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การเปรียบเทียบค่าจำเพาะการแยกสารของคะพิลลารีคอลัมน์ประเภทมอนอลิตที่ปรับปรุงพื้นผิวด้วยพอลิออกตะเดกซิลเมทะคริเลตและหมู่ออกตะเดกซิลสำหรับการแยกสารประกอบกลุ่มแฮโลเจนในลิควิดโครมาโทกราฟีแบบรีเวิร์สเฟส

Selectivity Comparisons of Monolithic Silica Capillary Columns Modified with Poly(octadecyl methacrylate) and Octadecyl Moieties for Halogenated Compounds in Reversed-Phase Liquid Chromatography

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

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ABSTRACT

Stationary phase selectivities for halogenated compounds in reversed-phase HPLC were compared using C18 monolithic silica capillary columns modified with poly(octadecyl methacrylate) (ODM) and octadecyl moieties (ODS). The preferential retention of halogenated benzenes on ODM was observed in methanol/water and acetonitrile/water mobile phases. In selectivity comparison of selected analytes on ODM and ODS, greater selectivities for halogenated compounds were obtained with respect to alkylbenzenes on an ODM column, while similar selectivities were observed with a homologous series of alkylbenzenes on ODM and ODS columns. These data can be explained by greater dispersive interactions by more densely packed octadecyl groups on the ODM polymer coated column together with the contribution of carbonyl groups in ODM side chains. 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 an ODS column in the same mobile phase. These results imply that the ODM column can be used as a better alternative to the ODS column for the separation of other halogenated compounds.

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 [1–5]. In previous work, hybrid monolithic silica capillary columns were prepared from a mixture of tetramethoxysilane (TMOS) and methyltrimethoxysilane (MTMS) [3]. The surface of monolithic silica capillary column can be modified to obtain various stationary phases in different chromatographic modes such as reversed-phase [1–4], ion-exchange [6] and hydrophilic interaction [7] 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 [8–12].

C18 monolithic silica capillary columns for reversed-phase HPLC can be obtained by chemical modification with octadecyl moieties (ODS column) [13] or coating with poly(octadecyl methacrylate) (ODM column) [14]. 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 [15]. 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 [21–23]. 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 [16,17]. These include a number of compound

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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 [18]. 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 [15,18] 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.

2. Experimental

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(ethyleneglycol) (PEG, MW = 10,000) from Sigma–Aldrich (Steinheim, Germany); octadecyl methacrylate from TCI (Tokyo, Japan). Methanol, hexane, 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).

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

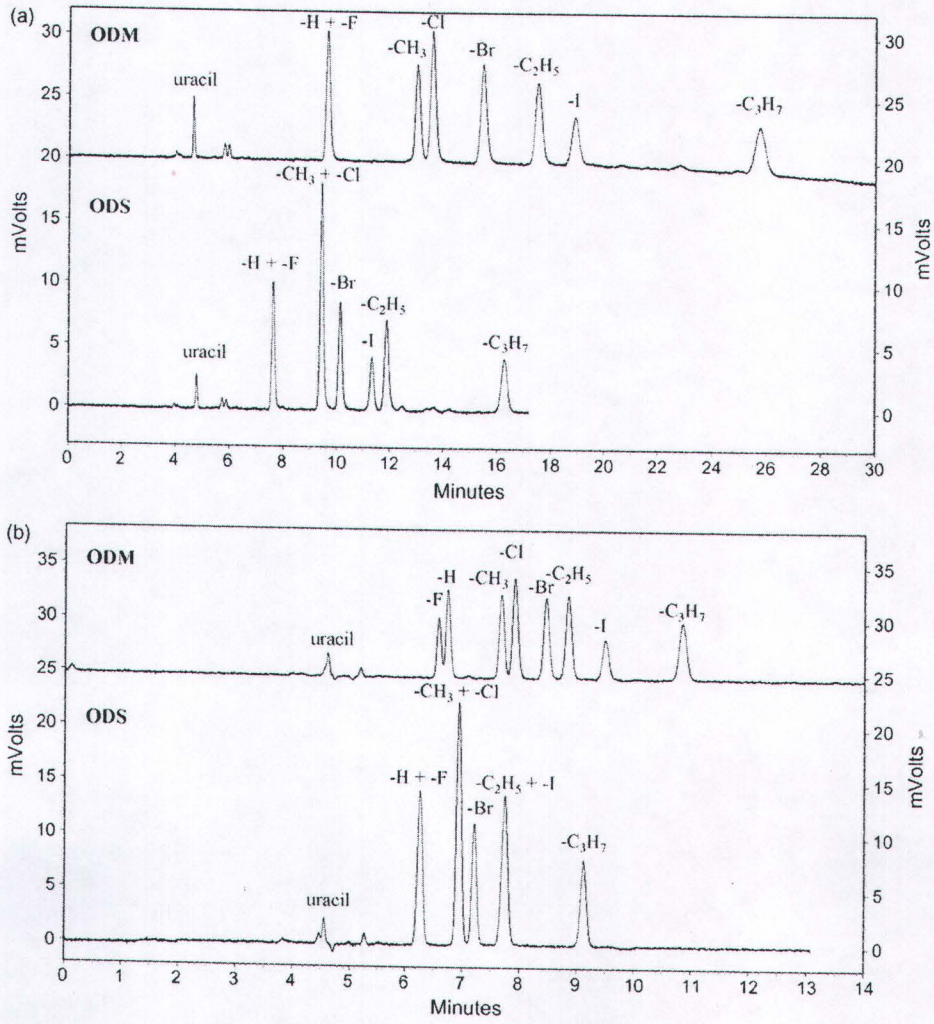

Fig. 1. Chromatograms of mixtures of monohalogenated benzenes and monoalkylbenzenes on ODM and ODS columns using (a) 70% (v/v) methanol and (b) 70% (v/v) acetonitrile. Uracil was used as an unretained marker.

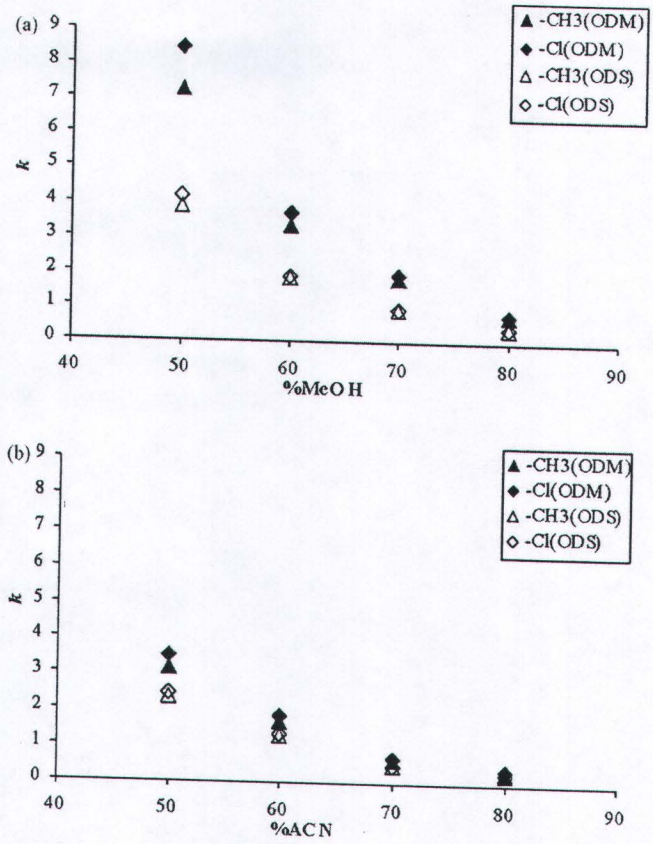

Fig. 2. Retention factor (k) of toluene and chlorobenzenes on ODM and ODS columns in a wide range of organic modifier concentrations: (a) methanol and (b) acetonitrile.

(–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-

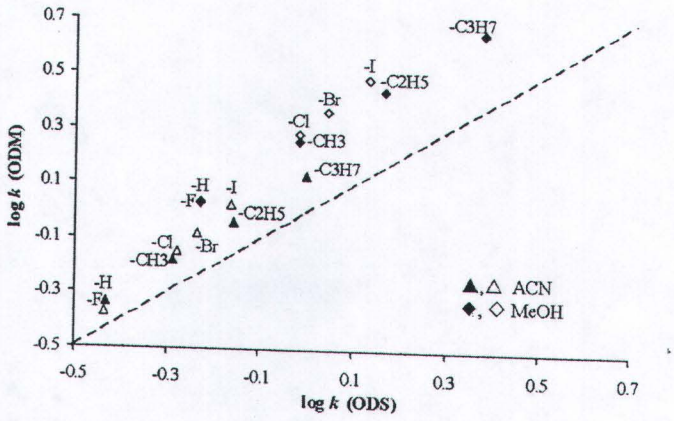

Fig. 3. Plots of log k of monosubstituted benzenes on an ODM column against those on an ODS column using (a) 70% (v/v) methanol and (b) 70% (v/v) acetonitrile as mobile phases.

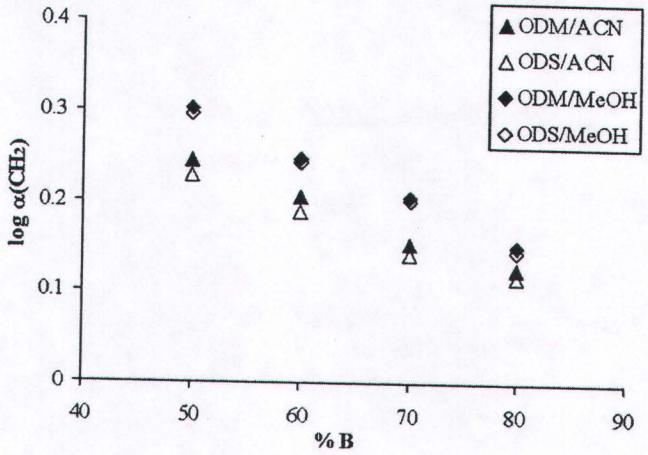

Fig. 4. Comparison of log $\alpha(CH_2)$ on ODM and ODS columns using acetonitrile and methanol mobile phases.

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).

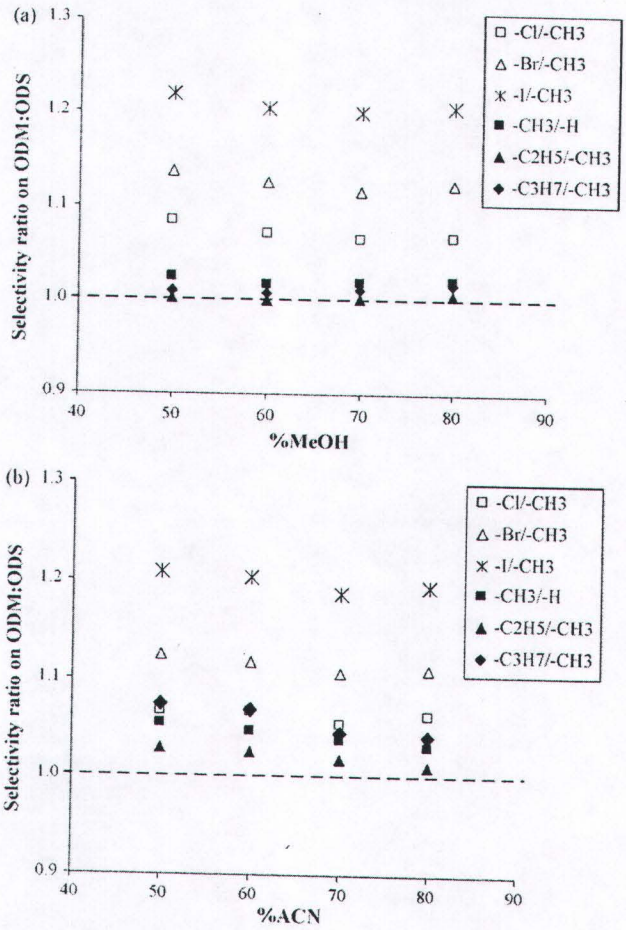

Fig. 5. The selectivity ratio on ODM:ODS columns for monosubstituted benzenes against selected reference analytes using (a) 50–80% (v/v) methanol and (b) 50–80% (v/v) acetonitrile as mobile phases.

2.2. 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.). The temperature of all systems was controlled in an air-circulating oven at 30 °C. 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.

2.3. Preparation and chemical modification of monolithic columns

Hybrid type monolithic silica capillary columns were prepared from a mixture of TMOS and MTMS in 200 μm I.D. fused silica capillaries, as previously reported [1]. The surface modification of ODM columns was obtained similar to previously reported procedures by free radical polymerization of octadecyl methacrylate using an AIBN initiator [14]. Briefly, monolithic silica capillary columns were washed with methanol for 24 h. Then, MOP bonding was carried out by rinsing columns with a solution of MOP:pyridine 1:1 for 48 h at 80 °C, followed by a methanol wash. 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 °C for 3 h. For ODS column preparation, monolithic silica capillary columns were washed with THF and then toluene. ODS columns were obtained 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 [13], 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 °C, followed by washes with toluene, THF and methanol, respectively.

The performance of prepared 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, $H_{amylbenzene}$ of 9.1 μm and K of 8.8×10^{-14} m^2 for ODS columns, while H_{uracil} of 10.9 μm, $H_{amylbenzene}$ 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,

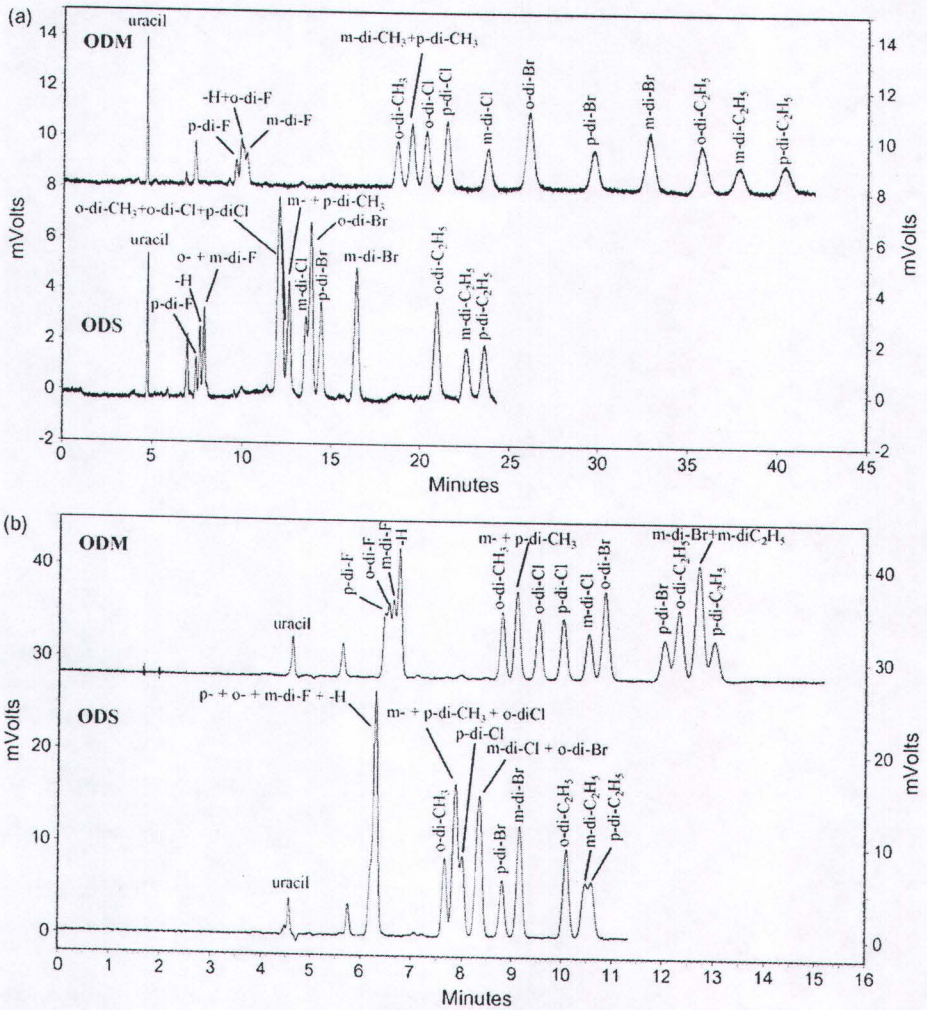

Fig. 6. Chromatograms of mixtures of dihalogenated benzenes and dialkylbenzenes on ODM and ODS columns using (a) 70% (v/v) methanol and (b) 70% (v/v) acetonitrile as mobile phases.

with RSD of 1.2% and 1.0% (n = two columns in the same batch of preparation).

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.

3.1. Monosubstituted benzenes

Fig. 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 Fig. 1a that the separation of –Cl and $–CH_3$, and –I and $–C_2H_5$ are achieved using the methanol mobile phase for the ODM column, but co-elution of –Cl and $–CH_3$ and reversed order of –I and $–C_2H_5$ were obtained on the ODS column under the same conditions. 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 (Fig. 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 (Fig. 1a) and ODS at 60% methanol (not shown) 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.

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 Fig. 2. 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 [14].

Fig. 3 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 Fig. 3, 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 (Fig. 3), 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 [12].

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. Fig. 4 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

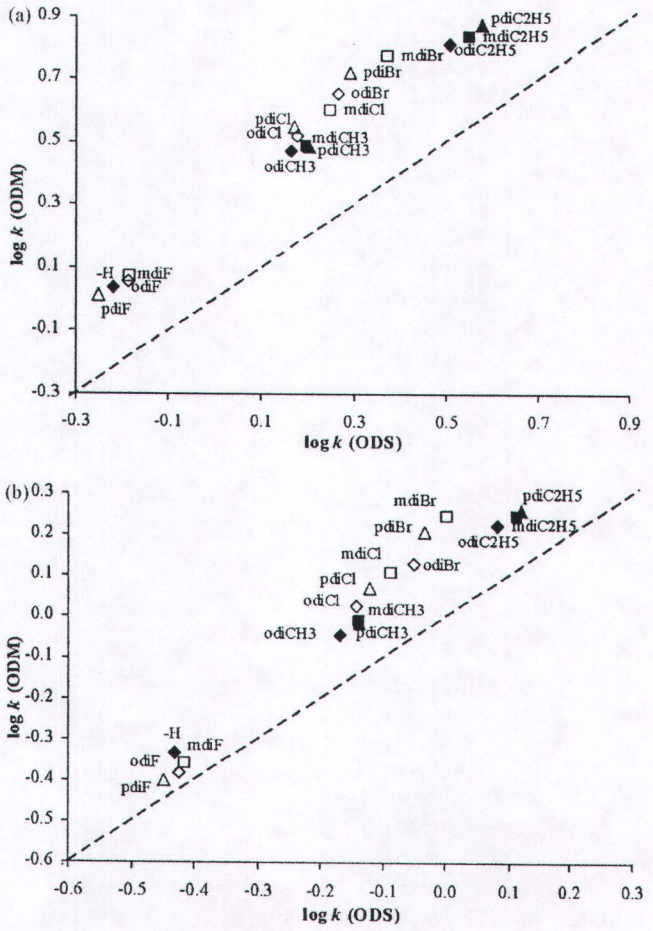

Fig. 7. Plots of log k of disubstituted benzenes on an ODM column against those on an ODS column using (a) 70% (v/v) methanol and (b) 70% (v/v) acetonitrile as mobile phases.

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) [14], 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 Fig. 3 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 [19].

Selectivity (α) is defined as the ratio of retention factors of 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 Fig. 5. 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 [20] 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 [21–23], 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.

3.2. Disubstituted benzenes

Fig. 6 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 are 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,

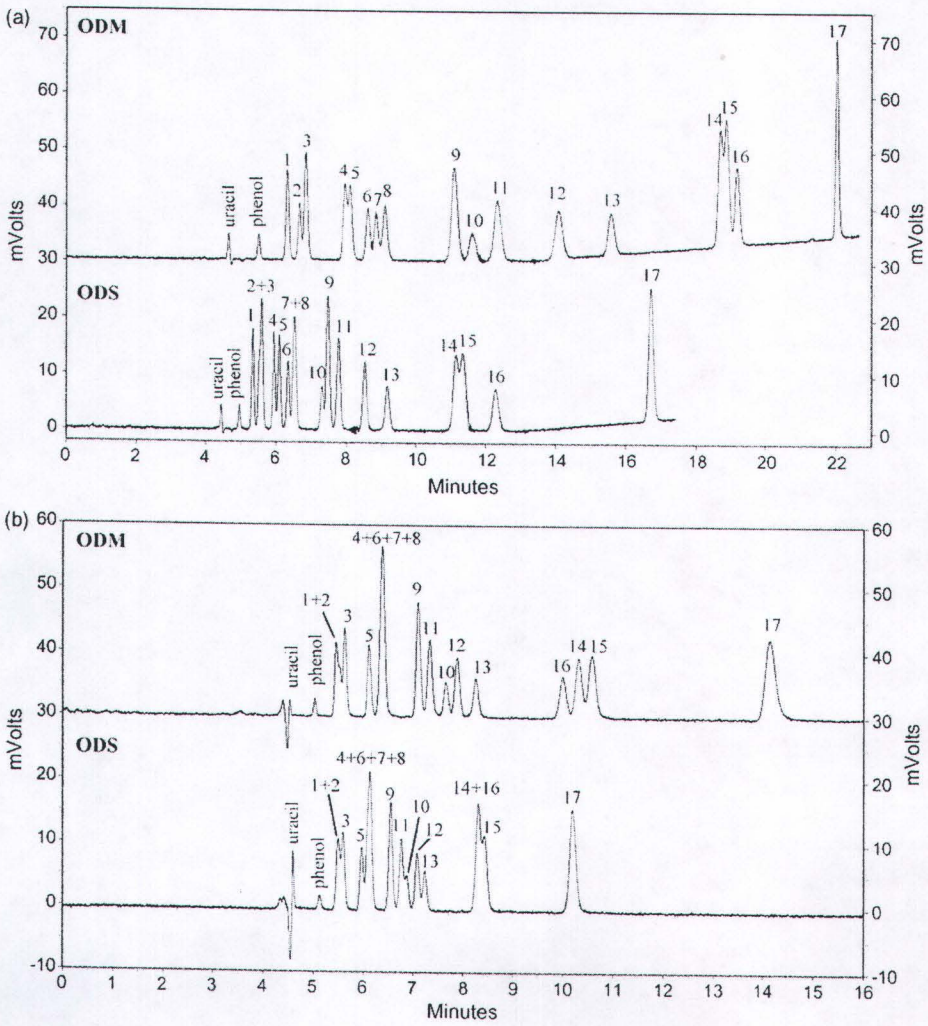

Fig. 8. Separation of 17 chlorophenols on ODM and ODS columns using (a) gradient elution of A:B with 70%B (0–8 min), 70–100%B (8–18 min) and 100%B (18–23 min), where A is water with 0.1% formic acid (FA), and B is methanol with 0.1% FA, and (b) isocratic elution of 40:60 A:B, where A is water with 0.1%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

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 Fig. 7. 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 [21–23].

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 are shown in Fig. 8, 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 [24]. As can be seen in Fig. 8a, 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 (Fig. 8b). 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 [21–23]. 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.

4. Conclusions

Monolithic silica capillary column modified with poly(octadecyl methacrylate) (ODM) have greater preference and greater selectivity for halogenated benzenes, in comparison with the monolithic silica capillary columns bonded using octadecyldimethyl-*N*,*N*-diethylaminosilane (ODS). This is due to the greater contribution of dispersive interactions on the ODM stationary phase with the contribution of carbonyl groups in ODM side chains. In most cases, 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 better separation of homologues and isomers of chlorophenols. The results imply that the ODM column can be used as an alternative to the conventional ODS column for separating other halogenated compounds.

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ผลงานวิจัยตีพิมพ์ในวารสาร

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การเปรียบเทียบและการทำนายรีเทนชันของสารประกอบแอโรแมติกที่มีหมู่แทนที่สองหมู่ในคะพิลลารีอิเล็กโทรไคเนทิกโครมาโทกราฟี

Comparison and Prediction of the Retention in Micellar Electrokinetic Chromatography and Microemulsion Electrokinetic Chromatography for Disubstituted Benzenes

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Research Article

Comparison and prediction of the retention in micellar electrokinetic chromatography and microemulsion electrokinetic chromatography for disubstituted benzenes

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

Keywords:
CE / Disubstituted benzenes / EKC / Retention / Separation
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1 Introduction

MEKC and MEEKC are widely used for CE separation of neutral as well as charged compounds [1–4], covering vitamins [5, 6], commercial pharmaceuticals [1, 7], bioanalysis [8] and natural products [9, 10]. Typically, MEKC is performed by adding a surfactant, such as SDS, to form a micellar phase [1, 11, 12], while MEEKC is performed by adding oil droplets (such as octane and ethyl acetate), a surfactant as an emulsifier (such as SDS) to stabilize the microemulsion and to generate charged oil droplets, and a cosurfactant (such as short chain alcohols) to lower the interfacial tension and to enhance the stability of the microemulsion system [3–5, 13–15]. The EKC separation mechanism is based on the difference in the partitioning of analytes between an aqueous phase and a pseudostationary phase (PSP), a micellar phase in MEKC [16, 17] and a microemulsion in MEEKC [18, 19].

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Abbreviations: BADGE, bisphenol-A-diglycidyl ether; **BZ,** alkylbenzenes; **DB,** dodecylbenzene; **PSP,** pseudostationary phase

In EKC, the retention factor (k), defined as the ratio of the total moles of analytes in PSP *versus* those in the aqueous phase, is one of the characteristics that express the retention behavior of analytes in PSP [13, 20, 21]. The retention mechanism in EKC may be explained using quantitative structure-relationships and quantitative structure–property relationships between the analytes and the PSP [22–24]. Theoretically, the retention factor is related to K and the phase ratio (V_m/V_{aq}) by the equation $k = K(V_m/V_{aq})$ [5, 25, 26], where K is the distribution constant of the analyte between the two phases, and V_m and V_{aq} are the PSP volume and the aqueous phase volume, respectively. At the same concentration of SDS in the buffers, higher values of log k were found in MEKC than MEEKC for six herbicides [25], bisphenol-A diglycidyl ether and its derivatives [26]. However, in comparison with MEKC at 50 mM SDS, MEEKC at 60 mM SDS gave a higher log k for test analytes [27].

Typically, the relative affinity of analytes in the same PSP and phase ratio under particular conditions can be compared using k. The higher the retention factor, the stronger the partitioning or the affinity of the analytes in PSP. In the case of the different phase ratios or PSP, such as the microemulsion and the micelle [27], it is more reasonable to compare the relative affinity of the analytes between two systems in terms of K rather than k, because k depends on the phase ratio, while K is independent of the phase ratio. However, with known k values derived experimentally, the value of K in MEEKC cannot be calculated exactly because of

an inexact value for the phase ratio as described in previous work [21, 28, 29].

The retention index (I) or migration index is another parameter to express the retention behavior of analytes, and is compared with the reference standards, such as a homologous series of alkylbenzenes (BZ) or alkyl aryl ketones [20, 21, 30]. The retention index is independent of the phase ratio and the concentration of the surfactant in MEKC [20, 30] and MEEKC [21], and, therefore, may be used for comparison of the relative affinity of analytes in different PSP and/or phase ratios.

In previous work [31], the values of k and K for disubstituted benzenes in MEKC were predicted based on the solute structure of monosubstituted benzenes using a group additive approach. The predicted values of log k for positional isomers (such as *ortho-*, *meta-* and *para-*) of disubstituted benzenes were assumed to be equal, while the observed values of log k for these compounds are different. Therefore, it is interesting to extend previous work [31] so as to be able to predict the retention behaviors of disubstituted benzenes.

Accordingly, the aims of this work are to investigate I for comparison of the relative affinity of disubstituted benzenes between the microemulsion in MEEKC and the micellar in MEKC at either the same or at different SDS concentrations, and to establish an alternative group-based additive approach for the prediction of k and I for disubstituted benzenes with different moieties from those for disubstituted benzenes with same moieties.

2 Materials and methods

2.1 Chemicals

Disodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$), 1-butanol and a homologous series of benzenes (BZ), such as benzene, toluene, ethylbenzene and propylbenzene, were obtained from Fluka (Buchs, Switzerland); SDS was from Sigma (St. Louis, MO, USA); ethyl acetate was from Merck (Darmstadt, Germany); dodecylbenzene (DB) and the following disubstituted benzenes were from Sigma-Aldrich (Steinheim, Germany): *o-*, *m-*, *p*-xylenes (diMe); *o-*, *m-*, *p*-dimethoxybenzenes (diOMe); *o-*, *m-*, *p*-dibenzaldehydes (diCHO); dihalogenated benzenes with same moieties (diX), such as *o-*, *m-*, *p*-difluorobenzenes (diF), *o-*, *m-*, *p*-dichlorobenzenes (diCl) and *o-*, *m-*, *p*-dibromobenzenes (diBr); dihalogenated benzenes with different moieties (X/Y), such as *o-*, *m-*, *p*-chlorofluorobenzenes (F/Cl), *o-*, *m-*, *p*-bromochlorobenzenes (Cl/Br) and *o-*, *m-*, *p*-bromofluorobenzenes (F/Br); halogenated toluenes (Me/X), such as *o-*, *m-*, *p*-chlorotoluenes (Me/Cl), *o-*, *m-*, *p*-fluorotoluenes (Me/F) and *o-*, *m-*, *p*-bromotoluenes (Me/Br); halogenated methoxybenzenes (OMe/X), such as *o-*, *m-*, *p*-chloromethoxybenzenes (OMe/Cl), *o-*, *m-*, *p*-fluoromethoxybenzenes (OMe/F) and *o-*, *m-*, *p*-bromomethoxybenzenes (OMe/Br); halogenated benzaldehydes (CHO/X), such as *o-*, *m-*, *p*-chlorobenzaldehydes (CHO/Cl), *o-*, *m-*, *p*-fluorobenzaldeshydes (CHO/F) and *o-*, *m-*, *p*-bromobenzaldehydes (CHO/Br); *o-*, *m-*, *p*-methoxytoluenes (Me/OMe); *o-*, *m-*, *p*-tolualdehydes (Me/CHO); *o-*, *m-*, *p*-methoxybenzaldehydes (OMe/CHO).

2.2 Preparation of buffers and analytes

The MEKC buffer contained 10 mM $Na_2B_4O_7$ at pH 9.2 and 50 mM SDS, while MEEKC buffer contained 10 mM $Na_4B_2O_7$ at pH 9.2, 50 or 60 mM SDS, 0.5% v/v ethyl acetate and 1.2% v/v 1-butanol. It should be noted that oils typically used in MEEKC include octane, heptane and ethyl acetate. However, ethyl acetate/water has lower interfacial tension and requires lower SDS concentration than octane/water or heptane/water to form stable microemulsions [4, 32]. In MEEKC with high EOF, the higher the SDS concentration, the longer the analysis time. Therefore, ethyl acetate with 50 and 60 mM SDS (1.4 and 1.7% w/v) was used in this work.

All buffers were sonicated for 30 min to obtain clear degassed solutions. Stock solutions of each analyte, thiourea (an EOF marker) and DB (a PSP marker), were separately prepared at a concentration of 10 000 ppm in methanol. In MEKC, the sample mixture containing the desired analytes, thiourea and DB at 100 ppm each, was obtained by pipetting each stock solution and then diluting the mixture with 50 mM SDS in water, while the sample mixture for MEEKC analysis was diluted with the solution containing 50 mM SDS in water, 0.5% v/v ethyl acetate and 1.2% v/v 1-butanol. All buffers and final test analytes solutions were filtered through a 0.45-μm PTFE membrane filters prior to CE analysis.

2.3 CE conditions

All the CE separations were performed using a Beckman Coulter MDQ-CE system equipped with a photo-DAD scanning from 190 to 300 nm and monitoring at 214 nm. An uncoated fused-silica capillary of 40.2 cm in length (30 cm to detector) × 50 μm id, thermostated at 25°C, was used. The voltage was set at 15 kV and the sample solution was introduced by 0.5 psi pressure injection for 3 s. Each experiment was carried out in triplicate. Between consecutive runs, the capillary was rinsed with methanol, 0.1 M NaOH for 2 min each and then with the buffer for 1 min.

3 Results and discussion

3.1 Retention and retention index in MEKC and MEEKC

In MEKC and MEEKC, the retention index of analytes can be estimated from the equation [20, 21]

$$I = \frac{100\,(\log k - b)}{a} \quad (1)$$

where a and b are the slope and the intercept, respectively, of a linear Eq. (2) between log k and z:

$$\log k = az + b \tag{2}$$

where z is the number of carbon atoms of a homologous series used as standard reference compounds. From an electropherogram of MEKC and MEEKC with high EOF, k for uncharged analytes can be calculated according to the equation:

$$k = \frac{t_m - t_{eo}}{t_{eo}(1 - t_m/t_{mc})} \tag{3}$$

where t_m, t_{mc} and t_{eo} are the migration times of an analyte, a PSP marker (dodecylbenzene) and an EOF marker (thiourea), respectively.

Table 1. Linear relationship between the log k and z values obtained from a homologous series of alkylbenzenes in MEEKC and MEKC

Mode	[SDS] mM	log $k = az + b$		
		a	b	r^2
MEKC	50	0.448 ± 0.011	−2.695 ± 0.085	0.9987
MEEKC	50	0.442 ± 0.012	−2.615 ± 0.087	0.9986
MEEKC	60	0.443 ± 0.013	−2.534 ± 0.096	0.9984

Using a homologous series of BZ (C6 to C9), the linear relationship between log k and z was obtained, as given in Table 1. Similar methylene selectivity, where $\alpha_{CH2} = a$ [29], indicates that the microemulsion and micellar phases have a similar hydrophobicity [21].

Figure 1 shows a comparison of the log k and I values for disubstituted benzenes, obtained from MEEKC and plotted against those obtained from MEKC, where I values were determined using BZ as standards. Comparison of I and k values between the same and different concentrations of SDS revealed a similar relative I for each analyte in MEEKC against MEKC (Fig. 1B and D) and thus the independence of I with respect to the SDS concentration, whilst a different relative log k value (Fig. 1A and C) was observed due to the dependence of k upon the SDS concentration. This implies that I can be used to compare the relative affinity of solutes in different PSP or phase ratios. Similar I values in MEKC and MEEKC were obtained for xylenes, dihalogenated benzenes and halogenated toluenes, while significantly smaller I values were observed in MEEKC than in MEKC for diCHO, diOMe and OMe/CHO. In addition, a slightly smaller I in MEEKC than in MEKC was observed for disubstituted benzenes containing a methoxy moiety or an aldehyde moiety, such as OMe/X, Me/OMe, CHO/X and Me/CHO. This may be explained as being due to the dominant hydrophobic interaction of the PSP-solute in the highly hydrophobic xylenes and dihalogenated benzenes resulting in a similar affinity of solutes in the micelles and the

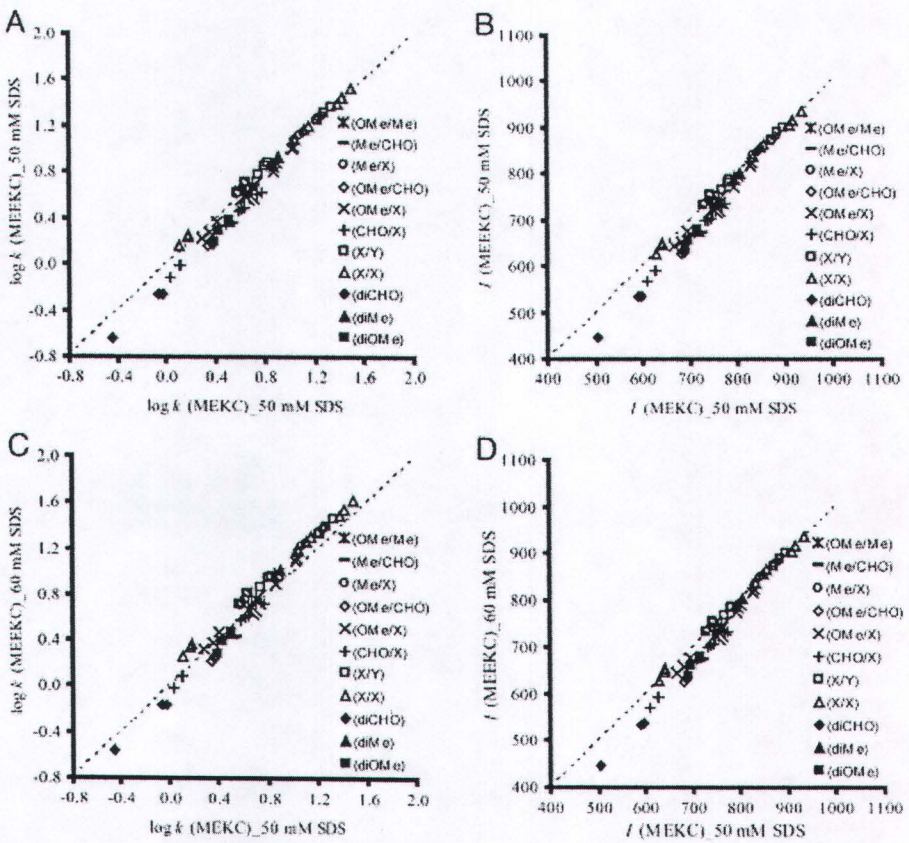


Figure 1. Plots of the observed log k (A and C) and I (B and D) values obtained for disubstituted benzenes in MEEKC against the same in MEKC.

microemulsion. Since the methoxy and aldehyde moieties are more polar than the alkyl and halogenated moieties, free 1-butanol in the aqueous solution and/or 1-butanol co-surfactant at the oil surface decreases K with different selectivity, resulting in a smaller I value in MEEKC than in MEKC. It should be noted that the retention for disubstituted benzenes are in the order of (i) diCHO < diOMe < diMe < diF < diCl < diBr, and (ii) F/Cl < F/Br < Cl/Br. The I order is similar to the log K_{ow} order given in Table 2.

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

The value of log K_{ow} may be determined from the linear relationship between log K_{ow} and log k [33–35] or log K_{ow} and I [21, 28]. In our previous work, no significant difference was found between K_{ow} values obtained from k or I. In addition, a calibration plot of log K_{ow} against I is simpler when known values of nominal I for BZ are used as I standards [21] according to Eq. 4

$$\log K_{ow} = cI + d \quad (4)$$

where c and d are the slope and the intercept, respectively.

Using C6–C9 BZ as standards [21, 36], a log K_{ow}-nominal I calibration gives the best fit as a linear equation (log $K_{ow} = 0.00516(\pm 0.0001)I - 0.970(\pm 0.080)$), with an r^2 value of 0.9992. The observed values of log K_{ow} for the disubstituted benzenes obtained from MEKC and MEEKC are shown in Table 2, with the literature values of the same compounds where available. Excellent agreement of log K_{ow} from MEKC and MEEKC was found for xylenes, dihalogenated benzenes

Table 2. Predicted and literature values of log K_{ow} values for the disubstituted benzenes in MEKC and MEEKC

Disubstituted benzene	log K_{ow}			Disubstituted benzene	log K_{ow}		
	Observed[a]		Literature [36]		Observed[a]		Literature [36]
	MEKC	MEEKC			MEKC	MEEKC	
o-diMe	3.12	3.10	3.12	*o*-Me/CHO	2.68	2.47	2.26
m-diMe	3.18	3.17	3.20	*m*-Me/CHO	2.74	2.51	NA
p-diMe	3.18	3.17	3.15	*p*-Me/CHO	2.71	2.48	NA
o-diF	2.33	2.38	NA	*o*-OMe/F	2.46	2.36	NA
m-diF	2.33	2.37	NA	*m*-OMe/F	2.61	2.53	NA
p-diF	2.26	2.27	NA	*p*-OMe/F	2.54	2.41	NA
o-diCl	3.42	3.45	3.38	*o*-OMe/Cl	2.69	2.50	NA
m-diCl	3.46	3.48	3.48	*m*-OMe/Cl	2.80	2.64	NA
p-diCl	3.34	3.34	3.38	*p*-OMe/Cl	2.84	2.64	NA
o-diBr	3.78	3.76	NA	*o*-OMe/Br	3.33	3.27	NA
m-diBr	3.87	3.85	NA	*m*-OMe/Br	3.32	3.24	NA
p-diBr	3.74	3.71	NA	*p*-OMe/Br	3.13	3.02	NA
o-F/Cl	2.87	2.91	NA	*o*-CHO/F	2.16	1.96	NA
m-F/Cl	2.88	2.91	NA	*m*-CHO/F	2.25	2.08	NA
p-F/Cl	2.79	2.80	NA	*p*-CHO/F	2.26	2.08	NA
o-F/Br	3.06	3.09	NA	*o*-CHO/Cl	2.95	2.86	NA
m-F/Br	3.09	3.11	NA	*m*-CHO/Cl	3.17	3.12	NA
p-F/Br	3.00	2.99	NA	*p*-CHO/Cl	3.14	3.08	NA
o-Cl/Br	3.61	3.62	NA	*o*-CHO/Br	2.87	2.66	NA
m-Cl/Br	3.68	3.68	NA	*m*-CHO/Br	2.99	2.80	NA
p-Cl/Br	3.55	3.54	NA	*p*-CHO/Br	3.03	2.82	NA
o-Me/F	2.85	2.86	NA	*o*-OMe/CHO	2.53	2.28	NA
m-Me/F	2.81	2.81	NA	*m*-OMe/CHO	2.56	2.32	NA
p-Me/F	2.80	2.80	NA	*p*-OMe/CHO	2.56	2.29	NA
o-Me/Cl	3.42	3.42	3.42	*o*-diOMe	2.59	2.33	NA
m-Me/Cl	3.38	3.38	3.28	*m*-diOMe	2.72	2.54	NA
p-Me/Cl	3.34	3.35	3.33	*p*-diOMe	2.63	2.42	NA
o-Me/Br	3.59	3.59	NA	*o*-diCHO	1.60	1.34	NA
m-Me/Br	3.55	3.56	NA	*m*-diCHO	2.09	1.80	NA
p-Me/Br	3.54	3.54	NA	*p*-diCHO	2.06	1.80	NA
o-Me/OMe	2.95	2.88	2.74				
m-Me/OMe	2.87	2.78	2.66				
p-Me/OMe	2.91	2.80	2.81				

NA = not available.
a) SD ≤ ±0.08.

and halogenated toluenes, while in contrast a smaller log K_{ow} value was derived from MEEKC than from MEKC for the disubstituted benzenes containing a mono- or di-substituent of methoxy or aldehyde. This difference can be explained in a similar way as that of the differences in I values above. In addition, the observed log K_{ow} from MEKC and MEEKC was found to be in good agreement with the available literature values of some compounds, except for *o*-methylbenzaldehyde, indicating that in most cases either MEKC or MEEKC can be used for determination of log K_{ow}.

3.3 Predicted and observed retention for disubstituted benzenes with different moieties

The group additive approach is based on the assumption of additive-constitutive properties [30, 37, 38], and is applied for calculation of log K_{ow} [29, 39, 40], from Eq. (5)

$$\log K_{ow}(PR) = \kappa(P) + \sum \kappa(R) \quad (5)$$

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

The values of log K_{ow} for disubstituted benzenes with the same and different *R*-values are given by Eqs. (6)–(8), where P in this work refers to C_6H_4.

$$\log K_{ow}(PR^1R^1) = \kappa(P) + 2\kappa(R^1) \quad (6)$$

$$\log K_{ow}(PR^2R^2) = \kappa(P) + 2\kappa(R^2) \quad (7)$$

$$\log K_{ow}(PR^1R^2) = \kappa(P) + \kappa(R^1) + \kappa(R^2) \quad (8)$$

It follows from Eqs. (6)–(8) that

$$\log K_{ow}(PR^1R^2) = \frac{\log K_{ow}(PR^1R^1) + \log K_{ow}(PR^2R^2)}{2} \quad (9)$$

Since log k and I are directly related to log K_{ow}, it follows from Eqs. (2), (4) and (8) that

$$I(PR^1R^2) = \frac{I(PR^1R^1) + I(PR^2R^2)}{2} \quad (10)$$

and so

$$\log k(PR^1R^2) = \frac{\log k(PR^1R^1) + \log k(PR^2R^2)}{2} \quad (11)$$

Therefore, with a known log K_{ow}, I and log k for the disubstituted benzenes with the same R moieties, the predicted values for disubstituted benzenes with a different R can be obtained from the average value for PR^1R^1 and PR^2R^2, without having to know the values of κ.

Figure 2 compares the observed and predicted values of I for different disubstituted benzenes, using I data obtained from disubstituted benzenes with the same R. For example, the predicted I for *p*-chlorofluorobenzene is obtained from the average I for *p*-dichlorobenzene and *p*-difluorobenzene. In general, the observed values of I were found to be consistent with the predicted ones, and especially for halogenated toluenes and dihalogenated benzenes. However, poor agreement between the observed and predicted I values was seen for the methoxyaldehydes and tolualdehydes, and particularly in the case of the *ortho*-isomers. This may be caused by electronic effects for all positional isomers, such as the electron withdrawing by aldehyde groups and electron releasing by methoxy groups [41], and intramolecular interactions of the *ortho*-substituents on a change in the polarity of these compounds. This implies that the group additive approach is suitable for the prediction of the reliable retention rates for disubstituted benzenes with small effects of electronic affecting groups. It should be noted that, in previous work [31], the group additive approach was used for prediction of the micelle–water distribution constant (K_{mw}) in MEKC using a similar relationship to that of Eq. (5). However, the known values of κ_{mw} for P and R were needed and, moreover, the predicted values of K_{mw} for *o*-, *m*- and *p*-PR^1R^2 were equal, yet the observed values of K_{mw} for these positional isomers are in fact different.

3.4 Predicted and observed values of log *k* for disubstituted aromatic compounds from our previous work

In our previous work, the retention of bisphenol-A-diglycidyl ether derivatives (BADGEs) [26] and curcuminoids [42] containing two different moieties were observed to be good between those containing the same two moieties. Tables 3 and 4 show the good agreement between the predicted and observed log k

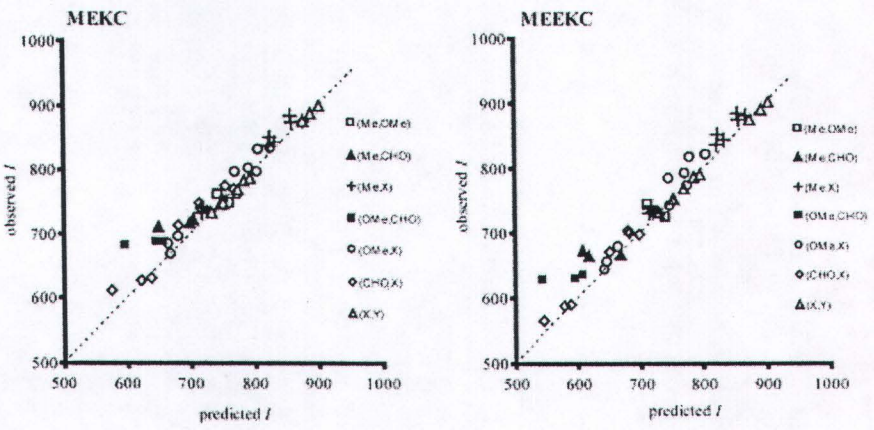
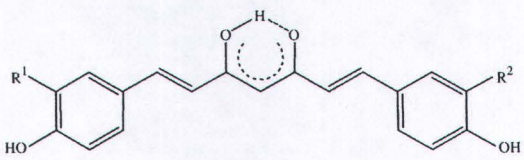


Figure 2. Plots of the observed and predicted I values obtained for disubstituted benzenes with different moieties in MEKC and MEEKC.

Table 3. Observed and predicted values of log *k* for curcuminoids containing two different R moieties (–OCH_3, –H)

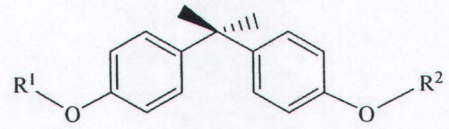


Demethoxycurcumin
(R^1 = –OCH_3, R^2 = –H)

MEEKC conditions	log *k*	
	Predicted[a)]	Observed[b)]
(a) *Various types and concentrations of organic co-solvents (+180 mM SDS)*		
25% v/v ACN	0.651	0.649
25% v/v EtOH	0.828	0.825
25% v/v 2-PrOH	0.700	0.698
30% v/v 2-PrOH	0.602	0.600
(b) *Various [SDS] and 25% v/v 2-PrOH*		
130 mM	0.646	0.643
180 mM	0.700	0.698

The MEEKC buffer contained 50 mM phosphate at pH 2.5, 1.1% v/v *n*-octane, 890 mM 1-butanol plus SDS and organic co-solvents as indicated in the table. Other CE conditions are as reported in [42].
a) Obtained from the average log *k* for bis-demethoxycurcumin ($R^1 = R^2 =$ –OCH_3) and curcumin ($R^1 = R^2 =$ –H).
b) Data from previous work [42]. RSD for $k < 1.0\%$.

Table 4. Observed and predicted values of log *k* for BADGE and BADGE.HCl containing two different substituting groups



BADGE.HCl.H_2O
(R^1 = 2,3-dihydroxypropyl, R^2 = 3-chloro-2-hydroxylpropyl)
BADGE.H_2O
(R^1 = 2,3-dihydroxypropyl, R^2 = glycidyl ether)

CE conditions	log *k* in MEEKC				log *k* in MEKC			
	BADGE.HCl.H_2O		BADGE.H_2O		BADGE.HCl.H_2O		BADGE.H_2O	
	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed
(a) *Various types of organic co-solvents at 25% v/v and 180 mM SDS*								
ACN	0.591	0.613	0.567	0.569	1.00	1.03	0.945	0.950
EtOH	0.571	0.582	0.538	0.531	1.44	1.48	1.36	1.36
2-PrOH	0.502	0.506	0.481	0.469	0.887	0.914	0.850	0.849
(b) *Various [SDS] and 25% v/v EtOH*								
180 mM	0.571	0.582	0.538	0.531	1.44	1.48	1.36	1.36
140 mM	0.511	0.524	0.474	0.467	1.38	1.41	1.29	1.28
100 mM	0.413	0.424	0.368	0.362	1.31	1.34	1.20	1.19

The MEKC buffer contained 50 mM phosphate at pH 2.5, plus SDS and organic co-solvents as indicated. The MEEKC buffer contained 50 mM phosphate at pH 2.5, 1.0% v/v *n*-octane, 890 mM 1-butanol, plus SDS and organic co-solvents as indicated. Other CE conditions were as reported in [26]. RSD for $k < 1.5\%$.

values obtained for the curcuminoid and bisphenol-A-diglycidyl ether each containing two different moieties, which indicates that the group additive approach can also be extended to be used for prediction of the retention of disubstituted aromatic compounds with a large parent molecule.

4 Concluding remarks

The values of *k* and *I* for disubstituted benzenes in MEKC and MEEKC were determined. Comparing the relative *I* values for each solute in MEEKC against those in MEKC, revealed very

similar values, and was independent of I with respect to the SDS concentration. In contrast, under the same comparisons, different relative k values were found. This implies that I, rather than k, can be used as the parameter to compare the relative affinity of compounds in MEEKC and MEKC that have different PSP and phase ratios. A similar I value in both the MEEKC and MEKC was observed for the disubstituted benzenes with $-CH_3$ and/or halogen moieties ($-F$, $-Cl$ and $-Br$). In contrast, the same disubstituted benzenes with $-CHO$ and $-OCH_3$ moieties revealed a smaller I value being obtained in MEEKC than in MEKC, presumably because free 1-butanol in the aqueous phase in MEEKC increases the solubility of the solutes and thereby, reduces the affinity or I of the solutes in the microemulsion. In addition, the predicted log k and I values for disubstituted benzenes with different moieties of $-CH_3$ and halogens were found to be in excellent agreement with the observed values, when the predicted values were obtained from the average for disubstituted benzenes with same moieties, according to a group additive approach. However, a significant difference between the observed and predicted retention was found for disubstituted benzenes with at least one moiety of $-CHO$ or $-OCH_3$, due to electronic effects of polar moieties causing a deviation in the actual affinity of solutes away from the predicted interactions.

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ภาคผนวก 3

ผลงานวิจัยตีพิมพ์ในวารสาร

Electrophoresis 32 (2011), 203-209

แบบจำลองทางทฤษฎีของค่าจำเพาะการแยกสำหรับสารที่มีประจุในไมเซลลาร์อิเล็กโทรไคเนทิโครมาโทกราฟี

Theoretical Models of Separation Selectivity for Charged Compounds in Micellar Electrokinetic Chromatography

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Research Article

Theoretical models of separation selectivity for charged compounds in micellar electrokinetic chromatography

Equations and theoretical models for MEKC separation selectivity (α_{MEKC}) were established to explain a change in separation and electrophoretic mobility order of fully charged analytes, in which α_{MEKC} is related to the dimensionless values of mobility selectivity in CZE (α_{CZE}) and retention selectivity (α_k) in MEKC, and where α_{CZE} and α_k are defined as the ratio of electrophoretic mobility in CZE and the ratio of retention factor (k) in MEKC for two charged analytes, respectively. Using four alkylparabens as test analytes, excellent agreement was found between the observed α_{MEKC} and the proposed α_{MEKC} models of test analytes in MEKC over a wide range of SDS concentrations and values of k. For example, in comparison with CZE separation of charged analytes, MEKC separation can enhance separation selectivity up to the maximum value when the selectivity ratio (ρ) is greater than 1.0 ($\rho = \alpha_k/\alpha_{CZE}$), while lower separation selectivity is obtained with $\rho < 1.0$ ($\alpha_{CZE} > \alpha_k > 1$).

Keywords:
Charged compounds / MEKC / Mobility selectivity / Retention selectivity / Separation selectivity
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1 Introduction

MEKC is one of the modes in CE in which the MEKC buffer contains a surfactant, such as SDS, to form micelles acting as a pseudo-stationary phase [1–5]. MEKC is widely used for separation of neutral and charged compounds as reported in reviews covering the literature up to 1999 [6], 2002 [7], 2003 [8], 2005 [3], and 2009 [9]. The MEKC separation of neutral analytes is performed using ionic surfactants [1, 3–6, 9–13], while charged analytes are separated using ionic, or non-ionic surfactants [1, 3, 4, 6, 9, 10, 14–16]. With different partitioning of analytes in the micelle, neutral as well as charged analytes show differences in effective electrophoretic mobility ($\Delta\mu$), and therefore these can be separated in MEKC [2, 3, 6, 14, 17, 18]. Typically, the resolution, R_s, of two analytes in CE is given by [19, 20]:

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

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Abbreviations: **BP**, butyl *p*-hydroxybenzoate; **EP**, ethyl *p*-hydroxybenzoate; **IP**, methyl *m*-hydroxybenzoate; **PP**, propyl *p*-hydroxybenzoate

where N is the number of theoretical plates or efficiency, μ is the effective electrophoretic mobility, $\Delta\mu$ is the difference in μ, $\mu_2-\mu_1$, for two analytes, and μ_{eo} is the electroosmotic mobility. For two analytes with same direction of μ, Eq. (1) may be rearranged to relate to the resolution R_s to the efficiency term, the selectivity term, and the mobility term as expressed in the following equation:

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha_m - 1}{\alpha_m}\right)\left|\frac{\mu_2}{\bar{\mu}+\mu_{eo}}\right| \quad (2)$$

where α_m is the separation selectivity or mobility selectivity, which is defined as the ratio of effective electrophoretic mobilities for two analytes such as μ_2/μ_1 for $|\mu_2| > |\mu_1|$.

In MEKC with normal elution mode, the same direction of electroosmotic velocity (v_{eo}), the observed velocity of analytes and observed velocity of micelles (v_{mc}), and $|v_{eo}| > |v_{mc}|$, the resolution equation for neutral analytes can be expressed to relate to the retention term as follows [21, 22]:

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha_k - 1}{\alpha_k}\right)\left(\frac{k_2}{1+\bar{k}}\right)\left(\frac{1 - t_{eo}/t_{mc}}{1+(t_{eo}/t_{mc})\bar{k}}\right) \quad (3)$$

where k is the retention factor, α_k is the retention selectivity defined as the ratio of k such as k_2/k_1, and t_{eo} and t_{mc} are the migration times of an EOF marker and a micelle marker, respectively.

With the normal elution mode of the MEKC separation of fully charged analytes due only to the difference in micellar partitioning and not in their electrophoretic

mobilities, the resolution equation is given as follows [21]:

$$R_s = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{\alpha_k - 1}{\alpha_k}\right)\left(\frac{k_2}{1+\bar{k}}\right) \times \left(\frac{1+t_{eo}/t_{ep} - t_{eo}/t_{mc}}{1+t_{eo}/t_{ep}+(t_{eo}/t_{mc})\bar{k}}\right) \quad (4)$$

where t_{ep} is the migration time corresponding to the electrophoretic mobility of the charged analyte, and $t_{ep} = t_{ep2} = t_{ep1}$ for this case.

It can be seen in Eqs. (3) and (4) that the separation selectivity may be now referred to the retention selectivity. In the case of analytes with a difference in their electrophoretic mobility (μ_0) at zero concentration of surfactant or CZE, the MEKC separation is based on the differences in both k and μ_0. In comparison with the CZE separation of charged analytes, the addition of surfactant to the MEKC buffer may result in improved separation for some analytes, but lowered degree of separation for other analytes [4, 10, 13–17, 23–26]. These dual effects are similar to those seen in chiral separation using dual cyclodextrins as reported in our previous work [27].

In order to explain the separation selectivity and electrophoretic mobility order in MEKC for charged analytes with differences in μ_0, the aims of this work are to establish theoretical models of MEKC separation selectivity which is related to the dimensionless values of mobility selectivity in CZE and retention selectivity in MEKC, and to compare the observed and predicted separation selectivity in MEKC. The MEKC separation with normal elution mode was carried out using SDS surfactant in a 10-mM disodium tetraborate buffer at pH 10.2, and the test analytes used were alkylparabens.

2 Materials and methods

2.1 Chemicals

All the test analytes were purchased from Sigma-Aldrich (Steinheim, Germany): isomethylparaben (methyl *m*-hydroxybenzoate; IP), ethylparaben (ethyl *p*-hydroxybenzoate; EP), propylparaben (propyl *p*-hydroxybenzoate; PP), and butylparaben (butyl *p*-hydroxybenzoate; BP). Sodium hydroxide and disodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$) were supplied by Fluka (Buchs, Switzerland), SDS from Sigma (St. Louis, MO, USA), all organic solvents obtained from Merck (Dramstadt, Germany), and dodecylbenzene/micellar marker (M) from Sigma-Aldrich.

2.2 CE conditions

CE experiments were carried out with a Beckman Coulter MDQ-CE system equipped with a photo-DAD scanning from 190 to 300 nm and monitoring at 220 nm. The data-handling system comprised an IBM PC and 32 Karat Software. The uncoated fused-silica capillary, 40.2 cm in length (30 cm to detector) × 50 μm id (Polymicro Technologies, AZ), was used for CZE and MEKC separations, thermostated at 25°C. Voltage was set at 15 kV. A sample solution was introduced by 0.5 psi pressure injection for 3 s. Prior to each daily analysis, the capillary was rinsed sequentially for 15 min with methanol, 0.1 M NaOH, water, and a running buffer. Between consecutive runs, the capillary was flushed with 0.1 M NaOH and then with a running buffer, each for 2 min. All experiment runs were performed in duplicate.

2.3 Buffering electrolytes

The electrophoretic mobilities were determined in two buffer systems: (1) CZE with 10 mM disodium tetraborate buffer, adjusted to pH 10.2 with 1.0 M NaOH, and (2) MEKC with 20 to 60 mM SDS in a pH 10.2 10 mM disodium tetraborate buffer. All buffers were prepared using Milli-Q water, sonicated for 30 min, and then filtered through 0.45 μm PTFE filters prior to use.

2.4 Stock and standard solutions

Each test analyte was separately dissolved in 5 mL of ACN, and then each analyte solution was diluted with Milli-Q water in a 10 mL volumetric flask to give 1000 ppm stock solutions. Stock solutions of DB and thiourea were also separately dissolved in ethanol, and then diluted to 20 000 ppm with Milli-Q water. A working standard solution containing 20–60 ppm of each test analyte, 30 ppm thiourea, and 150 ppm DB was prepared by diluting each of the stock solutions with a 1.0 mM borate buffer. All solutions were filtered through 0.45 μm PTFE filters prior to analysis.

3 Results and discussion

3.1 Theoretical models of separation selectivity in MEKC for charged compounds

In MEKC, effective electrophoretic mobilities of two fully charged analytes, μ_{MEKC}, are given by [21]

$$\mu_{(MEKC,1)} = \left(\frac{\mu_{0,1} + k_1\mu_{mc}}{1+k_1}\right) \quad (5)$$

$$\mu_{(MEKC,2)} = \left(\frac{\mu_{0,2} + k_2\mu_{mc}}{1+k_2}\right) \quad (6)$$

where μ_0 is the electrophoretic mobility at zero concentration of SDS or under CZE conditions, and μ_{mc} is the electrophoretic mobility of the micelle marker. Subscripts 1

and 2 refer to the analytes 1 and 2, respectively. The separation selectivity in MEKC (α_{MEKC}), the ratio of μ_{MEKC} with $k_2 \geq k_1 > 0$, may be rearranged to relate to the dimensionless values as follows:

$$\alpha_{MEKC} = \alpha_{CZE}\left(\frac{\beta + \frac{\alpha_k}{\alpha_{CZE}}k_1}{\beta + k_1}\right)\left(\frac{(1+k_1)}{1+\alpha_k k_1}\right) \quad (7)$$

where α_{CZE} is the mobility selectivity in CZE or the ratio of μ_0, *e.g.* $\mu_{0,2}/\mu_{0,1}$, α_k is the retention selectivity of the SDS surfactant for two analytes or the ratio of k, *e.g.* k_2/k_1, and β is the ratio for $\mu_{0,1}/\mu_{mc}$. It should be noted that α_k is always ≥ 1.0. The value of $\alpha_{CZE} > 1.0$ refers to the same order of $|\mu|$ in CZE and k in MEKC, *e.g.* $k_2 > k_1$ and $|\mu_2| > |\mu_1|$, whereas $\alpha_{CZE} < 1.0$ refers to the reversed order of $|\mu|$ in CZE and k in MEKC, *e.g.* $k_2 > k_1$ and $|\mu_2| < |\mu_1|$.

According to Eq. (7) and our previous work on theoretical models of separation selectivity of chiral separation using dual cyclodextrins [27], the proposed theoretical models of α_{MEKC} for two charged analytes in MEKC can be classified into four types as listed in Table 1, based on the ranges of α_{CZE}, α_k, ρ, and the order of $|\mu|$ in CZE and k in MEKC. In order to predict the value of α_{MEKC} for two charged analytes, the value of β in Eq. (7) is assumed to be equal to 0.5. Figure 1 shows plots of the α_{MEKC} model of Types I–III over a wide range of k_1. Practically, an increase in k may be obtained by an increase in the concentration of SDS ([SDS]) in an MEKC buffer.

According to the α_{MEKC} model for Type I in Fig. 1A, at a fixed value of ρ except in the case $\rho \approx 1.0$, the value of α_{MEKC} increases with an increase in k_1 to a maximum value, and then decreases at higher values of k_1. At a fixed value of k_1, the higher the value of α_k, the greater the value of α_{MEKC}. The k_1 giving the maximum α_{MEKC} value decreases as the value of α_k increases. Therefore, the α_{MEKC} model of Type I shows that, with the same order of $|\mu|$ in CZE and k in MEKC for charged analytes, higher α_k than α_{CZE} can improve α_{MEKC} of two solutes in MEKC. In contrast to Type I, the α_{MEKC} model for Type II ($\alpha_k \leq \alpha_{CZE}$) in Fig. 1B shows that the value of α_{MEKC} decreases with an increase in k_1, implying poorer separation for two charged analytes. For the reversed order of $|\mu|$ in CZE and k in MEKC for charged analytes as shown in Fig. 1C, the theoretical α_{MEKC} for the Type III model starts from less than 1.0 ($1/\alpha_{MEKC} > 1$) to near 1.0 (poorer separation) with increasing k_1 and then higher than 1.0 (better separation) at higher k_1 values. At an α_{MEKC} of 1.0, the value of k_1 is given by:

$$k_1 = \frac{(1 - \alpha_{CZE})}{(\alpha_{CZE} - \alpha_k) + (\alpha_k - 1)/\beta} \quad (8)$$

The small value of α_k gives a higher k_1 at α_{MEKC} 1.0, which is consistent with the bottom line for α_{MEKC} of 0.99 in Fig. 1C. It should be noted that for a theoretical value of α_{CZE} or $\alpha_{MEKC} < 1.0$, the practical separation selectivity is equal to $1/\alpha_{CZE}$ or $1/\alpha_{MEKC}$. Therefore, an increase in k_1 may result in a reversed order of electrophoretic mobility for two charged analytes in MEKC. The model Type IV ($\alpha_k = 1.0$ and $\alpha_{CZE} = 1.0$) indicates that no resolution is obtained for two solutes (plot not shown).

As can be seen in Fig. 1, the theoretical model of α_{MEKC} can be employed to describe the separation of two charged analytes. The greater the α_{MEKC} value ($\alpha_k > 1.0$), the greater the resolution. Better separation selectivity in MEKC over CZE can be obtained for the α_{MEKC} Type I ($\alpha_k > \alpha_{CZE}$), or Type III models ($\alpha_k >> \alpha_{CZE}$, or $\alpha_k > 1/\alpha_{CZE}$) at appropriate values of k_1.

It should be noted that the direction of EOF velocity and total velocity does not affect the electrophoretic mobility of analytes and micelles, and the retention factor of analytes in MEKC. Owing to independence of the values of α_m and α_k with the direction of these velocities, our proposed selectivity models can be used for MEKC with normal, reversed, and restricted modes classified by the direction of EOF and total velocity as details given in [22].

3.2 Observed and predicted α_{MEKC} for negatively charged compounds in MEKC with normal elution mode and anionic SDS surfactant

In this work, parabens such as IP, BP, PP, and EP, a weak acid with HO-C_6H_4COOR, were chosen as test analytes. In a basic buffer with pH > pK_a of parabens, these parabens can carry a negative charge. The apparent pK_a values were found to be 8.80, 7.98, 8.00, and 7.97, respectively (literature values of 9.2 for IP [28] and 8.4 for other parabens [29]). It should be noted that the apparent pK_a values were determined by measuring effective mobility (μ_{eff}) in CZE with a 10-mM $Na_2B_4O_7$ buffer at various pH values of 7.8–10.2, and plotting $1/\mu_{eff}$ versus 10^{-pH} ($[H_3O^+]$) to obtain pK_a from log (slope/*Y*-intercept) [30]. A pH 10.2 borate buffer was used to

Table 1. Types of theoretical models for α_{MEKC}

Type	Order of $\|\mu\|$ in CZE and k in MEKC	α_{CZE}	α_k	ρ	Assumed values		
					α_{CZE}	α_k	β
I	Same	$\alpha_{CZE} \geq 1$	$\alpha_k > \alpha_{CZE} \geq 1$	$\rho > 1$	1.1	1.2–3.3	0.5
II	Same	$\alpha_{CZE} > 1$	$\alpha_{CZE} \geq \alpha_k \geq 1$	$\rho \leq 1$	1.5	1.0–1.5	0.5
III	Reversed	$\alpha_{CZE} < 1$	$\alpha_k \geq 1 > \alpha_{CZE}$	$\rho > 1$	0.8	1.0–6.4	0.5
IV	Co-migration	$\alpha_{CZE} = 1$	$\alpha_k = \alpha_{CZE} = 1$	$\rho = 1$	—	—	0.5

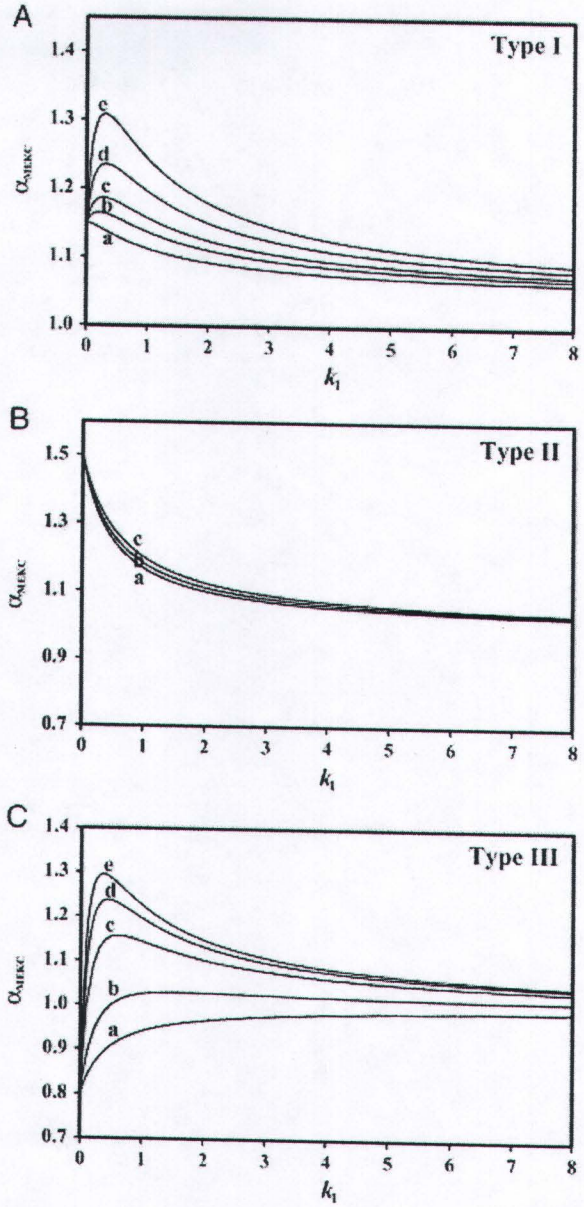

Figure 1. Predicted models of the separation selectivity (α_{MEKC}) for two charged analytes in MEKC. Calculation using Eq. (7) and data as listed in Table 1. a–e refer to the values of α_k, for (A) 1.2, 1.4, 1.7, 2.2 and 3.3, respectively, (B) 1.0, 1.2 and 1.5, respectively, and (C) 1.0, 1.6, 3.2, 4.8 and 6.4, respectively.

afford almost fully negatively charged analytes with degrees of ionization of 0.96 for IP and 0.99 for other parabens, calculated using apparent pK_a determined under our CE conditions and the equation for degrees of ionization reported in the textbook [31].

In order to obtain the predicted value of α_{MEKC} for two charged analytes in MEKC, the following parameters must be known: the mobility selectivity in CZE, the retention selectivity in MEKC, and the retention factor in MEKC. Figure 2 shows the separations of parabens using an SDS-free system (or a CZE system) and an MEKC system with various SDS concentrations (20–60 mM). From CZE separation (Fig. 2A), in which the electrophoretic mobility vectors of negatively charged parabens are opposite to an EOF vector, the migration time order EP>PP>BP>IP with the effective electrophoretic mobilities μ of −2.25, −2.13, −2.03 and $-1.97 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$, respectively, indicates the |μ| order EP>PP>BP>IP, in line with the charge-to-size ratio for homologous series EP>PP>BP. The smaller |μ| for IP than other parabens may be due to the smaller degree of ionization, and/or larger hydrodynamic size of IP.

Theoretically, for MEKC separation of neutral analytes with normal elution mode in ionic surfactant, the migration behavior depends only on the retention factors of the analytes. The higher the retention factor, the longer the migration time. However, for the MEKC separation of charged analytes with the above conditions, the migration behavior depends on the electrophoretic mobility and retention factor of each analyte, and therefore it is difficult to predict the order of the migration time or the effective electrophoretic mobility in MEKC.

The retention factor k is calculated from MEKC electropherograms using the following equation [18, 21, 32]:

$$k = \frac{\mu_{MEKC} - \mu_0}{\mu_{mc} - \mu_{MEKC}} \tag{9}$$

where all parameters are previously defined in Eqs. (5) and (6). From the MEKC electropherograms in Fig. 2B–D, the retention factors (Table 2) for negatively charged parabens were obtained in the order BP>PP>IP>EP, which are consistent with the magnitude order of octanol–water distribution constants in this series BP>PP>EP [33–35].

As seen in Fig. 2D for MEKC separation with 60 mM SDS, the order of t_m or $|\mu_{MEKC}|$ is obtained to be BP>PP>IP>EP, whereas different orders are obtained in MEKC at 20 mM SDS (Fig. 2B): BP>PP>EP>IP, and in CZE (Fig. 2A), EP>PP>BP>IP. These differences in migration behavior can be explained using the separation selectivity models in Section 3.1.

Figure 3 shows the observed and predicted values of α_{MEKC} for parabens in MEKC over a wide range of [SDS] (Fig. 3A) and k_1 values (Fig. 3B). The former is useful to consider the SDS concentration giving the achieve resolution of all solutes and the reversed migration, whereas the latter is useful to compare the observed and the predicted model without known [SDS]. The predicted values of α_{MEKC} at different [SDS] (6.0–60 mM) were calculated using data in Table 2 and Eq. (7). Table 2 also lists the mobility selectivity, retention selectivity, retention factor, selectivity ratio, and predicted models of α_{MEKC}. As previously mentioned, for MEKC separation of a particular analyte pair, such as PP and IP, k_1 refers to the retention factor for the solute with smaller k, such as k_{IP}. Using a wide range of [SDS] (20–60 mM), the observed k_1 can be plotted against [SDS] to derive a linear calibration plot, allowing predicted values of k_1 at various [SDS] to be obtained. Using data in Table 2 and Eq. (7), the observed values of α_{MEKC} in Fig. 3 were found to

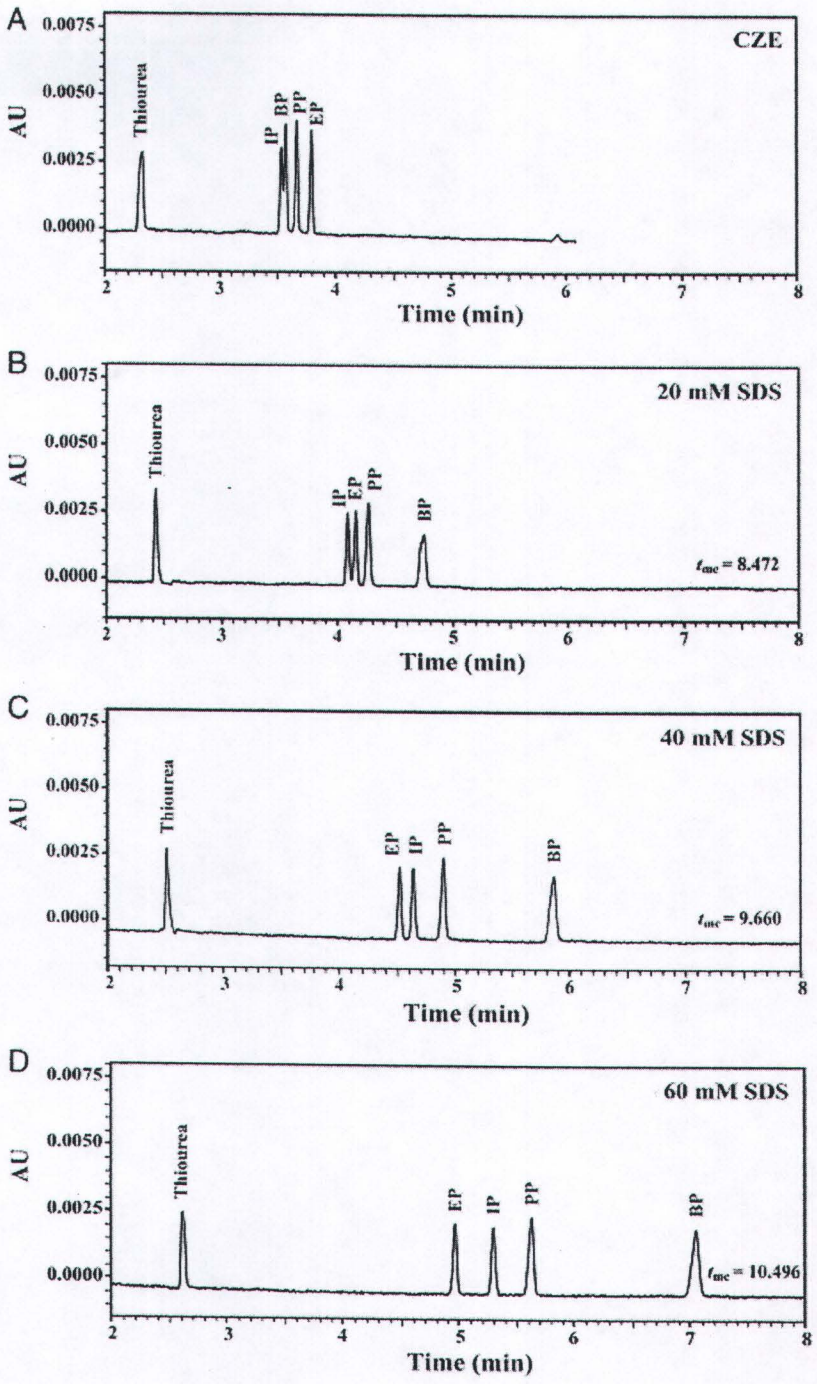

Figure 2. Electropherograms of parabens using (A) 0 (CZE), (B) 20, (C) 40, and (D) 60 mM SDS in a 10-mM $Na_2B_4O_7$ buffer adjusted to pH 10.2 with 1.0 M NaOH. Other CE conditions: uncoated fused-silica capillary, 50 μm id × 40.2 cm (30 cm to the detector), a temperature of 25°C, an applied voltage of 15 kV, UV detection at 220 nm, and 0.5 psi pressure injection for 3 s.

Table 2. Mobility selectivity (α_{CZE}), retention selectivity (α_k), retention factor (k_1), selectivity ratio (ρ), and types of α_{MEKC} model

Pair	Solute 1	$k_1 = a[SDS]+b$	α_{CZE}	α_k	β	ρ	Types of model for α_{MEKC} in MEKC
IP/EP	EP	0.00236[SDS]−0.013	0.888	k_{IP}/k_{EP}	0.575	ρ>1	III
PP/IP	IP	0.00871[SDS]−0.028	1.066	k_{PP}/k_{IP}	0.511	ρ>1	I
BP/PP	PP	0.01004[SDS]−0.035	0.953	k_{BP}/k_{PP}	0.544	ρ>1	III
	BP	0.03068[SDS]−0.057	—	—	—		—

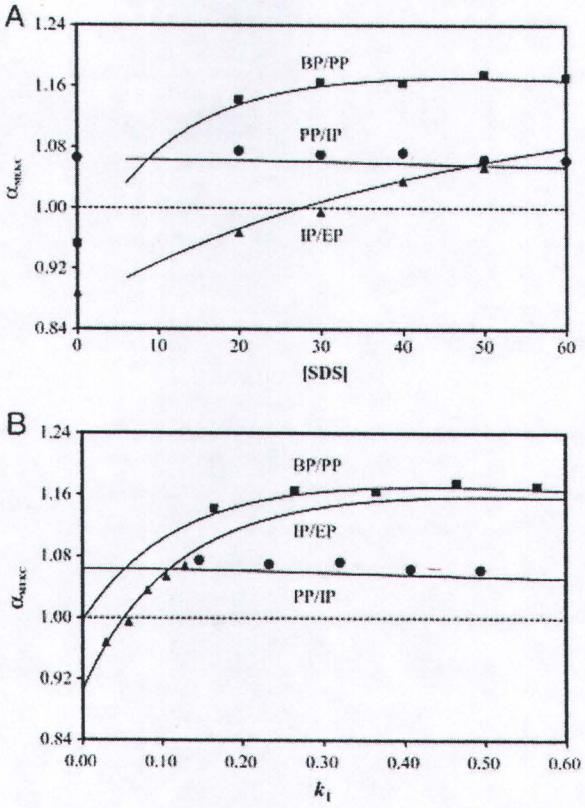

Figure 3. Observed (symbols) and predicted (solid lines) α_{MEKC} for two charged analytes in MEKC. (A) Various concentrations of SDS and (B) various values of k_1. Predicted values are obtained using Eq. (7) and data as listed in Table 2.

be in good agreement with the predicted values, indicating that Eq. (7) can be used for prediction of the α_{MEKC} values over a wide range of [SDS] and k_1.

According to electropherograms in Fig. 2 and data in Table 2, the same order of $|\mu|$ in CZE and k in MEKC was obtained, and therefore $\alpha_{CZE}>1.0$ and $\alpha_{MEKC}>1.0$. Although the ρ value is greater than 1.0, a slight decrease in the observed and predicted values of α_{MEKC} with an increase in [SDS] and k_1 is due to small calculated values of ρ between 1.032 and 1.072 for 10–60 mM SDS. Therefore, a change in α_{MEKC} for PP/IP in MEKC is consistent with the α_{MEKC} model of Type I described earlier with small values of ρ.

Owing to the reversed order of $|\mu|$ in CZE and k in MEKC for IP/EP and BP/PP, and the theoretical value of α_{CZE} being less than 1.0, the reversed $|\mu|$ order for IP/EP and BP/PP at high [SDS] and at low or zero [SDS] is consistent with the α_{MEKC} Type III model. At an α_{MEKC} value of 1.0, the predicted values of k_1 in Fig. 3B are estimated to be 0.003 for BP/PP at very low [SDS], and 0.052 for IP/EP which is in good agreement with the observed k_1 of 0.064. It should be noted that, employing Eq. (8) with an average α_k of 3.831 for IP/EP at 6.0–60 mM SDS, the predicted values of k_1 of 0.057 giving α_{MEKC} of 1.0 are found to be in good agreement with k_1 in Fig. 3B (0.052).

4 Concluding remarks

We have shown that a change in MEKC separation selectivity for two charged analytes over a wide range of [SDS] and k values can be explained and predicted using our proposed equations and theoretical models of separation selectivity, where the separation selectivity is related to the dimensionless values of the mobility selectivity in CZE and retention selectivity in MEKC. In comparison with CZE, the ability of MEKC to improve or reduce separation selectivity for two charged analytes depends on the model of separation selectivity. In addition, excellent agreement was found between the observed α_{MEKC} and the proposed α_{MEKC} models of test analytes in MEKC over a wide range of [SDS] and k values.

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ภาคผนวก 4

ผลงานวิจัยเตรียมส่งตีพิมพ์ในวารสาร

แบบจำลองทางทฤษฎีของค่าจำเพาะการแยกสำหรับสารที่มีประจุในเทคนิคไซโคลเด็กซ์ทรินอิเล็กโทรไคเนทิโครมาโทกราฟี

Theoretical Models of Separation Selectivity for Charged Compounds in Cyclodextrin Electrokinetic Chromatography

Theoretical models of separation selectivity for charged compounds in cyclodextrin electrokinetic chromatography

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Abstract: Simple equation and theoretical models, related to the dimensionless values of separation selectivity (α_{CZE}) in capillary zone electrophoresis (CZE), binding selectivity (κ), β, γ and K*C*, are proposed in order to explain a change in the separation selectivity (α_{CD}) in cyclodextrin electrokinetic chromatography (CD-EKC) for two charged analytes using neutral cyclodextrin (CD), and where α and κ are defined as the ratio of effective electrophoretic mobility in CZE (μ_0) and the ratio of binding constant (K), respectively, *C* the free CD concentration, K_2 the weaker CD binding constant, μ_∞ the electrophoretic mobility of analyte-CD complex, β_2 the $\mu_{0,2}/\mu_{\infty,2}$ ratio and γ the $\mu_{\infty,1}/\mu_{\infty,2}$ ratio. Using substituted benzoic acids and phenoxyacetic acid as test analytes with β-CD, the changes in the observed α_{CD} values were found to be in good agreement with the theoretical α_{CD} models over a wide range of K_2C and *C* values. For example, in comparison with CZE, the presence of CD in the buffer can enhance α_{CD} up to the maximum value with the selectivity ratio ($\rho = \kappa/\alpha_{CZE}$) > 1.0, while a worse α_{CD} is obtained with ρ < 1.0.

Keywords: Capillary electrophoresis, Capillary zone electrophoresis, Cyclodextrin electrokinetic chromatography, Separation selectivity

1. Introduction

Cyclodextrin electrokinetic chromatography (CD-EKC) is one of the modes in CE which is performed by the addition of cyclodextrin (CD) in the buffer to form an inclusion complex between the analyte and the CD. The effective electrophoretic mobility (μ) of the analyte in CD-EKC is given by Eq. (1) [1-3];

$$\mu = \frac{\mu_0 + KC\mu_\infty}{1 + KC} \quad (1)$$

where μ_0 and μ_∞ are the electrophoretic mobilities of the analyte and its complex at zero (capillary zone electrophoresis, CZE) and at infinite CD concentrations, 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 the initial CD concentration and (ii) that it is significantly greater than the analyte concentration.

CD-EKC is widely used for the separation of enantiomers [4-12] with identical μ_0 and μ_∞ values but with different K values and, therefore, CD-EKC separation is based on the differences in μ only that arise from the differences in K of each isomer to CD. The resolution, R_s, of two analytes in CE is given by Eq. (2) [3, 13];

$$R_s = \frac{\sqrt{\overline{N}}}{4} \cdot \frac{\Delta\mu}{\overline{\mu} + \mu_{eo}} \quad (2)$$

where $\overline{N}$ and $\overline{\mu}$ are the average efficiency and electrophoretic mobility, respectively, $\Delta\mu$ is the electrophoretic mobility difference, and μ_{eo} is the electroosmotic mobility. Generally, the resolution equation in CE (Eq. (2)) may be rearranged to relate to the efficiency, separation selectivity and mobility terms, as in Eq. (3) [14];

$$R_s = \left(\frac{\sqrt{\overline{N}}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left|\frac{\mu_2}{\overline{\mu} + \mu_{eo}}\right| \quad (3)$$

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

In addition to chiral separation, CD-EKC can be also used for separating achiral compounds [15-22], such as small molecules and positional isomers. Here, with a difference in μ_0, the separation in CD-EKC is then not only due to the difference in K but also to the difference in μ_0. In comparison with CZE, the use of CD may result in an enhancement of α for some analytes or a loss of α for other analytes, and consequentially the same or a reversed migration order of analytes may be obtained between CD-EKC and CZE [16-17, 19-20, 22]. These dual effects with the presence of a pseudo-stationary phase are similar to those in the chiral separation of charged enantiomers with dual neutral CDs [23], and in the micellar

electrokinetic chromatographic (MEKC) separation of charged compounds [14] in our previous works. A change in separation selectivity from dual effects can be clarified using theoretical models of separation selectivity for charged compounds, as detailed previously [14, 23].

Therefore, the aim of this study is to develop equation and theoretical models in order to explain a change in the neutral CD-EKC separation selectivity (α_{CD}) and electrophoretic mobility order for charged analytes with different μ_0 values. According to our previous work [14, 23], the equation and theoretical models of α_{CD} are related to the dimensionless values. In addition, the observed and predicted α_{CD} values were compared from various β-CD concentrations, using four negatively charged analytes of substituted benzoic acids and phenoxyacetic acid.

2. Experimental

2.1 Chemicals

All reagents were of analytical grade. Disodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), and sodium hydroxide were purchased from Fluka (Buchs, Switzerland), β-CD was from Sigma (St. Louis, MO, USA), mesityl oxide was from Aldrich (WI, USA). The following substituted benzoic acids were obtained from Sigma-Aldrich (Steinheim, Germany): 3-chlorobenzoic acid (3C), 4-chlorobenzoic acid (4C) and 4-methylbenzoic acid (4M). 4-Chloro-2-methylphenoxyacetic acid (MCPA) was supplied from Riedel de Haën (Seelze, Germany).

2.2 Preparation of running buffers and analytes

CE measurements were carried out in two buffer systems: CZE and CD-EKC. The CZE buffer contained 10 mM $Na_2B_4O_7$ at pH 9.2, whilst the CD-EKC buffers are derived from this by the addition of various concentrations of β-CD (2 to 16 mM, limited by the solubility of β-CD of 16 mM), in the 10 mM $Na_2B_4O_7$ at pH 9.2.

Stock solutions of each test analyte at 5.0 mM were separately dissolved in methanol and then diluted with purified water. 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 $Na_2B_4O_7$. All sample solutions and buffers were sonicated and filtered through 0.45 μm membrane filters prior to CE analysis.

2.3 CE 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 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 °C. The applied voltage was set at 15 kV. A sample solution was introduced by 0.5 psi pressure injection for 3 s. Prior to analysis each day, the capillary was rinsed with 0.1 M NaOH and then 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.

3. Results and discussion

3.1 Theoretical models of neutral CD-EKC separation selectivity

As can be seen in Eq. (3), the resolution mainly depends on α, and the separation selectivity in CD-EKC (α_{CD}), instead of α in Eq. (3), is defined as the ratio of μ for two analytes, *e.g.* μ_2/μ_1. From Eq. (1), in the case of charged analytes 1 and 2 to neutral CD with $K_1 \geq K_2$, the CD-EKC separation selectivity may be rearranged to relate to the dimensionless values of α_{CZE}, κ, β, γ and K*C* as Eq. (4);

$$\alpha_{CD} = \alpha_{CZE}\left(\frac{\beta_2 + K_2C}{\beta_2 + \alpha_{CZE}\kappa K_2 C\gamma}\right)\left(\frac{1+\kappa K_2 C}{1+K_2 C}\right) \quad (4)$$

where α_{CZE} is the separation selectivity in CZE (defined as the ratio of μ_0 for two analytes, *e.g.* $\mu_{0,2}/\mu_{0,1}$), κ is the CD binding selectivity (defined as the ratio of K for two analytes, *e.g.* K_1/K_2, $\kappa \geq 1.0$), β_2 is the $\mu_{0,2}/\mu_{\infty,2}$ ratio and γ is the $\mu_{\infty,1}/\mu_{\infty,2}$ ratio. It should be noted that the stronger binding of analyte 1 than 2 to CD results in smaller apparent $|\mu|$ of analyte 1 than 2 and, therefore, the separation selectivity (α_{CZE} or α_{CD}) was calculated from the ratio of μ for analyte 2/1, whereas κ was from the ratio of K for analyte 1/2.

Using a similar concept to our previous works on the chiral separation selectivity for charged enantiomers with dual neutral CDs [23], and the separation selectivity for charged compounds in MEKC [14], the separation selectivity for charged compounds in neutral CD-EKC in this work can be classified into four types, as given in Table 1, based on the selectivity ratio (ρ) which is defined as the κ/α_{CZE} ratio, and the orders of $|\mu_0|$ in CZE and K in CD-EKC. The same order refers to when $K_1 > K_2$ and $|\mu_{0,1}| > |\mu_{0,2}|$, while the reversed order refers to when $K_1 > K_2$ but $|\mu_{0,2}| > |\mu_{0,1}|$. It should be noted that Eq. (4) can be also used for $\mu_{0,1} \neq \mu_{0,2}$ or $\mu_{0,1} = \mu_{0,2}$ ($\alpha_{CZE} > 1.0$, < 1.0 or $= 1.0$), κ is always ≥ 1.0, and the practical separation selectivity is equal to $1/\alpha_{CZE}$ or $1/\alpha_{CD}$ for the theoretical value of α_{CZE} or $\alpha_{CD} < 1.0$. Using Eq. (4) and the data in Table 1, with $\beta = 3$ and $\gamma = 1$, Figure 1 shows the plots of α_{CD} models of Types I to III as a function of the K_2C products at various ρ values. At a K_2C value of 0, the α_{CD} value refers to α_{CZE}.

According to the α_{CD} model for Type I as shown in Fig.1A with $\kappa > \alpha_{CZE} \geq 1$, an increase in the concentration of CD in CD-EKC enhances α_{CD} to a maximum value at $K_2C \approx 1$, and then decreases at higher K_2C values. At a given K_2C, the higher the value of ρ, the greater the value of α_{CD}. This indicates that, in comparison with CZE, neutral CD-EKC with the Type I α_{CD} model can improve the resolution of charged compounds. This may simply be explained by that, for $|\mu_{0,2}| > |\mu_{0,1}|$ in CZE ($\alpha_{CZE} > 1$), analyte 1 with a stronger binding to CD ($K_1 > K_2$ with $\kappa > \alpha_{CZE}$) results in a much smaller $|\mu_1|$ than $|\mu_2|$ and, therefore, a higher α_{CD} or μ_2/μ_1 at the appropriate K_2C value.

In contrast to that observed with the Type I model, CD-EKC with the Type II α_{CD} model ($\alpha_{CZE} \geq \kappa \geq 1$), or when the CD binding selectivity is less than the $|\mu_0|$ selectivity (Fig. 1B), showed a gradual decrease in the α_{CD} over a wide range of K_2C values. The smaller the ρ value, the smaller the α_{CD} value. This implies an inferior separation ability of the two analytes in CD-EKC than in CZE for the case of the Type II α_{CD} model.

As can be seen in Fig. 1C, for the Type III α_{CD} model with $\kappa \geq 1 > \alpha_{CZE}$, the reversed order of $|\mu|$ in the CD-EKC, compared to that in CZE, results in a loss of the separation, starting from $1/\alpha_{CD} > 1$ ($\alpha_{CD} < 1$) to near 1 and then increases the separation ($\alpha_{CD} > 1$) at higher K_2C values. From Eq. (4), it follows that the value of K_2C giving α_{CD} of 1 is given by Eq. (5);

$$K_2C = \frac{\beta(1-\alpha_{CZE})}{\beta(\alpha_{CZE}\kappa - 1) + \alpha_{CZE}(1-\kappa)} \qquad (5)$$

With $\alpha_{CZE}\kappa = 1$ or $\kappa = 1/\alpha_{CZE}$ as shown in Fig. 1C, when $\kappa = 1.25$ the same order of $|\mu|$ in CD-EKC and $|\mu_0|$ in CZE is observed over a wide range of K_2C values, and K_2C gives α_{CD} of 1 at $-\beta$, implying that a α_{CD} of 1 occurs at an infinite K_2C value. The higher the value of ρ, the lower the value of K_2C at α_{CD} of 1. The K_2C value that gives a maximum α_{CD} value decreases with increasing ρ values. In comparison with CZE, CD-EKC with this α_{CD} model, especially at high κ values, can provide a better separation with $\kappa > 1/\alpha_{CZE}$. With $\kappa = \alpha_{CZE} = 1$ for the Type IV α_{CD} model (Table 1), there is no separation of the charged analytes under either the CZE or CD-EKC conditions (plot not shown).

3.2 Observed and predicted neutral CD-EKC separation selectivity for negatively charged compounds

In order to predict the theoretical models of α_{CD} for charged compounds in neutral CD-EKC, the values of α_{CZE}, κ, β and γ must be known. In initial experimental work, several weak acids were used in the CD-EKC separation. However, the substituted benzoic acids and phenoxyacetic acid, including 3C, 4C, 4M and MCPA, were chosen here to be representatives to cover three types of α_{CD} models. With $pK_a < 4.4$ for all test analytes [24, 25], then in the pH 9.2 buffer used here, each analyte carries a fully negative charge. Figure 2 shows an example of the obtained electrophoregrams for the simultaneous separation of 3C, 4C, 4M and MCPA in CZE buffer with 0 mM β-CD (Fig. 2A) and in the CD-EKC buffers with 2 and 12 mM β-CD (Fig. 2B and 2C, respectively). As can be seen in Fig. 2A, when using CZE the negatively charged analytes migrate against the electroosmotic flow (EOF) with the $|\mu_0|$ order of 3C > 4C > 4M > MCPA, depending on the ratio of charge to hydrodynamic radius of the analytes. From the CD-EKC electropherograms, the $|\mu|$ orders of the test analytes were obtained to be 3C > 4C ≈ 4M > MCPA in 2 mM β-CD (Fig. 2B), whereas 3C > 4M > 4C > MCPA in 12 mM β-CD (Fig. 2C). In comparison with CZE, the same migration order in CD-EKC was found for 4C/3C, 4M/3C and MCPA/4C over a wide range of CD concentrations from 2-16 mM β-CD, while a reversed order was obtained for 4C/4M at a CD concentration above 2 mM β-CD. This different migration behavior for these analytes can be explained by using the α_{CD} models in Section 3.1.

Table 2 shows the values of μ_0, μ_∞, α_{CZE}, K, κ, ρ, β, γ and types of α_{CD} model for each pair of test analytes. The K and μ_∞ values were determined using the CEfit Program of a nonlinear least-squares fit to the data points of the corrected electrophoretic mobilities, with a change in the buffer viscosity, as a function of the CD concentration [23, 26-27]. Accordingly, from the data in Table 2 and Eq. (4), the observed and predicted α_{CD} values were evaluated and plotted for the test analytes in various K_2C products (Fig. 3A) and β-CD concentrations (0-16 mM, Fig. 3B). The former can be used to compare the observed and predicted model without known CD concentrations, while the latter can be used to consider the CD concentrations giving the achieved resolution of the analytes and the reversed migration orders. As can be seen in Fig. 3, the observed values of α_{CD} were found to be in good agreement with the predicted values, indicating that Eq. (4) can be used for the prediction of the α_{CD} values over a wide range of K_2C and C values.

From the electropherograms in Fig. 2 and the data in Table 2, a reversed order of $|\mu_0|$ in CZE and K in CD-EKC was obtained for the 4C/3C, 4M/3C and MCPA/4C analyte pairs, but the same order was observed for $|\mu_0|$ in CZE and $|\mu|$ in CD-EKC. In comparison with CZE, a greater α_{CD} value was obtained at a higher K_2C value and C for 4C/3C and 4M/3C with $\rho > 1.0$ and, therefore, a change in α_{CD} for 4C/3C and 4M/3C is consistent with the Type I α_{CD} model. In contrast to that seen for 4C/3C and 4M/3C above, at

higher K_2C and C values, smaller α_{CD} is seen for MCPA/4C with $\rho < 1.0$, which is consistent with the Type II α_{CD} model.

Due to the same order of $|\mu_0|$ in CZE and K in CD-EKC for 4C/4M with $\alpha_{CZE} < 1.0$ and $\rho > 1.0$ (Table 2), the reversed order of $|\mu_0|$ in CZE and $|\mu|$ in CD-EKC was found at higher K_2C and C values, as shown in the Fig. 2. Therefore, the CD-EKC separation for 4C/4M is consistent with the Type III α_{CD} model. The α_{CD} of 1 for a pair of 4C/4M was observed at 2 mM β-CD and K_2C of 0.170, which is in good agreement with the calculated K_2C of 0.166 using Eq. (5).

4. Conclusions

The proposed equation and the theoretical models for CD-EKC separation selectivity presented herein, which are related to the dimensionless values of α_{CZE}, κ, β, γ and KC, can be used to explain and reliably predict the change in the CD-EKC separation selectivity and electrophoretic mobility order for charged analytes over a wide range of neutral CD concentrations and K_2C products. In comparison with CZE, the presence of CD in the buffer may improve or reduce separation selectivity for charged analytes in CD-EKC, depending on the CD-EKC separation selectivity models. When using substituted benzoic acids and phenoxyacetic acid as test analytes with β-CD, a good agreement was found between the observed and predicted values of CD-EKC separation selectivity across various CD concentrations and K_2C values.

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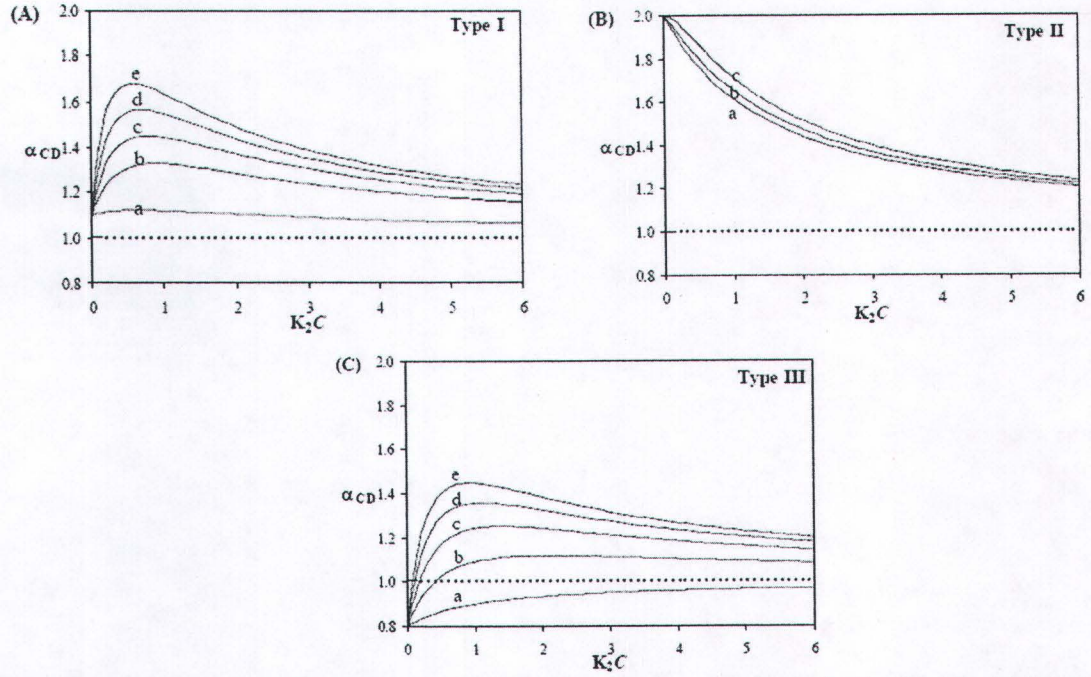

Figure 1 Predicted models of CD-EKC separation selectivity for charged analytes. Calculated using Eq. (4) and the data listed in Table 1. a to e refer to the binding selectivity (κ) values for (A) 1.2, 2.4, 3.5, 4.9 and 6.8, respectively, (B) 1.1, 1.4 and 2.0, respectively, and (C) 1.25, 2.3, 3.5, 4.8, 6.2, respectively.

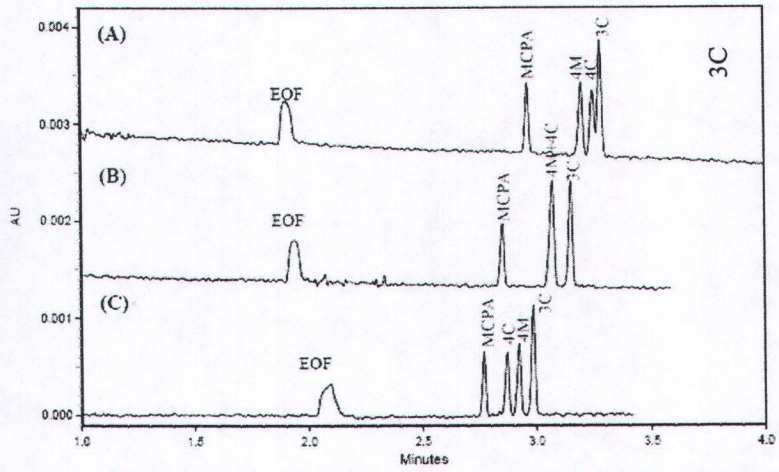

Figure 2 Electropherograms of substituted benzoic acids and phenoxyacetic acid using (A) 0 mM (CZE), (B) 2 mM and (C) 12 mM β-CD in 10 mM $Na_2B_4O_7$ at pH 9.2. Other CE conditions were: 50 μm I.D.×40.2 cm (30 cm to detector) uncoated fused-capillary, temperature of 25 °C, applied voltage of 15 kV, UV detection at 214 nm and 0.5 psi pressure injection for 3 s.

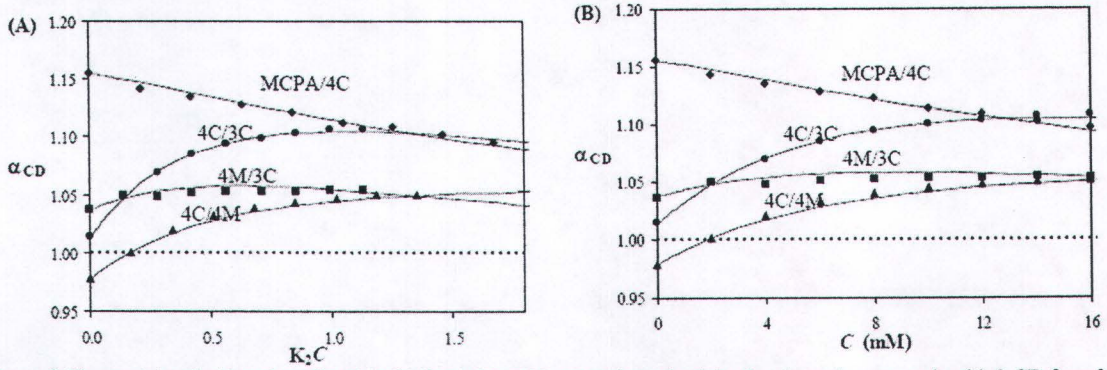

Figure 3 Observed (symbols) and predicted (solid lines) CD-EKC separation selectivity for charged compounds with β-CD from 0-16 mM. (A) Various K_2C values and (B) various β-CD concentrations (C). Predicted values are obtained using Eq. (4) and the data in Table 2.

Table 1 Types of theoretical models of α_{CD}

Type	Order of $\|\mu_0\|$ in CZE and K in CD-EKC	Order of $\|\mu_0\|$ in CZE and $\|\mu\|$ in CD-EKC	α_{CZE} ($\mu_{0,2}/\mu_{0,1}$)	κ (K_1/K_2)	ρ	Assumed values			
						α_{CZE}	κ	β	γ
I	Reversed	Same	$\alpha_{CZE} \geq 1$	$\kappa > \alpha_{CZE} \geq 1$	$\rho > 1.0$	1.1	1.2-6.8	3.0	1.0
II	Reversed	Same	$\alpha_{CZE} > 1$	$\alpha_{CZE} \geq \kappa \geq 1$	$\rho \leq 1.0$	2.0	1.1-2.0	3.0	1.0
III	Same	Same or Reversed	$\alpha_{CZE} < 1$	$\kappa \geq 1 > \alpha_{CZE}$	$\rho > 1.0$	0.8	1.25-6.2	3.0	1.0
IV	Comigration	Comigration	$\alpha_{CZE} = 1$	$\kappa = \alpha_{CZE} = 1$	$\rho = 1.0$	-	-	-	-

Table 2 Electrophoretic mobility (μ, 10^{-8} m^2 V^{-1} s^{-1}), CZE separation selectivity (α_{CZE}), binding constant (K, M^{-1}), binding selectivity (κ), selectivity ratio (ρ), the ratio of $\mu_{0,2}/\mu_{\infty,2}$ (β), the ratio of $\mu_{\infty,1}/\mu_{\infty,2}$ (γ), and types of α_{CD} models for test analytes.

Analyte	μ_0	μ_∞	K	Pair	α_{CZE}	κ	ρ	β	γ	Type of models for α_{CD}
3C	-2.86	-0.81	70.8±0.4	4C/3C[a]	1.01	1.48	1.47	3.53	1.08	I
4C	-2.82	-0.87	104.7±0.6	MCPA/4C[a]	1.16	1.07	0.92	3.23	1.05	II
4M	-2.76	-0.89	84.8±0.3	4C/4M[a]	0.98	1.23	1.26	3.11	0.98	III
MCPA	-2.44	-0.91	110.6±1.3	4M/3C[a]	1.04	1.20	1.15	3.53	1.09	I

[a]Analyte 2

ภาคผนวก 5

ผลงานวิจัยเตรียมส่งตีพิมพ์ในวารสาร

การเปรียบเทียบค่าจำเพาะการแยกในเทคนิคคะพิลลารีอิเล็กโทรฟอริซิสำหรับสารกลุ่มแอโรแมติกแอซิดที่มีหมู่แทนที่เป็นคลอโรและเมทิล

Comparison of Separation Selectivity in CZE, MEKC and CD-EKC for Chlorobenzoates/Methylbenzoates, and Application to Separation of Phenoxy Acid Herbicides

Comparison of Separation Selectivity in Capillary Zone Electrophoresis, Micellar Electrokinetic Chromatography and Cyclodextrin Electrokinetic Chromatography for Chlorobenzoates/Methylbenzoates, and Application to Separation of Phenoxy Acid Herbicides

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Abbreviations: **C⁻**, chlorobenzoate; **CD-EKC**, cyclodextrin electrokinetic chromatography; **DC⁻**, dichlorobenzoate; **DM⁻**, dimethylbenzoate; **DM-β-CD**, dimethyl-β-cyclodextrin; **MeOH**, methanol; **M⁻**, methylbenzoate

Keywords: Cyclodextrin electrokinetic chromatography, Capillary zone electrophoresis, micellar electrokinetic chromatography, Phenoxy acids, Separation Selectivity

Abstract: CE separation selectivity for monochlorobenzoates/monomethylbenzoates (C^-/M^-) and dichlorobenzoates/dimethylbenzoates (DC^-/DM^-), having similar electrophoretic mobilities, were compared in three CE modes including CZE, MEKC and cyclodextrin-EKC (CD-EKC). Small CZE separation selectivity (α_{CZE}) was obtained for each pair of C^-/M^- and DC^-/DM^-. The very slightly higher MEKC separation selectivity (α_{MEKC}) than α_{CZE} was obtained due to the small retention selectivity. The higher CD-EKC separation selectivity (α_{CD}) than α_{CZE} and α_{MEKC} was obtained for C^-/M^- and DC^-/DM^-, due to the great CD binding selectivity. In addition, simultaneous separation of ten phenoxy acid herbicides, especially 2,4-DB/MCPB and 2,4-D/MCPA having the difference in chloro and methyl substituents on a benzene ring, was also obtained using the CD-EKC buffer containing 3.0 mM DM-β-CD. Therefore, in comparison with CZE and MEKC, CD-EKC can be used as the better alternative to separate compounds having the different substituents only by chloro and methyl groups.

1 Introduction

CE is a high efficiency separation technique for separating charged and neutral compounds. Basically, the separation mechanism in CE is based on the difference in the electrophoretic mobility (μ) of the analytes. The resolution, R_s, of two analytes in CE is given by the equation [1, 2]:

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

where $\overline{N}$ and $\overline{\mu}$ are the average efficiency and the average electrophoretic mobility, respectively, $\Delta\mu$ is the electrophoretic mobility difference, and μ_{eo} is the electroosmotic mobility. Typically, the resolution (R_s) in CE (Eq. (1)) may be rearranged to relate to efficiency, separation selectivity and mobility terms, as follows [3]:

$$R_s = \left(\frac{\sqrt{\overline{N}}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left|\frac{\mu_1}{\overline{\mu} + \mu_{eo}}\right| \qquad (2)$$

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

The separation selectivity is a characteristic that simply describes separation in CE. This parameter shows the ability of the separation method to distinguish analytes from the others. Since the CZE separation for charged analytes arise only from the differences in charge-to-size ratio, it is difficult to reach baseline resolution for separating the analytes having very similar μ or small α ($\alpha \approx 1.0$). As a result, several methods to improve the CZE separation have been reported. The use of the pH of the buffers close to the pK_a of the analytes [4-9] is one of the methods to obtain the difference in μ for acid compounds. The optimum pH value is theoretically calculated to be the average pK_a values for two analytes [7-8]. However, this method is not impractical use for the simultaneous separation of several pairs of analytes having different average pK_a values. Moreover, the additives, such as ionic liquids [10] and organic solvents [4-5, 11-16], may be used to improve α because of their influence on μ and μ_{eo}. In addition to CZE, the addition of any pseudo stationary phases such as micelles in MEKC [11, 17-18] and CDs in CD-EKC [11, 19-23] has been reported to improve the separation due to the interaction of the analytes and pseudo stationary phases.

From the literature [24], chlorobenzoate (C^-) and methylbenzoate (M^-) show very similar measured absolute electrophoretic mobility (μ^0, 10^{-8} m^2 V^{-1} s^{-1}) of each positional isomer of C^-/M^-: -3.16/-3.07, -3.22/-3.11 and -3.17/-3.07 for $2C^-/2M^-$, $3C^-/3M^-$ and $4C^-/4M^-$, respectively. Therefore, the calculated separation selectivities are 1.029, 1.035 and 1.033 for 2-, 3- and 4-C^-/ M^- isomer, respectively, indicating low resolution of each pair.

The aims of this study are to investigate and to compare the separation selectivity in three CE modes for benzoic acids with 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-EKC with different types and concentrations of CDs. In addition, the CE separation for acidic herbicides having the difference in a chloro- and a methyl-substituent on the aromatic ring was also examined. In comparison with CZE, a change in separation selectivity in MEKC and CD-EKC will be also explained using our recent works on separation selectivity models [3, 25].

2 Materials and methods

2.1 Chemicals

All reagents were of analytical grade. Disodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), and sodium hydroxide were purchased from Fluka (Buchs, Switzerland); thiourea, methanol (MeOH) and ACN were from Merck (Darmstadt, Germany); SDS, α-CD, β-CD and dimethyl-β-CD (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). All herbicide standards were obtained from Riedel de Haën (Seelze, Germany) and Dr. Ehrenstorfer GmbH (Augsburg, Germany): 4-Chloro-2-methylphenoxyacetic acid (MCPA), 4-(2-methyl-4-chlorophenoxy) butyric acid (MCPB), 2,4-dichlorophenoxyacetic acid (2,4-D), 4-(2,4-dichlorophenoxy) butyric acid (2,4-DB), (2,4,5-trichlorophenoxy) acetic acid (2,4,5-T), 5-(2-chloro-4-trifluoromethylphenoxy)-2-nitrobenzoic acid (acifluorfen), 3,6-dichloro-pyridine-2-carboxylic acid (clopyralid), 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 2-(4-chlorophenoxyl)-2-methyl-propionic acid (mecroprop), and 4-amino-3,5,6-trichloro-picolinic acid (picloram).

2.2 Preparation of running buffers and analytes

CE measurements were carried out in three buffer systems: i) CZE, ii) MEKC, and iii) CD-EKC. BGEs used for CZE contained 10 mM $Na_2B_4O_7$ at pH 9.2 with or without the addition of organic modifiers (0 to 20% v/v) such as methanol (MeOH) and ACN. The MEKC buffers contained a 10 mM borate buffer at pH 9.2 and SDS (20–80 mM). The effect of organic modifiers in MEKC was studied using a 10 mM borate buffer at pH 9.2, 40 mM SDS and various concentrations of organic modifiers (0 to 20% v/v MeOH or ACN). For CD-EKC, BGEs containing α-CD (2-60 mM), β-CD (2-16 mM) or DM-β-CD (2-50 mM) were prepared at various concentrations by weighing an appropriate amount of CD and dissolving these in 10 mM $Na_2B_4O_7$ at pH 9.2.

Stock solutions of each test analyte at 5.0 mM were separately dissolved in MeOH and then diluted with purified water. The mixtures contained each analyte at 0.1 mM and mesityl oxide as EOF marker in CZE and CD-EKC, while thiourea as EOF marker and DB as micelle marker in MEKC. All sample solutions and buffers were filtered through 0.45 μm membrane filters prior to CE analysis.

2.3 CE conditions

All CE separations were performed on a Beckman Coulter MDQ CE system equipped with a photo-DAD scanning from 190 to 300 nm and monitoring at 214 nm. An uncoated fused-silica used (Polymicro Technologies, Phoenix, AZ, USA) was 40.2 cm in length (30 cm to detector) × 50 μm id, and thermostatted at 25 °C. The applied voltage was set at 15 kV. A sample solution was introduced by 0.5 psi pressure injection for 3 s. Prior to analysis each day, the capillary was conditioned as detailed in previous work [25]. Each experiment was carried out in duplicate.

3 Results and discussion

3.1 CZE separation of C^-/M^- and DC^-/DM^-

The CZE separation mechanism is based on the difference in μ of the analytes due to the difference in the ratio of charge to hydrodynamic radius of solutes [1]. Figure 1A shows the separated electropherograms of a pair of monosubstituted benzoates: $2C^-/2M^-$, $3C^-/3M^-$ and $4C^-/4M^-$, using 10 mM borate buffer at pH 9.2 without the addition of organic modifier. In the pH 9.2 buffer, each analyte, with pK_a < 4.5 [26-27], carries a fully negative charge and, therefore, each analyte migrates after an EOF marker with the migration time order or the electrophoretic mobility (|μ|) order: $C^- > M^-$. A pair of C^-/M^- in each positional isomer migrates closely for each other. The μ values (10^{-8} m^2 V^{-1} s^{-1}) of the C^-/M^- are shown in

Table 1, with %RSD < 1.0. As can be seen from Fig. 1A, the baseline resolution of C^-/M^- for all pairs was not achieved, R_s < 1.5. Figure 1B shows the CZE separations of disubstituted benzoates DC^-/DM^- (2,3-, 2,4-, 2,5-, 2,6-, 3,4 and 3,5-isomers). The |μ| values of disubstituted benzoates were found in the order $DC^- > DM^-$ (Table 1), similar to the |μ| order of monosubstituted benzoates $C^- > M^-$. The baseline resolution of DC^-/DM^- was achieved for 2,6-, 3,4- and 3,5-isomers (R_s = 1.53–1.72), while not achieved for 2,3-, 2,4- and 2,5-isomers (R_s = 0.88–1.42).

From Fig. 1A for the separation of monosubstituted benzoates, the CZE separation selectivities (α_{CZE}) were obtained to be 1.018, 1.025 and 1.023 for 2-, 3- and 4-C^-/M^- isomers, respectively, which have the same trend but slightly less than those of literature values calculated using μ^0 as previously mentioned in Section 1. The values of α_{CZE} for disubstituted benzoates were obtained in the range of 1.019-1.038. The α_{CZE} value close to 1.0 indicates small separation selectivity or small difference in μ. According to Eq. (2), 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.

The addition of organic solvent into BGEs was reported to improve the separation of sample mixtures in CZE due to a change in μ and μ_{eo} [4-5, 11-16]. In this work, the effect of organic solvents to α_{CZE} was studied by separately adding 10-20% v/v MeOH or ACN into 10 mM $Na_2B_4O_7$ buffer. With an increase in organic modifier concentrations, the same |μ| order was obtained with longer migration time (data not shown). Figure 2 shows an example of the effect of MeOH on α_{CZE} and R_s for monosubstituted benzoates (C^-/M^-). From Fig. 2A1, although a slight decrease in α_{CZE} was obtained with increasing the amount of MeOH in the buffer, the better R_s was observed (Fig. 2A2) because of the slightly higher values of the mobility term and the greater $\overline{N}$ (data not shown) according to Eq. (2). The similar result with MeOH was also observed using ACN as an organic modifier (data not shown).

The use of a buffer with suitable pH at the average pK_a values for two analytes [7-8] is one of the alternative methods to enhance α_{CZE}. For example, using the pK_a values reported from literature [26-27] for calculation of suitable pH of the buffers, the different pHs of 3.39, 4.05 and 4.17 may be used for separating each pair of 2-, 3- and 4-C^-/M^-, respectively. Another alternative method to improve α for benzoic acid derivatives is the addition of pseudo-stationary phases such as micelles in MEKC [11, 17-18] and CDs in CD-EKC [11, 19-23].

3.2 MEKC separation of C^-/M^- and DC^-/DM^-

Using an MEKC buffer containing 40 mM SDS in a 10 mM borate buffer at pH 9.2, anionic micelles and negatively charged solutes migrate toward the cathode with the presence of high EOF. As shown in Table 1, the migration of analytes in MEKC were found in the |μ| order $C^- > M^-$ and $DC^- > DM^-$, similar |μ| order in CZE. Typically, the charged analytes in MEKC are separated due to the differences in their micellar partitioning and their electrophoretic mobility (α_{CZE}). In our recently previous work [3], the separation selectivity for charge analytes in MEKC relates to α_k and α_{CZE} as the equation:

$$\alpha_{MEKC} = \alpha_{CZE}\left(\frac{\beta + \dfrac{\alpha_k}{\alpha_{CZE}}}{\beta + k_1}\right)\left(\frac{1 + k_1}{1 + \alpha_k k_1}\right) \qquad (7)$$

where α_{MEKC} is the mobility selectivity in MEKC, α_k is the retention selectivity defined as the ratio of retention factor (k) in MEKC for two analytes and β is the ratio of the analyte μ in CZE (μ_0) to the micelle marker μ in MEKC.

In comparison between MEKC and CZE as shown in Table 1, the very slightly higher α_{MEKC} than α_{CZE} is due to the small α_k giving the small ratio of α_k/α_{CZE} ranging from 1.013 to 1.063. According to theoretical models for α_{MEKC} [3], a slight increase in α_{MEKC} for C^-/M^- and DC^-/DM^- is consistent with the model of Type I ($\alpha_k > \alpha_{CZE} \geq 1.0$) with small values of α_k/α_{CZE}. 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), except for $2C^-/2M^-$ (R_s = 1.09) and $2,4DC^-/2,4DM^-$ (R_s = 1.25).

Over a wide range of SDS concentrations of 20–80 mM, the α_{MEKC} values for each pair of C^-/M^- or DC^-/DM^- were insignificantly different (data not shown). Figure 2B1 and 2B2 show an example of the effect of MeOH in the MEKC buffer on α_{MEKC} and R_s, respectively, for monosubstituted benzoates (C^-/M^-). As can be seen in Fig. 2B, an increase in organic solvent concentrations gives a very slight decrease in α_{MEKC} (Fig.

2B1) but the better R_s (Fig. 2B2) in MEKC, a similar trend found in CZE with the addition of organic solvents.

3.3 CD-EKC separation of C^-/M^- and DC^-/DM^-

In order to enhance 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 pH 9.2 borate buffer. It should be noted that 16 mM for β-CD is the saturated solubility in water, 60 mM α-CD gave the achieved baseline resolution with $R_s > 2.4$, except for the co-elution of $2,6DC^-/2,6DM^-$, and 50 mM DM-β-CD gave $R_s > 4.5$, except for the co-elution of $2C^-/2M^-$ and $2,6DC^-/2,6DM^-$. In the presence of CD, the CD-EKC separation for two charged analytes arises from the differences in their binding constant (K) to CD and their electrophoretic mobility (α_{CZE}). The different ability of CD to form inclusion complex with the analytes is governed by several factors such as the match between the cavity size of CD and the molecular size of the analytes, hydrophobic interaction and hydrogen bonding interactions [1, 2].

In our recently reported work [25], the CD-EKC separation selectivity for charge compounds was developed to relate to κ and α_{CZE} as the following equation:

$$\alpha_{CD} = \alpha_{CZE}\left(\frac{\beta_2 + K_2 C}{\beta_2 + \alpha_{CZE}\kappa K_2 C\gamma}\right)\left(\frac{1+\kappa K_2 C}{1+K_2 C}\right) \quad (3)$$

where α_{CD} is the mobility selectivity in CD (μ_2/μ_1), κ is the CD binding selectivity defined as the ratio of K for two analytes (K_1/K_2 with $K_1 \geq K_2$), β is the ratio of the analyte μ in CZE to the analyte-CD complex μ in CD-EKC ($\mu_{0,2}/\mu_{\infty,2}$), γ is the ratio of μ_∞ for two analytes ($\mu_{\infty,1}/\mu_{\infty,2}$), and C is CD concentration.

Table 2 shows the values of K, κ, observed maximum selectivities in CD-EKC ($\alpha_{CD,\,max}$) and types of α_{CD} model for each pair of C^-/M^- or DC^-/DM^-, using 2-60 mM α-CD, 2-16 mM β-CD or 2-50 mM DM-β-CD. The K and μ_∞ values were determined using the CEfit Program of a nonlinear least-squares fit to the data points of the corrected electrophoretic mobilities, with a change in the buffer viscosity, as a function of C [28-29]. The calculated μ_∞ of weakly-binding analytes was obtained from the average of μ_∞ values of other positional isomers and used to calculate K, as previously suggested [21]. The greater K for chlorosubstituted than methylsubstituted benzoates in each isomer indicates the strong CD binding to C^- than M^- and also DC^- than DM^-. This is possibly due to the stronger hydrophobicity of chlorosubstituted than methylsubstituted benzoates to CD cavity as seen from the higher k in MEKC for C^- than M^- and DC^- than DM^-. In addition, the steric effect of $-CH_3$ than $-Cl$ may reduce inclusion complexing for M^- or DM^- to CD. In comparison with other isomers, $2C^-$, $2M^-$, $2,6DC^-$ and $2,6DM^-$ have weaker binding to CD possibly due to the steric effect of substituents adjacent to a $-COO^-$ group.

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

Over a wide range of CD concentration, the values of α_{CD} increase to maximum values and then decrease and, therefore, the observed maximum α_{CD} as listed in Table 2 is the value for each pair of C^-/M^- or DC^-/DM^- at a given CD concentration in a working range used in this work. According to theoretical models for α_{CD} [25], in comparison with CZE, an increase in α_{CD} for each pair of C^-/M^- or DC^-/DM^- is consistent with the Type III α_{CD} model ($\kappa \geq 1 > \alpha_{CZE}$), except for the Type I α_{CD} model ($\kappa > \alpha_{CZE} \geq 1$) for $2C^-/2M^-$ with α-CD-EKC.

It should be noted that the analyte 2 in CD-EKC refers to the analyte with lower K, in order to fit with Eq.(3) and the Type of α_{CD} model [25]. For example, for $3C^-/3M^-$ with α-CD having K values of 80.7/22.3 M^{-1}, the selectivities are obtained from the following calculation; $\alpha_{CD} = \mu(3M^-)/\mu(3C^-)$, $\kappa = K(3C^-)/K(3M^-)$ and $\alpha_{CZE} = \mu_0(3M^-)/\mu_0(3C^-)$. Therefore, for pairs only with the Type III α_{CD} model, the theoretical α_{CZE} used in Eq.(3) for CD-EKC is equal to the reciprocal of the practical α_{CZE} in CZE shown in Table 1, such as 0.976 or 1/1.025 for $3C^-/3M^-$.

According to theoretical models for α_{CD} in our previous work [25], for the Type I α_{CD} model giving the same $|\mu|$ order in CD-EKC with CZE, an increase in C enhances α_{CD} to a maximum value and then decreases at higher C. Due to the opposite selectivities of α_{CZE} and κ for the Type III α_{CD} model giving the same $|\mu|$ order in CZE with CD-EKC at low C and possibly reversed $|\mu|$ order at high C, an increase in C results in a loss of the separation, starting from $1/\alpha_{CD} > 1$ ($\alpha_{CD} < 1$) to near 1, and then increases the separation ($\alpha_{CD} > 1$) at higher C.

In comparison with CZE, CD-EKC provides the better separation for a pair of $2C^-/2M^-$ with α-CD with the Type I α_{CD} model and other pairs with the Type III α_{CD} model. As previously explained [25], the Type III α_{CD} model can improve the separation with the larger κ than α_{CZE} values and high *C*. For the following four pairs, such as $2C^-/2M^-$ with DM-β-CD, and $2,6DC^-/2,6DM^-$ with α-CD, β-CD or DM-β-CD, worse separation in CD-EKC than CZE were observed due to the small ratio of κ/α_{CZE}.

As a result of most cases, the better R_s for C^-/M^- and DC^-/DM^- was achieved with CD-EKC than CZE and MEKC ($\alpha_{CD} > \alpha_{MEKC} > \alpha_{CZE}$). Using a wide range of *C*, such as 2–60 mM α-CD, 2–16 mM β-CD or 2–50 mM DM-β-CD, the baseline resolution was achieved for a 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.

3.4 Simultaneous separation of positional isomers of C^-/M^- and DC^-/DM^-

In CZE with 10 mM borate buffer (Fig. 3A), the co-elution of three pairs of C^-/M^- was observed due to similar μ for each positional isomer C^-/M^-. As can be seen from Fig. 3B using an MEKC buffer containing 40 mM SDS, the slightly better separation was obtained for positional isomeric separation C^-/M^- because of slightly higher α_k than α_{CZE} for each pair of C^-/M^- isomers. In comparison with CZE (Fig. 3A) and MEKC (Fig. 3B), the reversed apparent |μ| order in CD-EKC for $M^- > C^-$, particularly at 20 mM α-CD (Fig. 3C), 16 mM β-CD (Fig. 3D) or 5.0 mM DM-β-CD (Fig. 3E), 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, resulted in the lower apparent |μ| of analyte and its complex. In comparison with CZE and MEKC, CD-EKC gave the better simultaneous separation for similar μ compounds of C^-/M^- due to greater α_{CD} resulting from higher κ than α_{CZE} and α_k. Over a wide range of 15–60 mM α-CD, *e.g.* 20 mM (Fig. 3C), in the buffer, CD-EKC achieved the baseline resolution for simultaneous separation of C^-/M^-.

The similar trend was also found for the better simultaneous separation of DC^-/DM^- with CD-EKC (Fig. 3F-3H) than CZE and MEKC (data not shown for CZE and MEKC). Using 40 mM DM-β-CD (Fig. 3H), the baseline separation of positional isomeric separation of DC^-/DM^- was achieved, except for $2,3DM^-/2,5$ DM^- and $2,6DC^-/2,6DM^-$. 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.

3.5 Application to phenoxy acid herbicides

The separation selectivity was also compared for phenoxy acid herbicides using three CE modes: CZE, MEKC and CD-EKC. Initial study is focused on the separation of 2,4-DB/MCPB and 2,4-D/MPCA which have only one different substituent of chloro and methyl group on a benzene ring. For CZE separation in Fig. 4A, the co-elution peaks of 2,4-DB/MCPB and 2,4-D/MCPA were observed due to their similar μ or small α_{CZE}, which is consistent with the previous work [23]. In MEKC with 40 mM SDS in Fig. 4B, these two pairs were also co-eluted. With the addition of MeOH or ACN up to 20 %v/v in the CZE or MEKC buffer, the baseline resolution was not obtained for 2,4-DB/MCPB and 2,4-D/MCPA (data not shown). Using CD-EKC with various types and concentrations of CD, the values of *K*, κ and $\alpha_{CD,\,max}$ and types of α_{CD} model for 2,4-DB/MCPB and 2,4-D/MCPA are listed in Table 2. According to theoretical models for α_{CD} [25], in comparison with CZE, an increase in α_{CD} for 2,4-DB/MCPB and 2,4-D/MCPA with α-CD is consistent with the Type III α_{CD} model ($\kappa \geq 1 > \alpha_{CZE}$), whereas the Type I α_{CD} model ($\kappa > \alpha_{CZE} \geq 1$) for these analytes with β-CD or DM-β-CD. Due to 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 two pairs was observed (data not shown). In comparison with β-CD, α-CD and DM-β-CD have the much higher separation selectivity (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). Baseline resolution was achieved over a wide range of *C*, for example, 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. Using 2.0–15 mM α-CD, the simultaneous separation of 2,4-DB/MCPB, 2,4-D/MCPA and other six phenoxy acid herbicides was not achieved. With 2.0–8.0 mM DM-β-CD for simultaneous separation of ten phenoxy acid herbicides, 3.0 mM DM-β-CD gave the achieved baseline resolution for all analytes within 4.0 min as shown in Fig. 4D.

4 Concluding remarks

CE separation selectivity for two analytes having similar μ, such as C^-/M^- and DC^-/DM^-, were compared in three CE modes: CZE, MEKC and CD-EKC. Small α_{CZE} was obtained for each pair of C^-/M^- and DC^-/DM^-. 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 in the buffer of CZE or MEKC, a slight decrease in α_{CZE} and α_{MEKC} was observed. In comparison with CZE and MEKC, CD-EKC gave the higher α_{CD} than α_{CZE} and α_{MEKC} for C^-/M^- and DC^-/DM^- because of large different in binding constant of analytes to CD. The better simultaneous separation of positional isomers of C^-/M^- and DC^-/DM^- was also

observed using CD-EKC than CZE or MEKC. In addition, the baseline separation for 2,4-DB/MCPB and 2,4-D/MCPA, two pairs of phenoxy acid herbicides having the difference in chloro and methyl substituents on a benzene ring, was achieved in CD-EKC using α-CD or DM-β-CD, while not achieved in CZE and MEKC. The results imply that CD-EKC can be used as the better alternative to separate compounds having the different substituents only by chloro and methyl groups, in comparison with CZE and MEKC.

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Table 1 Mobilities (μ), mobility selectivities (α_{CZE} and α_{MEKC}) and retention selectivities (α_k) for C^-/M^- and DC^-/DM^- in CZE and MEKC without the addition of organic modifiers.

Analytes (2/1)	CZE[a]		MEKC[b]		
	μ (10^{-8} m^2 V^{-1} s^{-1})	α_{CZE} ($\mu_{0,2}/\mu_{0,1}$)	α_{MEKC} (μ_2/μ_1)	α_k (k_2/k_1)	α_{MEKC} Type
$2C^-/2M^-$	-2.83/-2.78	1.018	1.019	1.031	I
$3C^-/3M^-$	-2.86/-2.79	1.025	1.027	1.061	I
$4C^-/4M^-$	-2.82/-2.76	1.022	1.023	1.048	I
$2,3DC^-/2,3DM^-$	-2.74/-2.67	1.026	1.029	1.050	I
$2,4DC^-/2,4DM^-$	-2.67/-2.62	1.019	1.021	1.036	I
$2,5DC^-/2,5DM^-$	-2.73/-2.65	1.030	1.033	1.053	I
$2,6DC^-/2,6DM^-$	-2.70/-2.62	1.031	1.034	1.056	I
$3,4DC^-/3,4DM^-$	-2.75/-2.65	1.038	1.046	1.103	I
$3,5DC^-/3,5DM^-$	-2.72/-2.62	1.038	1.046	1.099	I

[a]CZE buffer contained a 10 mM borate buffer at pH 9.2. [b]MEKC buffer contained 40 mM SDS in a 10 mM borate buffer at pH 9.2.

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

Analytes[a]	α-CD (0 to 60 mM)				β-CD (0 to 16 mM)				DM-β-CD (0 to 50 mM)			
	K (M^{-1})	κ	$\alpha_{CD,\,max}$ ([CD][b])	Type	K (M^{-1})	κ	$\alpha_{CD,\,max}$ ([CD][b])	Type	K (M^{-1})	κ	$\alpha_{CD,\,max}$ ([CD][b])	Type
$2C^-/2M^-$	2.7±0.5 /2.9±0.5	1.07	1.036 (60)	I	6.5±0.5/4.8±0.3	1.35	1.021 (16)	III	< 1.0/<1.0	~1.0	1.018 (0)	x
$3C^-/3M^-$	80.7±2.0/22.3±0.6	3.62	1.408 (50)	III	70.8±0.4/29.7±0.2	2.38	1.183 (16)	III	30.7±0.8/8.4±0.2	3.65	1.404 (50)	III
$4C^-/4M^-$	129.0±3.0/27.5±0.6	4.69	1.444 (30)	III	104.7±0.6/84.8±0.3	1.23	1.048 (14)	III	45.4±1.1/29.5±0.5	1.54	1.264 (40)	III
$2,3DC^-/2,3DM^-$	3.5±0.9/2.0±0.8	1.75	1.074 (60)	III	52.9±0.5/45.0±0.9	1.18	1.081 (16)	III	15.2±0.4/8.2±0.5	1.85	1.417 (50)	III
$2,4DC^-/2,4DM^-$	204.5±3.8/35.1±0.8	5.83	1.452 (20)	III	79.7±0.8/50.0±0.6	1.59	1.141 (16)	III	22.7±0.5/15.7±0.5	1.45	1.260 (50)	III
$2,5DC^-/2,5DM^-$	97.3±2.9/37.2±1.1	2.62	1.247 (30)	III	80.5±0.6/43.3±0.7	1.86	1.206 (16)	III	21.0±0.6/2.2±0.3	9.55	1.241 (50)	III
$2,6DC^-/2,6DM^-$	1.2±0.2/1.1±0.4	1.09	1.031 (0)	III	4.5±0.5/4.3±0.7	1.05	1.031 (0)	III	<1.0/<1.0	~1.0	1.031 (0)	x
$3,4DC^-/3,4DM^-$	14.5±2.8/2.1±0.5	6.90	2.025 (60)	III	404.1±3.1/167.6±1.2	2.41	1.217 (6)	III	203.6±3.5/68.5±1.2	2.97	1.679 (50)	III
$3,5DC^-/3,5DM^-$	191.9±5.8/38.8±1.3	4.95	1.444 (20)	III	66.5±0.1/6.2±1.4	10.7	1.298 (16)	III	40.3±0.9/1.9±0.3	21.2	1.627 (50)	III
2,4-DB/MCPB	381.5±12.0/235.9±4.9	1.61	1.157 (6)	III	434.8±9.8/460.4±7.1	1.06	1.027 (10)	I	211.8±4.2/275.4±6.6	1.30	1.077 (6)	I
2,4-D/MCPA	274.3±7.8/197.3±6.0	1.39	1.072 (6)	III	108.2±1.6/110.6±1.3	1.02	1.037 (10)	I	41.9±1.1/59.1±1.1	1.41	1.178 (50)	I

[a]Analyte 2 refers to the former for the α_{CD} Type I, while the latter for the α_{CD} Type III.
[b]CD concentrations (mM) giving the observed $\alpha_{CD,\,max}$ in parenthesis.
x refers to non-identified Type due to very small K.

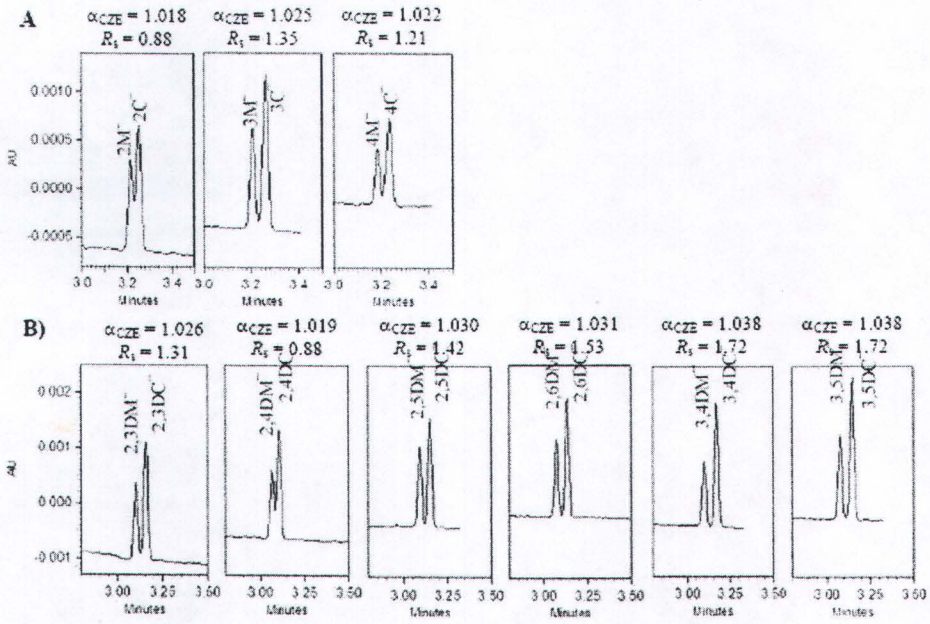

Figure 1 Electropherograms for CZE separation of (A) chlorobenzoate/methylbenzoate; C^-/M^- and (B) dichlorobenzoate/dimethylbenzoate; DC^-/DM^-. CE conditions: a 10-mM borate ($Na_2B_4O_7$) buffer at pH 9.2, 50 μm id×40.2 cm (30 cm to detector) uncoated fused-silica capillary, temperature of 25 °C, applied voltage of 15 kV, UV detection at 214 nm and 0.5 psi pressure injection for 3 s.

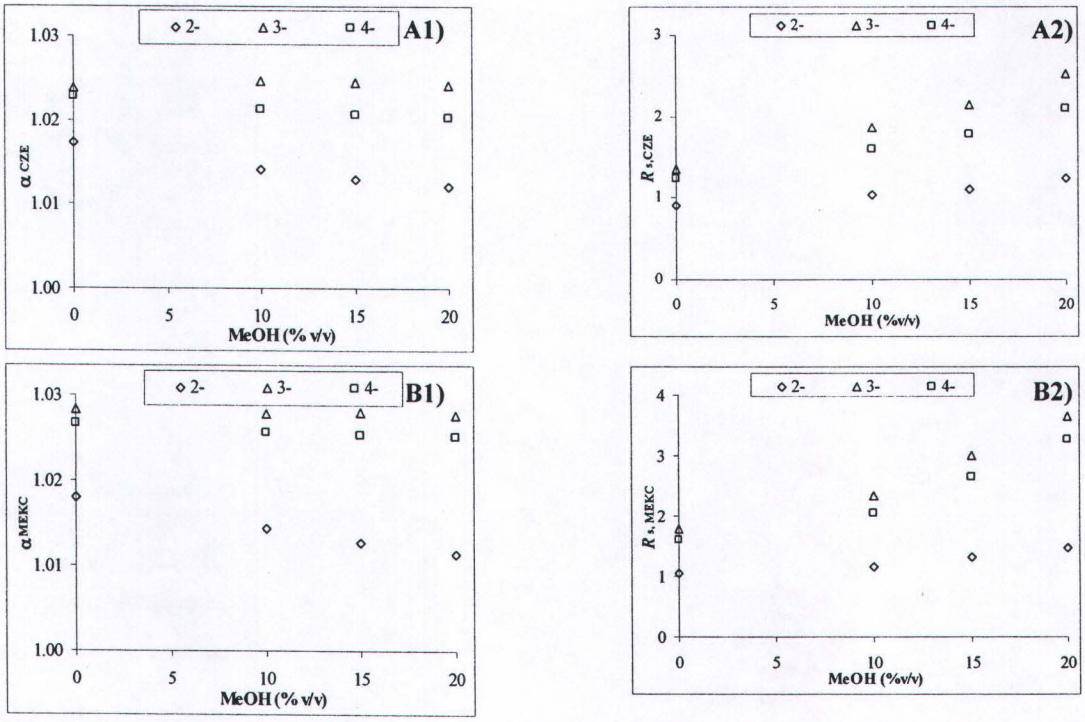

Figure 2 Effect of MeOH in the buffer on mobility selectivity (A1, B1) and resolution (A2, B2) of C^-/M^- in CZE and MEKC: (A1) mobility selectivity in CZE (α_{CZE}), (A2) resolution in CZE ($R_{s,\,CZE}$), (B1) mobility selectivity in MEKC (α_{MEKC}) and (B2) resolution in MEKC ($R_{s,\,MEKC}$). A CZE buffer contained a 10-mM borate buffer at pH 9.2 and MeOH. An MEKC buffer contained 40 mM SDS and MeOH in a 10-mM borate buffer at pH 9.2. Other CE conditions as shown in Fig. 1.

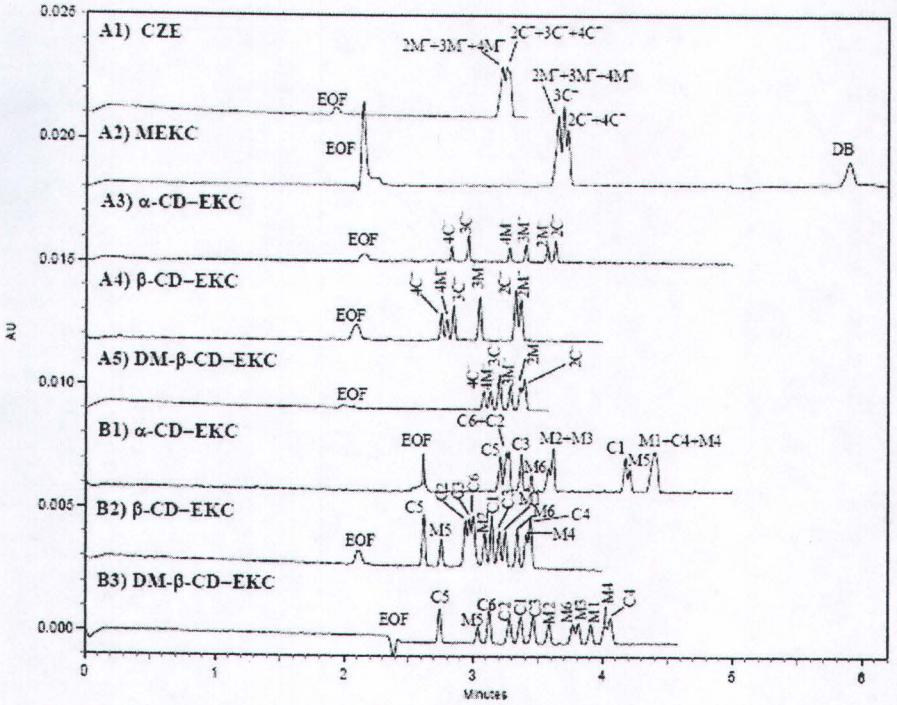

Figure 3 Simultaneous separation of positional isomers of C^-/M^- (A) and DC^-/DM^- (B) in three CE modes at a pH 9.2 buffer: (A1) CZE with 10 mM $Na_2B_4O_7$, (A2) MEKC with 40 mM SDS in 10 mM $Na_2B_4O_7$, and CD-EKC with 10 mM $Na_2B_4O_7$ containing (A3) 20 mM α-CD, (A4) 16 mM β-CD, (A5) 5 mM DM-β-CD, (B1) 60 mM α-CD (B2) 12 mM β-CD and (B3) 40 mM DM-β-CD. (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). Other CE conditions as shown in Fig. 1.

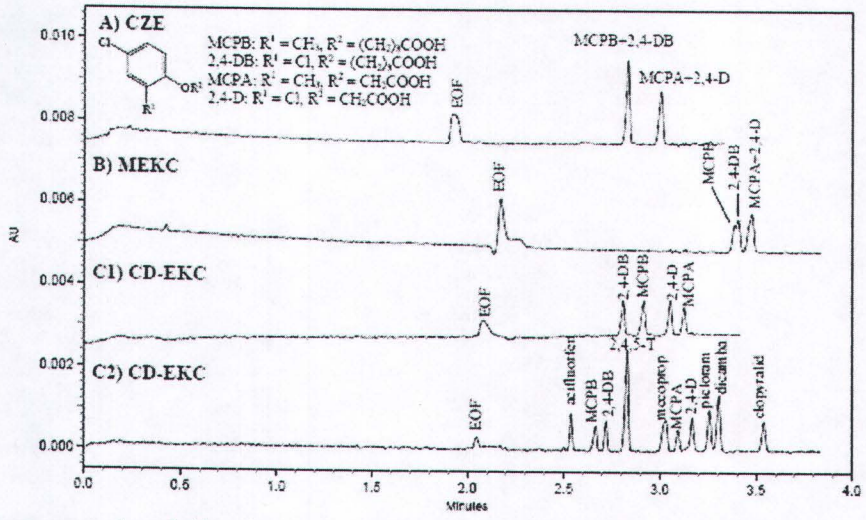

Figure 4 Simultaneous separation of chlorophenoxy acid herbicides in three CE modes at a pH 9.2 buffer: (A) CZE with 10 mM $Na_2B_4O_7$, (B) MEKC with 40 mM SDS in 10 mM $Na_2B_4O_7$ and (C) CD-EKC with 10 mM $Na_2B_4O_7$ containing (C1) 2.0 mM α-CD and (C2) 3.0 mM DM-β-CD. Other CE conditions as shown in Fig. 1.

ภาคผนวก 6

สรุป Output จากโครงการวิจัย

ผลงานวิจัยตีพิมพ์ในวารสารระดับนานาชาติ

1) 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*, 1216 (2009) 5868-5874. IF = 4.101.
2) S. Angkanasiriporn, W. Singsung, A. Petsom, T. Nhujak, "Comparison and prediction of the retention in micellar electrokinetic chromatography and microemulsion electrokinetic chromatography for disubstituted benzenes" *Electrophoresis*, 31 (2010) 695-701, IF = 3.077.
3) C. Puangpila, A Petsom, T. Nhujak "Theoretical models of separation selectivity for charged compounds in micellar electrokinetic chromatography *Electrophoresis*, 32 (2011), 203-209. IF = 3.077
4) W. Soonthorntuntikul, M. Srisa-art, N. Leepipatpiboon, T. Nhujak "Theoretical models of separation selectivity for charged compounds in cyclodextrin electrokinetic chromatography" In preparation for submission.
5) W. Soonthorntantikul, N. Leepipatpiboon, M. Srisa-art, T. Nhujak "Comparison of Separation Selectivity in CZE, MEKC and CD-EKC for chlorobenzoates/methylbenzoates, and application to separation of phenoxy acid herbicides" In preparation for submission.

ผลงานนำเสนอในที่ประชุมวิชาการระดับนานาชาติ

1) C. Puangpila, A. Petsom, T. Nhujak "Theoretical models of separation selectivity for charged compounds in micellar electrokinetic chromatography" *Poster Presentation,* 10th Tenth Asia-Pacific International Symposium on Microscale Separation and Analysis, December, 10-13, 2010, Hong Kong.
2) W. Soonthorntuntikul, M. Srisa-art, N. Leepipatpiboon, T. Nhujak "Theoretical models of separation selectivity for charged compounds in cyclodextrin electrokinetic chromatography", *Poster Presentation,* 10th Asia-Pacific International Symposium on Microscale Separation and Analysis, December, 10-13, 2010, Hong Kong.
3) W. Soonthorntantikul, N. Leepipatpiboon, M. Srisa-art, 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,* 10th Asia-Pacific International Symposium on Microscale Separation and Analysis, December, 10-13, 2010, Hong Kong.
4) C. Puangpila, A. Petsom, T. Nhujak "Theoretical models of separation selectivity for charged compounds in micellar electrokinetic chromatography" *Poster Presentation,* 17th International Symposium on Electro- and Liquid Phase-separation Techniques, 29 August- 1 October, 2010, Maryland, USA.
5) S. Angkanasiriporn, W. Singsung, A. Petsom, T. Nhujak, "Retention of disubstituted benzenes micellar electrokinetic chromatography and microemulsion electrokinetic chromatography, *Poster Presentation*, 24th International Symposium on Microscales Bioseparation, 12-28 October 2009, Dalian, China.
6) 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" *Poster Presentation,* 33rd International Symposium on High-Performance Liquid Phase Separation and Related Techniques, 2-5 December 2008, Kyoto, Japan.