polarizability. In comparison with the slope of log *k* for monosubstituted benzenes on ODM against ODS stationary phases (Figure 4.4), the slope from monohalogenated benzenes is greater in mobile phases with either acetonitrile or methanol because of additional interactions affecting the retention behavior of halogenated benzenes on the ODM column compared with alkylbenzenes [Turowski *et al*. 2001].

The logarithm of the methylene group selectivity (log $\alpha(CH_2)$) can be obtained from the value of the slope of a linear plot between log *k* of the alkylbenzenes against the number of alkyl carbons. Figure 4.5 shows plots of log $\alpha(CH_2)$ of alkylbenzenes in a range of 50-80 % (v/v) organic solvents including acetonitrile and methanol on ODM and ODS columns. The value of $\alpha(CH_2)$ reflects the stationary phase hydrophobicity. The higher the value of $\alpha(CH_2)$, the greater the hydrophobicity of the stationary phase. In comparison with the ODS column, the ODM column shows a higher hydrophobicity using either acetonitrile or methanol mobile phases. This is caused by the difference in bonding density or the amount of C18 bonded to silica support. Due to the lower preference for planar solutes on the ODS column (the higher C18 chain density on the ODM column) [Núñez *et al*. 2007a], the greater distance between the C18 chains on the ODS stationary phase allows for a higher amount of organic solvent to be adsorbed onto the ODS stationary phase than that on the ODM stationary phase. A significant difference in log $\alpha(CH_2)$ or hydrophobicity on ODM and ODS columns in Figure 4.5 was observed in acetonitrile in comparison to methanol mobile phases, probably due to the greater amount of adsorbed acetonitrile on the reversed-phased stationary phase [Kazakevich *et al*. 2001].

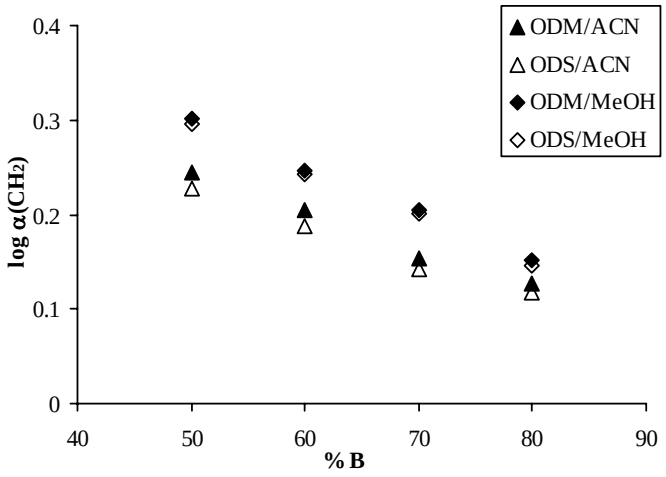


Figure 4.5 Comparison of log $\alpha(CH_2)$ on ODM and ODS columns using acetonitrile and methanol mobile phases.

As previously mentioned, selectivity (α) in LC is defined as the ratio of retention factors for two analytes. In this work, the selectivity of ODM and ODS columns for monosubstituted benzenes was compared by plotting the ratio of the selectivity of ODM relative to ODS columns as shown in Figure 4.6. A similar selectivity, the α ratio $\approx$ 1, on ODM and ODS columns for homologous series of alkylbenzenes was observed. When the α value is obtained from halogenated benzenes with respect to toluene, the selectivity of the stationary phases for analytes is influenced by the structural fragments. The ODM column provides a greater selectivity for monohalogenated benzenes relative to toluene in both acetonitrile and methanol mobile phases. An increase in the selectivity of halogenated benzenes relative to toluene was observed with an increasing size in the halogenated atom. The difference in the selectivity of halogenated compounds on these two columns could involve dispersive interactions [Turowski *et al*. 2003] to a greater extent for a large halogen atom as a substituent than in the case of alkylbenzenes on the ODM column. According to previous works on selectivity of poly(octadecyl acrylate) stationary phase grafted on silica particles in a conventional HPLC column [Fukumoto *et al.* 1994, Chowdhury *et al*. 2000, Malik *et al.* 2008], it can be mentioned in our work that the contribution of carbonyl groups in the side chain of the ODM stationary phase can be involved through carbonyl-π interactions between the stationary phase and solute to cause the enhanced retention of halogenated compounds with the ODM phase.

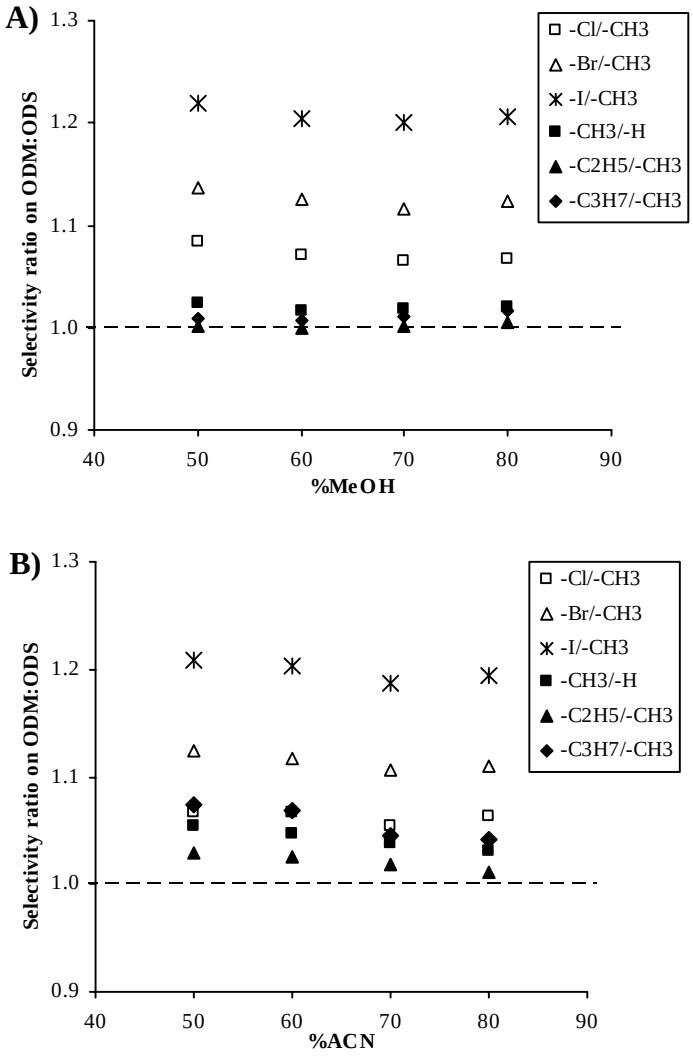


Figure 4.6 The selectivity ratio on ODM:ODS columns for monosubstituted benzenes against selected reference analytes using (A) 50-80 % (v/v) methanol and (B) 50-80 % (v/v) acetonitrile as mobile phases.

4.3.2 Disubstituted benzenes

The chromatographic performance of the analytes on ODM and ODS columns was also investigated using dihalogenated benzenes and dialkylbenzenes as test analytes. Figure 4.7 shows chromatograms of disubstituted benzenes on ODM and ODS stationary phases using 70% methanol and 70% acetonitrile mobile phases. The general elution order of *ortho*-, *meta*- and *para*-disubstituted benzenes has similar tendencies as in the case of monosubstituted benzenes. The elution order on ODM and ODS columns is the same. These test analytes elute in the order of the following retention times di–F ≈ –H < di–CH_3 < di–Cl < di–Br < di–C_2H_5. The elution order varies systematically with a change in substituents, but it is not systematic within these groups. For dialkylbenzenes, the elution order of each isomer is *ortho* < *meta* < *para*. Dichlorobenzene and dibromobenzene isomers elute in the retention time order of *ortho*- < *para*- < *meta*-, while *para*- < *ortho*- < *meta*- for diflurobenzenes on both ODM and ODS columns. It is obvious that the ODM column provides better separation of positional isomers of dihalogenated benzenes for the adjacently eluted isomers that cannot be separated under the same conditions on the ODS column.

Plots of log *k* values for disubstituted benzenes on the ODM column were plotted against those on the ODS column using 70% methanol and 70% acetonitrile mobile phases as in Figure 4.8. The greater retention of disubstituted benzenes on ODM than ODS columns was obtained as the similar trends of monosubstituted benzenes. The effect of the stationary phase on the retention for each positional isomer of halogenated benzenes was greater on the ODM column with an increasing size of the halogen atom owing to greater dispersion interactions together with the contribution of carbonyl groups on the ODM stationary phase [Fukumoto *et al.* 1994, Chowdhury *et al*. 2000, Malik *et al.* 2008].

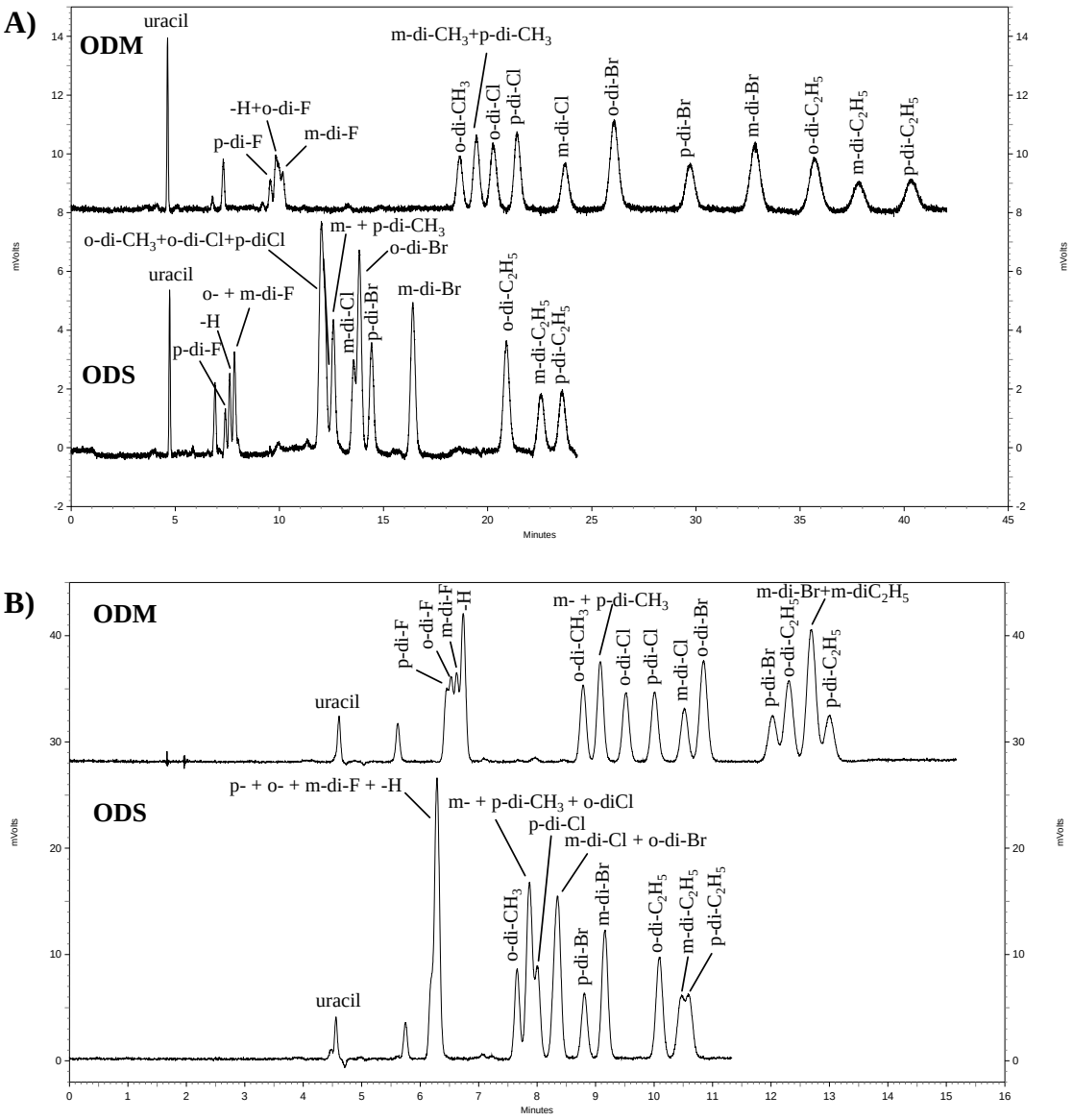


Figure 4.7 Chromatograms of mixtures of dihalogenated benzenes and dialkylbenzenes on ODM and ODS columns using (A) 70% (v/v) methanol and (B) 70% (v/v) acetonitrile as mobile phases. Other CLC conditions were as shown in Section 4.2.3.

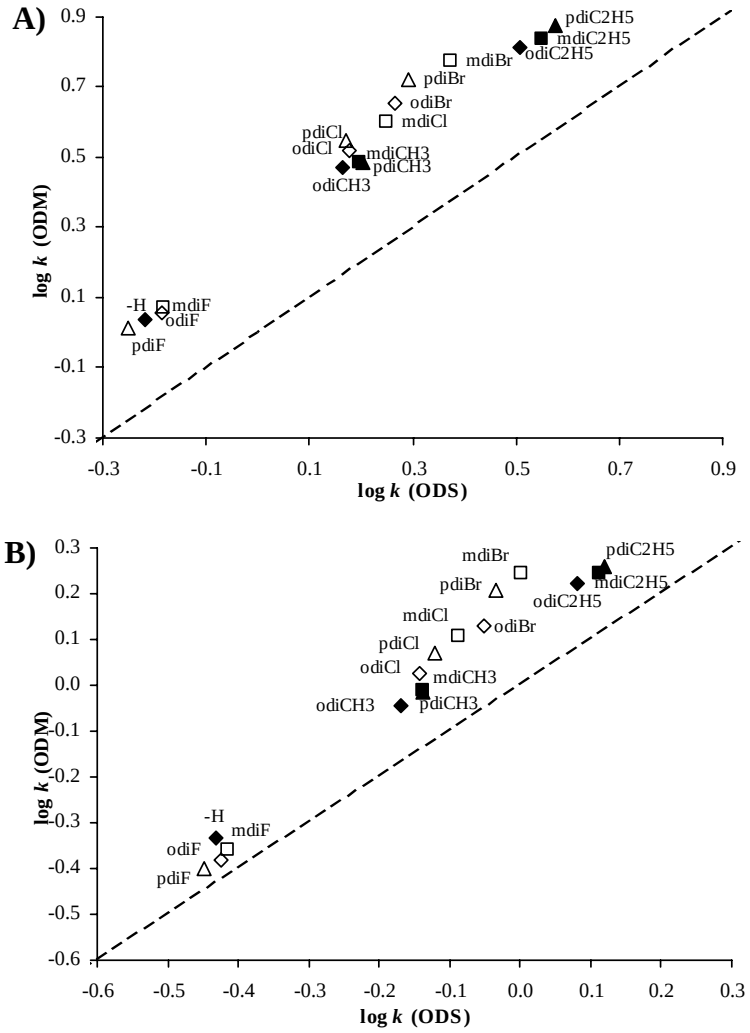


Figure 4.8 Plots of log *k* of disubstituted benzenes on an ODM column against those on an ODS column using (A) 70% (v/v) methanol and (B) 70% (v/v) acetonitrile as mobile phases.

4.3.3 Chlorophenols

The performance of ODM and ODS columns for separating halogenated compounds was demonstrated using chlorophenols as test analytes. These compounds contain a number of homologues and isomers. The separation of mono- and polychlorophenols on ODM and ODS columns is shown in Figure 4.9, using methanol and acetonitrile mobile phases. The retention of chlorophenols was longer with increasing the number of the chlorine atom on phenol due to the higher hydrophobicity of solutes. When chlorine was substituted on the isolated substitution, these compounds gave higher retentions compared to the congested substitution due to higher hydrophobic areas [Tanaka *et al*. 1993]. As can be seen in Figure 4.9A, the separation of 4-chlorophenol/3-chlorophenol (2/3) and 2,4-dichlorophenol/3,4-dichlorophenol (7/8) pairs was achieved on the ODM column, while co-elution of these pairs was observed on the ODS column under the same methanol mobile phase composition with comparable times. In addition, the reversed retention order of 3,5-dichlorophenol/2,3,6-trichlorophenol (9/10) on ODM and ODS columns was obtained. The better separation of chlorophenols on the ODM column was also observed using the same mobile phase as isocratic elution of 60% acetonitrile (Figure 4.9B). The higher retention and better resolution of 17 chlorophenols on the ODM than ODS columns was obtained due to greater dispersive interactions of analytes on the ODM column. The retention is also enhanced by the carbonyl-π interactions on the ODM stationary phase [Fukumoto *et al.* 1994, Chowdhury *et al*. 2000, Malik *et al.* 2008]. It should be noted that the optimized separation of chlorophenols with the ODM or ODS column may also be performed by appropriate gradient elution with the mobile phase.

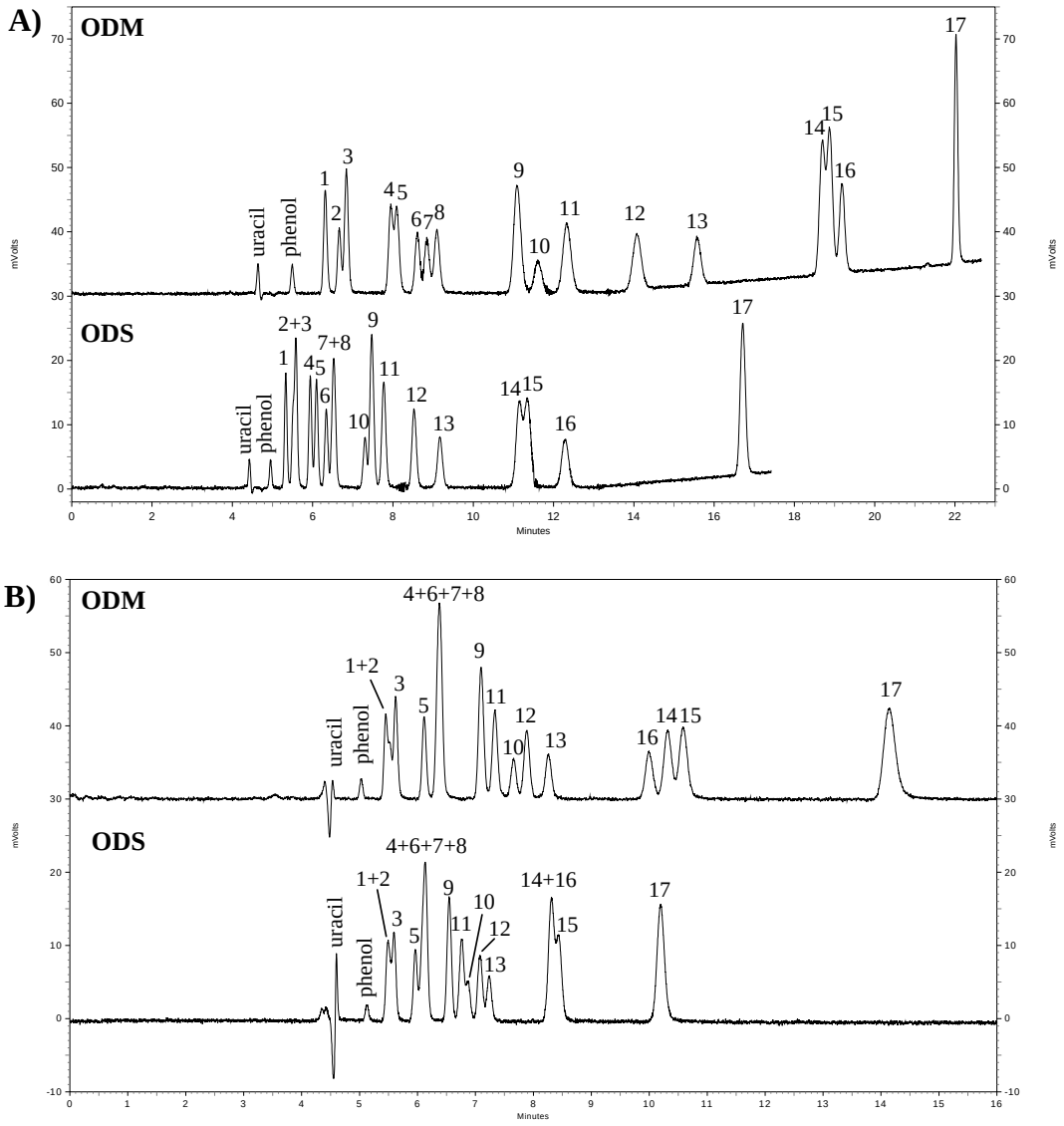


Figure 4.9 Separation of 17 chlorophenols on ODM and ODS columns using (A) gradient elution of A:B with 70 % B (0-8 min),70-100 % B (8-18 min) and 100% B (18-23min), where A is water with 0.1% formic acid, and B is methanol with 0.1 % formic acid, and (B) isocratic elution of 40:60 A:B, where A is water with 0.1% formic acid, and B is acetonitrile with 0.1% formic acid.

4.4 Conclusions

Selectivities of two reversed-phase stationary phases for halogenated compounds were compared using monolithic silica capillary columns modified with poly(octadecyl methacrylate) (ODM) and octadecyl moieties (ODS) in CLC. Halogenated compounds, including mono- and di-substituted benzenes and chlorophenols, showed the preferential retention on the ODM stationary phase in either methanol/water or acetonitrile/water mobile phases. In selectivity comparisons of selected analytes on ODM and ODS columns, greater selectivities for halogenated benzenes were obtained with respect to alkylbenzenes on the ODM column, while similar selectivities were observed with a homologous series of alkylbenzenes on ODM and ODS columns. These results can be explained by greater dispersive interactions by more densely packed octadecyl groups on the ODM stationary phase together with the contribution of carbonyl-π interactions in side chains of the ODM column. For the positional isomeric separation of dihalogenated benzenes (*ortho*-, *meta*-, *para*-), the ODM column also provided better separation of these isomers for the adjacently eluted isomers that cannot be completely separated on the ODS column under the same condition. The better resolution of seventeen chlorophenols on the ODM column was also achieved in comparison with the ODS column. Therefore, the results imply that the ODM column can be used as an alternative to the conventional ODS column for separating other halogenated compounds.

CHAPTER V

SEPARATION SELECTIVITY OF MIXED-MODE MONOLITHIC SILICA CAPILLARY COLUMNS IN CAPILLARY ELECTROCHROMATOGRAPHY

5.1 Introduction

Capillary electrochromatography (CEC) has become an interesting miniaturized separation technique that combines the high efficiency of capillary electrophoresis (CE) and the high selectivity of high-performance liquid chromaotography (HPLC). These unique characteristics of CEC have contributed to its rapid development in a wide variety of application from small molecules to large biological molecules such as peptides and proteins. The differential migration of the analytes in CEC is due to the combination of chromatographic partition and electromigration mechanisms. Both charged and neutral can be separated simultaneously in CEC [Xie *et al.* 2004].

The column is the heart of a CEC system because it not only serves as the separation media but also generates EOF to drive the mobile phase through the column. Typically, CEC columns used are conventional packed capillary columns with commercially available HPLC silica particles. However, they possess some inherent limitations such as tedious packing procedure, bubble formation, frit fabrication and lack of reproducibility. In recent years, monolithic columns have become an attractive separation column in CEC because they can effectively overcome the difficulties associated with traditional packed capillary columns. The continuous monolith beds can be prepared by *in situ* polymerization and chemically boned to the inner walls of the capillaries. Therefore, this eliminates the need of retaining frits which are responsible for bubble formation encountered with packed capillaries [Yan *et al.* 2004].

Both polymer-based and silica-based monolithic columns have been widely used in CEC. Polymer-based monoliths have excellent pH stability and wide application range of pH values, however, they suffer from swelling in organic solvent which lead to the lack of mechanical stability. The silica-based monoliths offer high mechanical strength and good solvent resistance. Moreover, the popularity of the silica monoliths can be linked to the availability of different chemistries that can be used for surface modification [Ye *et al.* 2007].

Similar to HPLC, reversed-phase (RP) monolithic silica columns, such as C18, are widely used for the separation of a variety compounds in CEC. However, highly polar and basic analytes are difficult to be separated on conventional RP silica-based column in CEC [Ye *et al.* 2010]. For silica-based monoliths, the negative charge of residual silanol groups on the surface of monolith is essential for the generation of EOF. Due to the weak acidity of silanol groups, silica monoliths can only work in the mobile phase at pH > 5.0 to maintain high sufficient EOF. In addition, the separation of basic compounds on traditional silica-based stationary phases often suffers from peak broadening and tailing due to the interaction between the basic solutes and residual silanol groups. Therefore, the stationary phases with positively charged functionalities suitable for the separation of basic analytes were designed, which include ligand comprising both ionizable typically polar functional groups to generate EOF and hydrophobic chain to provide RP separation [Ye *et al.* 2006, Ye *et al.* 2007, Allen and Rassi 2003, Ding *et al.* 2010].

CEC with two or more kinds of chromatographic retentions, so called mixed-mode column (*i.e.* RP/cation exchange [Chen *et al.* 2010], RP/anion exchange [Ye *et al.* 2009, Ding *et al.* 2006, Jemale *et al.* 2011]), can be used as an alternative column to overcome the previously mentioned problems. Mixed mode columns have attracted more intention recently because the presence of charged groups can provide both stable EOF and also ion-exchange sites for charged compounds. Recently [Ye *et al.* 2009], mixed-mode of reversed-phase (RP) and weak anion exchange (WAX) for CEC columns has been reported by chemical modification of the silica monolith with hexadecyl and aminopropyl moieties. Due to the presence of amino groups, the

column can be generated either cathodic EOF (the direction of EOF from anode to cathode) or anodic EOF (the direction of EOF from cathode to anode). The electrochromatographic performance was evaluated using alkylbenzenes, benzoic acids and aniline as test analytes. This mixed-mode column exhibited RP chromatographic behavior for neutral solutes. The separation of anionic analytes is based on the RP, WAX and electrophoresis. In addition, symmetrical peaks can be observed for the separation of basic analytes due to the presence of positively charged amino groups. So far, the performance of the mixed-mode columns was mainly tested by using alkylbenzenes and a few other compounds as solutes in terms of electrochromatographic efficiency, ion-exchange mechanism and peak shape improvement for basic solutes.

Therefore, it is interesting to extend the previous work of Ye *et al.* [2009] to investigate the electrochromatographic behavior and separation selectivity for some polar and nonpolar compounds on mixed-mode columns. Two types of RP/WAX monolithic columns were prepared by bonding simultaneously with octadecyl and aminopropyl moieties (ODS/AP_1), and with octadecyl and 3-(2-aminoethylamino)propyl moieties (ODS/AP_2). Then, the electrochromatographic performance for some polar and nonpolar aromatic compounds was investigated on ODS/AP_1 and ODS/AP_2, compared with the monolithic column modified with octadecyl moieties (ODS) column.

5.2 Experimental

5.2.1 Chemicals and materials

All reagents were of analytical grade. Tetramethoxysilane (TMOS) was purchased from ShinEtsu Chemicals (Tokyo, Japan); methyltrimethoxysilane (MTMS) was from Aldrich (WI, USA); poly(ethylene glycol) (PEG, MW = 10,000) and urea were from Sigma-Aldrich (Steinheim, Germany). Acetic acid, methanol, acetonitrile (ACN), phosphoric acid and toluene were obtained from Merck (Darmstadt, Germany); sodium dihydrogenphosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and sodium hydroxide were

from Fluka ((Buchs, Switzerland); octadecyltrimethoxysilane (ODS), aminopropyltrimethoxysilane (AP_1) and 3-(2-aminoethylamino)propyl] trimethoxysilane (AP_2) were from Acros Organic (Geel, Belgium). Toluene was distilled before further use. All solutions were prepared with ultrapure water using a Milli-Q water system (18 MΩ·cm, Millipore, MA, USA). Fused silica capillaries of 100 μm i.d. and 375 μm o.d. was obtained form Polymicro Technologies (Phoenix, AZ, USA).

All chemical standards were purchased from Aldrich (WI, USA), Merck and Fluka. The following benzenes derivatives used in the study: thiourea, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene, hexylbenzene, *o*-terphenyl, triphenylene, fluorobenzene, chlorobenzene, bromobenzene, phenol, catechol, resorcinol, hydroquinone, *o*-cresol, *p*-cresol, *p*-chlorophenol, methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate, methyl benzoate, ethyl benzoate, propyl benzoate, butyl benzoate, *o*-methoxyphenol, nitrobenzene, aniline, *p*-toluidine.

5.2.2 Preparation and chemical modification of monolithic columns [Motokawa *et al.* 2002, Ye *et al.* 2009]

Hybrid type monolithic silica capillary columns were prepared from a mixture TMOS: MTMS (3:1 v/v) in100 μm i.d. fused silica capillaries using the procedures as previously described in Section 3.2.2.1. The on-column bonding reaction was employed using a solution of 10% (v/v) of ODS, a mixture of ODS and AP_1 9:1 (v/v), or a mixture of ODS and AP_2 9:1 (v/v), in toluene. The monolithic silica capillary column was flushed with a reaction solution at 3 bar for 1 h, and then allowed to reacted at 110 ºC for 1 h. This step was repeated three times, and the final reaction was done overnight. Finally, the columns were rinsed with toluene followed by methanol. The schematic reaction pathway for surface modification was shown in Figure 5.1 for ODS, ODS/AP_1 and ODS/AP_2 columns.

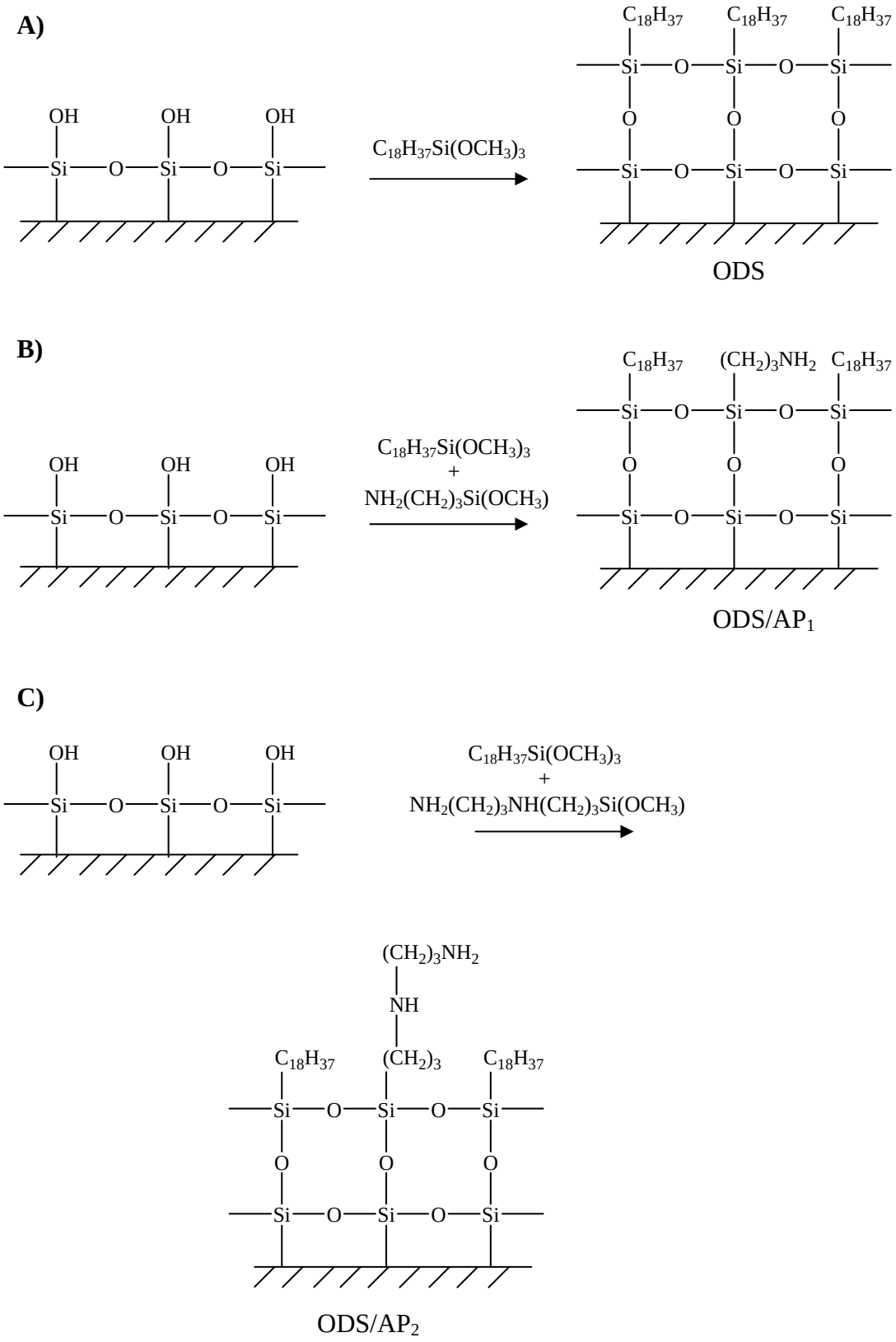


Figure 5.1 Surface modification of monolithic silica columns with silanization reaction of ODS (A), ODS/AP_1 (B) and ODS/AP_2 (C).

5.2.3 CEC instrumentation and conditions

All CEC experiments were performed on a Beckman Coulter MDQ CE system equipped with a photo-diode array detector. The CEC column of 100 μm i.d. had a total length of 31.2 cm (21.0 cm to detector). A detection window was made on the continuous bed by removing the polyimide coating using a capillary burner for on-column detection. The applied voltage was set at 15 or -15 kV unless otherwise stated, and the temperature was controlled at 25 ºC. UV detection was monitoring at 214 nm. Each sample solution was introduced into the column through electrokinetic injection method with 3 or -3 kV 3s.

Stock solution of phosphate buffer were prepared by dissolving appropriate amount of sodium dihydrogenphosphate or diluting phosphoric acid with water, then adjusting to desired pH by NaOH. The mobile phase was prepared by mixing the desired amount of phosphate buffer and ACN. Stock solutions of each test analyte were dissolved in ACN and then diluted with mobile phase. All sample solutions and mobile phases were filtered through 0.45 μm membrane and sonicated for 30 min before use. Prior to the CEC analysis each day, the monolithic column was rinsed with a mobile phase by a syringe pump with a flow rate of 0.3 mL/h for 1 h, and then was electrokinetically conditioned with applied voltage of 10 or -10 kV until a stable current was achieved. The apparent retention factor (*k*) in CEC can be calculated using Equation 2.31 which reflected the elution of analytes from the combination of both chromatographic and electrophoretic process. In this work, thiourea was used as an EOF marker.

5.3 Results and discussion

5.3.1 Column performance of prepared monolithic silica capillary columns

As previously mentioned in Section 5.1, in case of ODS column, a cathodic EOF (EOF has the direction to the cathode at the detection end) is generated from the ionization of residual silanol groups on the surface of silica monolith, usually at pH >

5.0. In case of ODS/AP_1 and ODS/AP_2, these mixed-mode silica monoliths contain amino groups and residual silanol groups on the surface, and therefore, they can be generated either anodic or cathodic EOF. The direction of EOF on mixed-mode column depends on the net surface charge density of the monolith surface which can be easily controlled by the pH value of mobile phase. With the buffer at acidic pH, the protonation of amino groups on the silica monolith surface is dominated, and therefore, the net positive charge density is obtained to produce the anodic EOF. With the buffer at basic pH, the full ionization of residual silanol groups was obtained together with the suppression in the protonation of amino groups, and therefore, the net negatively charged density is obtained to generate the cathodic EOF. In previous work [Ye *et al.* 2009], the mixed-mode column containing hexadecyltrimethoxysilane (C_{16}) and AP_1 generated the anodic EOF in the buffer pH in the range of 2.5 to 7.3 and the cathodic EOF in the buffer pH in the range of 7.3 to 9.0.

Figure 5.2 shows the electrochromatographic separation of thiourea and a mixture of alkylbenzenes obtained on ODS (Figure 5.2A), ODS/AP_1 (Figure 5.2B), and ODS/AP_2 (Figure 5.2C) columns using 50% ACN in 10 mM phosphate buffer at pH 7.0 for ODS column and at pH 2.5 for ODS/ AP_1 and ODS/AP_2. At these condition, cathodic EOF was generated on ODS column while anodic EOF on ODS/ AP_1 and ODS/AP_2 columns. All these three columns provided good separation for all compounds with symmetrical peaks (peak asymmetry at 10% between 0.9 and 1.2). As can be seen, the retention of alkylbenzenes is different on these three columns. Table 5.1 lists the retention factors (k) of some analytes, methylene group selectivity ($\alpha(CH_2)$ and shape selectivity ($\alpha_{T/O}$). As previously mentioned in Section 3.3.1, the k values of alkylbenzenes can be used to measure the amount of alkyl-bonded stationary phase bonded to the silica. In addition, hydrophobic selectivity of monolithic silica capillary columns can be estimated from the slope of linear relationship between log k and z (z is the carbon number in aliphatic chain of alkylbenzenes), as illustrated in Figure 5.3.

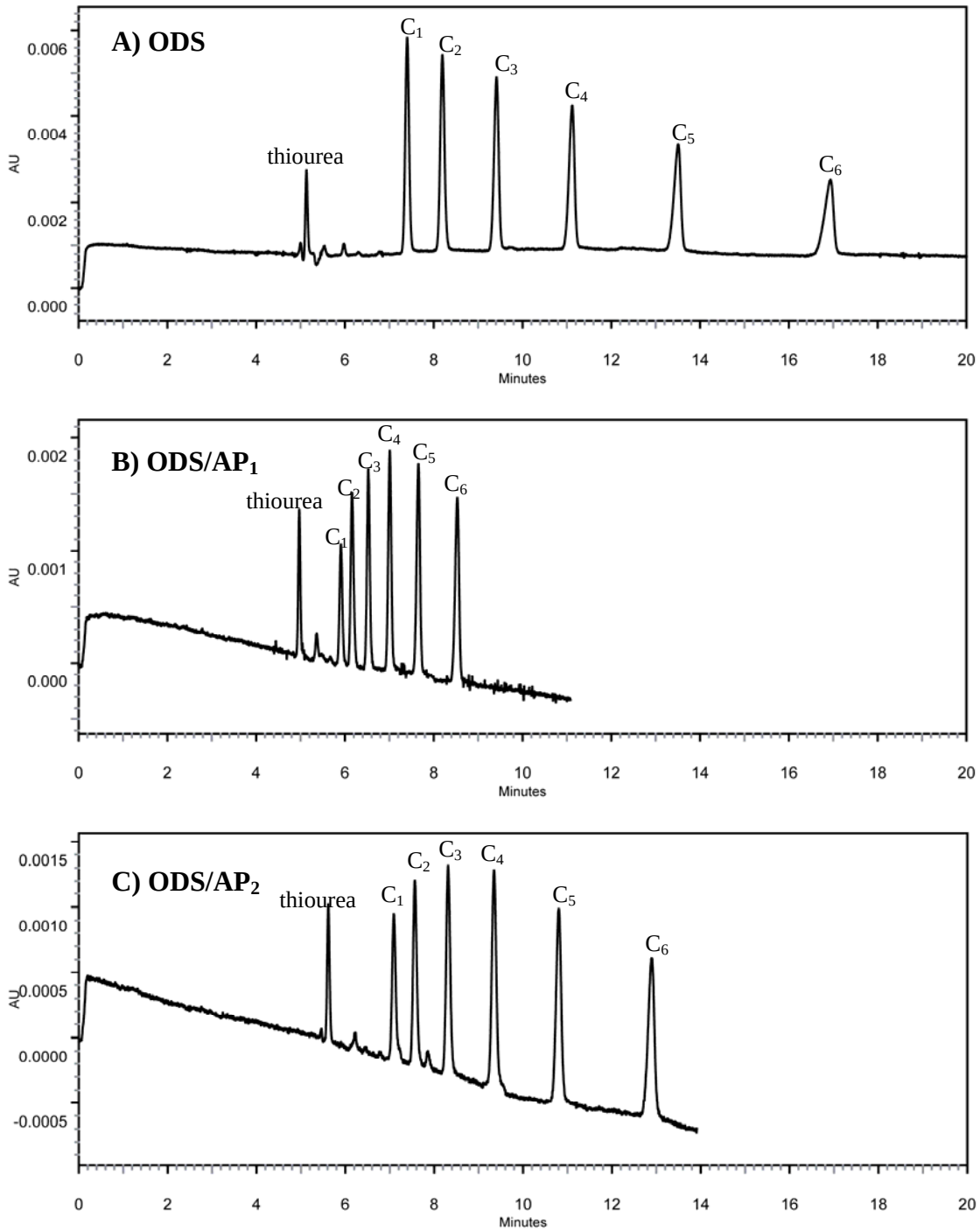


Figure 5.2 Electrochromatograms of a mixture of thiourea (an unretained compound) and alkylbenzenes, $C_6H_5C_nH_{2n+1}$ (*n* =1-6) on ODS (A), ODS/AP_1 (B), and ODS/AP_2 (C) columns using 50% ACN in 10 mM phosphate buffer at pH 7.0 mobile phase for ODS column, and 50% ACN in 10 mM phosphate buffer at pH 2.5 mobile phase for ODS/AP_1 and ODS/AP_2 columns. Other CEC conditions were shown in Section 5.2.3.

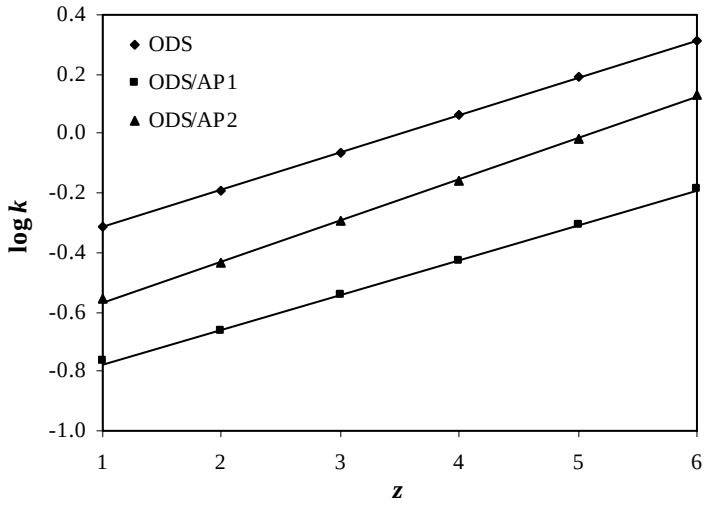


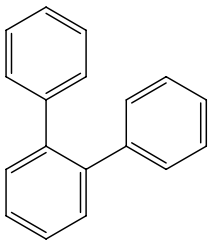
Figure 5.3 Plots of logarithmic retention factor (log *k*) against carbon number in aliphatic chain of alkylbenzenes. CEC conditions are same as in Figure 5.2.

As can be seen in Table 5.1, the greater retention factor of alkylbenzenes on ODS column than ODS/AP_2 and ODS/AP_1 columns, respectively, was obtained. This is because the higher amount of C18 chain bonded to the surface on ODS column compared with ODS/AP_2 and ODS/AP_1, and also the longer alkyl chain on ODS/AP_2 compared with ODS/AP_1. In addition, the slightly greater $\alpha(CH_2)$ value was obtained on ODS than ODS/AP_2 and ODS/AP_1, respectively, indicating the slightly higher hydrophobicity on ODS than ODS/AP_2 and ODS/AP_1. Column-to-column reproducibility of the prepared monolithic silica columns was also investigated in terms of $k_{\text{alkylbenzenes}}$. The fair reproducibility (n = 3 columns) was observed, for example, RSD values for $k_{\text{hexylbenzene}}$ were 8.6, 6.0 and 7.8% for ODS, ODS/AP_1 and ODS/AP_2, respectively.

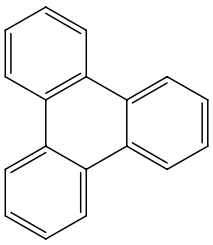
Table 5.1 Retention factor (*k*), methylene selectivity ($\alpha(CH_2)$), shape selectivity ($\alpha_{T/O}$) on ODS, ODS/AP_1 and ODS/AP_2 columns. Mobile phase: 50% ACN in 10 mM phosphate buffer at pH 7.0 for ODS column, and 50% ACN in 10 mM phosphate buffer at pH 2.5 for ODS/AP_1 and ODS/AP_2 columns.

Column	*k* of alkylbenzenes						$\alpha(CH_2)$	*k*		$\alpha_{T/O}$
	C1	C2	C3	C4	C5	C6		*o*-terphenyl	triphenylene	
ODS	0.51±0.02 (4.2)	0.68±0.03 (4.7)	0.92±0.05 (5.9)	1.24±0.08 (6.4)	1.67±0.12 (7.5)	2.26±0.19 (8.6)	1.35±0.01	1.76±0.17	2.09±0.14	1.19±0.10
ODS/AP_1	0.18±0.01 (4.9)	0.23±0.01 (5.1)	0.30±0.01 (4.9)	0.40±0.02 (5.2)	0.52±0.03 (5.5)	0.70±0.04 (6.0)	1.31±0.01	0.66±0.05	0.82±0.07	1.24±0.01
ODS/AP_2	0.30±0.02 (5.4)	0.38±0.01 (3.5)	0.51±0.01 (2.0)	0.68±0.02 (2.9)	0.91±0.05 (5.9)	1.24±0.10 (7.8)	1.33±0.03	1.02±0.06	1.26±0.03	1.23±0.08

%RSD in parenthesis



o-terphenyl



triphenylene

Figure 5.4 Chemical structures of *o*-terphenyl and triphenylene.

Shape selectivity ($\alpha_{T/O}$) defines the capability of stationary phase to diffentiate between planar and nonplanar compounds of similar molecular weights and sizes. The $\alpha_{T/O}$ values can be evaluated from the ratio of *k* for triphenylene (planar) and *o*-terphenyl (nonplanar) (k_T/k_O) due to the similar hydrophobic properties but different planarity of triphenylene and *o*-terphenyl [Nogueira *et al.* 2006]. The structure of these two compounds was shown in Figure 5.4. As shown in Table 5.1, the $\alpha_{T/O}$ values on ODS/AP_1 and ODS/AP_2 columns seem to be slightly greater than that on ODS columns. As suggested in Nogueira *et al.* [2006], the greater $\alpha_{T/O}$ does not necessarily indicate a better steric recognition capability of the RP/WAX phases, but rather may reflect reinforced electrophilic interactions of amino groups with π-electrons of aromatic compounds.

Figure 5.5 compares the plots of *k* of alkylbenzenes over a wide range of *u* on ODS, ODS/AP_1 and ODS/AP_2 columns. The *u* value of mobile phase was varied by changing the applied voltage from 5 to 22.5 kV for the ODS column but -5 to -22.5 kV for the latter two columns. At higher than ± 22.5 kV, the CEC separation cannot be performed due to the current breakdown, probably caused by the bubble formation from much higher Joule heating. Therefore, the *u* above 1.0 mm/s did not obtain for some columns. From Figure 5.5, the *k* values are not significantly different with an increase in *u*. To evaluate the column performance, Figure 5.6 shows the van Deemter plots of ODS, ODS/AP_1 and ODS/AP_2 columns. All three type columns gave similar plots with good efficiencies, for example, $H_{thiourea}$ of 3.7, 4.0 and 4.2 μm at *u* of about 0.9 mm/s for ODS, ODS/AP_1 and ODS/AP_2 columns, respectively. The slightly better column performance was observed with *u* in the range of 0.5-1.0 mm/s. Moreover, in

comparison with *H* of monolithic silica columns obtained in CLC (Figure 3.10 and Table 3.2), better *H* was observed in CEC due to a flat flow profile of the mobile phase in CEC instead of a parabolic flow profile in CLC [Ishizuka *et al.* 2000], as previously mentioned in Section 2.2.5.1.

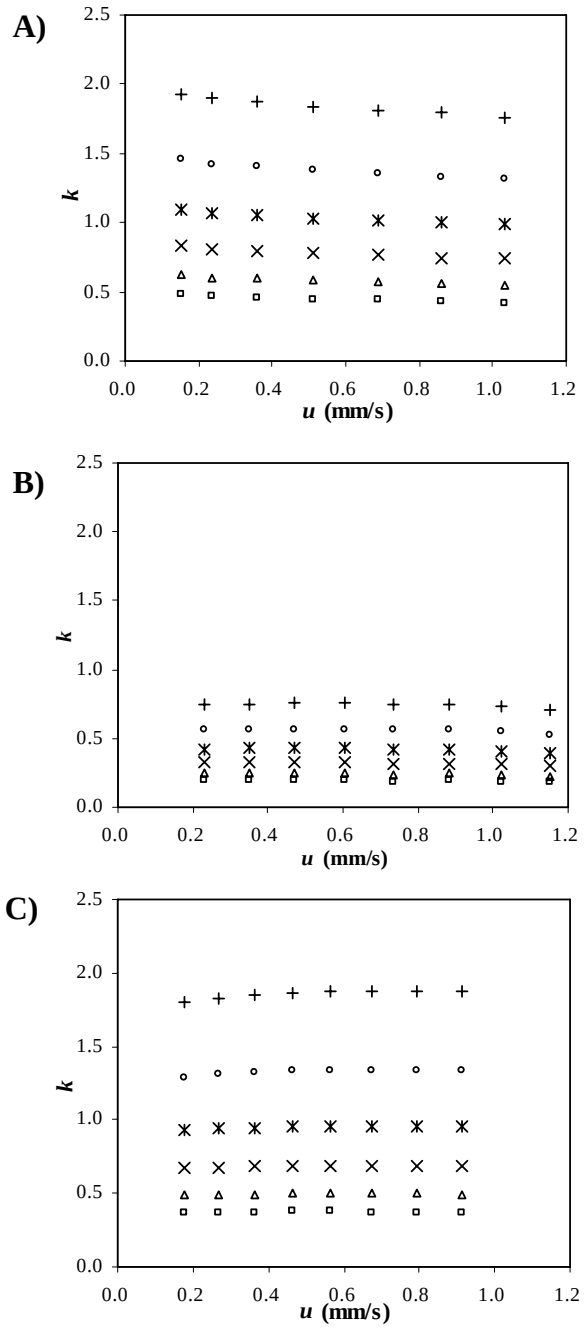


Figure 5.5 Plots of retention factor (*k*) of alkylbenzenes over a wide range of linear velocity (*u*) on ODS (A), ODS/AP_1 (B), and ODS/AP_2 (C) columns using 50% ACN in 10 mM phosphate buffer at pH 7.0 mobile phase for ODS column, and 50% ACN in 10 mM phosphate buffer at pH 2.5 mobile phase for ODS/AP_1 and ODS/AP_2 columns. Analytes: toluene (□), ethylbenzene (△), propylbenzene (×), butylbenzene (∗), amylbenzene (○) and hexylbenzene (+) were used as test analytes. The applied voltage is in a range of (-) 5 to (-) 22.5 kV. Other CEC conditions were shown in Section 5.2.3.

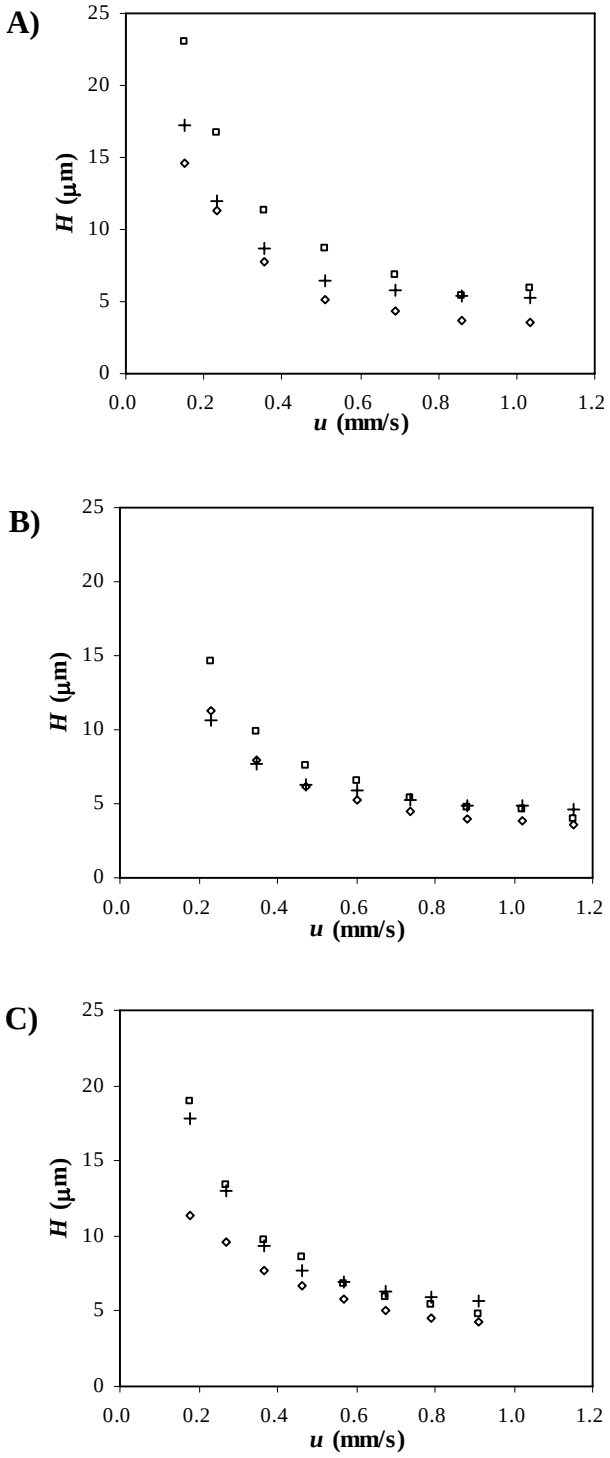


Figure 5.6 The van Deemter plots for ODS (A), ODS/AP_1 (B), and ODS/AP_2 (C) columns using 50% ACN in 10 mM phosphate buffer at pH 7.0 mobile phase for ODS column, and 50% ACN in 10 mM phosphate buffer at pH 2.5 mobile phase for ODS/AP_1 and ODS/AP_2 columns. The applied voltage is in a range of (-) 5 to (-) 22.5 kV. Other CEC conditions were shown in Section 5.2.3. Thiourea (◇), an unretained compound), toluene (□) and hexylbenzene (+) were used as test analytes.

5.3.2 Electrochromatographic behaviors of ODS, ODS/AP_1 and ODS/AP_2 for benzenes derivatives

To investigate and compare the electrochromatographic behavior of various compounds under the same condition, some polar and nonpolar benzene derivatives was analyzed on ODS, ODS/AP_1 and ODS/AP_2 using 40% (v/v) ACN in 10 mM phosphate buffer pH 6.0. Under this condition, phenol compounds (pK_a values of about 9, [Ye *et al.* 2006]) are present in their neutral form, while amine compounds (pK_a values of about 5, [Ye *et al.* 2009]) are partially protonated. The plots of log *k* values for these aromatic compounds on ODS/AP_1 and ODS/AP_2 against those on ODS are shown in Figure 5.7. The solid lines were drawn based on alkylbenzenes with slope of ≈ 1, indicating the similar contribution of the hydrophobic property. As can be seen in Figure 5.7A, aromatic compounds containing hydroxyl groups such as phenol (no. 7), dihydroxybenzenes (no. 8-10), *p*-cresol (no. 11), *p*-chlorophenol (no. 12), *o*-methoxyphenol (no. 13) and also alkyl *p*-hydroxybenzoates (no. 14-17) gave the upper deviation from the solid line. This indicates that ODS/AP_1 column has the preferential retention of the aromatic compounds containing hydroxyl groups due to the dipole-dipole interaction between the hydroxyl groups of the analytes and amino groups on the ODS/AP_1 in addition to only dispersion interaction. Other compounds such as halogenated benzenes (no. 4-6) and alkyl benzoates (no. 18-21) shows linear correlation between two columns, indicating similar behavior on ODS/AP_1 and ODS columns. In comparison with ODS column, the electrochromatographic behavior of the analytes on the ODS/AP_2 column shows the similar trends with the ODS/AP_1 column but the greater retention on the ODS/AP_2 column was observed (Figure 5.7B).

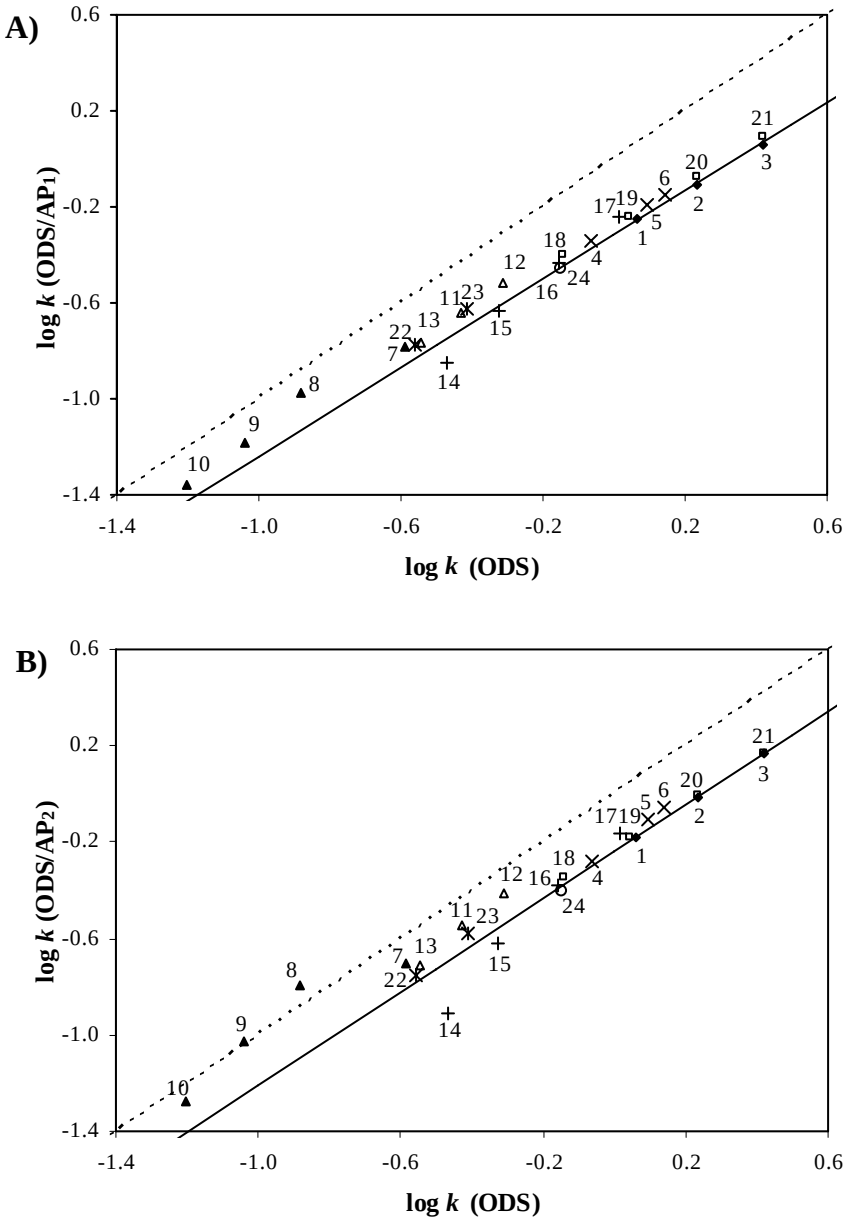


Figure 5.7 Plots of log *k* values for some polar and nonpolar aromatic compounds on ODS/AP_1 (A) and ODS/AP_2 (B) against those on ODS, using 40% ACN in 10 mM phosphate buffer at pH 6.0 mobile phase. Other CEC conditions were shown in Section 5.2.3. The solid lines were drawn based on alkylbenzenes, while the dashed line is diagonal. *Alkylbenzenes*: 1) toluene, 2) ethylbenzene, 3) propylbenzene; *halogenated benzenes*: 4) fluorobenzene, 5) chlorobenzene, 6) bromobenzene; *phenol compounds*: 7) phenol, 8) catechol, 9) resorcinol, 10) hydroquinone, 11) *p*-cresol, 12) *p*-chlorophenol, 13) *o*-methoxyphenol; *alkyl p-hydroxybenzoates*: 14) methyl *p*-hydroxybenzoate, 15) ethyl *p*-hydroxybenzoate, 16) propyl *p*-hydroxybenzoate, 17) butyl *p*-hydroxybenzoate; *alkyl benzoates*: 18) methyl benzoate, 19) ethyl benzoate, 20) propyl benzoate, 21) butyl benzoate; *aromatic amines*: 22) aniline, 23) *p*-toluidine; *other*: 24) nitrobenzene.

5.3.3 Separation of phenol compounds

As previously mentioned in Section5.3.2, the aromatic compounds containing hydroxyl groups seem to have more preference on the mixed-mode ODS/AP_1 and ODS/AP_2 columns. Some phenols compounds including phenol, *o*-methylphenol and three positional isomers with two phenolic hydroxyl groups of hydroquinone, recorsinol and catechol were used as test analytes. Figure 5.8 compares the electrochromatographic separation of these compounds on ODS/AP_1 and ODS/AP_2 columns with that on ODS column under the same condition. Good peak symmetry and comparable column efficiency was observed for the separation of phenol compounds on all three CEC columns. Baseline separation for all five analytes was obtained on ODS/AP_2 column (Figure 5.8C), while partial overlap of three peaks of dihydroxybenzenes was observed on ODS column (Figure 5.8A). This is due to the preferential retention and greater selectivities for these phenol compounds on ODS/AP_2 than ODS columns. For example, the α values of 1.94 and 1.58 on ODS/AP_2 and ODS columns, respectively, for resorcinol/hydroquinone (2/1), and 1.71 and 1.46 on ODS/AP_2 and ODS columns, respectively, for catechol/ resorcinol (3/2). Although ODS/AP_1 column has the preference for phenol compounds with the α values of 1.39 and 1.52 for resorcinol/hydroquinone (2/1) and catechol/ resorcinol (3/2), respectively, partial separation of first two peaks of dihydroxybenzenes was obtained (Figure 5.8B), probably due to the smaller retention factors of these compounds on ODS/AP_1.

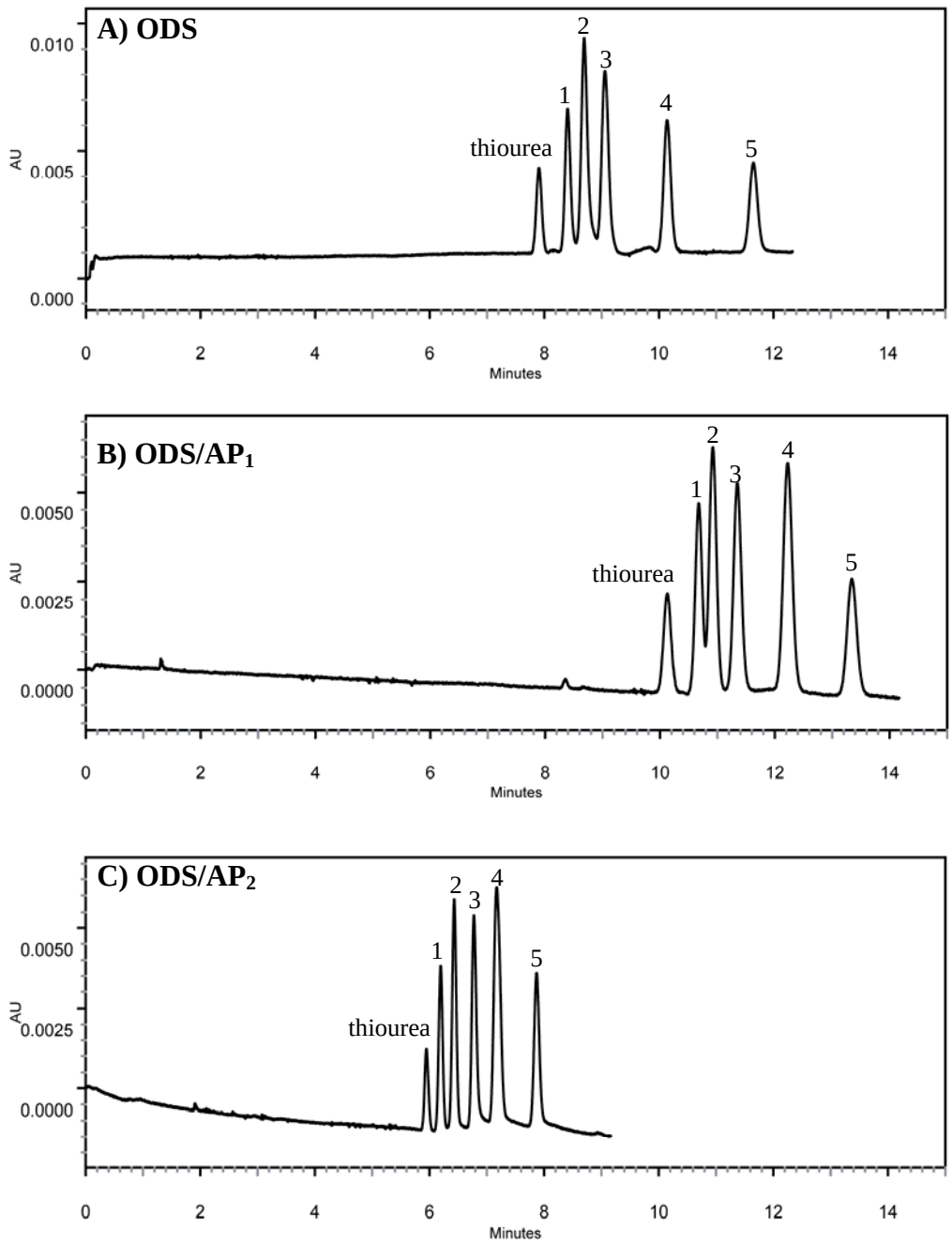


Figure 5.8 Electrochromatograms of a mixture of thiourea (an unretained compound) and phenol compounds on ODS (A), ODS/AP_1 (B), and ODS/AP_2 (C) columns using 35% ACN in 10 mM phosphate buffer at pH 6.0 mobile phase. Analytes: 1) hydroquinone, 2) resorcinol, 3) catechol, 4) phenol and 5) *o*-cresol. Other CEC conditions were shown in Section 5.2.3.

5.4 Conclusions

The monolithic silica capillary columns bonded with the following moieties were prepared: octadecyl (ODS), octadecyl and aminopropyl (ODS/AP_1), and octadecyl and 3-(2-aminoethylamino)propyl (ODS/AP_2). The electrochromatographic performance on these columns was also evaluated in terms of column efficiency and retention properties. For example, the monolithic silica capillary columns gave the plate heights of 3.7, 4.0 and 4.2 μm for ODS, ODS/AP_1 and ODS/AP_2 columns, respectively, using thiourea as a test analyte at *u* of 0.9 mm/s. The greater retention factor of alkylbenzenes on ODS column than ODS/AP_2 and ODS/AP_1 columns, respectively, was obtained. In addition, the slightly greater hydrophobicity was observed on ODS column than that on ODS/AP_1 and ODS/AP_2, while the slightly higher shape selectivity was obtained on ODS/AP_1 and ODS/AP_2 than that on ODS. In comparison with the ODS column, the mixed-mode columns of ODS/AP_1 and ODS/AP_2 showed the preferential retention for aromatic compounds containing hydroxyl groups in CEC. The better separation of some phenol compounds was observed on ODS/AP_2 column than on ODS and ODS/AP_1 columns under the same condition. This can be explained by the dipole-dipole interactions between hydroxyl groups of the analytes and amino groups on ODS/AP_2 columns. Therefore, ODS/AP_2 can be used as a good alternative to reversed-phase ODS columns for the separation of other compounds having hydroxyl groups.

CHAPTER VI

SELECTIVITY OF FULLY CHARGED ACHIRAL COMPOUNDS IN CAPILLARY ELECTROPHORESIS WITH A NEUTRAL CYCLODEXTRIN

6.1 Introduction

CE has become a highly effective laboratory tool for separating charged compounds because of its advantages over HPLC, such as high separation efficiency, automation ability, reduced analysis time, and small solvent and sample consumptions [Chankvetadze 1997a, Khaledi 1998]. According to Equation 2.26, separation selectivity is one of most important characteristics of the separation. This parameter can be expressed by the difference in μ ($\Delta\mu$) or the ratio of μ (α) [Chankvetadze 1997b]. As previously mentioned in Chapter I, CZE separation mechanism is based on the difference in electrophoretic mobility (μ) of the analytes due to the difference in charge-to-size ratios. In case of the separation of two weak acids or weak bases with the same or small different μ, and so a small separation selectivity ($\alpha \approx 1$), it is difficult to reach baseline resolution. The separation in CZE is usually tunable by changing the pH of BGEs [Terabe *et al.* 1988, Wren 1991, Khaledi 1998], resulting in a change in the degree of ionization of the analytes. Theoretically, the optimum pH value is calculated as the average of the pK_a values for two analytes [Terabe *et al.* 1988, Wren 1991]. However, this method is impractical to use for the simultaneous separation of several pairs of analytes having different average pK_a values. The change of the ionic strength of buffers [Harrold *et al.* 1995, Bahga *et al.* 2010], or the use of buffer additives such as ionic liquid [Yu *et al.* 2005], organic solvents [Sarmini and Kenndler 1997, Kenndler 2009], may also be used to modify the separation selectivity because of their influences on the electrophoretic mobility of the analytes and EOF. In addition, MEKC, one of the modes in CE, can be used as a

complementary technique to CZE for a greater control of the separation selectivity [Terabe *et al.* 1984, de Boer *et al.* 1999], as described in Chapter I.

The addition of cyclodextrin(s) into the buffer is one of the methods used to improve CZE separation, so called cyclodextrin modified CZE or CD-CZE. This selector is widely used for CE separation of chiral [Chankvetadze 1997b, Scriba 2008, Chankvetadze 2009, Fanali 2009, Dubský *et al.* 2010] and also achiral compounds [Hsieh and Huang 1996, Luong and Nguyen 1997, Česla *et al.* 2007]. Cyclodextrins (CDs) are cyclic oligosaccharide built of 6, 7, or 8 *D*-(+)-glucopyranose subunits bonded via α-(1,4) linkages, and termed α-, β- and γ-CD, respectively. The structure of CD is shown in Figure 6.1. The physical properties and characteristics of native CDs are summarized in Table 6.1. The structure of a CD molecule in three dimensions resembles a truncated cone with a non-polar cavity. The rim at the top of the cone is lined with secondary (C2, C3) hydroxyl groups, and at the bottom with primary (C6) hydroxyl groups. The central hydrophobic cavity can form inclusion complexes with a variety of guest molecules, such as alkyl or aromatic moieties protiens and peptides, aromatic hydrocarbons and other hydrophobic compounds, as long as there is a match between size and shape of the solute and the CD cavity size [Luong and Nguyen 1997]. Therefore, the separation mechanism in the presence of CD mainly depends on analyte-CD complexation with the different binding constant of analyte to CD and/or the different electrophoretic mobility of the analyte in complex forms.

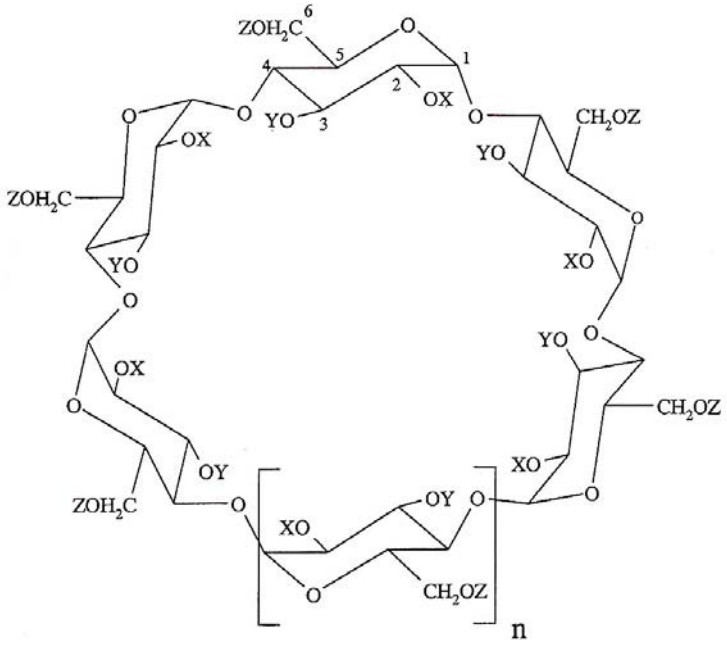


Figure 6.1 Sturcture of cyclodextrins, when n = 1, 2, 3 for α-, β- or γ-CD, respectively, and X, Y and Z = H or CH_3 for native forms or methyl ether derivatives. One ring has carbon numbers.

Table 6.1 Some important physical properties of native cyclodextrins. [Luong and Nguyen 1997]

Characteristics	α	β	γ
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Internal cavity diameter (nm)	0.47-0.52	0.60-0.64	0.75-0.83
External cavity diameter (nm)	1.46±0.05	1.54±0.04	1.75±0.04
Height of torus (nm)	0.79±0.1	0.79±0.1	0.79±0.1
pK_a range of hydroxyl groups	12.1-12.6	12.1-12.6	12.1-12.6
Solubility in water (g/100 mL)	14.5	1.85	23.20
Typical guest molecules	benzene	napthalene	anthracene

From the literature values of the slightly different absolute electrophoretic mobilities (μ^0, 10^{-8} m^2 V^{-1} s^{-1}) for each positional isomer of monochlorobenzoate (C^-) and monomethylbenzoate (M^-) [Li and Lucy 2001], such as -3.16/-3.07, -3.22/-3.11 and -3.17/-3.07 for $2C^-/2M^-$, $3C^-/3M^-$ and $4C^-/4M^-$, respectively, the calculated separation selectivities (μ_2/μ_1) were obtained as 1.03, 1.04 and 1.03 for 2-, 3- and 4-C^-/ M^- isomers, respectively. This indicates the low resolution of each C^-/M^- pair in CZE. Moreover, only partial or no resolution was reported for some pairs of chlorophenoxy acids that differ in only one chloro and methyl substituent on a benzene ring, such as 2,4-dichlorophenoxyacetic acid (2,4-D)/4-chloro-2-methylphenoxyacetic acid (MCPA) and 4-(2,4-dichlorophenoxy) butyric acid (2,4-DB)/ 4-(2-methyl-4-chlorophenoxy) butyric acid (MCPB) [Hsieh and Huang 1996, Farran *et al.* 1999]. Therefore, separation selectivity enhancement is important for the simultaneous separation of these herbicides. Previously, the separation of these chlorophenoxy acid herbicides was reported using different CE approaches: CZE with the addition of an organic modifier for 2,4-D/MCPA [Farran *et al.* 1999], CD-CZE with the addition of different CDs for 2,4-D/MCPA, 2,4-DB/MCPB and other three chlorophenoxy acids [Hsieh and Huang 1996, Farran *et al.* 1999, Nielen 1993], MEKC with the addition of surfactants and an organic modifier for 2,4-D/MCPA [Farran *et al.* 1999], and CD-MEKC for 2,4-D/MCPA and other two phenoxy acids [Fung and Mak 2001].

In order to predict the optimum CE condition for chiral separation with the presence of CD, most of previous works have been focused on the theoretical models of separation selectivity (α) using either a neutral [Wren and Rowe 1992, Rawjee *et al.* 1993a, Rawjee *et al.* 1993b] or a charged CD [Williams and Vigh 1997, Yang *et al.* 2001]. In most case for fully charged enantiomers with a neutral CD, the separation selectivity increases to a maximum value, and, then decrease at higher CD concentrations (*C*) [Wren and Rowe 1992, Rawjee *et al.* 1993b, Penn *et al.* 1993, Penn *et al.* 1994, Hammitzsch-Wiedemann and Scriba 2009]. At low and high *C*, the reversal of $|\mu|$ order of some enantiomers may be obtained depending on the effects of the binding constants and μ of analyte-CD complexes [Hammitzsch-Wiedemann and

Scriba 2009]. For the neutral or charged enantiomers with a charged CD, the optimization of the separation can be predicted using the charged resolving agent migration model [Williams and Vigh 1997]. Previously, the charged resolving agent migration model was generalized in order to explore the possible separation selectivity patterns for cationic enantiomers [Zhu and Vigh 2000a], noncharged enantiomers [Zhu *et al.* 2000], and anionic enantiomers [Zhu and Vigh 2000b]. The optimization separation approach is different for different separation selectivity patterns.

For CE separation of achiral compounds, the use of a CD may result in an enhancement of the separation for some pairs of analytes or a loss of separation for other pairs, and consequentially the same or the reversed $|\mu|$ order of analytes may be obtained [Hsieh and Huang 1996, Česla *et al.* 2007], in comparison with CZE without CD. Several theoretical models for enantiomeric separation cannot be directly applied for achiral CE separation due to the typically different μ_0 for achiral compounds but the same μ_0 for enantiomers, where μ_0 is the effective electrophoretic mobility of free analyte. Recently [Zakaria *et al.* 2002], a migration model for CE separation of the aromatic bases has been described as a function of the pH and the neutral CD concentration.

This chapter consists of two main objectives: i) to compare of separation selectivity of CE for selected analytes and ii) to develop selectivity patterns of fully charged achiral compounds in CE with neutral CD. Using different additives in a borate buffer at pH 9.2, the separation selectivity was investigated and then compared for a pair of benzoic acid derivatives that differ only in the chloro and methyl substituents: i) CZE with different types and concentrations of organic solvents, ii) MEKC with different SDS concentrations, and different types and concentrations of organic modifiers, and iii) CD-CZE with different types and concentrations of CDs. In addition, the simultaneous CE separation was also optimized for these benzoic acid derivatives and ten chlorophenoxy acid herbicides, including 2,4-D/MCPA, 2,4-DB/MCPB and other six chlorophenoxy acids. The structures of chlorophenoxy acids are shown in Table 3.1. In addition, a general separation selectivity equation was developed and

separation selectivity patterns was also predicted for fully charged achiral compounds with a neutral CD in order to explain a change in separation selectivity and |μ| order for fully charged achiral compounds over a wide range of *C*. Moreover, the existence of these patterns was experimentally verified using positional isomers of chlorobenzoic acids and methylbenzoic acids, and phenoxy acids as test analytes and α-CD as a neutral CD.

6.2 Experimental

6.2.1 Chemicals

All reagents were of analytical grade. Disodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), and sodium hydroxide (NaOH) were purchased from Fluka (Buchs, Switzerland); thiourea, methanol (MeOH) and acetonitrile (ACN) were from Merck (Darmstadt, Germany); sodium dodecyl sulfate (SDS), α-CD, β-CD and heptakis-(2,6-di-*O*-methyl)-β-CD (dimethyl-β-CD or DM-β-CD) were from Sigma (St. Louis, MO, USA); mesityl oxide was from Aldrich (WI, USA); dodecylbenzene (DB) and the following benzoic acid derivatives with chloro and methyl groups were from Sigma-Aldrich (Steinheim, Germany): 2-, 3-, 4-methylbenzoic acid (M); 2-, 3-, 4-chlorobenzoic acid (C); 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, 3,5-dimethylbenzoic acid (DM); 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, 3,5-dichlorobenzoic acid (DC). Four phenoxy acid standards were obtained from Riedel de Haën (Seelze, Germany) and Dr. Ehrenstorfer GmbH (Augsburg, Germany): MCPA, MCPB, 2,4-D, 2,4-DB, 2,4,5-T, acifluorfen, clopyralid, dicamba, mecoprop, and picloram.

6.2.2 Preparation of running buffers and analytes

CE measurements were carried out in two buffer systems: CZE and MEKC. CZE buffers contained 10 mM $Na_2B_4O_7$ at pH 9.2, and included the addition of the organic modifiers, MeOH and ACN, at 0-20% (v/v) or the addition of different CD: α-CD (2-60 mM), β-CD (2-16 mM) or DM-β-CD (2-50 mM), while MEKC buffer contained

10 mM $Na_2B_4O_7$ at pH 9.2 and included the addition of SDS (20-80 mM), or the addition of 40 mM SDS and various concentration (0-20% (v/v)) organic modifiers (MeOH or ACN).

Stock solutions of each test analyte at 5.0 mM were separately dissolved in methanol and then diluted with purified water. For CZE separation, the sample mixtures containing each analyte at 0.1 mM and 50 ppm mesityl oxide were obtained by pipetting each stock solution and then diluting the mixture with 1.0 mM $Na_2B_4O_7$. For MEKC separation, 50 ppm thiourea was used as the EOF marker and 100 ppm DB as the micelle marker in the sample mixtures. All sample solutions and buffers were sonicated and filtered through 0.45 μm membrane filters prior to CE analysis.

6.2.3 CE instrumentation and conditions

All CE separations were performed on a Beckman Coulter MDQ CE system equipped with a photo-diode array scanning from 190 to 300 nm and monitoring at 214 nm, and with an uncoated fused-silica column (Polymicro Technologies, Phoenix, AZ, USA) of 40.2 cm in length (30 cm to detector) × 50 μm i.d., and thermostatted at 25 ºC. The applied voltage was set at 15 kV. Each sample solution was introduced by 0.5 psi pressure injection for 3 s. Prior to analysis each day, the capillary was first rinsed with 0.1 M NaOH and then with the buffer for 10 min each. Between consecutive runs, the capillary was rinsed with 0.1 M NaOH and then the buffer for 3 min each. Each experiment was carried out in duplicate.

6.2.4 Determination of binding constants of CD:analytes complexes [Chankvetadze 1997a]

CE has been used as a method for determining binding constants of host:guest complexes through a measurement of the change in μ of the guest molecules as a function of the concentration of host present in the BGE.

In the host:guest binding process between analyte A (guest) and CD (host), binding equilibrium is represented by

$$A + CD \rightleftharpoons CDA \tag{6.1}$$

The binding constant, *K*, for this process is defined as;

$$K = \frac{[CDA]}{[CD][A]} \tag{6.2}$$

where [*i*] is the concentration of species *i* at equilibrium. The mass balance equations are

$$C_{CD} = [CD] + [CDA] \tag{6.3}$$

$$C_A = [A] + [CDA] \tag{6.4}$$

where C_i is the total concentration of species *i*. In the presence of CD in the BGE, the net electrophoretic mobility of the analyte, μ, is given by

$$\mu_A = x_A\mu_A + x_{CDA}\mu_{CDA} \tag{6.5}$$

where x_i is the mole fraction and μ_i is the electrophoretic mobility of species *i*. It follows that

$$\mu = \frac{\mu_A + K[CD]\mu_{CDA}}{1 + K[CD]} \tag{6.6}$$

With known μ_A and [CD], the binding constant may be calculated by fitting the mobilities of the analyte ion as a function of CD concentration.

6.2.5 Relative viscosity

In CE system, relative viscosities of the BGE were determined by measuring the time needed under pressure flushing for each solution, containing benzoic acid as an absorbance marker, to travel from the injection end to the detector. The ratio of the times, t_c/t_0, for BGE with a given CD concentration to BGE without CD gives η_c/η_0 [Peng *et al.* 1997]. These values were subsequently fitted to a power series in CD concentration (mM) as follow;

$$(\eta_c/\eta_0) = 1 + 2.303\times10^{-3}[\alpha\text{-CD}] + 1.72\times10^{-5}[\alpha\text{-CD}]^2 \quad ; r^2 = 0.9974 \quad (6.7)$$

$$(\eta_c/\eta_0) = 1 + 2.638\times10^{-3}[\beta\text{-CD}] + 6.80\times10^{-6}[\beta\text{-CD}]^2 \quad ; r^2 = 0.9943 \quad (6.8)$$

$$(\eta_c/\eta_0) = 1 + 3.624\times10^{-3}[\text{DM-}\beta\text{-CD}] + 1.41\times10^{-5}[\text{DM-}\beta\text{-CD}]^2 \quad ; r^2 = 0.9997 \quad (6.9)$$

Equations 6.8 and 6.9 were obtained from Nhujak [2001]. Since the observed electrophoretic mobility (μ_{obs}, calculated using Equation 2.4) depends on the viscosity of the BGE containing CD [Penn *et al.* 1995, Nhujak *et al.* 2001], relative viscosities were used as a factor for correction of electrophoretic mobility as the following equation,

$$\mu = \mu_{obs}\frac{\eta_c}{\eta_0} \quad (6.10)$$

6.3 Results and discussion

6.3.1 Comparison of separation selectivity of CE for chlorobenzoates and methylbenzoates, and its application to separation of chlorophenoxy acid herbicides

6.3.1.1 CZE with the addition of organic solvent

Figure 6.2A shows representative CZE electropherograms of three individual pairs of monosubstituted benzoates: $2C^-/2M^-$, $3C^-/3M^-$ and $4C^-/4M^-$, using 10 mM borate buffer at pH 9.2 without the addition of any organic modifier. With pK_a < 4.9 for all test analytes [Cessna and Grover 1978, Dean 1987, Hsieh and Huang 1996], each analyte in the pH 9.2 buffer used here carries a fully negative charge and, therefore, each analyte migrates after the EOF marker with an effective electrophoretic mobility ($|\mu|$) order of $C^- > M^-$. In accordance with the slight difference in the observed μ values (10^{-8} m^2 V^{-1} s^{-1}, %RSD < 1.0) of the C^-/M^- (Table 6.2), each pair of C^-/M^- positional isomers migrate closely to each other. Indeed, the baseline resolution of C^-/M^- for all pairs was not achieved, R_s < 1.5 (Figure 6.2A). The CZE separations of the disubstituted benzoates DC^-/DM^- (2,3-, 2,4-, 2,5-, 2,6-, 3,4 and 3,5-isomers) (Figure 6.2B) revealed $|\mu|$ values in the order $DC^- > DM^-$ (Table 6.2), similar to the $|\mu|$ order of $C^- > M^-$. The baseline resolution of DC^-/DM^- was achieved for the 2,6-, 3,4- and 3,5-isomers (R_s = 1.53-1.72), while in contrast it was not achieved for the 2,3-, 2,4- and 2,5-isomers (R_s = 0.88-1.42) (Figure 6.2B).

From Figure 6.2A, the obtained CZE separation selectivities (α_{CZE}, defined as the ratio of μ in CZE for two analytes) were 1.018, 1.025 and 1.022 for the 2-, 3- and 4-C^-/M^- isomers, respectively, which show the same trend but slightly lower values than those of in the literature calculated using μ^0, as previously mentioned in Section 6.1. The values of α_{CZE} for disubstituted benzoates were obtained in the range of 1.019-1.038, which being close to 1.0 indicates a very small separation selectivity or small difference in μ. According to Equaion 2.26, the most important parameter to

enhance R_s is the separation selectivity. The higher the value of α, the better the value of R_s. The resolution also scales with the mobility term ($\left|\mu_2/(\overline{\mu}+\mu_{eo})\right|$) and the square root of the efficiency.

In this work, the effect of organic solvents upon α_{CZE} was studied by separately adding 10-20% (v/v) MeOH or ACN into 10 mM $Na_2B_4O_7$ buffer. With increasing organic modifier concentrations, the same $|\mu|$ order was obtained but with a longer migration time. The effect of MeOH on α_{CZE} and R_s for monosubstituted benzoates (C^-/M^-) is shown in Figure 6.3A. Although a slight decrease in α_{CZE} was obtained with increasing levels of MeOH in the buffer (Figure 6.3A1), a better R_s was observed (Figure 6.3A2) because of the slightly higher values of the mobility term and the greater $\overline{N}$. A similar result with ACN was also observed using ACN (Figure 6.3B).

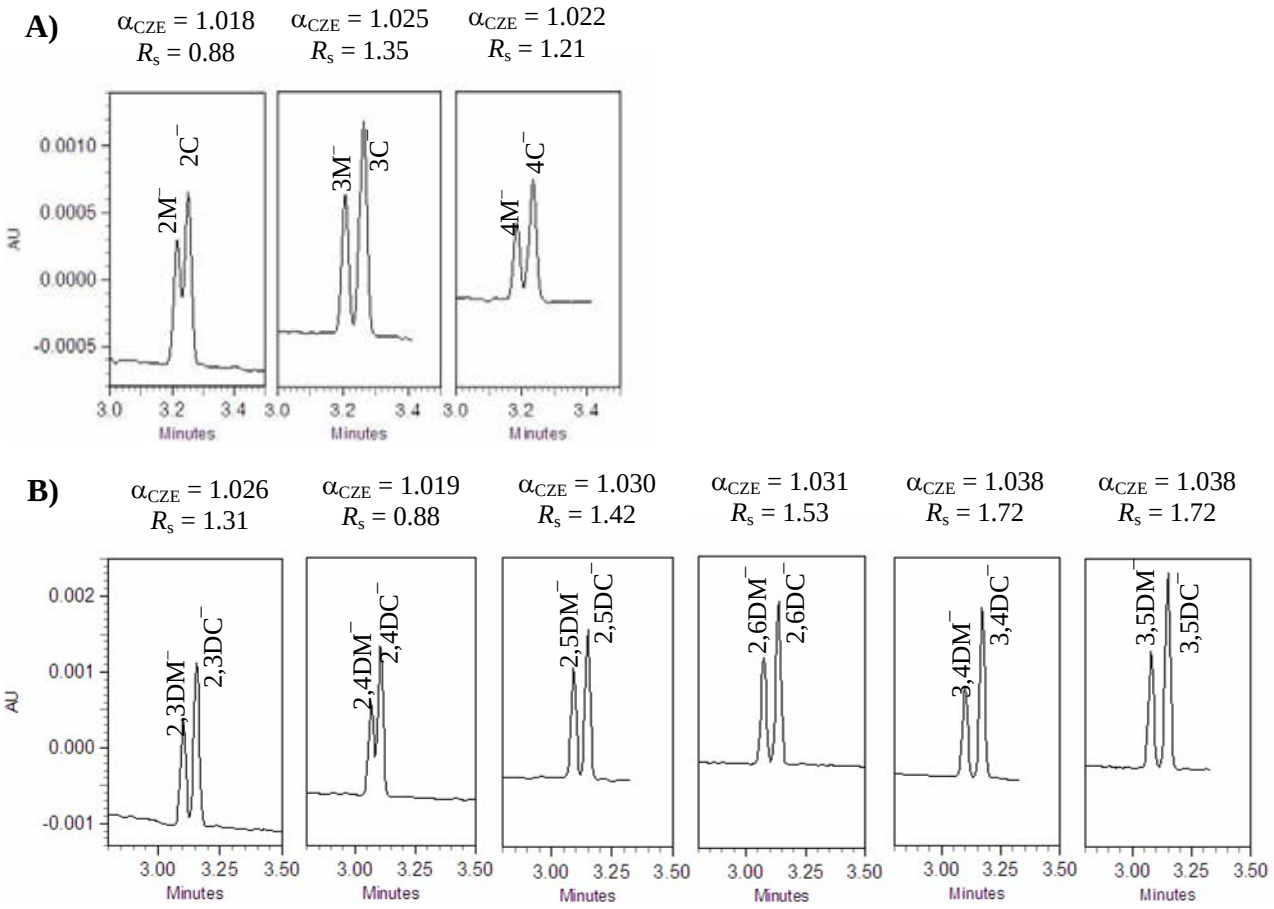


Figure 6.2 Electropherograms for CZE separation of (A) chlorobenzoate/methylbenzoate; C^-/M^- and (B) dichlorobenzoate/dimethylbenzoate; DC^-/DM^-. CZE buffer contained a 10-mM borate ($Na_2B_4O_7$) buffer at pH 9.2. Other CE conditions were shown in Section 6.2.3.

Table 6.2 Mobilities (μ, 10^{-8} m^2 V^{-1} s^{-1}), CZE mobility selectivities ($\alpha_{CZE} = \mu_{CZE,\,2}/\mu_{CZE,\,1}$), MEKC mobility selectivities ($\alpha_{MEKC} = \mu_{MEKC,\,2}/\mu_{MEKC,\,1}$) and retention selectivities ($\alpha_k = k_2/k_1$) for C^-/M^-, DC^-/DM^-, 2,4-DB/MCPB and 2,4-D/MCPA in CZE and MEKC without the addition of organic modifiers.

Analytes	CZE[a]		MEKC[b]			
	μ	α_{CZE}	μ_{MEKC}	α_{MEKC}	k[c]	α_k
$2C^-/2M^-$	-2.83/-2.78	1.018	-2.64/-2.59	1.019	0.47/0.45	1.031
$3C^-/3M^-$	-2.86/-2.79	1.025	-2.67/-2.60	1.027	0.47/0.44	1.061
$4C^-/4M^-$	-2.82/-2.76	1.022	-2.64/-2.57	1.023	0.46/0.44	1.048
$2,3DC^-/2,3DM^-$	-2.74/-2.67	1.026	-2.47/-2.40	1.029	0.40/0.38	1.050
$2,4DC^-/2,4DM^-$	-2.67/-2.62	1.019	-2.41/-2.36	1.021	0.38/0.37	1.036
$2,5DC^-/2,5DM^-$	-2.73/-2.65	1.030	-2.48/-2.40	1.033	0.41/0.38	1.053
$2,6DC^-/2,6DM^-$	-2.70/-2.62	1.031	-2.45/-2.37	1.034	0.41/0.39	1.056
$3,4DC^-/3,4DM^-$	-2.75/-2.65	1.038	-2.52/-2.41	1.046	0.43/0.39	1.103
$3,5DC^-/3,5DM^-$	-2.72/-2.62	1.038	-2.50/-2.39	1.046	0.36/0.33	1.099
2,4-DB/MCPB	-2.23/-2.24	1.004	-2.25/-2.23	1.009	0.73/0.71	1.049
2,4-D/MCPA	-2.51/-2.49	1.008	-2.35/-2.32	1.011	0.76/0.74	1.005

[a]CZE buffer contained a 10 mM borate buffer at pH 9.2 (%RSD of μ < 1.0).

[b]MEKC buffer contained 40 mM SDS in a 10 mM borate buffer at pH 9.2 (%RSD of μ < 1.0).

[c]The retention factor, *k*, in MEKC can be calculated using the equation,

$k = \dfrac{t_R - t_{eo}}{t_{eo}\left(1 - t_R / t_{mc}\right)}$, where t_R, t_{eo} and t_{mc} are the migration time of the analytes, EOF marker and micelle marker, respectively.

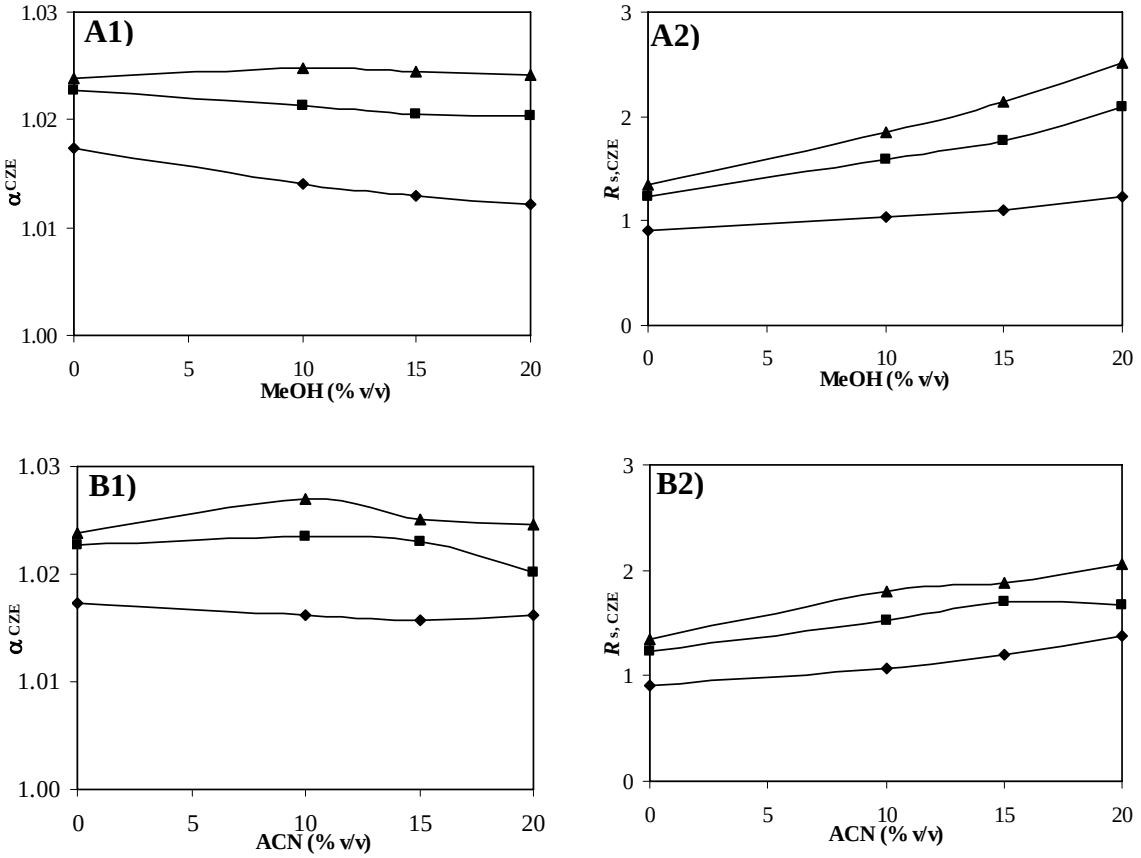


Figure 6.3 Effect of organic modifier in the CZE buffer on CZE mobility selectivity (α_{CZE}) (A1, B1) and resolution (A2, B2) of C^-/M^- ($2C^-/2M^-$ (◆), $3C^-/3M^-$ (▲) and $4C^-/4M^-$ (■)): (A) MeOH and (B) ACN. The indicated organic solvent concentration (% (v/v)) was added to 10 mM $Na_2B_4O_7$ buffer (pH 9.2). All other CE conditions were shown in Section 6.2.3.

6.3.1.2 MEKC with the addition of organic solvent

Using an MEKC buffer containing 40 mM SDS in a 10 mM $Na_2B_4O_7$ buffer at pH 9.2, anionic micelles and negatively charged solutes migrate towards the cathode with the presence of high EOF. As shown in Table 6.2, the migration of analytes in this MEKC buffer were found in the $|\mu|$ order $C^- > M^-$ and $DC^- > DM^-$, which is a similar $|\mu|$ order to that seen in CZE. Typically, the charged analytes in MEKC are separated due to the differences in their micellar partitioning and their electrophoretic mobility (α_{CZE}).

Comparing the μ and α values between MEKC and CZE (Table 6.2), the very slightly higher α_{MEKC} than α_{CZE} is due to small difference in the partitioning of the analytes between the micellar and aqueous phases or the small α_k. In comparison with CZE, the slightly better R_s for C^-/M^- and DC^-/DM^- in MEKC was obtained due to the slightly higher α_{MEKC} than α_{CZE} and the greater mobility term for MEKC with comparable N. In most cases, the baseline resolution in MEKC was achieved for a pair of C^-/M^- (R_s = 1.59-1.77) and DC^-/DM^- (R_s = 1.73-2.67), with the exception of $2C^-/2M^-$ (R_s = 1.09) and $2,4DC^-/2,4DM^-$ (R_s = 1.25).

Over a 20-80 mM range of SDS, the α_{MEKC} values for each pair of C^-/M^- was insignificantly different (Figure 6.4). As an example, Figure 6.5 shows the effect of increasing MeOH concentrations in MEKC buffer on α_{MEKC} and R_s, respectively, for C^-/M^-. An increase in the MeOH concentration gives a very slight decrease in α_{MEKC} (Figure 6.5A1) but a better R_s (Figure 6.5A2) in MEKC, which is similar to the trend found in CZE with the addition of organic solvents. A similar result with ACN was also observed using ACN (Figure 6.5B).

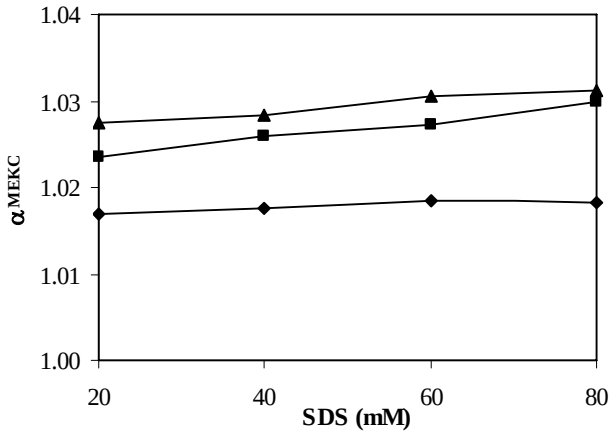


Figure 6.4 Effect of SDS concentration (20-80 mM) in the MEKC buffer on MEKC mobility selectivity (α_{MEKC}) of C^-/M^- ($2C^-/2M^-$ (◆), $3C^-/3M^-$ (▲) and $4C^-/4M^-$ (■)). All other CE conditions were shown in Section 6.2.3.

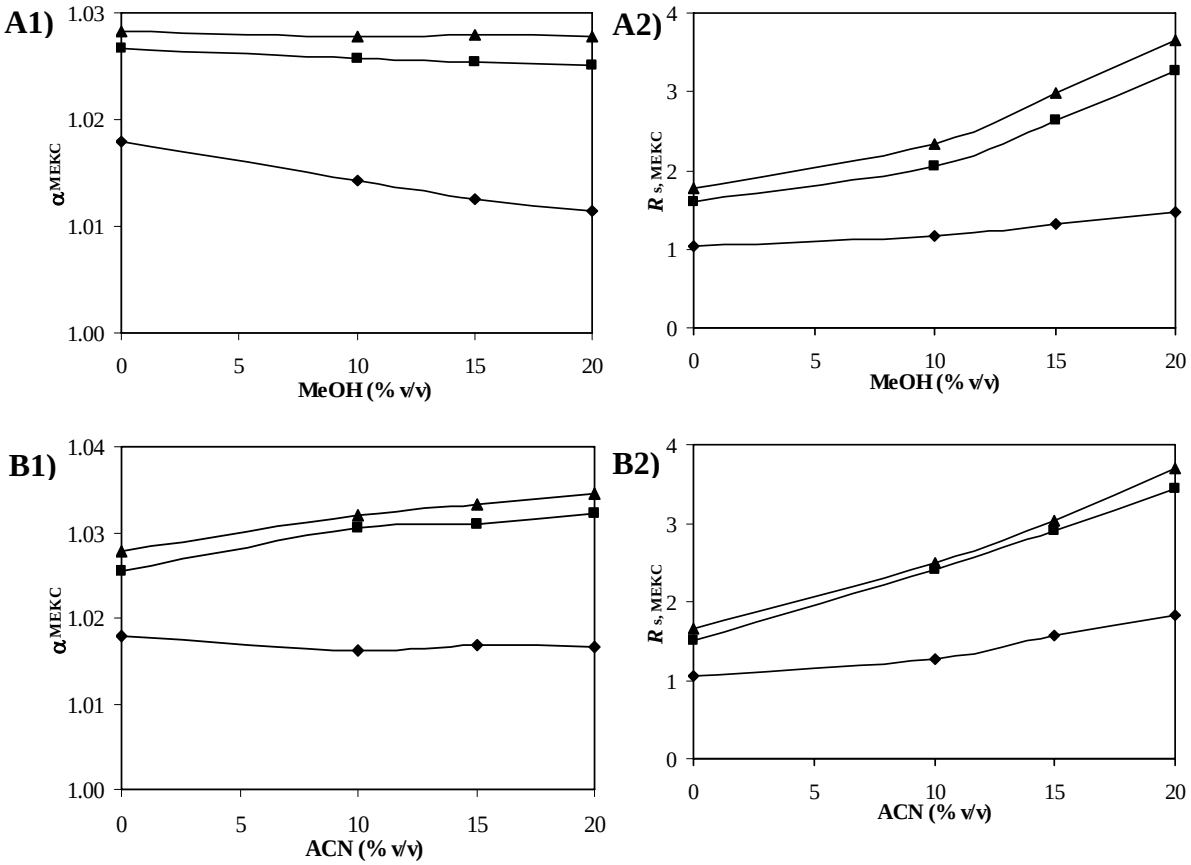


Figure 6.5 Effect of organic modifier in the MEKC buffer on MEKC mobility selectivity (α_{MEKC}) (A1, B1) and resolution (A2, B2) of C^-/M^- ($2C^-/2M^-$ (◆), $3C^-/3M^-$ (▲) and $4C^-/4M^-$ (■)): (A) MeOH and (B) ACN. The indicated organic solvent concentration (% (v/v)) was added to 10 mM $Na_2B_4O_7$ buffer (pH 9.2) with 40 mM SDS. All other CE conditions were shown in Section 6.2.3.

6.3.1.3 CD-CZE with different types and concentrations of CD

In order to enhance the separation selectivity of the charged analytes, α-CD (2-60 mM), β-CD (2-16 mM) and DM-β-CD (2-50 mM) were separately added into the 10 mM borate buffer (pH 9.2). It should be noted that 16 mM for β-CD is the saturated solubility in water. The addition of 60 mM α-CD or 50 mM DM-β-CD achieved a baseline resolution for all isomers pairs, with a R_s > 2.4 and > 4.5, except for the co-elution of 2,6DC$^-$/2,6DM$^-$ in 60 mM α-CD, and the co-elution of 2C$^-$/2M$^-$ and 2,6DC$^-$/2,6DM$^-$ in 50 mM DM-β-CD. In the presence of CDs in the buffer, CD-CZE separation of the two charged analytes arises from the differences in their binding constants (*K*) to CD and/or their electrophoretic mobilities. The different abilities of CDs to form an inclusion complex with each analytes is governed by several factors such as the match between the cavity size of the CD and the molecular size of the analytes, hydrophobic interactions and hydrogen bonding interactions [Chankvetadze 1997a].

Table 6.3 shows the values of *K*, CD binding selectivity (κ, defined as the ratio of *K* for two analytes *e.g.* K_2/K_1, κ>1) and the observed maximum selectivities in CD-CZE ($\alpha_{CD,\ max}$) for each pair of C$^-$/M$^-$ or DC$^-$/DM$^-$ analytes, using 2-60 mM α-CD, 2-16 mM β-CD or 2-50 mM DM-β-CD. As previously described in Section 6.2.4, the *K* and μ_{CDA} values were determined using the CEfit program of a nonlinear least-squares fit to the data points of the corrected electrophoretic mobilities after correction for the changes in the buffer viscosity, as a function of the CD concentration [Penn *et al.* 1995, Nhujak and Goodall 2001]. The calculated μ_{CDA} for weakly-binding analytes was obtained from the average of μ_{CDA} values of other positional isomers and was then used to calculate *K*, as previously suggested [Ferguson *et al.* 1997]. The greater observed *K* values for the chlorosubstituted than the methylsubstituted benzoates in each isomer indicates the stronger CD binding to C$^-$ or DC$^-$ than to M$^-$ or to DM$^-$, respectively. This is possibly due to the stronger hydrophobicity of the chlorosubstituted than the methylsubstituted benzoates to the CD cavity, as seen from the higher *k* values in MEKC for C$^-$ than M$^-$ and for DC$^-$ than DM$^-$. In addition, the

steric effect of large methyl group than chloro group may reduce the inclusion complexing for M^- or DM^- to CD. In comparison with other isomers, that $2C^-$, $2M^-$, $2,6DC^-$ and $2,6DM^-$ have a weaker binding to CD is possibly due to the steric effect of the substituents adjacent to the $-COO^-$ group.

Depending on the match between the interior size of CD (noted that β-CDs are larger than α-CDs) and the molecular size of the analytes, a higher *K* in α-CD than β-CD for C^- and DC^- was observed, except for $2C^-$, $2,3DC^-$, $2,6DC^-$ and $3,4DC^-$, while a higher *K* in β-CD for M^- and DM^- was obtained except for $3,5DM^-$. For native β-CD and its derivative, the smaller *K* value in DM-β-CD than β-CD for all analytes was observed and this is likely to be due to the steric hindrance of the methyl groups on the upper rim of the DM-β-CD cone. In most cases, α-CD and DM-β-CD gave higher binding selectivities ($\kappa = K_2/K_1$) than did β-CD, implying that α-CD and DM-β-CD are better selectors for separation of C^-/M^- and DC^-/DM^-. The κ values range from 1.0 to 21.2, which indicates that the position of the substituents affects on the difference in the CD binding of the substituted benzoates.

Figure 6.6 shows an example of mobility selectivity in CD-CZE (α_{CD}, defined as the ratio of μ in CD-CZE for two analytes) for $4M^-/4C^-$ and $3,5DM^-/3,5DC^-$ at various concentrations of added α-, β- or DM-β-CDs. It should be noted that the values of α_{CD} in these plots were calculated from the ratio of μ for M^-/C^- and that for DM^-/DC^- over a wide range of CD concentrations, and therefore, α_{CD} values of ≥ 1.0 and ≤ 1.0 can be obtained. As can be seen, the values of α_{CD} start from less than 1.0 in the absence of CD, due to the higher $|\mu|$ for C^- than M^- or for DC^- than DM^-, and then increase to more than 1.0 as the concentration of CD in the buffer increases. With respect to CZE, the CD-CZE resulted in a reversed order of $|\mu|$ for M^-/C^- and DC^-/DM^- because of the stronger binding of C^- or DC^- than M^- or DM^-, respectively, that leads to a greater decrease in the apparent $|\mu|$ of C^- or DC^- than M^- or DM^- [Hammitzsch-Wiedemann and Scriba 2009]. With increasing CD concentrations, the

values of α_{CD} increased to a maximum value and then decrease at higher CD concentrations.

In comparison with CZE and MEKC, CD-CZE gave a higher separation selectivity ($\alpha_{CD} > \alpha_{MEKC} > \alpha_{CZE}$) for C^-/M^- and DC^-/DM^-, except for $2C^-/2M^-$ with DM-β-CD, and $2,6DC^-/2,6DM^-$ with α-CD, β-CD or DM-β-CD. In most cases, a better R_s for C^-/M^- and DC^-/DM^- was achieved with CD-CZE than with CZE and MEKC. Using a wide range of CD concentrations (2-60 mM α-CD, 2-16 mM β-CD or 2-50 mM DM-β-CD), the baseline resolution was achieved for each isomer pair of C^-/M^- (R_s = 1.51-15.8) and DC^-/DM^- (R_s = 1.66-28.5), except for $2C^-/2M^-$ and $2,6DC^-/2,6DM^-$ with β-CD or DM-β-CD.

Table 6.3 Binding constants (K, M^{-1}), binding selectivities ($\kappa = K_1/K_2$), maximum mobility selectivities ($\alpha_{CD,\,max} = \mu_{CD,\,2}/\mu_{CD,\,1}$) for C^-/M^-, DC^-/DM^-, 2,4-DB/MCPB and 2,4-D/MCPA in CD-CZE.

Analytes	CD-CZE[a]								
	α-CD (0 to 60 mM)			β-CD (0 to 16 mM)			DM-β-CD (0 to 50 mM)		
	K	κ	$\alpha_{CD,\,max}$ ([CD][b])	K	κ	$\alpha_{CD,\,max}$ ([CD][b])	K	κ	$\alpha_{CD,\,max}$ ([CD][b])
$2C^-/2M^-$	2.7±0.5 / 2.9±0.5	1.07	1.036 (60)	6.5±0.5 / 4.8±0.3	1.35	1.021 (16)	< 1.0 / <1.0	≈1.0	1.018 (0)
$3C^-/3M^-$	80.7±2.0 / 22.3±0.6	3.62	1.408 (50)	70.8±0.4 / 29.7±0.2	2.38	1.183 (16)	30.7±0.8 / 8.4±0.2	3.65	1.404 (50)
$4C^-/4M^-$	129.0±3.0 / 27.5±0.6	4.69	1.444 (30)	104.7±0.6 / 84.8±0.3	1.23	1.048 (14)	45.4±1.1 / 29.5±0.5	1.54	1.264 (40)
$2,3DC^-/2,3DM^-$	3.5±0.9 / 2.0±0.8	1.75	1.074 (60)	52.9±0.5 / 45.0±0.9	1.18	1.081 (16)	15.2±0.4 / 8.2±0.5	1.85	1.417 (50)
$2,4DC^-/2,4DM^-$	204.5±3.8 / 35.1±0.8	5.83	1.452 (20)	79.7±0.8 / 50.0±0.6	1.59	1.141 (16)	22.7±0.5 / 15.7±0.5	1.45	1.260 (50)
$2,5DC^-/2,5DM^-$	97.3±2.9 / 37.2±1.1	2.62	1.247 (30)	80.5±0.6 / 43.3±0.7	1.86	1.206 (16)	21.0±0.6 / 2.2±0.3	9.55	1.241 (50)
$2,6DC^-/2,6DM^-$	1.2±0.2 / 1.1±0.4	1.09	1.031 (0)	4.5±0.5 / 4.3±0.7	1.05	1.031 (0)	<1.0 / <1.0	≈1.0	1.031 (0)
$3,4DC^-/3,4DM^-$	14.5±2.8 / 2.1±0.5	6.90	2.025 (60)	404.1±3.1 / 167.6±1.2	2.41	1.217 (6)	203.6±3.5 / 68.5±1.2	2.97	1.679 (50)
$3,5DC^-/3,5DM^-$	191.9±5.8 / 38.8±1.3	4.95	1.444 (20)	66.5±0.1 / 6.2±1.4	10.7	1.298 (16)	40.3±0.9 / 1.9±0.3	21.2	1.627 (50)
2,4-DB/MCPB	381.5±12.0 / 235.9±4.9	1.61	1.157 (6)	434.8±9.8 / 460.4±7.1	1.06	1.027 (10)	211.8±4.2 / 275.4±6.6	1.30	1.077 (6)
2,4-D/MCPA	274.3±7.8 / 197.3±6.0	1.39	1.072 (6)	108.2±1.6 / 110.6±1.3	1.02	1.037 (10)	41.9±1.1 / 59.1±1.1	1.41	1.178 (50)

[a]CD-CZE buffer contained different CDs in a 10 mM borate buffer at pH 9.2.

[b]CD concentrations (mM) giving the observed $\alpha_{CD,\,max}$ in parenthesis.

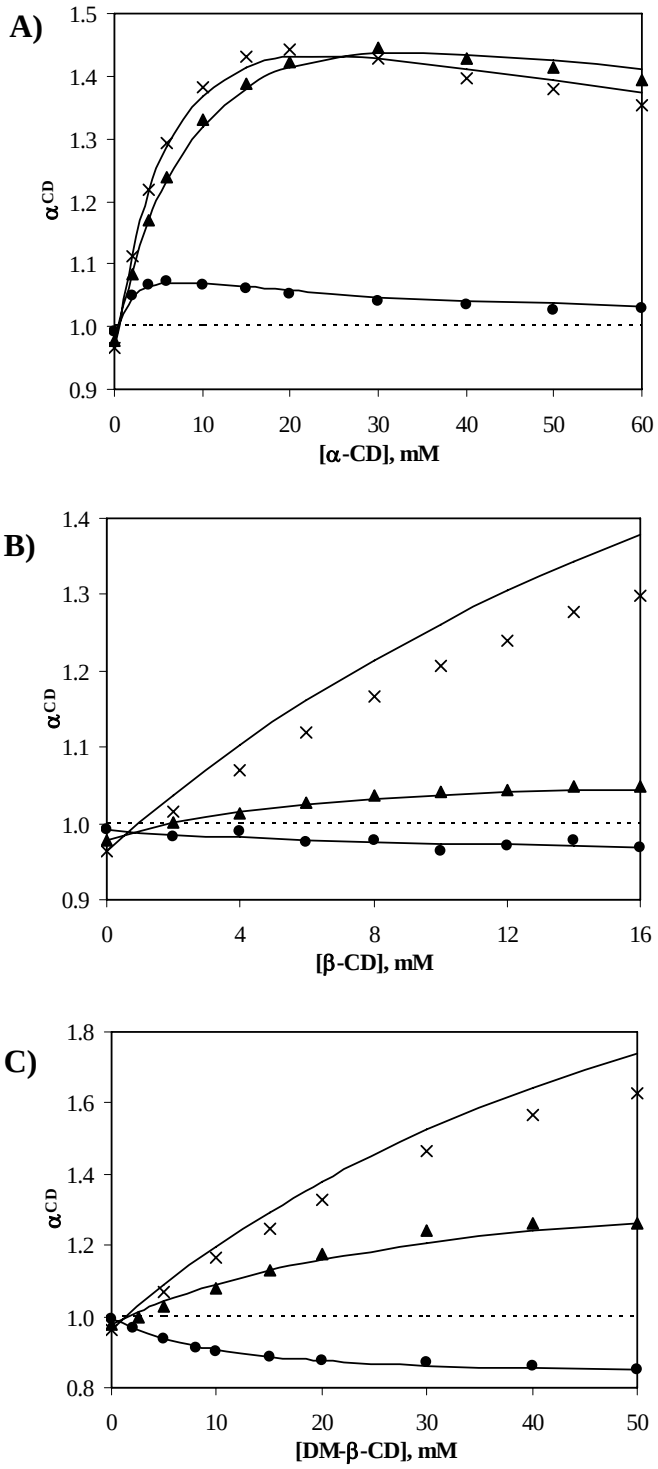


Figure 6.6 Observed (symbols) and predicted (solid) values of CD-CZE mobility selectivity (α_{CD}) for $4M^-/4C^-$ (▲), $35DM^-/35DC^-$ (×) and MCPA/24D (●) in CD-CZE using various CD types and concentrations: (A) α-CD, (B) β-CD and (C) DM-β-CD. A CD-CZE buffer contained a 10-mM borate buffer at pH 9.2 and CD. Other CE conditions are shown in Section 6.2.3. Predicted α_{CD} values were obtained by the ratio of μ for two analytes, using the calculated μ as given in Equation 2.24.

6.3.1.4 Simultaneous separation of positional isomers of chlorobenzoates and methylbenzoates

In CZE with 10 mM borate buffer (Figure 6.7A), the co-elution of three pairs of C^-/M^- was observed due to a similar μ for each positional isomer C^-/M^-. As can be seen from Figure 6.7B using an MEKC buffer containing 40 mM SDS, a slightly better separation was obtained for positional isomeric separation of the C^-/M^- analytes because of the slightly higher α_k than α_{CZE} for each pair of C^-/M^- isomers. In comparison with CZE (Figure 6.7A) and MEKC (Figure 6.7B), the reversed apparent $|\mu|$ order in CD-CZE for $M^- > C^-$, particularly at 20 mM α-CD (Figure 6.7C1), 16 mM β-CD (Figure 6.7C2) or 5.0 mM DM-β-CD (Figure 6.7C3), was observed, except for $2C^-/2M^-$ with 20 mM α-CD and 5.0 mM DM-β-CD. This is due to the stronger binding of C^- than M^- to neutral CD, results in a lower apparent $|\mu|$ of the analyte and its complex. In comparison with CZE and MEKC, CD-CZE gave a better simultaneous separation for C^-/M^- isomers with a similar μ due to the greater α_{CD} that results from the higher κ than α_{CZE} and α_k. Over the range of 15-60 mM α-CD (that for 20 mM is shown in Figure 6.7C1) in the buffer, CD-CZE achieved a baseline resolution for the simultaneous separation of C^-/M^-.

A better simultaneous separation of DC^-/DM^- was also found with CD-CZE (Figure 6.8C1-6.8C3) than with CZE (Figure 6.8A) and MEKC (Figure 6.8B). Using 40 mM DM-β-CD (Figure 6.8A3), the baseline separation of the positional isomers of DC^-/DM^- was achieved, except for $3,5DM^-/2,5DM^-$ (M6/M3) and $2,6DC^-/2,6DM^-$ (C4/M4). It should be noted that simultaneous separation of positional isomers of DC^-/DM^- may be improved by the addition of organic solvents, the change of CD types and concentrations or the use of dual CDs.

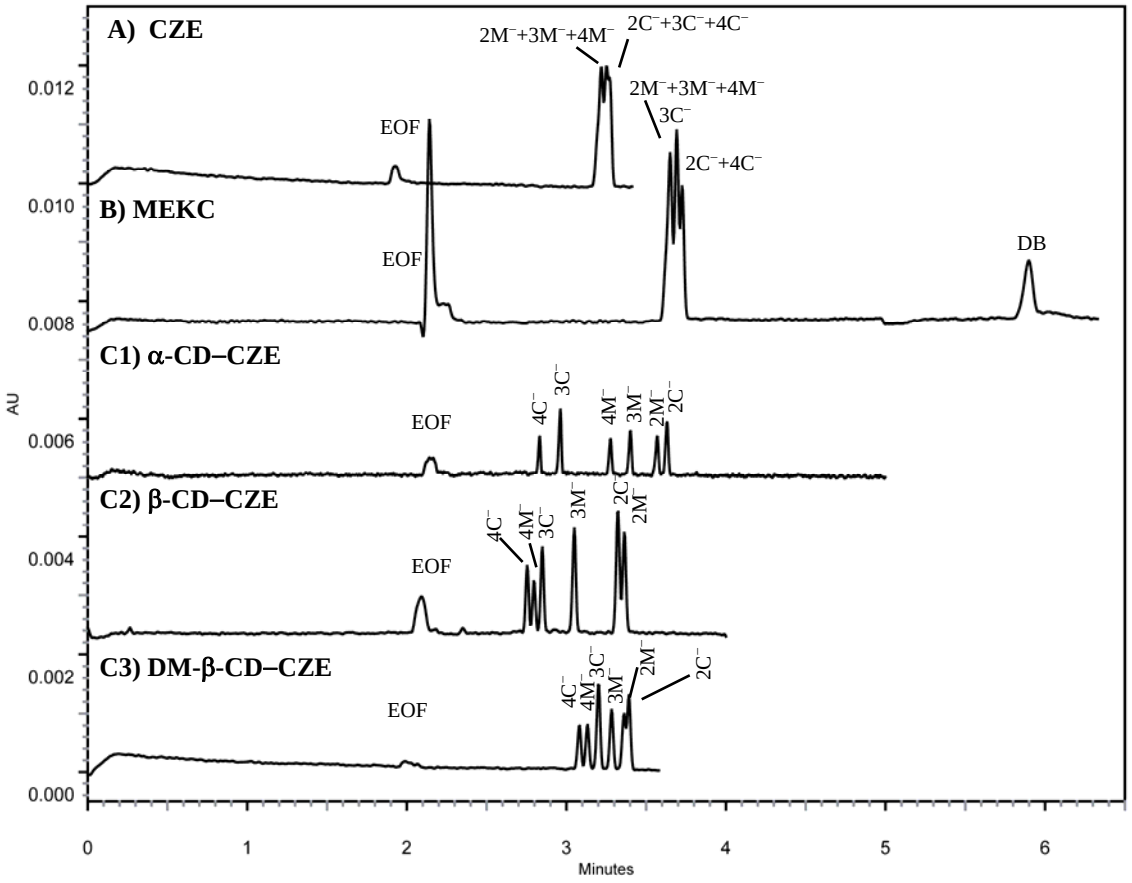


Figure 6.7 Simultaneous separation of positional isomers of C^-/M^- in 10 mM $Na_2B_4O_7$ at a pH 9.2 buffer and supplemented with: (A) CZE, no supplemented, (B) MEKC with 40 mM SDS, and CD-CZE with 10 mM $Na_2B_4O_7$ containing (C1) 20 mM α-CD, (C2) 16 mM β-CD and (C3) 5 mM DM-β-CD. Other CE conditions were shown in Section 6.2.3.

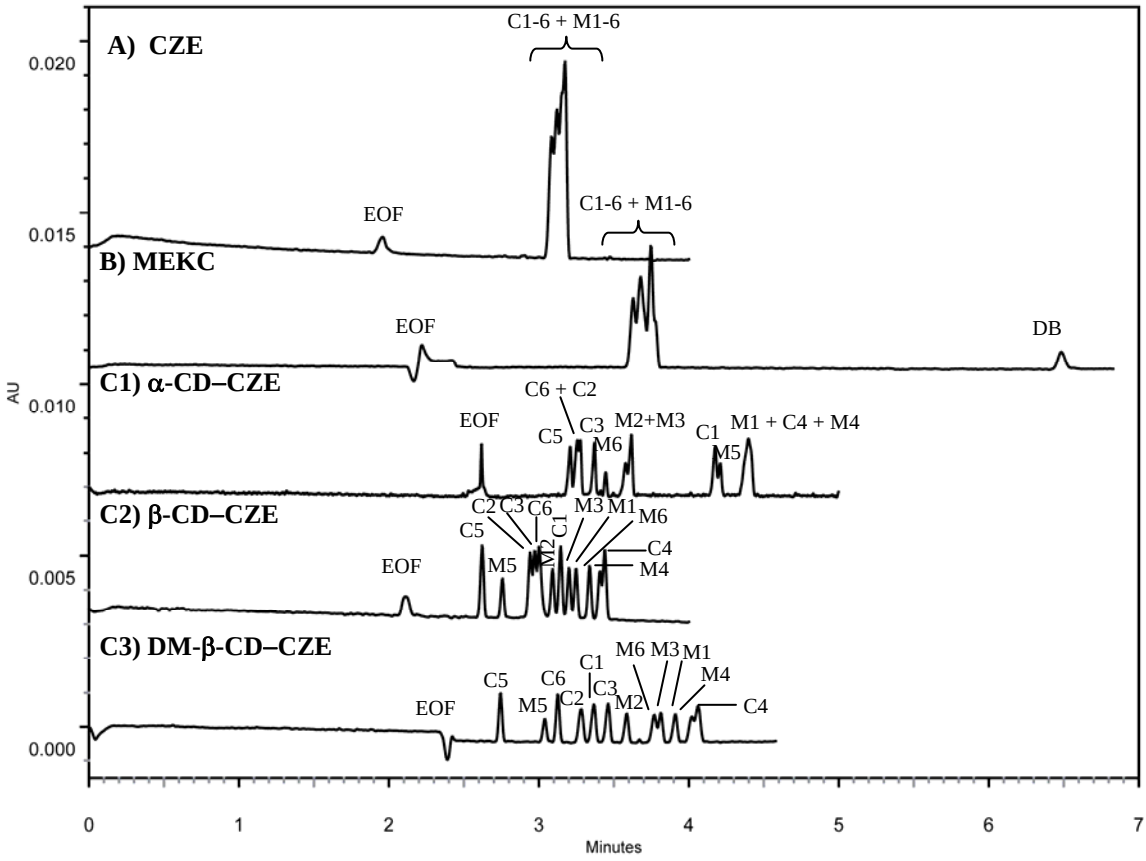


Figure 6.8 Simultaneous separation of positional isomers of DC^-/DM^- in 10 mM $Na_2B_4O_7$ at a pH 9.2 buffer and supplemented with: (A) CZE, no supplemented, (B) MEKC with 40 mM SDS, and CD-CZE with 10 mM $Na_2B_4O_7$ containing (C1) 60 mM α-CD, (C2) 12 mM β-CD and (C3) 40 mM DM-β-CD. Other CE conditions were shown in Section 6.2.3. Analytes: $2,3DC^-$ (C1), $2,4DC^-$ (C2), $2,5DC^-$ (C3), $2,6DC^-$ (C4), $3,4DC^-$ (C5), $3,5DC^-$ (C6), $2,3DM^-$ (M1), $2,4DM^-$ (M2), $2,5DM^-$ (M3), $2,6DM^-$ (M4), $3,4DM^-$ (M5), $3,5DM^-$ (M6).

6.3.1.5 Application to 10 chlorophenoxy acid herbicides

The separation selectivity was also compared for chlorophenoxy acid herbicides using CZE, MEKC and CD-CZE. This initial study focused on the separation of 2,4-DB/MCPB and 2,4-D/MPCA, which differ in only a single chloro and methyl group on a benzene ring. For CZE separation (Figure 6.9A), the co-elution peaks of 2,4-DB/MCPB and 2,4-D/MCPA showed no resolution between them due to their similar μ or small α_{CZE}, which is consistent with previous reports [Farran *et al.* 1999, Hsieh and Huang 1996]. In MEKC with 40 mM SDS (Figure 6.9B), these two pairs were also essentially co-eluted. With the addition of MeOH or ACN up to 20 % (v/v) in the CZE or MEKC buffer, the baseline resolution was still not obtained for 2,4-DB/MCPB and 2,4-D/MCPA (Figure 6.10).

Using CD-CZE with various types and concentrations of CD, in comparison with CZE, a reversed |μ| order in CD-CZE for MCPA/2,4-D as well as MCPB/2,4-DB was obtained using α-CD (Figure 6.6A) , while the same |μ| order was observed using β-CD (Figure 6.6B) and DM-β-CD (Figure 5.6C). This is probably due to the match between the cavity size of CD and the molecular size of the analytes, resulting in a stronger CD binding to the chloro substituent than the methyl substituent in α-CD, but a weaker CD binding of the methyl substituent than the chloro substituent to the β-CD and DM-β-CD. The values of K, κ and $\alpha_{CD,\,max}$ for 2,4-DB/MCPB and 2,4-D/MCPA are listed in Table 6.3. Consistent with the small κ of the analytes to β-CD (1.06 for 2,4-DB/MCPB and 1.02 for 2,4-D/MCPA), co-elution of the two pairs was observed (Figure 6.11). In comparison with β-CD, α-CD and DM-β-CD have much higher separation selectivities (α-CD with 1.61 for 2,4-DB/MCPB and 1.39 for 2,4-D/MCPA, and DM-β-CD with 1.30 for 2,4-DB/MCPB and 1.41 for 2,4-D/MCPA). Indeed, baseline resolution was achieved over a wide range of CD concentrations, for example, with 2.0-30 mM α-CD for 2,4-DB/MCPB, 2.0-15 mM α-CD for 2,4-D/MCPA, 2.0-8.0 mM DM-β-CD for 2,4-DB/MCPB, and 2.0-50 mM DM-β-CD for 2,4-D/MCPA. Figure 6.9 shows an example of the electropherograms obtained for simultaneous separation of 2,4-DB/MCPB and 2,4-D/MCPA in CD-CZE using 2.0

mM α-CD (Figure 6.9C1) and 3.0 mM DM-β-CD (Figure 6.9C2). Using 2.0-15 mM α-CD, the simultaneous separation of 2,4-DB/MCPB, 2,4-D/MCPA and other six chlorophenoxy acid herbicides was not achieved. With 2.0-8.0 mM DM-β-CD for simultaneous separation of ten chlorophenoxy acid herbicides, 3.0 mM DM-β-CD gave the achieved baseline resolution for all analytes within 4.0 min as shown in Figure 6.9C2.

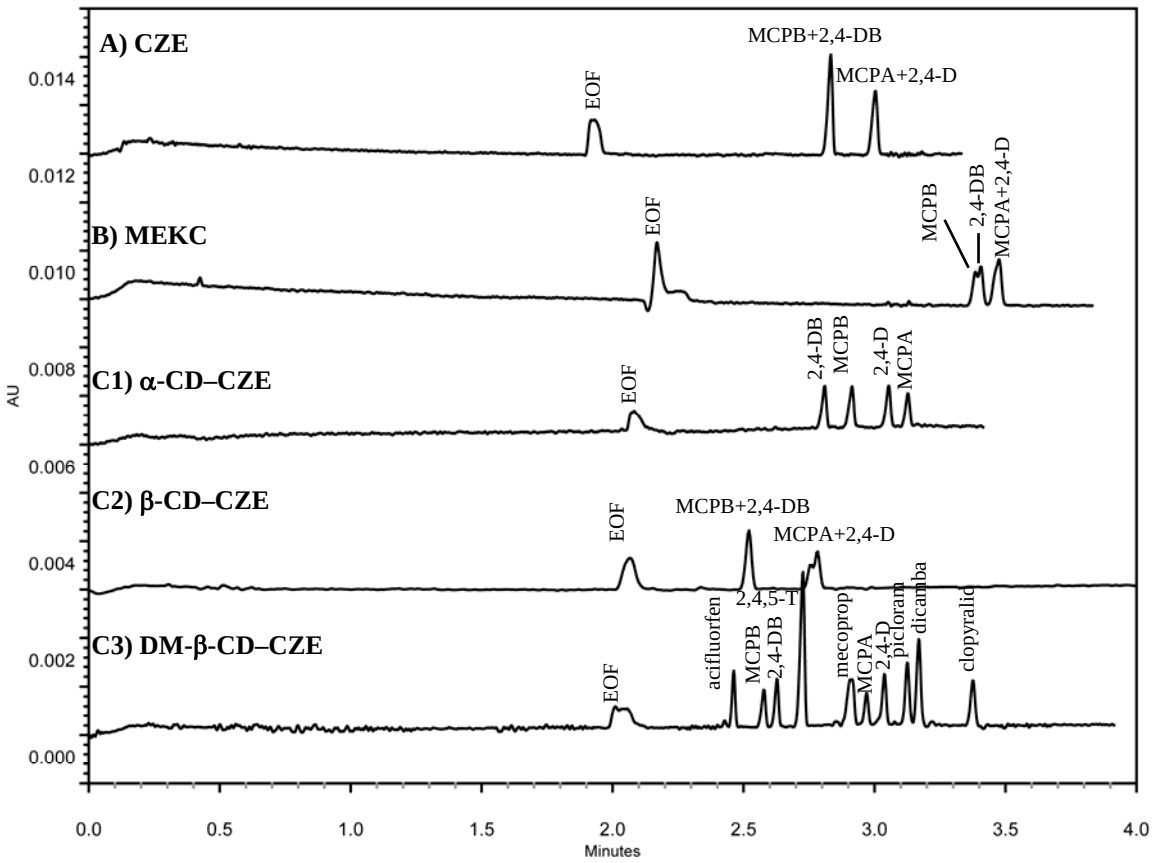


Figure 6.9 Simultaneous separation of chlorophenoxy acid herbicides in 10 mM $Na_2B_4O_7$ at a pH 9.2 buffer and supplemented with: (A) CZE, no supplemented, (B) MEKC with 40 mM SDS in 10 mM $Na_2B_4O_7$, and (C) CD-CZE with 10 mM $Na_2B_4O_7$ containing (C1) 2.0 mM α-CD, (C2) 10.0 mM β-CD, and (C3) 3.0 mM DM-β-CD. Other CE conditions were shown in Section 6.2.3.

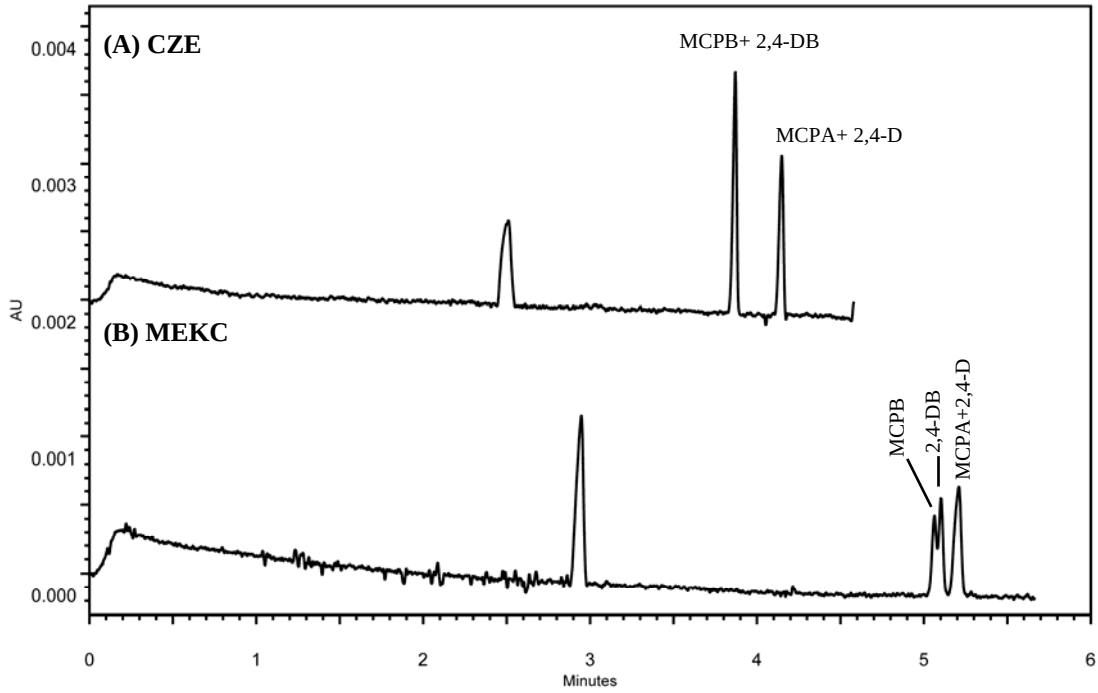


Figure 6.10 Simultaneous separation of 2,4-D, MCPA, 2,4-DB and MCPB in 10 mM $Na_2B_4O_7$ buffer at pH 9.2 and 10 % (v/v) methanol supplemented with: (A) CZE, no supplement and (B) MEKC with 40 mM SDS. Other CE conditions are as shown in Section 6.2.3.

6.3.2 Separation selectivity patterns of fully charged achiral compounds in CE with a neutral CD

6.3.2.1 Theory

According to Equation 6.6, in the presence of CD in the buffer, the effective electrophoretic mobility (μ) of the fully charged achiral analytes 1 and 2 are given by Equation 6.11-6.13,

$$\mu_1 = \frac{\mu_{0,1} + K_1 C \mu_{\infty,1}}{1 + K_1 C} \tag{6.11}$$

$$\mu_2 = \frac{\mu_{0,2} + K_2 C \mu_{\infty,2}}{1 + K_2 C} \tag{6.12}$$

where μ_0 and μ_∞ are the electrophoretic mobilities of the analyte and its complex at zero and at infinite *C*, respectively, *K* is the binding constant of the analyte to CD, and *C* is the free CD concentration at equilibrium that is assumed to (i) equal to the initial concentration and (ii) that it is significantly greater than the analyte concentration. Subscripts 1 and 2 refer to the analytes 1 and 2, respectively.

The separation selectivity (α), defined as the ratio of μ, is given by the equation;

$$\alpha = \frac{\mu_2}{\mu_1} = \left(\frac{\mu_{0,2} + K_2 C \mu_{\infty,2}}{\mu_{0,1} + K_1 C \mu_{\infty,1}} \right) \left(\frac{1 + K_1 C}{1 + K_2 C} \right) \tag{6.13}$$

In order to simplify Equation 6.13 to relate to the dimensionless values of α_0, κ, β, α_∞ and χ, using similar concept to previous work on the separation selectivity patterns for chiral compounds using neutral or anionic CD [Zhu and Vigh 2000, Zhu *et al*. 2000, Zhu and Vigh 2001, Nhujak *et al.* 2005], these parameters are defined as;

$$\alpha_0 = \frac{\mu_{0,2}}{\mu_{0,1}},\ \alpha_0 \geq 1.0 \tag{6.14}$$

$$\kappa = \frac{K_2}{K_1} \tag{6.15}$$

$$\beta = \frac{\mu_{0,2}}{\mu_{\infty,2}} \tag{6.16}$$

$$\alpha_\infty = \frac{\mu_{\infty,2}}{\mu_{\infty,1}} \tag{6.17}$$

$$\chi = K_1 C \tag{6.18}$$

where α_0 is separation selectivity of the analytes in free solution (at zero *C*), κ is the CD binding selectivity, α_∞ is separation selectivity of analyte-CD complexes, β reflects a change in electrophoretic mobility of the analytes in free and complex forms, and χ is the product of *K* and *C*. It should be noted that $|\mu_{0,2}|$ is denoted to be greater than $|\mu_{0,1}|$.

Therefore, from Equation 6.14-6.18, the separation selectivity in Equation 6.13 may be rearranged to link with five dimensionless parameters as,

$$\alpha = \alpha_0 \left(\frac{1 + \frac{\kappa}{\beta}\chi}{1 + \frac{\alpha_0}{\beta\alpha_\infty}\chi} \right) \left(\frac{1+\chi}{1+\kappa\chi} \right) \tag{6.19}$$

Under CE conditions of achiral anionic compounds and a neutral CD, the five dimensionless parameters are possibly in the ranges of $1.0 \leq \alpha_0 < \infty$, $0 < \kappa < \infty$, $0 < \beta < \infty$, $0 < \alpha_\infty < \infty$ and $0 \leq \chi < \infty$. In most cases, the β values are usually higher

than 1.0 due to a decrease in the electrophoretic mobility of the analyte-CD complexes (the greater hydrodynamic radius of the complexes than that of free analytes), and therefore, the β values for charged compounds with a neutral CD were considered in a limit range of $1 < \beta < \infty$ in this work. The different values of five dimensionless parameters are assumed for calculating a set of α curve (Equation 6.19) in order to investigate the effect of these parameters to α. In this work, the separation selectivity for achiral anionic analytes with a neutral CD can be classified into seven patterns (IA, IB, IB*, IIA, IIB, IIA* and IIB*), as given in Figure 6.11. The theoretical value of α can be obtained in a range of $0 < \alpha < \infty$. At χ (or K_1C) of 0, the α value refers to α_0, while the α value limits to α_∞ at infinite χ. It should be noted that the theoretical value of $\alpha < 1.0$ indicates the reversed |μ| order of two analytes at a given and zero *C*, and then the practical separation selectivity is equal to 1/α. Patterns I and II are assigned for an increase and a decrease in α, respectively, with increasing *C* in an initial range. "A" refers to the α value that monotonously and asymtotically approaches a limiting value, while "B" refers to the α value that reaches to the maximum point for Pattern I or the minimum point for Pattern II. Superscript "*" refers to the reversed |μ| order for two analytes at high CD concentrations, compared with their |μ| order at zero *C*.

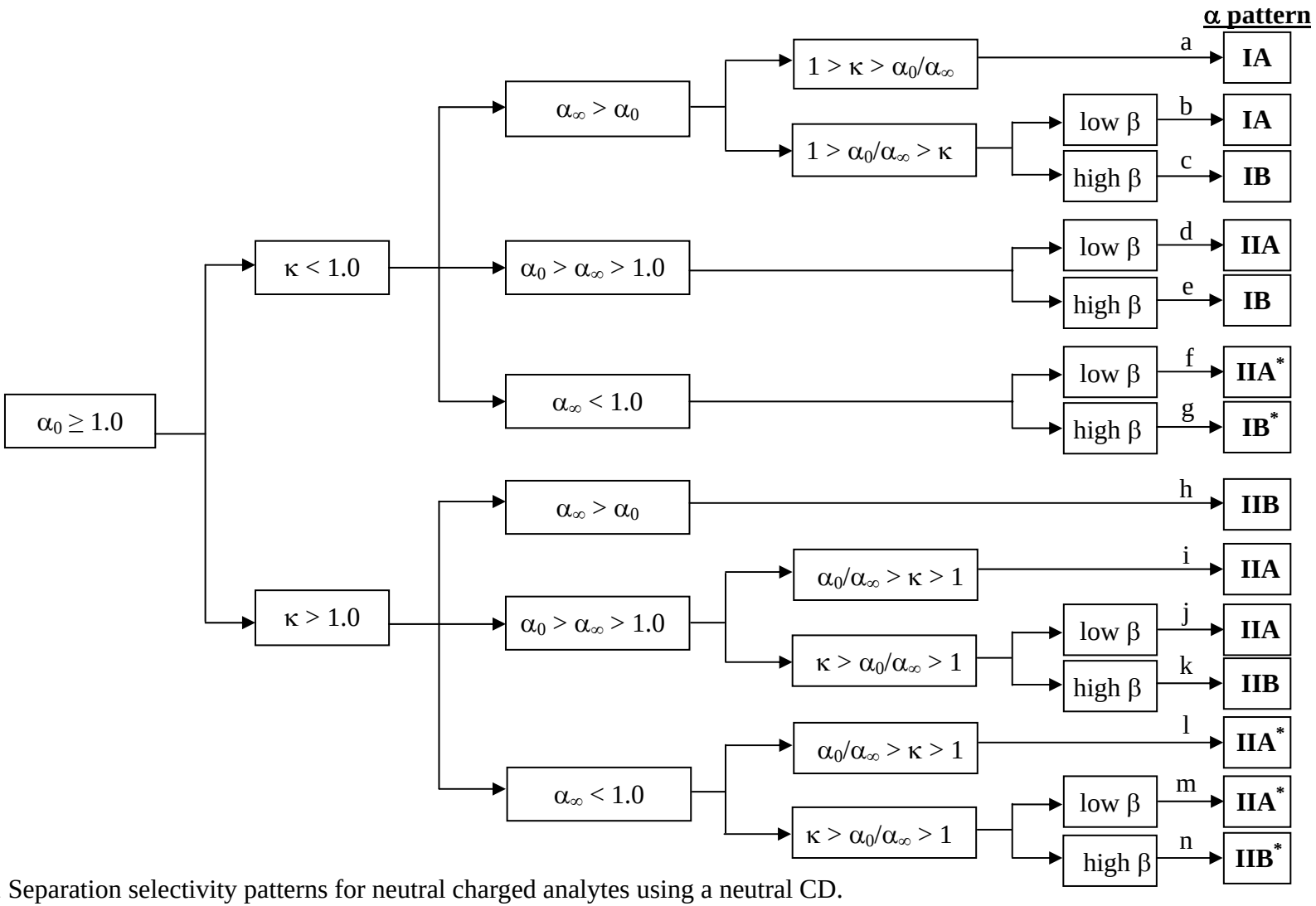


Figure 6.11 Separation selectivity patterns for neutral charged analytes using a neutral CD.

Figure 6.12 shows the possible seven separation selectivity patterns by plotting of the α values as a function of parameter χ, calculated using Equation 6.19 and the data presented in each panel. For pattern IA with the case (a) $\{\gamma > \alpha_0 > 1.0\}$, $\{\kappa < 1.0\}$, and $\{1 > \kappa > \alpha_0/\alpha_\infty\}$ or (b) $\{ \alpha_\infty > \alpha_0 > 1.0\}$, $\{\kappa < 1.0\}$, and $\{1 > \alpha_0/\alpha_\infty > \kappa\}$ at low β values, the α values increase to reach a α_∞ value neither any maximum point nor reversed $|\mu|$ orders for two analytes over a wide range of *C*. This selectivity shape indicates the better separation with either low or high *C* than that with zero *C*, and therefore, the separation optimization is simpler than other patterns [Zhu and Vigh 2001]. For pattern IB with the case (c) $\{\alpha_\infty > \alpha_0 > 1.0\}$, $\{\kappa < 1.0\}$, and $\{1 > \alpha_0/\alpha_\infty > \kappa\}$ at high β values, or (e) $\{\alpha_0 > \alpha_\infty > 1.0\}$ and $\{\kappa < 1.0\}$ at high β values, the α values increase to a maximum value, and, then decreases to a limiting value of α_∞ at high *C* with same $|\mu|$ orders. Therefore, in comparison with zero *C*, the better separation selectivity in pattern IB will be obtained at particular *C*, while a better or a worse separation at high *C* may be observed depending on the α_∞ values, for example, a better separation for $\alpha_\infty > \alpha_0$ but a worse separation for $\alpha_0 > \alpha_\infty$. Pattern IB*, with the case (g) $\{\alpha_0 > 1.0 > \alpha_\infty\}$ and $\{\kappa < 1.0\}$ at high β values, gives similar selectivity shape to pattern IB but with the reversed $|\mu|$ order ($\alpha < 1.0$) of the analytes at higher *C*. It should be noted that, when $\alpha_\infty < 1.0$, the analytes 1 and 2 give the reversed $|\mu|$ orders at high and zero *C*. Due to $\alpha_\infty < 1.0$ in the case of pattern IB*, the better separation at high *C* may be obtained for $1/\alpha_\infty > \alpha_0$ but the worse separation for $1/\alpha_\infty < \alpha_0$.

In contrast to pattern IA, the separation selectivity in pattern IIA, with the case (d) $\{\alpha_0 > \alpha_\infty > 1.0\}$ and $\{\kappa < 1.0\}$ at low β values, or (i) $\{\alpha_0 > \alpha_\infty > 1.0\}$, $\{\kappa > 1.0\}$, and $\{\alpha_0/\alpha_\infty > \kappa > 1\}$, or (j) $\{\alpha_0 > \alpha_\infty > 1.0\}$, $\{\kappa > 1.0\}$, and $\{\kappa > \alpha_0/\alpha_\infty > 1\}$ at low β values, shows a gradual α_{CD} decrease to approach a α_∞ value with increasing *C*, and the same $|\mu|$ order will be observed over a wide range of *C*. This can be implied that the worse separation in the presence of CD will be obtained, in comparison with zero *C*. For pattern IIB with (h) $\{\alpha_\infty > \alpha_0 > 1.0\}$ and $\{\kappa > 1.0\}$, or (k) $\{\alpha_0 > \alpha_\infty > 1.0\}$, $\{\kappa > 1.0\}$, and $\{\kappa > \alpha_0/\alpha_\infty > 1\}$ at high β values, the α value gradually decreases to a

minimum point, and then increases to a limiting value of α_∞ with same $|\mu|$ orders at high and zero *C*. However, the reversed $|\mu|$ orders may be obtained around *C* giving the minimum α, such as curve (h).

Pattern IIA*, with the case (f) { $\alpha_0 > 1.0 > \alpha_\infty$} and {$\kappa < 1.0$} at low β values, or (l) {$\alpha_0 > 1.0 > \alpha_\infty$}, {$\kappa > 1.0$}, and {$\alpha_0/\alpha_\infty > \kappa > 1$}, or (m) {$\alpha_0 > 1.0 > \alpha_\infty$}, {$\kappa > 1.0$}, and {$\kappa > \alpha_0/\alpha_\infty > 1$} at low β values, shows similar selectivity shape with pattern IIA but a reversal $|\mu|$ order for two analytes is obtained at high *C*. In comparison with CZE without CD, the better separation in the presence of CD may be observed when $1/\alpha_\infty > \alpha_0$. For pattern IIB* with the case (n) {$\alpha_0 > 1.0 > \alpha_\infty$}, {$\kappa > 1.0$}, and {$\kappa > \alpha_0/\alpha_\infty > 1$} at high β values, the separation selectivity decreases to a minimum value of < 1.0 resulting in a worse separation, then increases to a limiting value α_∞ with reversed $|\mu|$ order for two analytes. For the reversed order analytes with $1/\alpha > \alpha_0$, the better separation in CZE with CD ($\alpha < 1.0$) than without CD ($\alpha_0 > 1.0$) may be observed.

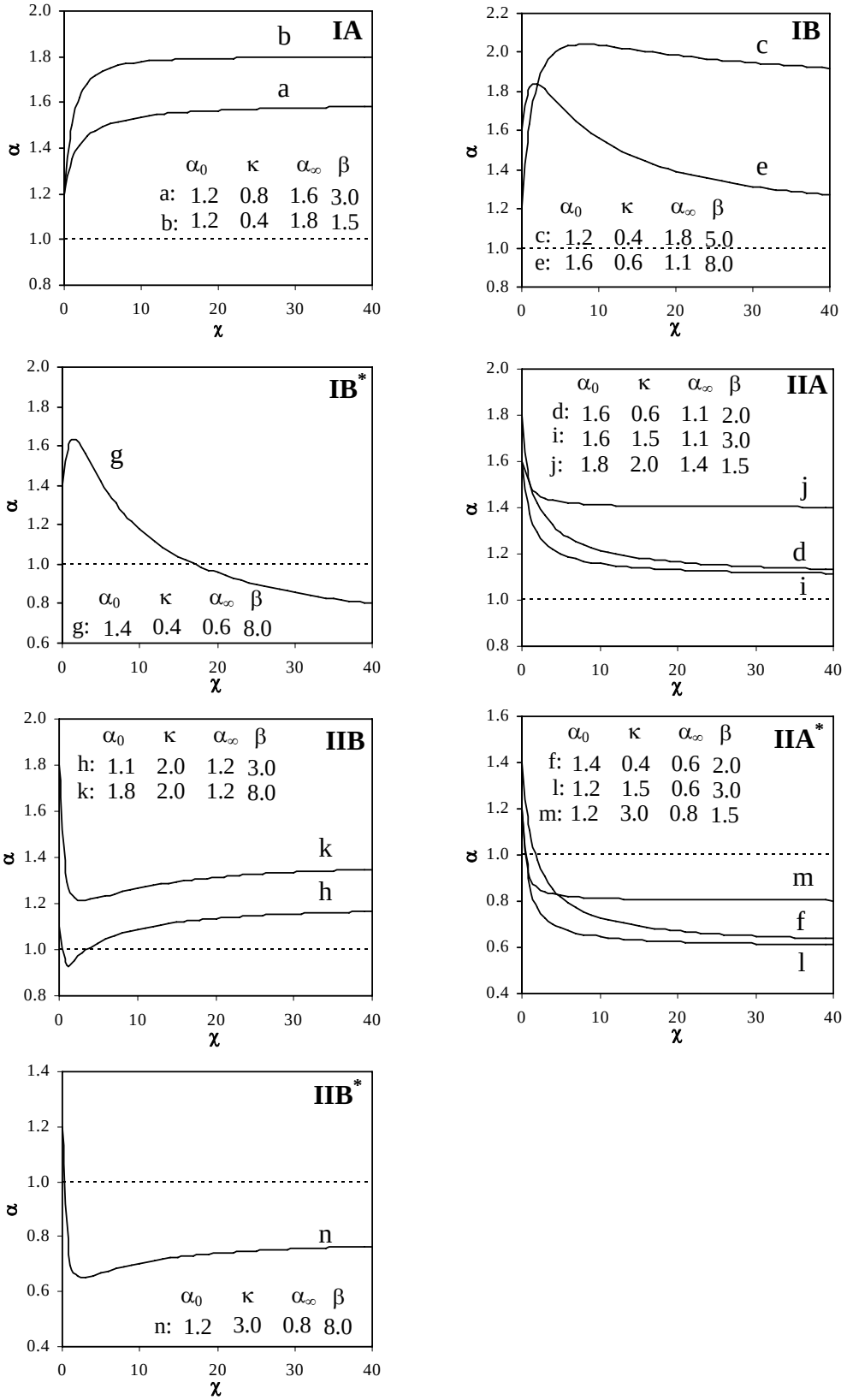


Figure 6.12 Predicted separation selectivity (α) patterns with the presence of a neutral cyclodextrin for fully charged achiral compounds, calculated using Equation 6.19 and the data listed in each panel.

6.3.2.2 Observed α patterns for negatively charged achiral compounds using a neutral CD

In initial experimental work as shown in Section 6.3.1.3, three sets of acidic compounds were used in the CE separation using α-CD: (i) positional isomers of C^- and M^-, (ii) positional isomers of DC^- and DM^-, and (iii) four chlorophenoxy acids (MCPB, 2,4-DB, MCPA and 2,4-D). Figure 6.13 shows an example of the obtained electropherograms for simultaneous separation of each set of the analytes, C^-/M^-, DC^-/DM^- and chlorophenoxy acids, with and without the addition of α-CD. At 0 mM α-CD (Figure 6.13A), the simultaneous separation of positional isomers of C^- and M^- showed the co-elution of the analytes due to very slightly different μ of these structurally related compounds. Over a range of 15-60 mM α-CD (that for 20 mM is shown in Figure 6.13A) in the buffer, a baseline separation was achieved for the simultaneous separation of these analytes. Although the simultaneous separation of DC^- and DM^- isomers was not achieved a baseline separation using α-CD, a better separation of DC^- and DM^- was also found with the presence of α-CD (Figure 6.13B with 60 mM α-CD), in comparison with the 0 mM α-CD in buffer (Figure 6.13B). It should be noted that the separation of DC^- and DM^- may be improved by the addition of organic solvents, the change of CD types and concentrations or the use of dual CDs. For the simultaneous separation of four phenoxy acids, the co-elution of MCPB/2,4-DB and MCPA/2,4-D was observed in the buffer without the addition of 0 mM α-CD (Figure 6.13C), while a baseline resolution was achieved in a range of 2.0-15 mM α-CD (that for 2.0 mM is shown in Figure 6.13C).

Since many pairs of the analytes in each set gave the similar separation selectivity patterns, some substituted benzoic acids and phenoxyacetic acids, including $4C^-$, $4M^-$, $3C^-$, $3M^-$, $25DC^-$, $25DM^-$, $35DC^-$, $35DM^-$, and all four chlorophenoxy acids, were chosen here as representatives to cover the various α patterns. Figure 6.16 shows plots of the corrected effective electrophoretic mobilities (μ) of the selected analytes against the α-CD concentrations (0-60 mM). With an increase in α-CD concentrations, the μ values of the analytes gradually decrease to approach a μ_∞ value due to the

greater hydrodynamic radius of the analytes-CD complexes. In order to use Equation 6.19 for predicting the separation selectivity curves for fully charged achiral analytes using a neutral CD, the following values of μ_0, μ_∞, α_0, K, κ, β, and α_∞ for each pair of test analytes must be known, as listed in Table 6.4. Accordingly, from the data in Table 6.4 and Equation 6.19, Figure 6.14 shows a good agreement between the observed (symbols) and the predicted (solid lines) α for the test analytes in a wide range of C.

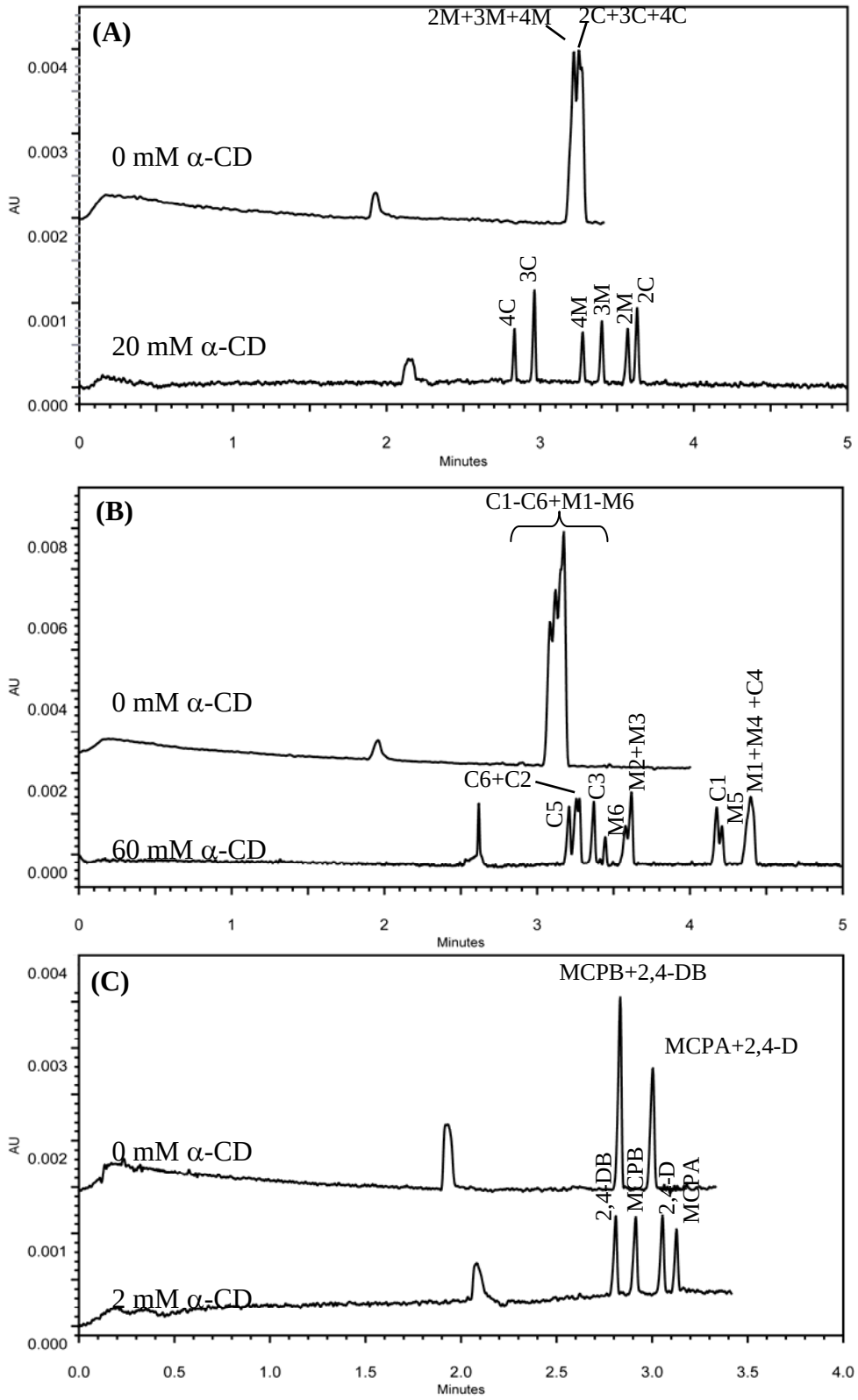


Figure 6.13 Simultaneous separation of acid compounds with and without the addition of α-CD in 10 mM $Na_2B_4O_7$ buffer at pH 9.2 for (A) C^-/M^-, (B) DC^-/DM^-, and (C) chlorophenoxy acids. Other CE conditions were as shown in Section 6.2.3. See Figure 6.8 for peak identifications.

Table 6.4 Electrophoretic mobility (μ, 10^{-8} m^2 V^{-1} s^{-1}), binding constant (K, M^{-1}) and the dimensionless parameters of α_0, κ, β and α_∞ for some test analytes with α-CD.

Analyte	μ_0	μ_∞	K	Pair	α_0	κ	α_∞	β
$4C^-$	-2.92	-1.12	129.0±3.0	4C/4M[a]	1.02	4.69	0.88	2.61
$4M^-$	-2.85	-1.28	27.5±0.6	3C/4C[a]	1.01	0.63	1.02	2.60
$3C^-$	-2.96	-1.14	80.7±2.0	3M/4M[a]	1.01	0.81	1.13	1.99
$3M^-$	-2.89	-1.45	22.3±0.6					
$2,5DC^-$	-2.81	-1.15	97.3±2.9	25DC/25DM[a]	1.03	2.62	0.91	2.44
$2,5DM^-$	-2.73	-1.26	37.2±1.1	35DC/35DM[a]	1.03	4.95	0.87	2.54
$3,5DC^-$	-2.79	-1.10	191.9±5.8	25DC/35DC[a]	1.01	0.51	1.05	2.44
$3,5DM^-$	-2.70	-1.26	38.8±1.3					
MCPB	-2.29	-0.88	235.9±4.9	MCPA/MCPB[a]	1.11	0.84	1.26	2.29
2,4-DB	-2.28	-0.83	381.5±12.0	MCPA/24DB[a]	1.11	0.52	1.34	2.29
MCPA	-2.54	-1.11	197.3±6.0	24D/MCPB[a]	1.12	1.16	1.25	2.33
2,4-D	-2.56	-1.10	274.3±7.8					

[a]Analyte 1

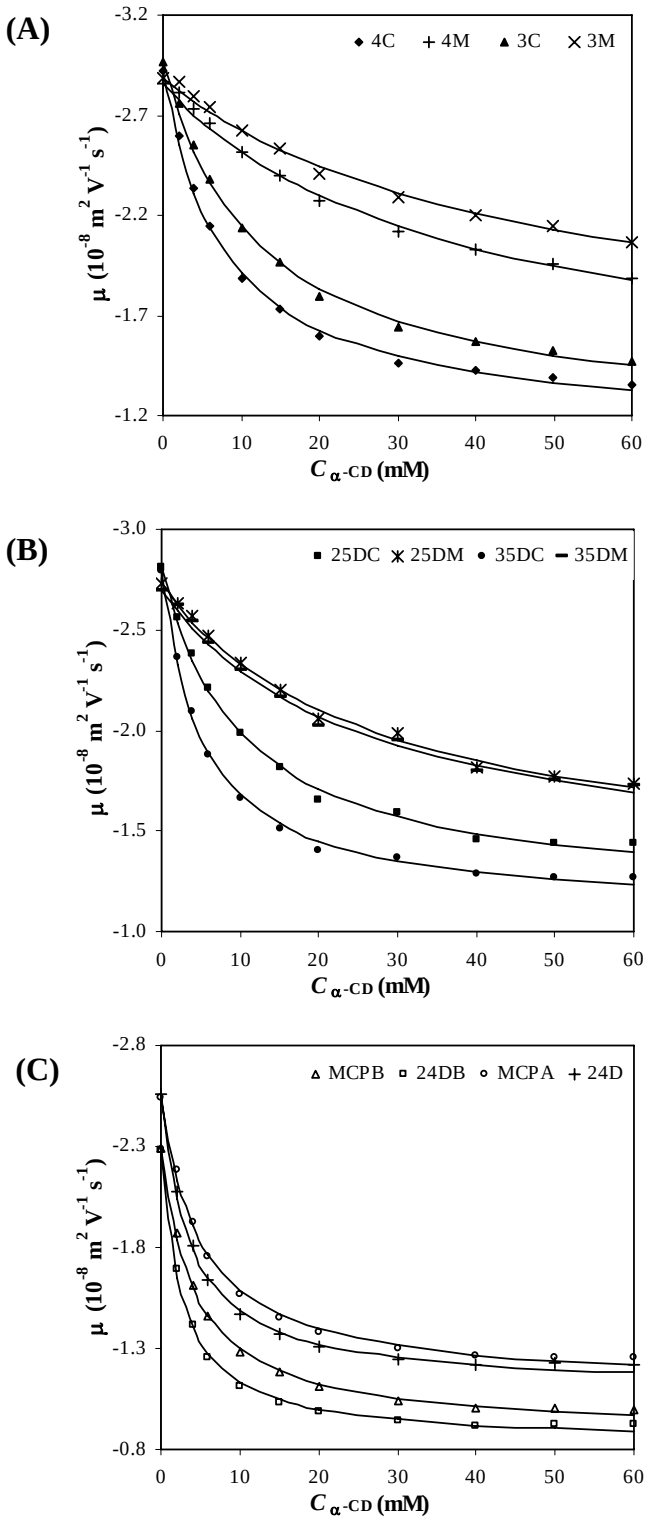


Figure 6.14 Observed (symbols) and predicted (solid lines) effective electrophoretic mobilities (μ) of the some anionic analytes with α-CD (0-60 mM): (A) C^- and M^- isomers, (B) DC^- and DM^- isomers, and (C) chlorophenoxy acids. Predicted values are obtained using Equation 6.6 and the data in Table 6.4.

As can be seen in Figure 6.15A with an increase in *C*, the better separation selectivity for $3M^-/4M^-$ and MCPA/MCPB monotonously and asymtotically approaches to a limiting α_∞ value, where the observed α values of 1.10 and 1.26 at 60 mM α-CD, and the α_∞ values of 1.13 and 1.26 for the former and the latter pairs, respectively. This observed α is consistent with pattern IA. In Figure 6.15B, the separation selectivity for $3C^-/4C^-$, $25DC^-/35DC^-$ and MCPA/2,4-DB was obtained to fit with pattern IB, where the α value increases to a maximum values, then decreases to a α_∞ value. For 2,4-D/MCPB with no change of $|\mu|$ order, the observed separation selectivity in Figure 6.15C was found to decrease to a minimum value at low *C*, and then to increase to a α_∞ value at high *C*, with values of 1.23 (observed at 60 mM α-CD) and 1.25 for α and α_∞, respectively. This observed α is consistent with pattern IIB. Unlike other analytes, $4C^-/4M^-$, $25DC^-/25DM^-$ and $35DC^-/35DM^-$ showed a decrease in α to 1.0 and then a reversed order ($\alpha < 1.0$) with a decrease in α to a minimum value and then a gradual increase in α to a α_∞ value. This observed α is consistent with pattern IIB^*. Although the α resulted in the reversed order of these pairs, the better separation was observed in a wide range of *C*, particularly nearby or above *C* giving the minimum α due to $\kappa \geq \alpha_0$, $1/\alpha_\infty \geq \alpha_0$, and a high β value, as listed in Table 6.4.

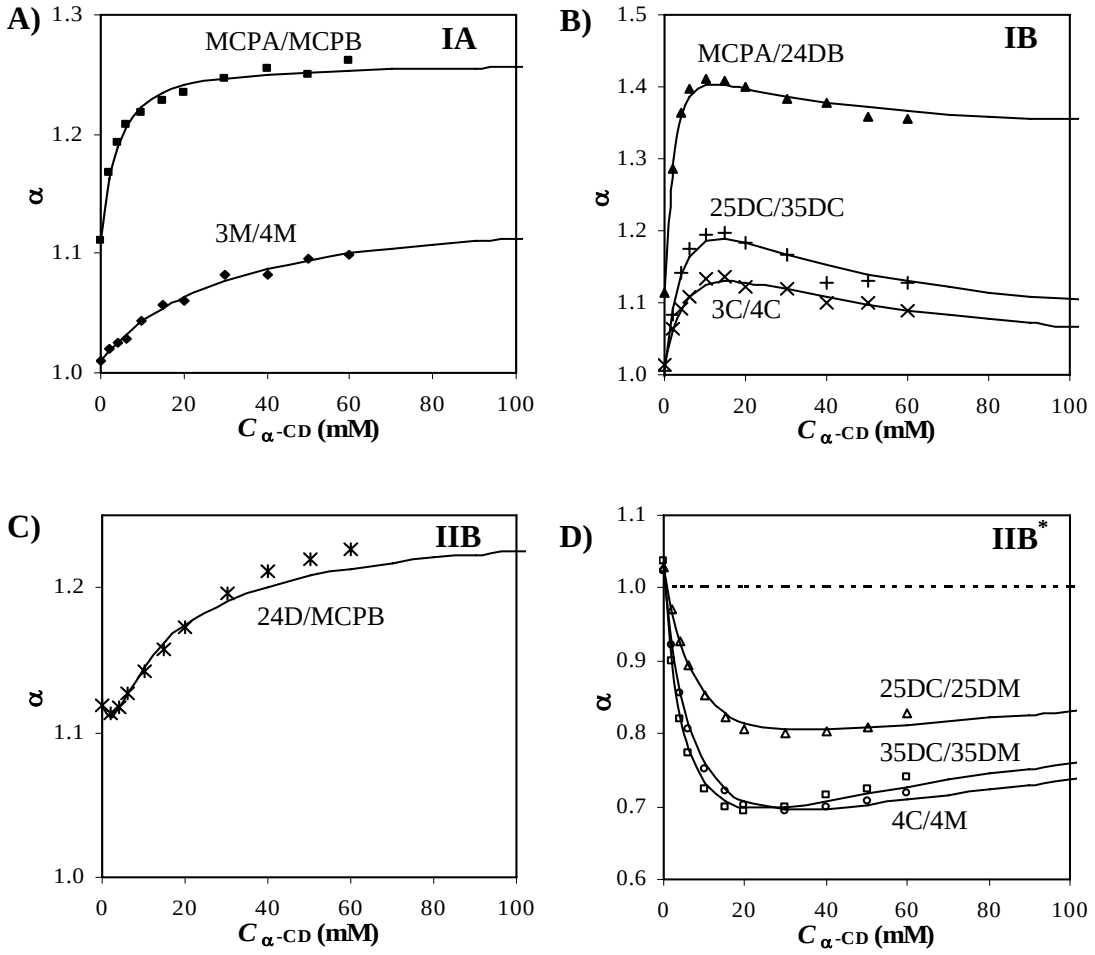


Figure 6.15 Observed (symbols) and predicted (solid lines) separation selectivity (α) of the some analyte pairs with α-CD. Experimentally observed α curves are consistent with: (A) pattern IA, (B) pattern IB, (C) pattern IIB, and (D) pattern IIB*. Predicted values are obtained using Equation 6.19 and the data in Table 6.4.

6.4 Conclusions

The selectivity of the CE separation for two analytes having a similar μ, such as C^-/M^- and DC^-/DM^-, were compared in three CE system: CZE, MEKC and CD-CZE. A small α_{CZE} was obtained for each pair of C^-/M^- and DC^-/DM^-, but a slightly higher α_{MEKC} than α_{CZE} was observed due to the small difference in partitioning of C^-/M^- and DC^-/DM^- into micelles. With the addition of organic solvents into the buffer of CZE or MEKC, a slight decrease in α_{CZE} and α_{MEKC} was observed. In comparison

with CZE and MEKC, CD-CZE gave a higher α_{CD} than α_{CZE} and α_{MEKC} for C^-/M^- and DC^-/DM^- because of the large difference in the binding constant of each analyte to CD. A better simultaneous separation of the positional isomers of C^-/M^- and DC^-/DM^- was also observed using CD-CZE compared to that with CZE or MEKC. In addition, baseline separation for 2,4-DB/MCPB and 2,4-D/MCPA, two pairs of phenoxy acid herbicides that differ only in a single chloro and methyl substituent on a benzene ring, was achieved in CD-CZE using α-CD or DM-β-CD, but not in CZE and MEKC. These results imply that CD-CZE is a better alternative to separate compounds that differ in only chloro and methyl groups, compared to CZE and MEKC.

Moreover, a separation selectivity equation, related to the dimensionless parameters such as α_0, κ, β, α_∞ and χ, was simplified for the CE separation of achiral anionic analytes using a neutral CD in order to classify the separation selectivity patterns to explain a change in separation selectivity and electrophoretic mobility order, where α_0 is the separation selectivity of the analytes in CZE without CD (μ_2/μ_1); κ the CD binding selectivity (K_2/K_1); α_∞ the separation selectivity of analyte-CD complexes ($\mu_{\infty,2}/\mu_{\infty,1}$); β the ratio of electrophoretic mobility of the analytes in free and complexes forms ($\mu_{0,2}/\mu_{\infty,2}$); χ the product of K_1 and CD concentration (C), and where μ_0 and μ_∞ are the electrophoretic mobilities of the analyte and its complex at zero and at infinite C, respectively, K is the binding constant of the analyte to CD, and subscripts 1 and 2 refer to the analytes 1 and 2, respectively. With an increase in C, seven separation selectivity patterns were predicted, where α for the first three patterns increases from α_0 monotonously and asymtotically to α_∞ for pattern IA, α_0 to a maximum value and then falls down with the same $|\mu|$ order for pattern IB, and α_0 to a maximum value and then fall down with the reversed $|\mu|$ order for pattern IB*, while α for the last four patterns decreases from α_0 monotonously and asymtotically to α_∞ with the same $|\mu|$ order for pattern IIA, and with the reversed $|\mu|$ order for pattern IIA*, α_0 to a minimum value and then goes up with the same $|\mu|$ order for pattern IIB, and with the reversed $|\mu|$ order for pattern IIB*. The separation selectivity patterns can be used to optimize the separation. For example, patterns IA and IB can improve the

separation in a wide range of *C* except for possibly worse separation with $\alpha_\infty < \alpha_0$ at high *C* for pattern IB, while worse separation in wide range of *C* for patterns IIA and IIB except for possibly better separation with $\alpha_\infty > \alpha_0$ at high *C* for pattern IIB. Due to the reversed $|\mu|$ order in patterns IB^*, IIA^* and IIB^* with $1/\alpha_\infty > \alpha_0$, a possibly better separation can be obtained at high *C*. In addition, the existence of some separation selectivity patterns was observed using positional isomer of chlorobenzoates and methylbenzoates and also phenoxy acids as test analytes with α-CD in the buffer.

CHAPTER VII

SUMMARY AND RECOMMENDATIONS

This work involves high efficiency separation in capillary liquid chromatography (CLC) and capillary electrochromatography (CEC) covering the preparation, evaluation and selectivity comparisons of monolithic silica capillary columns for a reversed-phase mode in CLC and for a mixed-mode (reversed-phase/anion exchange chromatography) in CEC. In addition, the separation selectivity was compared in different modes in capillary electrophoresis (CE) such as capillary zone electrophoresis (CZE) with and without the addition of cyclodextrin (CD) and also micellar electrokinetic chromatography (MEKC).

7.1 Preparation and performance evaluation of monolithic silica capillary columns for reversed-phase in CLC, and application to chlorophenoxy acid herbicides and bisphenol-A-diglycidyl ether, bisphenol-F-diglycidyl ether and theirs derivatives

The monolithic silica capillary columns were prepared and chemically modified to be reversed-phase chromatography: one phase functionalized with octadecyl moieties (ODS columns) and the other one with poly (octadecyl methacrylate) (ODM columns). The performance of these two columns was evaluated in either 80% (v/v) methanol or 80% (v/v) acetonitrile according to the following chromatographic properties: retention, hydrophobicity of the stationary phase, plate height, van Deemter plot and column permeability. The uracil was used as an unretained compound and test analytes used are homologous series of alkylbenzenes. Under the same condition, higher retention factor on ODM columns was observed than that on ODS columns. In comparison with methanol mobile phase, the slightly higher retention on ODM was observed when using acetonitrile mobile phase due to the better solvation of acetonitrile on ODM stationary phase. The hydrophobicity of the stationary phase was

found to be slightly higher on ODM than ODS columns. ODS and ODM columns showed the high efficiency separation with plate height (*H*) nearly or less than 10 μm. For example, *H* of 8.6-8.9 μm and 11.1 μm was obtained for amylbenzene on ODS and ODM columns, respectively, using 80% (v/v) methanol mobile phase at *u* of 1.0 mm/s. In addition, high permeability (*K*) of ODS and ODM columns was obtained, such as 8.8×10^{-14}-9.4×10^{-14} and 7.3×10^{-14}-8.0×10^{-14} m^2 on ODS and ODM columns, respectively, in 80% methanol mobile phase.

Using ODS column as an analytical column, the baseline separation of 13 chlorophenoxy acid herbicides was obtained in a gradient elution of either acetonitrile or methanol mobile phase. In addition, the CLC method for the separation of bisphenol-A-diglycidyl ether (BADGE), bisphenol-F-diglycidyl ether (BFDGE) and theirs derivatives was developed on ODS column. All 12 BADGE, BFDGE and their derivatives can be achieved the baseline separation when using methanol mobile phase, while the co-elution of some analytes was observed in acetonitrile mobile phase. Although isomers of some BFDGE derivatives could not be separated, it can easily enhance the efficiency separation by using the longer ODS column.

7.2 Selectivity comparisons of monolithic silica capillary columns modified with poly(octadecyl methacrylate) and octadecyl moieties for halogenated compounds in reversed-phase liquid chromatography

The retention of halogenated and alkyl substituted benzenes was investigated and the selectivity of those compounds was also compared on monolithic silica capillary columns modified with poly(octadecyl methacrylate) (ODM) and with octadecyldimethyl-*N*,*N*-diethylaminosilane (ODS). The chromatographic performance of these compounds was investigated using either methanol or acetonitrile mobile phase in a range of 50-80% (v/v). To investigate the retention of the interested analytes, log *k* values of mono- and di-substituted benzenes on ODM column were plotted against those on ODS column. Both alkylbenzenes and halogenated benzenes gave a linear tendency increase with an increase in the number of carbon atoms in the aliphatic chain for alkylbenzenes and size and polarizability of halogen atom. The

slope of log *k* for halogenated benzenes on ODM against ODS stationary phases is greater in mobile phases with either acetonitrile or methanol. This indicates that the preferential retention of mono- and di-halogenated benzenes on ODM was observed. The separation selectivity of each monolith for the halogenated and alkylbenzenes is determined from the ratio of retention factor of the analyte of interest with respect to that of toluene. The greater selectivities for halogenated compounds were obtained on ODM than ODS columns, while similar selectivities were observed with a homologous series of alkylbenzenes on ODM and ODS columns. This is due to the greater contribution of dispersion interactions on the ODM stationary phase with the contribution of carbonyl groups in ODM side chains. For the isomeric separation of dihalogenated benzenes, the separation of adjacently eluted positional isomers of halogenated compounds was achieved on the ODM column which cannot be separated on the ODS column using the same mobile phase. The ODM column also provides the greater retention and better separation of homologues and isomers of chlorophenols than ODS column under the same condition. Therefore, the results imply that the ODM column can be used as a better alternative to the conventional ODS column for separating other halogenated compounds.

7.3 Separation selectivity of mixed-mode monolithic silica capillary columns in capillary electrochromatography

The monolithic silica capillary CEC columns were prepared and chemically modified with octadecyl moieties (ODS) for reversed-phase CEC, and with a mixture of octadecyl and aminopropyl moieties (ODS/AP_1) or a mixture of octadecyl and 3-(2-aminoethylamino)propyl moieties (ODS/AP_2) for mixed-mode CEC. The electrochromatographic performance on these three columns was also evaluated for some polar and non-polar compounds in terms of column efficiency and retention properties. For example, the modified monolithic silica capillary columns gave the plate heights of 3.7, 4.0 and 4.2 μm for ODS, ODS/AP_1 and ODS/AP_2 columns, respectively, using thiourea as a test analyte at *u* of 0.9 mm/s. The retention factor of alkylbenzenes was higher on ODS than that on ODS/AP_2 and ODS/AP_1, respectively. This is due to the different amount of alkyl-bonded stationary phase bonded to the

silica. The slightly higher hydrophobicity on ODS column was obtained, while the slightly greater shape selectivity on ODS/AP_1 and ODS/AP_2 columns was observed. In addition, log *k* values of some polar and nonpolar compounds, such as alkylbenzenes, alkyl benzoates, halogenated benzenes, phenols, alkyl *p*-hydroxybenzoates and anilines, on mixed-mode columns were plotted against those on ODS column in order to investigate the retention of various analytes. Aromatic compounds containing hydroxyl groups, such as phenols and alkyl *p*-hydroxybenzoate, shows the upper deviation from linear correlation of alkylbenzenes, indicating that the mixed-mode columns of ODS/AP_1 and ODS/AP_2 show the preferential retention for aromatic compounds containing hydroxyl groups, in comparison with ODS column. The better separation on ODS/AP_2 column than ODS and ODS/AP_1 columns was observed for a mixture of phenol compounds under the same condition. This can be explained by the additional interaction of dipole-dipole interactions between hydroxyl groups of the analytes and amino groups on ODS/AP_2 columns. Therefore, mixed-mode ODS/AP_2 columns can be used as a good alternative to reversed-phase ODS columns for the separation of other compounds having hydroxyl groups.

7.4 Selectivity of fully charged achiral compounds in capillary electrophoresis with a neutral cyclodextrin

The selectivity of the CE separation for two analytes having a similar μ, such as chlorobenzoates (C^-)/methylbenzoates (M^-) and dichlorobenzoates (DC^-)/ dimethylbenzoates (DM^-), were compared in three CE system: CZE, MEKC and CD-CZE. Using CZE with a 10-mM borate buffer at pH 9.2 without the addition of organic modifiers, small CZE separation selectivity (α_{CZE} of 1.018 to 1.038) was obtained for each pair of C^-/M^- (2-, 3- and 4-isomers) and DC^-/DM^- (2,3-, 2,4- ,2,5-, 2,6-, 3,4- and 3,5-isomers). Using an MEKC buffer containing 40 mM SDS in a 10-mM borate buffer at pH 9.2 without the addition of organic solvents, the very slightly higher MEKC separation selectivity (α_{MEKC} of 1.019 to 1.046) than α_{CZE} was obtained due to the small retention selectivity (defined as the ratio of retention factor for the analytes in micelle). With the addition of organic solvents (0-20% methanol or acetonitrile) in the buffer of CZE or MEKC, a slight decrease in α_{CZE} and α_{MEKC} was

observed. In CD-CZE with the presence of α-CD (2-60 mM), β-CD (2-16 mM) or dimethyl β-CD (2-50 mM DM-β-CD) in a 10-mM borate buffer, the higher CD-CZE separation selectivity (α_{CD}) than α_{CZE} and α_{MEKC} was obtained for C^-/M^- and DC^-/DM^- (α_{CD} up to 2.02), except for $2C^-/2M^-$ with DM-β-CD, and $2,6DC^-/2,6DM^-$ with β-CD or DM-β-CD. This can be explained by the difference in binding selectivity (defined as the ratio of binding constants, κ) of the analytes to CD, for example, α_{CD} of 1.44 for $4C^-/4M^-$ with 30 mM α-CD (κ = 4.69) and α_{CD} of 1.63 for $3,5DC^-/3,5DM^-$ with 50 mM DM-β-CD (κ = 21.2). For simultaneous separation of positional isomers of C^-/M^- and DC^-/DM^-, CD-CZE also gave the better separation, in comparison with CZE and MEKC. In addition, the baseline separation for two pairs of phenoxy acid herbicides having the difference in chloro and methyl substituents on a benzene ring was achieved in CD-CZE using α-CD or DM-β-CD, while not achieved in CZE and MEKC. Simultaneous separation of ten phenoxy acid herbicides was also obtained using the CD-CZE buffer containing 3 mM DM-β-CD in a 10-mM borate buffer at pH 9.2 within 4.0 min. These results imply that CD-CZE is a better alternative to separate compounds that differ in only chloro and methyl groups, compared to CZE and MEKC.

Moreover, based on the separation selectivity equation, related to the dimensionless parameters for fully charged achiral analytes using a neutral cyclodextrin (CD), the separation selectivity can be classified into seven patterns (IA, IB, IB^*, IIA, IIB, IIA^* and IIB^*). Patterns I and II are assigned for an increase and a decrease in α, respectively, with increasing CD concentrations (*C*) in an initial range. "A" refers to the α value that monotonously and asymtotically approaches a limiting value, while "B" refers to the separation selectivity (α) value that reaches to the maximum point for Pattern I or the minimum point for Pattern II. Superscript "*" refers to the reversed |μ| order for two analytes at high *C*, compared with their |μ| order at zero *C*. In comparison with CZE without CD, the presence of CD in the buffer may improve or reduce the separation selectivity accompanied by same or reversed electrophoretic mobility order for charged analytes, depending on the separation selectivity of the two analytes in free solution, the binding selectivity, the separation selectivity of analyte-

CD complexes and the ratio of electrophoretic mobility of the analytes in free and complexes forms. With an increase in *C*, the α for the first three patterns increases from the separation selectivity of the analytes in CZE without CD (α_0) monotonously and asymptotically to the separation selectivity of analyte-CD complexes (α_∞) for pattern IA, α_0 to a maximum value and then falls down with the same $|\mu|$ order for pattern IB, and α_0 to a maximum value and then fall down with the reversed $|\mu|$ order for pattern IB*, while α for the last four patterns decreases from α_0 monotonously and asymptotically to α_∞ with the same $|\mu|$ order for pattern IIA, and with the reversed $|\mu|$ order for pattern IIA*, α_0 to a minimum value and then goes up with the same $|\mu|$ order for pattern IIB, and with the reversed $|\mu|$ order for pattern IIB*. Using positional isomers of benzoic acids and phenoxy acids as test analytes and α-CD as a selector, the observed separation selectivity shapes was found to be excellent agreement with the predicted separation selectivity.

7.5 Recommendations for future research

In future research, the monolithic capillary column modified with octadecyl moieties (ODS column) or poly(octadecyl methacrylate) (ODM column) may be extended to separation with interested analytes in CLC coupled with mass spectrometry in order to achieve high efficiency separation from monolithic columns and high efficiency detection from the mass spectrometer. Since the ODM columns have the preferential for halogenated compounds, it is also interesting to apply for the CLC separation other halogenated compounds, such as polychlorinated biphenyls, using ODM columns. For mixed-mode CEC columns, it is interesting to extend study the electrochromatographic behavior for other polar compounds such as nitro, cyano and aldehyde groups. The separation of other analytes containing hydroxyl groups may also be applied. In addition, separation selectivity patterns for fully charged achiral compounds may be used as a guide for the optimization of complex mixtures that have similar electrophoretic mobility in CZE.

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W. Soonthorntantikul, N. Leepipatpiboon, T. Ikegami, N. Tanaka, T. Nhujak **"**Selectivity Comparisons of Monolithic Silica Capillary Columns Modified with Poly(octadecyl methacrylate) and Octadecyl Moieties for Halogenated Compounds in Reversed-Phase Liquid Chromatography**"** Journal of Chromatography A 2009, 1216, 5868-5874.

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1) W. Soonthorntantikul, M. Srisa-art, N. Leepipatpiboon, T. Nhujak "Theoretical models of separation selectivity for charged compounds in neutral cyclodextrin-electrokinetic chromatography" *Poster Presentation*, The 10th Asian-Pacific International Symposium on Microscale Separations and Analysis (APCE 2010), 10-13 December 2010, University of Hong Kong, Hong Kong, China.

2) W. Soonthorntantikul, M. Srisa-art, N. Leepipatpiboon, T. Nhujak "Comparison of separation selectivity in CZE, MEKC and CD-EKC for chlorobenzoates/ methylbenzoates, and application to separation of phenoxy acid herbicides" *Poster Presentation*, The 10th Asian-Pacific International Symposium on Microscale Separations and Analysis (APCE 2010), 10-13 December 2010, University of Hong Kong, Hong Kong, China.

3) W. Soonthorntantikul, N. Leepipatpiboon, T. Ikegami, N. Tanaka, T. Nhujak "Selectivity Comparison of Monolithic Silica Capillary Columns Modified with Poly(octadecyl methacrylate) and Octadecyl Moiety for Halogenated Compounds in Reversed-Phase HPLC" *Poster Presentation*, The 33rd International Symposium on

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1) W. Soonthorntantikul, M. Srisa-art, N. Leepipatpiboon, T. Nhujak "Comparison of separation selectivity in CZE, MEKC and CD-EKC for chlorobenzoates/ methylbenzoates, and application to separation of phenoxy acid herbicides" *Oral Presentation*, RGJ Seminar Series LXXXV "Contemporary Analytical Chemistry and Analytical Technology", 9 September 2011, Faculty of Science, Mahidol University, Bangkok, Thailand.

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3) W. Soonthorntantikul, N. Leepipatpiboon, T. Ikegami, N. Tanaka, T. Nhujak "Selectivity Comparison of Monolithic Silica Capillary Columns Modified with Poly(octadecyl methacrylate) and Octadecyl Moiety for Halogenated Compounds in Reversed-Phase liquid chromatography" Poster Presentation, RGJ-Ph.D. Congress XI "Research Towards Sustainability", 1-3 April, 2010, Jomtien Palm Beach Hotel & Resort, Pattaya, Chonburi, Thailand.

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