Studies on Separation of Closely Related Compounds

by Capillary Electrophoresis

Ryo Koike

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Chapter 1

General Introduction

1-1 Separation in CE

Capillary electrophoresis (CE) has been designed as a high efficiency separation technique. So far, various separation modes have been developed in CE, e.g., capillary zone electrophoresis (CZE) [1–5], capillary gel electrophoresis (CGE) [6,7], electrokinetic chromatography (EKC) [8–12], capillary electrochromatography (CEC) [13–15], capillary isoelectric focusing (CIEF) [16,17], and capillary isotachophoresis (CITP) [18,19]. In addition, CE often provides the highest separation efficiency among liquid-phase separation techniques due to its plug flow profile of the electroosmotic flow (EOF). Therefore, CE is currently one of the most important techniques for the separation and determination of numerous substances in various fields.

Among various modes in CE, CZE and EKC are popular because of their high applicabilities. In CZE, charged analytes can be separated according to the difference in their electrophoretic mobilities, which are determined by the charge and size of the ion, in the interior of a narrow capillary (10–100 μm I.D.) filled with a buffer solution. On the other hand, EKC, originally introduced by Terabe et al. [8], is a highly efficient separation mode for both of ionic and neutral compounds. In EKC, the separation mechanism is based on the interaction between an analyte and a pseudostationary phase (PSP) in a background solution (BGS). To attain high efficiency and selectivity,

various PSPs such as ionic surfactant micelle [8,9,20–22], microemulsion [23,24], liposome [25,26], polymer [27–30], cyclodextrin (CD) derivatives [31–32] and particles [33–37] have been applied to EKC.

It is well known that several factors affect the selectivity in CZE and EKC: pH and concentration of BGS, temperature and additives such as PSPs and organic modifiers. In particular, the addition of the organic modifiers into the BGS would allow the analysis of a wider range of analytes due to an enhancement of the solubility of various hydrophobic compounds [38–40]. Moreover, the addition of the modifiers is useful for enhancing the separation selectivity and the resolution of various compounds, and also alters the EOF rate and the electrophoretic mobility of analytes by changing the zeta potential of the capillary surface and the solvation states of ions, respectively. Therefore, it is very important to understand the function of the organic modifiers in CE.

On the other hand, in EKC, an ionic surfactant micelle is generally used as the PSP (micellar EKC, MEKC), which provides excellent separations in most cases. In MEKC, the separation occurs on the basis of mainly the hydrophobic interactions and/or the partition of an analyte between the surfactant micelle and a bulk solution or BGS. In surfactant–BGS systems, however, the micelle formation is interrupted by adding organic modifiers into the BGS, which often restricts the flexibility for adjusting the selectivity. Although several investigations dealing with the use of alternative PSPs have been reported in EKC [23–37], most of these additives hardly offer completely new selectivities compared to the surfactant micelles. In this thesis, studies on the improvement of the analytical performances in CE and the development of the analytical methods for closely related compounds by CZE and EKC are described.

1-2   Improvement of Analytical Performance in CE

1-2-1   Use of novel PSPs in EKC

As it is not possible to separate neutral analytes in CZE, EKC have been developed as a new effective separation mode in 1984.   Since the introduction of sodium dodecyl sulfate (SDS) micelle in EKC [8], many compounds have been applied as the PSPs.   In addition, significant advances in theories and applications have been reported.   As a result, EKC not only allows the separation of neutral analytes but also provides added selectivity in the separation of charged analytes with same mobilities such as enantiomers that are difficult to be separated by conventional CZE.   Recently, the application of various PSPs such as chiral surfactants, mixed micelles, polymers and particles has been investigated to improve the separation performance and versatility of EKC.

Although various ionic PSPs, such as micelles [8,9,20–22], microemulsion [23,24] and liposome [25,26], have been employed in EKC, ionic micelles are still the most often-used PSPs.   Among the ionic surfactants, SDS, which exhibits a low critical micelle concentration, provides good selectivity and efficiency.   Furthermore, many other commercial surfactants are available for the PSPs with varying the selectivity.

However, there are several limitations in EKC; the conventional ionic surfactants can not be applied to the MEKC analysis employing mass spectrometry (MS) detection [41–44].   For instance, SDS is known to decrease the signal response in electrospray ionization (ESI)-MS detection since the ionization of analyte ions is suppressed in the micellar system and the mass spectrometer is contaminated by low-volatile ionic micelles [42].   In addition, surfactants designed for obtaining specific chromatographic

selectivities are not commercially available, so that either the use of additives or the synthesis of selective surfactants should be required for unique or desired selectivities. These limitations have led many researchers to seek alternative PSPs. Recently, non-conventional PSPs have been employed in EKC to overcome such limitations and improve the selectivities. For example, polymer- and silica-based particles have been applied to the separation of catechols, phenols, polycyclic aromatic hydrocarbons (PAHs) and alkali metals [33–37]. However, up to now the application field of the particle PSPs is still limited. In this thesis, the author has studied on the applicability of an inorganic layered compound (synthetic smectite) as a novel PSP in EKC for improving the analytical performance. Furthermore, to overcome low concentration sensitivity that is main drawback in the EKC analysis, fundamental studies on on-line sample preconcentration by sweeping [45,46] using a smectite as a sweeping carrier have been also carried out. The obtained results are shown in Chapter 2.

On the other hand, one of the attractive applications of MEKC is chiral separations. The enantioseparation is very important issue especially in pharmaceutical, medical and biomedical fields because another enantiomer may produce critically different efficacy. So far, HPLC had been mainly used for the separation of enantiomers. In HPLC, the chiral separations are achieved by using chiral selector immobilized stationary phases, the addition of the selector to a mobile phase, or a precolumn derivatization for separating diastereoisomers. However, the design of the chiral stationary phases is complicated and such phases are usually applied to only a limited number of enantiomers. Besides, the use of the chiral mobile phase is also limited due to the consumption of large amounts of the chiral selectors.

In EKC, the optical resolution is achieved by adding the chiral selectors to the BGS.

Cyclodextrins (CDs) are the most common chiral additives for the separation of enantiomers in EKC.   In the chiral EKC analysis, CDs has been employed in the neat or micellar system, in which CDs are mixed with achiral surfactants (CD–modified MEKC; CD–MEKC) [47–50].   On the other hand, several chiral surfactants, e.g., sodium *N*-dodecanoyl-L-valinate (SDVal) [51–55], bile salts [56–59], digitonin [53] and saponins [60] have been also used for effective chiral discriminations in normal or modified MEKC.   Besides many chiral surfactants are available from natural sources, several synthesized chiral surfactants can be employed as the selectors in MEKC. Hence, the author has studied on the optical resolution by MEKC using sodium *N*-dodecanoyl-L-glutamate (SDGlu) as a new chiral selector and the results are shown in Chapter 6.

1-2-2   Use of organic modifiers in CE

Organic modifiers are used in CE to increase the solubility of analytes in BGS and enhance the separation selectivity.   The addition of the organic modifiers strongly affects the effective mobility of the analytes and the EOF rate.   Sarmini and Kenndler [38] investigated the effect of common organic solvents such as methanol, ethanol, acetonitrile, dimethyl sulfoxide (DMSO), acetone and tetrahydrofuran on the CZE and CITP separations in detail.   They summarized selectivity enhancement in the CE analysis of several amino acids, peptide, proteins and inorganic ions by the addition of the organic modifiers to aqueous BGSs.   Although the organic modifiers are often used in these two separation modes, studies on the role of organic modifiers in MEKC have been scarcely reported.

Since MEKC is generally operated with an aqueous running solution, hydrophobic

compounds cannot be separated adequately due to poor solubility in the aqueous BGS. Furthermore, it is difficult to dissolve a sufficient amount of highly hydrophobic compounds in the aqueous micellar solution to allow the use of UV detector, which is a conventional detection scheme in CE.   Organic modifiers are often added to the aqueous BGS used in the separation of ionic compounds by CE to improve the solubility of various sample components.   Hence, several researchers have investigated the effect of the addition of the organic modifiers, i.e., methanol [61,62], acetonitrile [62], and 2-propanol [63], to the aqueous micellar solutions.   However, it is generally recognized that the surfactant micelle is not stable in the aqueous solution containing more than 20–30% of the organic solvents [40].   Nevertheless, it has been reported that the addition of 80–100% methanol to buffer solutions in MEKC gave a good separation of hydrophobic compounds [64–67].   Thus, the author has studied on the MEKC separation of hydrophobic compounds such as PAHs with DMSO and acetone.   The obtained results are shown in Chapter 5.

1-2-3   Use of other additives in CE

Numerous approaches have been devoted to improve the CE separations of ionic analytes with similar electrophoretic mobilities by using various additives.   One of the most effective and simple approaches is the addition of ion-pair reagents to the BGS. Conventional ion-pair reagents developed for reversed-phase HPLC are frequently employed for improving the selectivity in CE [68].   For instance, alkylsulfonic acids and alkyl quaternary ammonium salts are used for the separation of cationic and anionic analytes, respectively.   In addition, polymeric ion-pair reagents, e.g., polybrene (hexadimethrine bromide) and poly(diallyldimethylammonium chloride) [69], and metal

cations such as Cu^{2+}, Zn^{2+} and Mg^{2+} [70] have been effectively employed to enhance the resolution and selectivity in CE through a formation of complex with ionic analytes.

High concentration of urea is often added to the aqueous BGS to increase the solubility of hydrophobic analytes [71]. The addition of high concentration of urea is also known to reduce the electroosmotic mobility and the migration velocity of the micelle, and slightly affects the separation selectivity and the migration order of analytes. Kaneta and co-workers [72] reported that the addition of glucose to the BGS in SDS–MEKC provided an extension of the migration window and improved the separation selectivity. Although a detailed mechanism is not fully clarified, the addition of glucose caused the decrease in the distribution coefficients between the micellar phase and analytes, especially with hydrophilic groups.

Reversal of the EOF in CE is essential to achieve a rapid separation of anionic analytes, which is commonly attained by the addition of cationic surfactants such as cetyltrimethylammonium bromide to the BGS [73]. The reversal of the flow direction is caused by the adsorption as a hemi-micelle or bilayer of cationic surfactants onto the capillary surface through electrostatic and/or hydrophobic interactions, and thus cationic surfactants effectively change the zeta potential of the capillary surface from negative to positive.

1-3   Application of High Performance CE

The analytical performance of CE has been advanced by the development of several separation modes and the application of various additives such as the organic modifiers and PSPs. Nowadays, CE has attracted considerable attention in various fields of

industry because of many advantages including high separation efficiency, short analysis time and little reagent consumption.　However, the separation and determination of surfactants in complicated formulations such as detergents, cosmetics, food and other consumers, which requires powerful separation techniques, is still a challenging task even in CE.

Surfactants are widely applied in various consumers and industrial formulations such as washing agents, emulsifiers, solubilizers and foaming agents [74].　These formulations consist of various surfactants, i.e., amphoteric, anionic and nonionic surfactants, and other additives, which consequently makes the analysis of the surfactant contents in real products difficult.　Furthermore, most technical products of the surfactant used for the formulations contain complexed homologous mixtures with varying the degree of alkyl chain length (C_8–C_{18}) and/or ethoxylation.　So far, several research groups have studied the HPLC separation of the surfactants [75–78]. However, it was difficult to separate various surfactants and additives in a single run.

Recently, CE has been increasingly employed for the separation and the determination of surfactants in household products [79–89].　However, the adequate separation for the precise analysis of the surfactants, especially for the nonionic and amphoteric surfactants, has not been achieved in the previous studies.　Recently, the successful electrochromatographic separation of these surfactants using a packed capillary have been reported but the fabrication of the capillaries is very difficult [90,91]. Thus, the introduction of a simple CE analysis method for these surfactants is still needed.　Hence, the author has studied on the development of high performance electrophoretic analysis methods for amphoteric surfactants and fatty alcohol ethoxylates in detail.　The obtained results are shown in Chapters 3 and 4.

1-4　Purpose and Contents of the Thesis

The aims of this thesis are the development of the separation methods for the closely related compounds such as PAHs, enantiomers and surfactant homologues with improving the analytical performances, i.e., efficiency, resolution, selectivity and applicability, in CE.　To realize these requirements, the author has investigated the effect of the organic modifiers, i.e., DMSO and acetone, on the MEKC separation of hydrophobic compounds, and the use of SDGlu and an inorganic layered compound as novel PSPs for the separation of PTH–DL-amino acids and nonionic compounds containing repeating ethylene oxide (EO) groups, respectively.　In addition, to expand the application area of CE, the author has studied on the separation and the determination of complicated surfactant samples containing homologues which are important materials in detergent industry.

In Chapter 2, the use of an inorganic layered compound as the PSP in the EKC analysis of polyoxyethylene mono phenyl ethers (PPEs) with the different degree of ethoxylation is described.　In EKC, SDS is the most popular PSP due to its selectivity, low cost, availability with high purity, optical absorption characteristics, and intrinsic micellar properties.　Although other surfactants have been applied to the PSP, the use of conventional micelles limits the applicability to the separation of highly hydrophobic compounds and MS detection [41–44].　Thus, several researchers have investigated the use of particles as alternative PSPs.　The characteristic features of particles which is suitable for the PSP in EKC are as follows: (i) surface functional groups provide the selectivity by the interaction with the analytes, (ii) surface ionizing groups give

electrophoretic mobility, (iii) small particles with high surface areas are useful for the sufficient retention and avoiding light scattering, (iv) homogeneous size distribution provides uniform mobility to prevent the peak broadening.   The author investigated the use of a synthetic smectite in EKC.   The retention characteristic of the smectite and the influence of the smectite concentration, analyte structure, ionic strength and organic modifiers on the separation of PPEs were studied.   Furthermore, the application to the analysis of the PPEs in household products and fundamental studies on on-line sample preconcentration by sweeping using smectite were also conducted to increase the detectability.

In Chapter 3, the determination method for four types of amphoteric surfactants and their homologues by CE with indirect UV detection is described.   Surfactants are widely used in consumer and industrial formulations as complex mixtures of homologues and isomers.   Therefore, efficient separation and determination methods are required for the selective analysis of target surfactants.   Although amphoteric surfactants account for only ~5% of the total surfactant trade among the four classes of surfactants, i.e., anionic, cationic, nonionic and amphoteric surfactants, the amphoterics are considered to be characteristic surfactants due to their ability to suppress the stimulation of the formulations.   Thus, the demand of the determination methods for the amphoterics will grow significantly in the near future.   However, very few studies have been reported on the HPLC and CE analyses of the amphoteric surfactants [92–95]. Although the 2D–HPLC separation of complex surfactant mixtures have been already reported [96], the application of this technique to the real formulations was not described.   Hence, the author investigated the development of a simultaneous

separation method for amphoteric surfactants and their homologues using CE with indirect UV detection. The effect of pH of the BGS, organic modifier and chromophore on the separation was studied. As a result, highly effective separation of four amphoteric surfactants and their homologues could be achieved in a short time. It was confirmed that the developed method provided high resolution and selective separations of the amphoterics not only in standard mixtures but also complex formulations such as detergent and cosmetics.

In Chapter 4, the application of CZE and MEKC to the separation of complicated fatty alcohol ethoxylate (FAE) homologues is described. The FAEs are the most important nonionic surfactants widely used in consumer and industrial products. Most technical products of the FAEs are complex mixtures of homologues with different numbers of the EO groups. Furthermore, the physical and chemical properties of the FAEs are considerably changed by the distribution of the EO groups.

The difficulties in the CE analysis of the FAEs are derived from their electrically neutral states and the lack of the UV absorption. One of the possibilities to make these analytes suitable for the CE analyses is a derivatization with a charged chromophore. Several research groups have reported on the CZE separation of the FAEs derivatized with carboxylic anhydride [97–101]. In these studies, it was demonstrated that CE was an excellent method for rapid fingerprint analysis of the FAEs in household formulations. However, the resolution of the homologues with long EO groups was insufficient, so that the detailed analysis of the EO distribution could not be attained in the previous studies. Thus, the author studied on the development of the effective separation methods for the EO homologues of the FAEs on the basis of the CE

technique with UV detection.   To attain this purpose, the FAEs were derivatized with cationic chromophore 2-fluoro-1-methylpyridinium p-toluenesulfonate [102,103], and CZE and MEKC with SDS and dodecyltrimethylammonium chloride were investigated in detail.

In Chapter 5, a fundamental study on the effect of organic modifiers on the separation of lipophilic compounds in MEKC employing UV detection is described.   Organic modifiers can improve the separation performances and selectivity in CE.   Thus, several concentrations of the organic modifiers, DMSO and acetone, were employed in the SDS–MEKC separation of PAHs.   As a result, highly effective separation of PAHs could be achieved at higher concentration of these modifiers.   Obtained results indicated that DMSO and acetone were effective for the separation of PAHs. Therefore, these modifiers will enhance the applicability of MEKC to the separation of hydrophobic compounds.

In Chapter 6, the chiral separation of PTH–DL-amino acids by MEKC with SDGlu and with digitonin–sodium taurodeoxycholate (STDC) mixed micelles is described. Since the enantioseparation was first achieved by CZE [11,104], numerous papers on the chiral separation by CE have appeared.   Although CDs have been considered as the most widely used chiral selectors in CE, the use of native CDs is restricted by their limited aqueous solubility and electrically neutral nature.   Thus, charged CDs and chiral surfactants have attracted great interest for the chiral separation in CE since these compounds are advantageous in higher solubility in water.   Furthermore, high enantioselectivity and resolution can be obtained at a low concentration of charged CDs

and chiral surfactants compared with neutral CDs.   Hence, SDGlu was employed as a chiral selector for the first time in MEKC for the enantioseparation of PTH–DL-amino acids.   The author evaluated the separation characteristics in SDGlu and digitonin–STDC micellar systems.

1-5   References

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Chapter 2

Separation of Nonionic Compounds by Electrokinetic Chromatography Using an Inorganic Layered Compound as a Pseudostationary Phase

2-1 Introduction

Electrokinetic chromatography (EKC), originally introduced by Terabe et al. in 1984, is a highly efficient separation method for neutral compounds [1]. In EKC, the separation mechanism is based on the interaction between analytes and a pseudostationary phase (PSP) in a background solution (BGS). To attain high efficiency and selectivity, various PSPs such as ionic surfactant micelle [1–4], microemulsion [5,6], liposome [7,8], polymer [9–12] and cyclodextrin [13–14] have been applied to EKC. In addition, it has been demonstrated that particles, which possesses charged surface and/or functional groups to interact with analytes, can be also used as the PSP. For example, polymer and silica-based particles are employed for the separation of catechols, phenols, polycyclic aromatic hydrocarbons and alkali metals [15–19].

It is well known that clay minerals, which are typical inorganic layered compounds, show a high adsorptivity especially for polar molecules [20,21]. Clay minerals consist of many layers of microscopically small crystal plates. These plates are called silicate layers which contain one or two sheets of silicon tetrahedrons and one sheet of aluminum or magnesium octahedrons. In general, these layers are negatively charged

due to their isomorphous substitution of cations in tetrahedral and/or octahedral sites. Since inorganic ions, e.g., Na^+, K^+, Ca^{2+} and Mg^{2+} ions, are sandwiched between the negatively charged silicate layers, the charge balance of the clay mineral is maintained. It has been assumed that ion-dipole interactions between the interlayer cations of clay minerals and the polar functional groups are the dominant bonding. Furthermore, clay minerals swell up by an interaction between interlayer cations and water molecules and this phenomenon makes clay minerals high dispersibility in an aqueous medium. Thus, the inorganic layered compounds are attractive to be employed as the PSP in EKC similar to polymer and silica-based particles.

On the other hand, sweeping is known as one of the most effective on-line sample concentration techniques for enhancing the concentration sensitivity especially in micellar EKC (MEKC) [22–26]. Sweeping is defined as a phenomenon where analytes in a solution containing no PSP are picked up and concentrated by the PSP dissolved in the BGS at the boundary between the sample matrix and BGS. According to the principle of the concentration technique, the PSPs described above have a potential as the sweeping carrier in EKC. In the previous papers, however, surfactant micelles have been mainly used as the sweeping carrier except for several reports [27–29]. To our knowledge, the application of the PSP of microparticles to sweeping has not been reported.

In the present paper, the EKC separation of nonionic compounds using an inorganic layered compound as the PSP is described. Polyoxyethylene mono phenyl ethers (PPEs), which have repeating ethylene oxide (EO) groups, were chosen as analytes. PPEs are used as solubilizers in household products such as laundry bleaches. Since their physical and chemical properties are greatly changed by the average number and

the distribution of the EO groups, the determination of the PPEs components is very important.   Generally, the EO groups of PPEs are determined by gas chromatography (GC), but the derivatization process, i.e., trimethylsilylation or acetylation, is required for the precise analysis.   In addition, samples should be evaporated to dryness prior to the derivatization because most household products are aqueous solution.   Therefore, it is important to develop simple and effective techniques for the analysis of PPEs.   The EO groups are expected to interact with the layered compounds in BGS.   The author chose a commercially available synthetic smectite as the PSP whose basic structure is 2:1 silicate layers consisting of two sheets of silicon tetrahedrons and an intermediate sheet of magnesium octahedrons.   The retention characteristics of the smectite for the compounds with the EO groups and the application to on-line sample preconcentration by sweeping will be discussed.

2-2   Experimental Section

2-2-1   Materials

A synthetic smectite, commercially available as Lucentite SWN, was kindly supplied by CO-OP Chemical (Tokyo, Japan) and used as received.   The structural formula of Lucentite SWN is $Na_{0.33}(Mg_{2.67}Li_{0.33})Si_4O_{10}(OH)_2$.   The surface of the smectite is negatively charged due to isomorphous substitution of Mg^{2+} ions by Li^+ ions in the sheets of magnesium octahedrons. Na^+ ions are sandwiched between the silicate layers of the smectite as exchangeable cations.   PPEs were obtained from Nippon Nyukazai (Tokyo, Japan).   PPEs are complex mixtures of homologues with the general formula of $C_6H_5O(CH_2CH_2O)_xH$, where x is the number of the EO groups and the average x of

2.0 (PhG-2.0), 3.0 (PhG-3.0) and 5.5 (PhG-5.5) were used in this study.   The highly

ethoxylated components ($x \geq 5$) in PhG-5.5 were obtained by a TOSOH preparative size

exclusion chromatography system (Tokyo, Japan) for the sweeping experiments.

Polyoxyethylene mono 2-naphthyl ether (PNE, average $x = 5$) was supplied by KAO

(Tokyo, Japan), methanol of HPLC grade from Kanto Chemical (Tokyo, Japan).

Methanol was used as an electroosmotic flow (EOF) marker.   All other reagents were

of analytical grade.   The BGSs containing the smectite were prepared by stirring for 12

h at room temperature.   All samples were diluted to appropriate concentrations with

deionized water.   The pH and conductivities of sample solutions for sweeping were

adjusted to be identical with those of the BGS with NaOH and NaCl, respectively, for

neglecting a stacking effect.   Prior to analyses, the BGSs and sample solutions were

filtrated with 5 μm (Sartorius, Goettingen, Germany) and 0.45 μm (Gelman Sciences,

Ann Arbor, MI, USA) pore size membrane syringe filters, respectively.

   In the GC measurements of PPEs, the sample was derivatized by the following

procedures.   0.1 g of household products were evaporated to dryness under nitrogen,

and then 1 mL of trimethylsilylation reagent (TMSI-H, GL Sciences, Tokyo, Japan) was

added.   The mixed solution was shaken vigorously and subsequently kept for 30 min at

room temperature.   The clear upper solution was used as the sample in the GC

analysis.

2-2-2   Apparatus

   All electrokinetic chromatograms were obtained with a Hewlett-Packard [3D]CE system

(Waldbronn, Germany) equipped with a diode-array UV detector combined with [3D]CE

Chemstation for system control, data collection and data analysis.   EKC and sweeping

experiments were performed on fused-silica capillaries of 40 or 72 cm effective length × 50 μm I.D. obtained from Agilent Technologies (Palo Alto, CA, USA).   The capillaries, which were conditioned with 1 M NaOH (15 min), followed by methanol (5 min), deionized water (5 min), and finally a BGS (5 min) prior to use, were flushed with the BGS for 2 min between each separation.   Samples were hydrodynamically injected under the pressure of 50 mbar.   Unless otherwise noted, UV detection was carried out at 197 nm and temperature was maintained 25 °C.   Water was deionized with a Milli-Q purification system (Millipore, Bedford, MA, USA).   The measurement of pH and conductivity were performed with a HORIBA D-51 pH meter (Kyoto, Japan) and HANNA instruments HI 98130 conductivity meter (Padova, Italy), respectively.   The size distribution of the smectite was measured with an ELS-8000 (Otsuka Electronics, Osaka, Japan).

   GC analyses of PPEs were performed on an Agilent 6890N equipped with a flame ionization detector.   A capillary column DB-1 30 m × 0.25 mm I.D. × 0.25 μm film thickness (Agilent Technologies) was used for the separation.   The initial temperature was 80 °C, and then increased to 300 °C at 10 °C/min.   The injected sample volume was 1 μL.

2-3   Results and Discussion

2-3-1   Electrophoretic feature of smectite

Prior to the EKC experiments, the electrophoretic mobility of the smectite was evaluated.   Since the smectite was negatively charged in water, the peak of the smectite was obtained in the conventional capillary zone electrophoresis (CZE) mode.   From the migration times of the EOF marker and smectite, the electrophoretic mobility of the smectite was determined to be 3.4×10^{-4} cm^2 V^{-1} s^{-1}.   Even though the obtained electrophoretic mobility was lower than that of the sodium dodecyl sulfate micelle, i.e., typically 4–5×10^{-4} cm^2 V^{-1} s^{-1}, that was higher than those of conventional low molecular weight compounds.   Thus, the smectite could be employed as the PSP in the EKC analysis of neutral compounds.   However, the peak observed in the CZE analysis of the smectite was broader as its width of 0.3 min.   This would be due to the heterogeneity in the size and/or zeta potential of the smectite.   Actually, the broad size distribution of the smectite was obtained with the light-scattering measurement and their size was estimated to be 132 ± 103 nm.   In other words, their distribution was ranging from 29 to 235 nm.   Although the size heterogeneity of the smectite might reduce the separation efficiencies, apparent decreases in the plate numbers were not observed as described later.

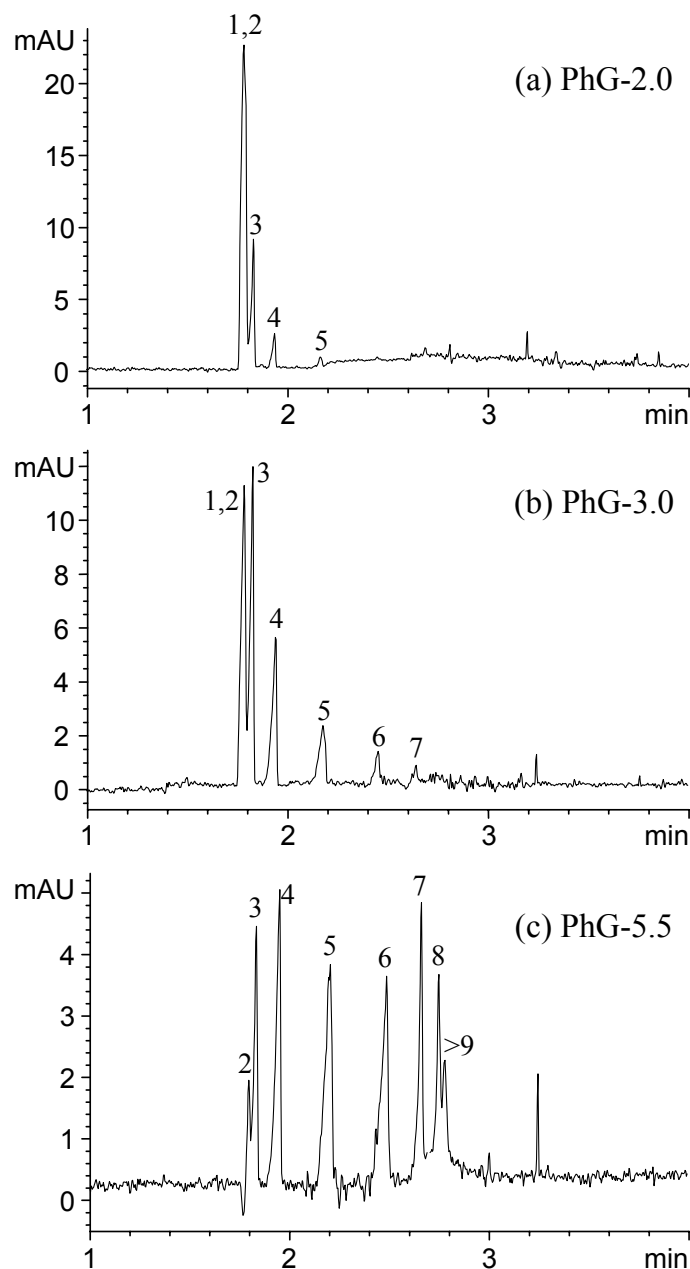**Figure 2-1.** Smectite–EKC separations of PPEs: (a) PhG-2.0, (b) PhG-3.0, (c) PhG-5.5. Capillary, 40 cm effective length, 48.5 cm total length; BGS, 0.1% (w/v) smectite, 5 mM NaCl (pH 10.3); sample concentration, 250 µg/mL; detection, 197 nm; applied voltage, 20 kV; injection, 2 s at 50 mbar; temperature, 25 °C. The numbers above the peaks correspond to the number of the EO groups of the PPE homologues.

2-3-2   Retention characteristics of smectite

Figure 2-1 shows the smectite–EKC analysis of PPEs.   The homologue peaks could be identified by comparing their migration times with those of pure standards. Although the separation window was apparently narrow ranging from 1.7 to 2.8 min, the neutral PPEs homologues with x from 3 to 8 were clearly separated.   The migration time was increased with increasing x.   This indicated that the interaction force between the analytes and smectite depended on the number of the EO groups of the PPE homologues.   It has been reported that the interaction between the EO groups and exchangeable cations in a smectite is caused by the hydrogen bonding of oxygen atoms with water in the hydration shell of exchangeable cations, the ion–dipole interactions and direct coordination between exchangeable cations and oxygen atoms of the EO groups [30–34].   In any case, the binding force between the EO groups and smectite will be high for highly ethoxylated PPEs, which provides large retention factors.   To evaluate the effect of the retention factor on the separation, the retention factors for the PPEs were plotted against x as shown in Figure 2-2.   The retention factors were dramatically increased from 0.05 to 50 with increasing x from 2 to 8, which clearly showed the strong interaction between the EO group and smectite.   In the EKC theory, the retention factor giving the best resolution (k_{opt}) is determined by the following equation [35],

$$k_{opt} = \sqrt{t_{PSP}/t_0} \qquad\qquad (2\text{-}1)$$

where t_{PSP} and t_0 are the migration times of the PSP and EOF marker, respectively.   In the experimental condition, k_{opt} was determined to be 1.28, so that the PPEs with x of

4–6 should be well separated.   Since the results in Figure 2-1 agreed well with this consideration, the separation behavior in smectite–EKC could be explained by the conventional MEKC theory.   On the other hand, the influence of the hydrophobic group of the EO compounds on the retention was investigated.   Comparing with PhG-5.5, the retention factors of more hydrophobic PNE homologues were apparently increased as shown in Figure 2-2.   As a result, the separation of PNEs could not be achieved for the components having more than seven EO groups.   Thus, the retention in smectite–EKC was dominated by both the electrostatic interaction of oxygen atoms in the EO groups and the hydrophobic interaction of the aryl groups with the smectite.
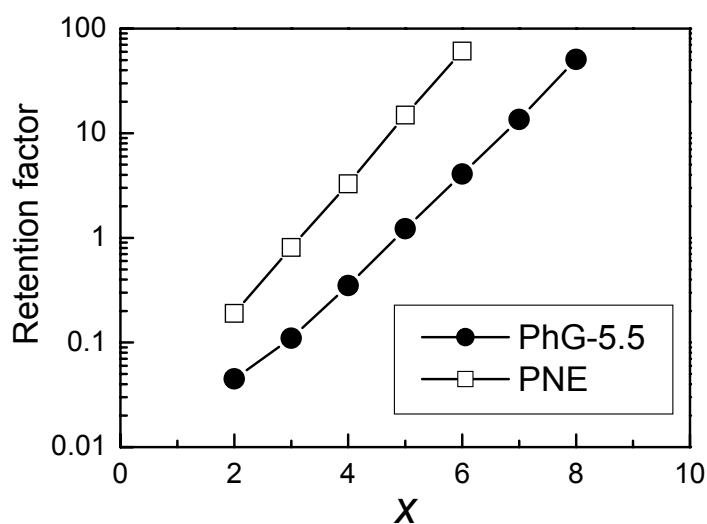

Figure 2-2.   Retention factor of PhG-5.5 and PNE in smectite–EKC.   Detection of PNE was conducted at 225 nm.   Other conditions are as in Figure 2-1.
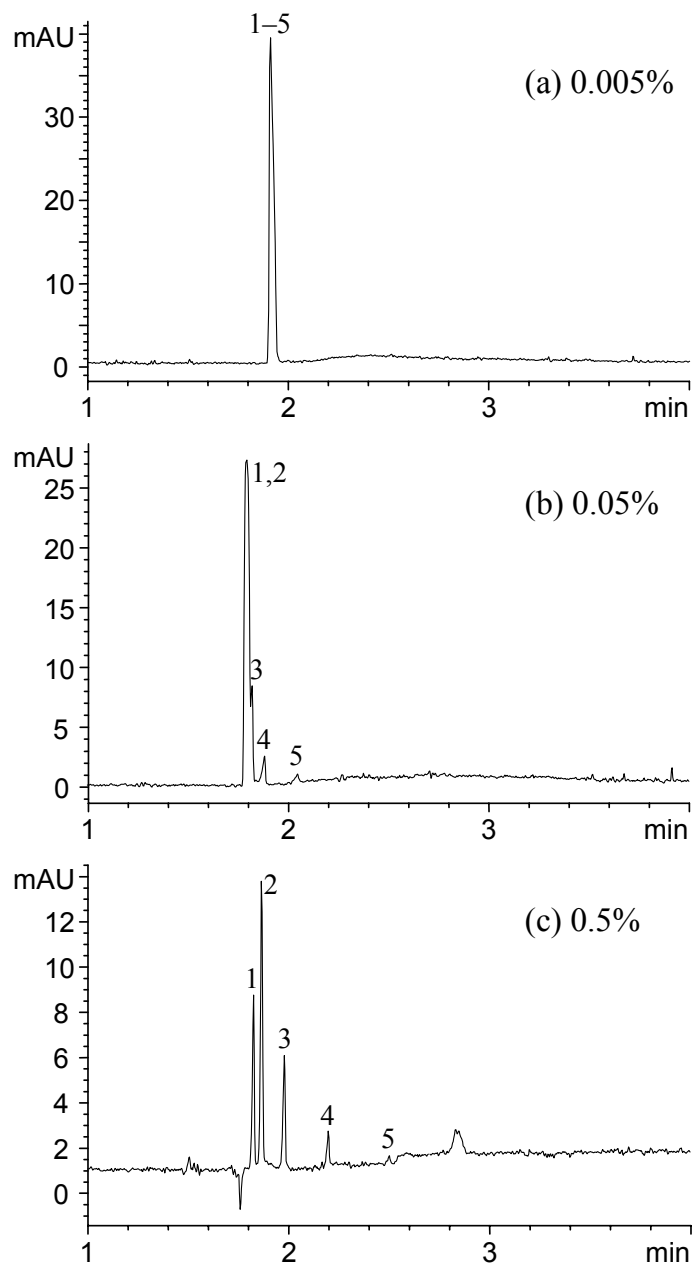
Figure 2-3. Effect of smectite concentration on the separation of PPE homologues: (a) 0.005%, (b) 0.05%, (c) 0.5%. Sample: PhG-2.0. Other conditions and peak identifications are as in Figure 2-1.

The behavior of the peak resolution of the PPE homologues was investigated with varying the smectite concentration and the results are shown in Figure 2-3.   When the concentration of the smectite was 0.005% (w/v), the single peak appeared at the migration time of the EOF marker (Figure 2-3a).   At the concentration of 0.05% (w/v), the peak of the higher ethoxylated homologues ($x \geq 3$) were observed (Figure 2-3b). With increasing the concentration of the smectite, the migration times of the homologues became progressively longer and the sufficient separation of the lower ethoxylated homologues was achieved at the concentration of 0.5% (w/v) as shown in Figure 2-3c.   Therefore, the retention factor could be adjusted by the concentration of the smectite as well as the conventional MEKC, which is effective for improving the resolution.

2-3-3   Effect of ionic strength and organic modifier

Since it has been reported that the addition of electrolytes reduced the swelling of smectite [36–38], the influence of the ionic strength on the separation was investigated by using NaCl as an electrolyte additive.   The separations of PhG-5.5 obtained with the NaCl concentrations of 0 and 7.5 mM are shown in Figure 2-4.   When the NaCl concentration was higher than 10 mM, the BGS became viscous owing to the aggregation of the smectite.   It should be noted that the viscosity of 0.1% smectite containing 5.0 mM NaCl was almost coincident with deionized water.   The migration time was increased with increasing the NaCl concentration since the EOF was suppressed with increasing the ionic strength.   However, the peak resolution and separation selectivity were not affected by the ionic strength, i.e., the degree of swelling of the smectite, below 7.5 mM.   Taking into account of the baseline stability, the NaCl

concentration of 5.0 mM was selected as the standard BGS composition.

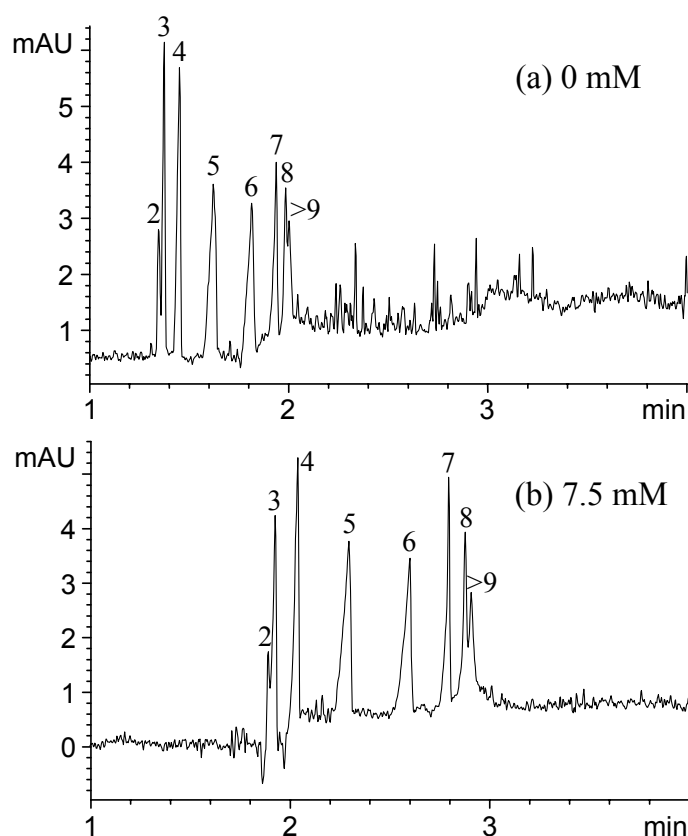
Figure 2-4. Effect of ionic strength on the separation of PPE homologues. Sample: PhG-5.5; BGS, 0.1% (w/v) smectite containing (a) 0 mM and (b) 7.5 mM NaCl (pH 10.3). Other conditions and peak identifications are as in Figure 2-1.

It has been reported that organic modifiers affect both the dispersibility and adsorptivity of the inorganic layered compounds [39]. Thus, the effect of organic

solvents on the separation was investigated. Organic modifiers, such as methanol and acetone, could be added to the BGS only up to 20% (v/v) with keeping the dispersibility of the smectite, which improved the plate numbers of the PPEs. It should be emphasized that the smectite aggregated and precipitated at the organic modifier concentrations of above 30% (v/v). Figure 2-5 shows the optimal separation of PhG-5.5 obtained with the BGS containing 10% (v/v) methanol. The migration times were increased due to slower EOF caused by the increase in the viscosity and the decrease in the dielectric constant of the BGS. However, a more effective separation was achieved in comparison with Figure 2-1c, especially for higher ethoxylated components. The plate numbers of the PPEs were improved from 17,000–99,000 to 47,000–227,000 by adding 10% (v/v) methanol. Therefore, organic modifiers were effective for improving the separation efficiencies in smectite–EKC.
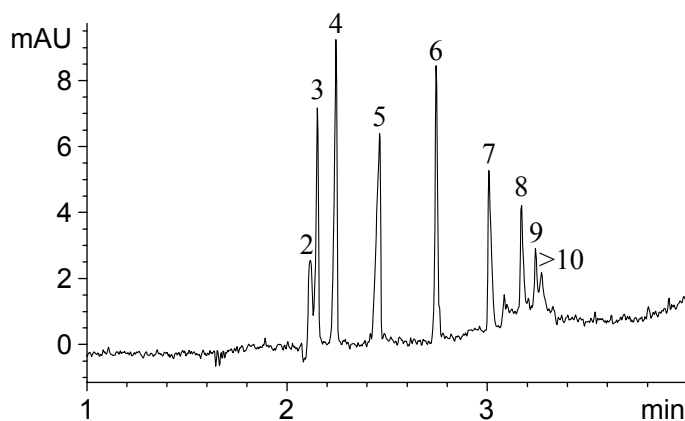

Figure 2-5. Optimal separation of PhG-5.5 with adding 10% (v/v) methanol in BGS. BGS, 0.1% (w/v) smectite, 5 mM NaCl (pH 10.3), 10% (v/v) methanol. Other conditions and peak identifications are as in Figure 2-1.

2-3-4 Reproducibility of smectite–EKC

The reproducibility in smectite–EKC was evaluated by using PhG-5.5 as test analytes. The run-to-run repeatability with the same and different BGS and the day-to-day reproducibility for the homologue peaks detected under the optimal separation condition are summarized in Table 2-1.

Table 2-1. RSDs for the smectite–EKC analysis of PhG-5.5 [a]

	%RSD		
	migration time	efficiency	peak area
run-to-run with same BGS	0.6	8.3	2.7
run-to-run with different BGS [b]	1.3	7.7	4.1
day-to-day with same BGS	1.7	7.5	3.7

[a] %RSDs ($n = 5$) were calculated from the mean values for PhG-5.5 homologues. Separation conditions are as in Figure 2-5.

[b] The BGS was prepared for each runs.

The reproducibility of the migration time was good with the relative standard deviation (RSD) values ranging from 0.6–1.7%. The RSDs for the plate numbers were acceptable with approximately 8% and the peak area reproducibility was in the range of 2.7–4.1%. It should be emphasized that the repeatabilities obtained with the different BGS prepared for each run were as good as those with the same BGS, which indicated

that the reproducible preparation of the smectite dispersion could be performed and the aggregation of the smectite was negligible under the present condition.   Therefore, the accurate and precise determination of the distribution of the EO groups of PPEs could be conducted in smectite–EKC.   It was confirmed that the average EO numbers (moles of EO groups/mol of sample) calculated from the weighted average of the corrected peak areas were 1.94 ± 0.08, 3.14 ± 0.08, and 5.48 ± 0.15 ($n = 5$) for PhG-2.0, PhG-3.0 and PhG-5.5, respectively.   These results clearly demonstrated the utility of the smectite as the PSP in the EKC analysis of nonionic compounds containing repeating EO groups.

2-3-5   Applications

The analysis of PPEs in commercially available laundry bleaches containing surfactants such as alcohol ethoxylate and other additives, e.g., bleach activator and chelating agents, was performed.   The samples were diluted to appropriate concentrations with water and analyzed without further purification except for filtration. Although a few peaks of other ingredients were detected, Figures 2-6a and 2-6b show the good similarity to the peak patterns of PhG-2.0 shown in Figure 2-3c and PhG-3.0 (data not shown), respectively, under the higher smectite concentration of 0.5%. Moreover, the average EO numbers of the detected PPEs determined from the weighted average of the corrected peak areas yielded 1.94 and 2.98 for the product-A and product-B, respectively.   Since the information about the EO distribution of PPEs in these products was not given by the manufactures, the validity of the component analysis could not be clarified.   However, the average EO numbers of the PPEs in these samples determined by the GC measurement with trimethylsylil derivatization

were 2.04 and 3.04 for the product-A and B, respectively.   Thus, the EO distribution obtained with smectite–EKC agreed with that with GC.   Considering the good quantitative ability of the developed method for PPEs as shown in the previous section, furthermore, the author believes that the present determination was almost validated. Thus, the developed method can be applied to the analysis of PPEs in real products.
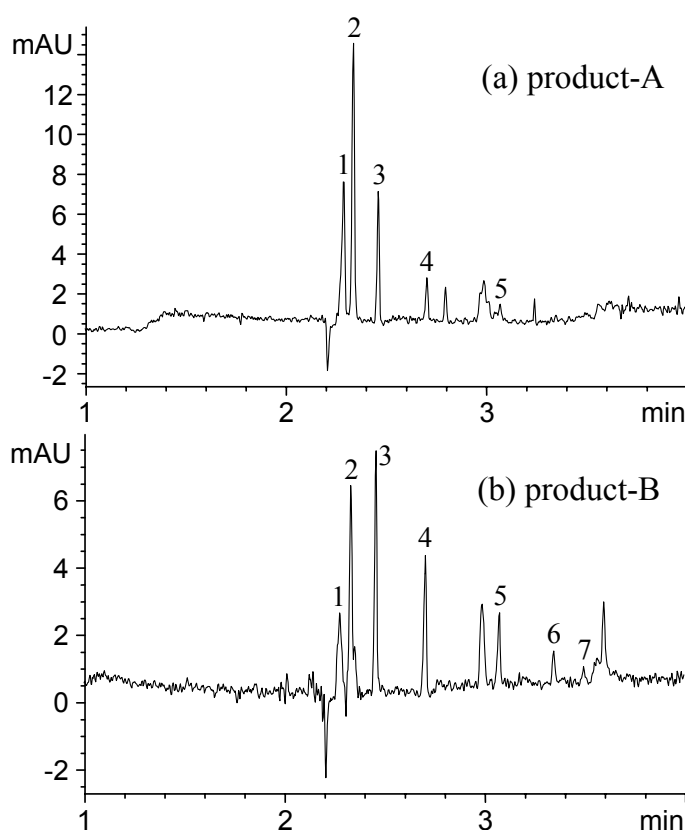

Figure 2-6. Typical EKC analyses of the commercially available laundry bleaches: (a) product-A (1:200), (b) product-B (1:100).   Value in the parenthesis represents a dilution ratio of the sample with water.   BGS, 0.5% (w/v) smectite, 5 mM NaCl (pH 10.3), 10% (v/v) methanol.   Other conditions and peak identifications are as in Figure 2-1.

2-3-6 On-line sample concentration by sweeping

The low concentration sensitivity is the main drawback in the EKC analysis employing UV detection. Thus, fundamental studies on on-line sample preconcentration by sweeping in smectite–EKC were conducted. The sweeping phenomenon in EKC, first reported by Quirino and Terabe [22], utilizes the interaction of the PSP in the BGS and sample solution that is free of the PSP. Theoretically, the length of the concentrated zone can be predicted by the following equation [22, 23],

$$l_{\text{sweep}} = l_{\text{inj}}\left(1/1 + k\right) \qquad\qquad (2\text{-}2)$$

where l_{inj}, l_{sweep} and k are the injection length, swept zone length and retention factor, respectively. Thus, the preconcentration efficiency for analytes with large retention factors should be high in the sweeping experiments. In the present study, the highly ethoxylated components ($x \geq 5$) in PhG-5.5 fractionated by preparative size exclusion chromatography were employed as test samples. Figure 2-7a shows the relationship between l_{inj} and l_{sweep}. As predicted by Eq. 2-2, l_{sweep} was decreased with increasing the retention factor. Since the theoretical straight lines accorded well with the experimental data points, on-line preconcentration of PPEs with the smectite was attained by the sweeping mechanism.

Figure 2-7b shows the separation obtained by conventional smectite–EKC with normal injection of 1 s (l_{inj}, 0.055 cm) for 500 ppm highly ethoxylated components ($x \geq 5$) in PhG-5.5. The PPEs with x of 5–9 were clearly separated. Under the sweeping condition with the injection time of 400 s (l_{inj}, 22.0 cm), an 100-fold diluted sample could be detected with almost same peak heights obtained with the normal

smectite–EKC analysis except for the PPE with x of 6 as shown in Figure 2-7c. The broader peaks for the PPEs with x of 6 and 7 were according to the low retention factor as demonstrated in Figure 2-7a. To evaluate the concentration efficiency of sweeping in smectite–EKC, the value of sensitivity enhancement factor (SEF_{height}) was calculated by comparing the peak height obtained in the sweeping condition with that in conventional EKC taking into account the dilution factor regardless of the injection volume of the sample solution [26]. As a result, the best SEF_{height} of 125 was obtained for the PPE with x of 8. These results clearly demonstrated that the smectite is useful as the sweeping carrier for enhancing the detectability. Although the highly ethoxylated PPEs were selected as the analytes in the present sweeping experiments, the application of higher concentration smectite will provide an effective concentration of the PPEs with a low number of the EO groups.

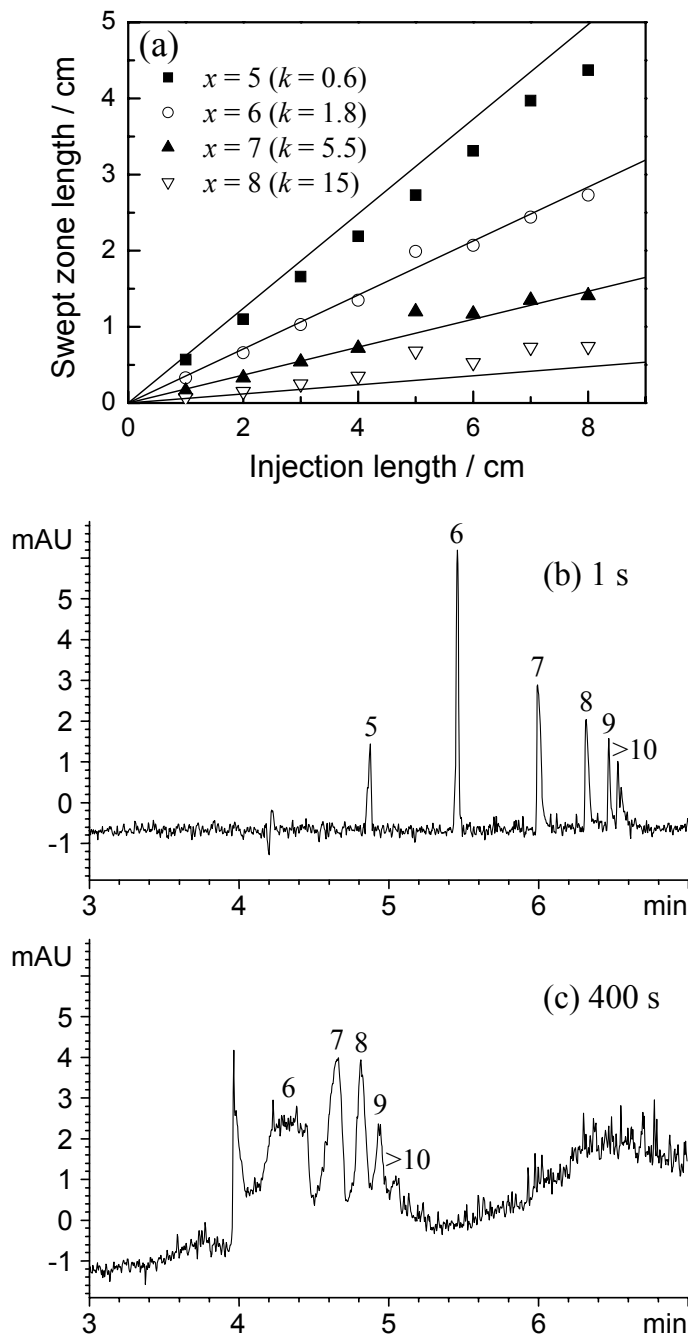**Figure 2-7.** Sweeping EKC analysis of PPE homologues: (a) Injection length versus swept zone length plot, separations with (b) conventional smectite–EKC and (c) sweeping. Capillary, 72 cm effective length, 80.5 cm total length; applied voltage, 30 kV; injection: (a) 1 s (0.055 cm), (b) 400 s (22 cm); sample concentration: (a) 500 μg/mL, (b) 5 μg/mL. Other conditions and peak identifications are as in Figure 2-5.

2-4 Conclusions

The application of the smectite as the PSP in EKC allowed the separation of nonionic compounds having repeating EO groups.   It was confirmed that the retention by the smectite depended on the number of the EO groups and the sufficient separation of the homologues was achieved with the plate numbers in the range of 47,000–227,000 under the optimal condition.   The proposed method showed satisfactory reproducibility of the migration time, separation efficiency and peak area.   Furthermore, the identification of the PPE homologues in household products was also possible by comparison with the peak patterns of the sample with known composition and thus the average EO number was accurately calculated with their peak areas.   Under the sweeping condition, SEF_{height} of 125 for the PPE with high retention factor was achieved in smectite–EKC.   Although data were not shown in this paper, similar results were obtained in terms of the selectivity, separation efficiency and sensitivity enhancement by using other commercially available synthetic clay minerals.   It is well known that the clay minerals interact with various polar compounds, including alcohols and amines.   Therefore, these compounds can be separated by using the inorganic layered compounds as PSPs, which will expand the application range of EKC.   In the present stage, the retention and separation characteristics of the smectite have not been fully clarified, so that further investigations, e.g., effects of structural factors and adsorptivity of the smectite, should be needed to improve the analytical performance in smectite–EKC.

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Chapter 3

Simultaneous Determination of Amphoteric Surfactants in Detergents by Capillary Electrophoresis with Indirect UV Detection

3-1   Introduction

Surfactants are widely used as detergents, solubilizers and foaming agents.   They are usually classified into anionic, cationic, nonionic and amphoteric surfactants according to their ionic properties.   Among these surfactants, amphoteric surfactants have good foaming property and high detergency especially in the presence of anionic surfactants. Additionally, they also show good surface-active functions over a wide range of pH, and are less irritating to skin and eyes compared to other surfactants.   These characteristics of the amphoteric surfactants are very suitable for household products and cosmetics such as hand dishwashing detergents, household cleaners, facial wash and shampoos [1,2], so that the amphoterics are the most growing group of surfactants in detergent industry and various amphoteric surfactants are now commercially available.   In particular, alkyldimethylamine *N*-oxide (AO), alkylamidopropylamine *N*-oxide (APAO), alkylbetaine (Bt) and alkylamidopropylbetaine (APB) are major amphoteric surfactants (Figure 3-1).   Their hydrophobic group is made up of a linear alkyl chain with 8–18 carbon atoms.   Since they have different surface-active properties, understandably, it is important to develop effective techniques for the separation and determination of these amphoterics especially in application products for quality control.

Household products and cosmetics consist of many different surfactants, i.e.,

amphoteric, anionic and nonionic surfactants that are usually mixtures of their homologues, which consequently cause a difficulty in the analysis of amphoteric compounds contents in real products.   Although many investigations have been reported on the analysis of various surfactants, few studies have been published on that of amphoteric compounds.   In past two decades, several research groups studied the separation of amphoteric surfactants by high performance liquid chromatography (HPLC) [3–6].   Although reversed-phase and ion-exchange chromatography have been the most popular techniques, it was difficult to separate anionic, nonionic surfactants, and other additives in single run.   Recently, Haefliger has reported an excellent separation of complex surfactant mixtures by using the two-dimensional HPLC technique [7].   Though a simultaneous analysis of all four classes of surfactants, i.e., cationic, amphoteric, nonionic and anionic, was succeeded, a drawback is that three HPLC pumps are required as mentioned by the author.   In addition, the application of this technique to the real products was not unfortunately described.

On the other hand, capillary electrophoresis (CE) which is one of the powerful separation techniques for surfactants have been applied to the analysis of many household and industrial products [8–18].   Since most amphoteric compounds have positive charge at lower pH, it is expected to be easily separated from other ionic surfactants without any complicated sample pretreatment procedures for these products. To our knowledge, however, very few studies have been reported on applying CE to the analysis of the amphoteric surfactants [19–22].   Furthermore, the separation ability for their homologues, that is the applicability for detailed analysis of alkyl chain distribution for the amphoterics, has not been investigated in CE.   Recently, Shamsi and co-workers has reported the successful electrochromatographic separation of

zwitterionic and nonionic surfactants but the fabrication of the packed capillaries seems to be difficult [21,22]. Thus, the introduction of a simple CE analysis method for amphoteric surfactants is still needed.

The purpose of this study was to develop a simultaneous separation method for AO, APAO, Bt, APB and their C_8–C_{18} homologues on the basis of the CE technique. Since AO and Bt exhibit no or weak UV absorption, indirect UV detection was applied. In the CE–indirect UV analysis, it is well known that peak shapes and sensitivities depend on the relative mobilities of the chromophores and analytes. When they have the same mobility, a symmetrical and highest peak is obtained; "mobility matching" [23,24]. For indirect UV detection of surfactants which have no chromophoric group, various UV absorbing compounds have been employed [9,24,25], and several authors have reported that alkylbenzyldimethylammonium compounds provided the best separation and sensitivity for alkyl quaternary ammonium compounds [15,18]. In this study, therefore, alkylbenzylammonium chloride compounds were selected as chromophores. The author optimized the analytical conditions for the determination of the amphoteric surfactants by the CE–indirect UV analysis, and validated the developed method. Furthermore, the analytical method was applied to the analysis of the amphoteric compounds in commercial products and effects of sample matrix and other additives on the analytical performance were also evaluated.

3-2　Experimental Section

3-2-1　Chemicals

Benzyltrimethylammonium chloride (BTMAC), benzyltripropylammonium chloride (BTPAC), phosphoric acid, hydrogen peroxide and *N,N*-dimethyl-1,3-propanediamine (DMAPA) were purchased from Wako (Osaka, Japan), decylbenzyldimethylammonium chloride (DBDMAC) from Fluka (Buchs, Switzerland). BTMAC, BTPAC and DBDMAC were used as chromophores without any further purification. Lauryltrimethylammonium chloride (LTMAC) was from Tokyo Chemical Industry (Tokyo, Japan), tetrahydrofuran (THF), methanol, acetone, acetonitrile and sodium chloroacetate (SMCA) from Kanto (Tokyo, Japan). Water was purified with a Milli-Q purification system from Millipore (Bedford, MA, USA). All reagents were of HPLC or analytical grade.

Structures of sample amphoteric surfactants are shown in Figure 3-1. Lauryldimethylamine *N*-oxide (C_{12}-AO), laurylbetaine (C_{12}-Bt), laurylamidopropylbetaine (C_{12}-APB) and cocoylbetaine (coco-Bt) were supplied by KAO (Tokyo, Japan), laurylamidopropylamine *N*-oxide (C_{12}-APAO) from Kawaken (Tokyo, Japan), cocoyldimethylamine *N*-oxide (coco-AO) from TOHO (Tokyo, Japan). Coco-AO and coco-Bt are industrial mixtures of C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}-AO (C_8–C_{18}-AO) and C_8–C_{18}-Bt, respectively, which have the alkyl chain length distribution of coconut fatty acid. These surfactants were obtained as 25–35% (w/w) aqueous solutions. *N,N*-Dimethylalkylamines (C_8–C_{18}) and fatty acid (C_8–C_{18}) were also supplied by KAO.

$$\begin{array}{c} CH_3 \\ | \\ R-N \longrightarrow O \\ | \\ CH_3 \end{array}$$

$$\begin{array}{c} CH_3 \\ | \\ R-CONH-CH_2CH_2CH_2-N \longrightarrow O \\ | \\ CH_3 \end{array}$$

(R : C_8–C_{18})

(R : C_7–C_{17})

C_8–C_{18}-AO

C_8–C_{18}-APAO

$$\begin{array}{c} CH_3 \\ | \\ R-\overset{+}{N}-CH_2COO^- \\ | \\ CH_3 \end{array}$$

$$\begin{array}{c} CH_3 \\ | \\ R-CONH-CH_2CH_2CH_2-\overset{+}{N}-CH_2COO^- \\ | \\ CH_3 \end{array}$$

(R : C_8–C_{18})

(R : C_7–C_{17})
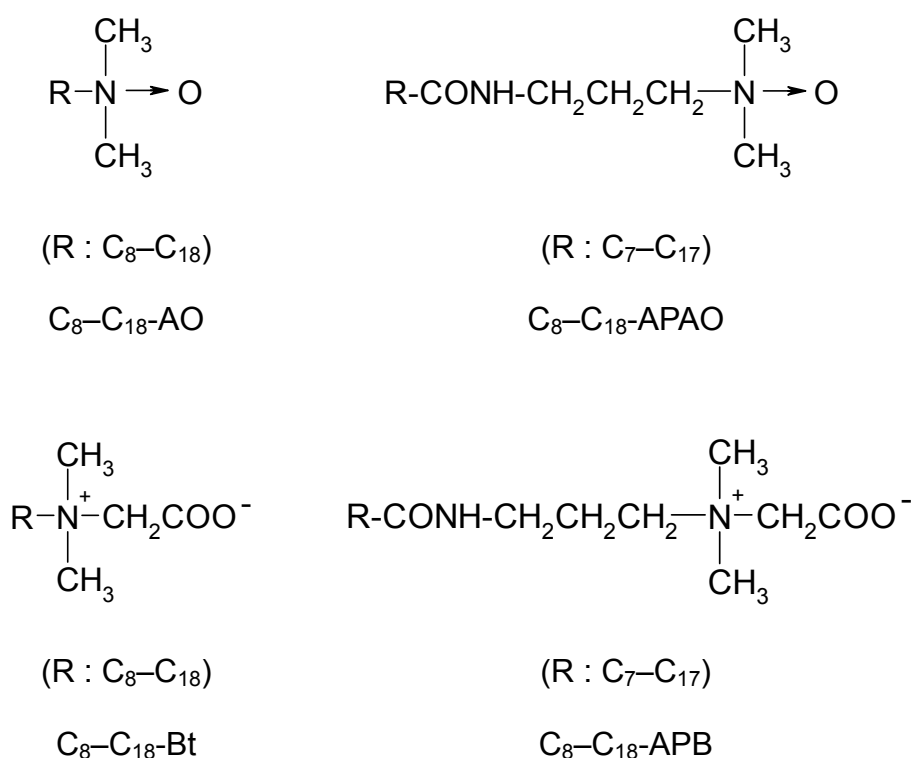
C_8–C_{18}-Bt

C_8–C_{18}-APB

Figure 3-1. Chemical structures of the AO, APAO, Bt and APB homologues used in this study.

3-2-2 *Preparation of amphoteric surfactant sample*

Amphoteric surfactants with alkyl chain length of C_8–C_{18} were prepared by the following procedures.   AO homologues were synthesized by oxidation of *N,N*-dimethylalkylamines with H_2O_2 at 80 °C for 5 h [26].   APAO homologues were also prepared by the same procedure using alkylamidodimethylpropylamines synthesized from C_8–C_{18}-fatty acids and DMAPA [27].   APB and Bt homologues were synthesized by quaternization of alkylamidodimethylpropylamines and *N,N*-dimethylalkylamines, respectively, with SMCA at 80 °C for 10 h [27].   These

syntheses were performed in water or water/ethanol. Extreme caution should be exercised to avoid an explosion when working with a heated solution of hydrogen peroxide. The conversions of these reactions were confirmed by ^{1}H NMR spectra recorded on a Varian MERCURY-400 spectrometer (Palo Alto, CA, USA), and all their conversions were over 98%.

For the sample preparation, the standard mixture of the amphoteric surfactants (C_8–C_{18}-AO, C_8–C_{18}-APAO, C_8–C_{18}-Bt and C_8–C_{18}-APB; 5%, w/w, concentration of each homologues), hand dishwashing detergents, shampoos and household cleaners were diluted to appropriate concentrations with methanol. All sample solutions were filtrated with a 0.45 μm pore size membrane filter (Gelman Sciences, Ann Arbor, MI, USA) prior to analysis.

3-2-3   CE measurements

All CE experiments were performed on a Hewlett-Packard [3D]CE system (Waldbronn, Germany) equipped with a diode array UV detector. Separations were carried out on fused-silica capillaries (Agilent Technologies, Palo Alto) of 56 cm effective length × 50 μm I.D. Samples were hydrodynamically injected at 50 mbar for 2 s. The applied voltage and the temperature were set at 25 kV and 25 °C, respectively. Indirect UV detection was performed with signal wavelength of 350 nm and reference one of 214 nm. The capillaries were flushed successively with 0.1 M NaOH for 2 min, water for 2 min, 0.1 M HCl for 2 min, water for 2 min, methanol for 2 min and background solution (BGS) for 4 min between each separation. Unless otherwise noted, the pH of BGS was adjusted to desired values with 85% H_3PO_4 before adding organic modifiers.

3-3 Results and Discussion

3-3-1 Effect of pH

Generally, ionic compounds are separated based on the charge and size in the CE analysis. Unlike other ionic surfactants, the charge of the amphoteric compounds is strongly affected by the pH value, so that the dependence of the electrophoretic mobilities (μ_{ep}) of the analytes on pH was investigated. Figure 3-2 shows the dependence of μ_{ep} on pH for C_{12}-AO, C_{12}-APAO, C_{12}-Bt, C_{12}-APB and LTMAC estimated from the migration time of the analytes and neutral marker methanol with direct or indirect UV detection. A typical cationic surfactant, LTMAC, was used as a reference sample for the amphoteric compounds. Since AO has no absorption in the UV region, the evaluation of μ_{ep} was carried out with indirect UV detection by using BTMAC as a chromophore. To prevent the adsorption of the analytes onto the inner surface of the capillary and the formation of micelles, 50% (v/v) acetonitrile was added to the BGS. As shown in Figure 3-2, μ_{ep} of the amphoteric compounds increased with decreasing pH, while that of LTMAC showed no change under acidic and neutral conditions. The observed mobility changes of C_{12}-AO and C_{12}-APAO is due to the protonation of amine N-oxide group which gives the cationic species in an equilibrated way ($-N=O \leftrightarrow -N-OH^+$) characterized by the proton dissociation constant of ~5.0 [28]. On the other hand, C_{12}-Bt and C_{12}-APB provide positively charged species below pH 4 according to the protonation of carboxyl groups. Apparently, the largest difference of μ_{ep} of the four amphoteric surfactants was obtained around pH 2.
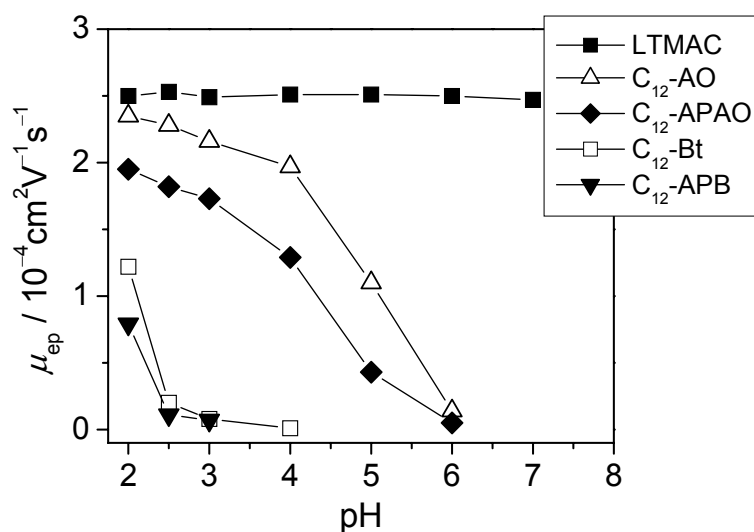
Figure 3-2. Dependence of μ_{ep} on pH of C_{12}-AO, C_{12}-APAO, C_{12}-Bt, C_{12}-APB and LTMAC. Capillary, 56.0 cm effective length, 64.5 cm total length, 50 μm I.D.; BGS, 20 mM phosphoric acid (pH 2.0) or 20 mM phosphate buffer (pH 2.5–7.0) containing 50% (v/v) acetonitrile and 5 mM BTMAC; applied voltage, +25 kV; injection, 2 s at 50 mbar; temperature, 25 °C; direct UV detection, 200 nm; indirect UV detection, signal, 350 nm, reference, 214 nm.

Figure 3-3 shows the CE–indirect UV analysis of the amphoteric surfactants around pH 2. Regardless of the pH value, all the surfactants were detected at the cathodic end and the migration order was first AO, followed by APAO, then Bt, finally APB homologues, which agrees well with that of μ_{ep} shown in Figure 3-2. Since μ_{ep} decreases with increasing the molecular size, the amphoteric homologues migrated in order of the increase in the alkyl chain length. By changing pH unit of only 0.2, the migration time and peak resolution was dramatically changed. At pH 1.8, the increases in μ_{ep} of the Bt homologues brought the reversal migration order of C_{18}-APAO and C_8-Bt compared to that at pH 2.0. In addition, the resolution among AO and

APAO homologues decreased as indicated by incomplete separation. On the other hand, the resolutions of the APB homologues were reduced at higher pH 2.2 due to their lower μ_{ep} and the resulting longer analysis time. Therefore, the optimal pH value of the BGS for the separation of the amphoteric analytes was determined to be 2.0.
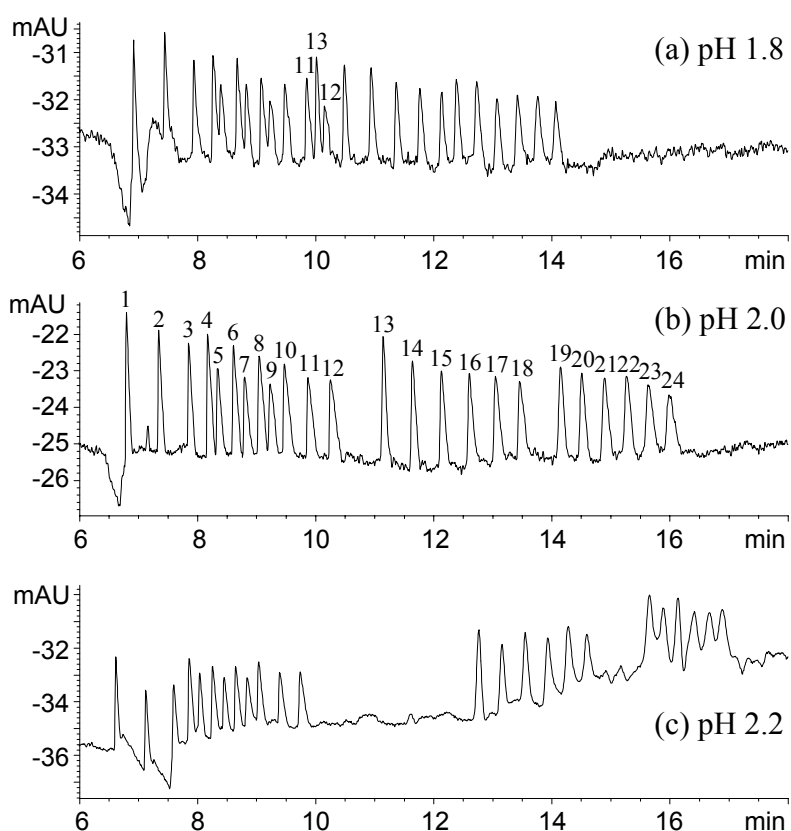

Figure 3-3. CE separation of the amphoteric surfactants at pH (a) 1.8, (b) 2.0 and (c) 2.2. Sample concentration: C_8–C_{18}-AO, 75 μg/mL; C_8–C_{18}-APAO, 100 μg/mL; C_8–C_{18}-Bt, 100 μg/mL; C_8–C_{18}-APB, 150 μg/mL; BGS, phosphoric acid (pH 1.8–2.2) containing 50% (v/v) acetonitrile and 5 mM BTMAC. Peak identification: 1, C_8-AO; 2, C_{10}-AO; 3, C_{12}-AO; 4, C_8-APAO; 5, C_{14}-AO; 6, C_{10}-APAO; 7, C_{16}-AO; 8, C_{12}-APAO; 9, C_{18}-AO; 10, C_{14}-APAO; 11, C_{16}-APAO; 12, C_{18}-APAO; 13, C_8-Bt; 14, C_{10}-Bt; 15, C_{12}-Bt; 16, C_{14}-Bt; 17, C_{16}-Bt; 18, C_{18}-Bt; 19, C_8-APB; 20, C_{10}-APB; 21, C_{12}-APB; 22, C_{14}-APB; 23, C_{16}-APB; 24, C_{18}-APB. Other conditions are as in Figure 3-2.
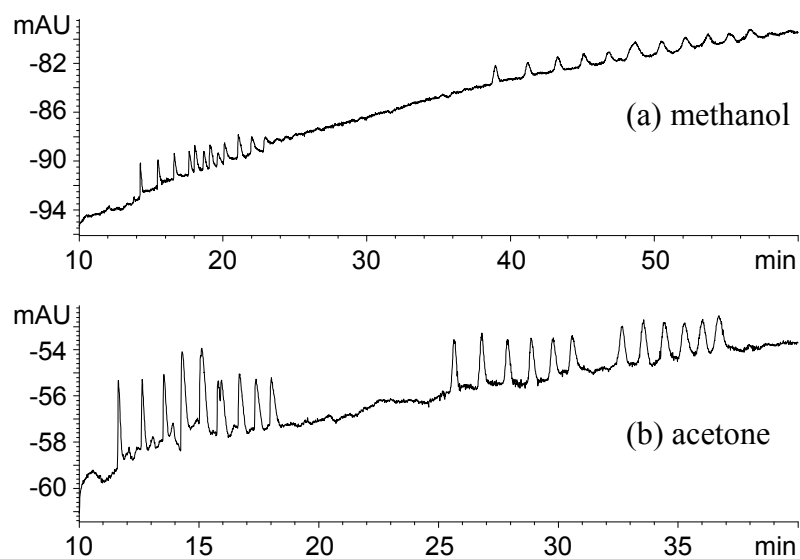
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Figure 3-4. Effect of organic modifiers on the separation of the amphoteric surfactants. BGS, 20 mM phosphoric acid (pH 2.0) containing 5 mM BTMAC and 50% (v/v): (a) methanol and (b) acetone. Other conditions are as in Figure 3-3.
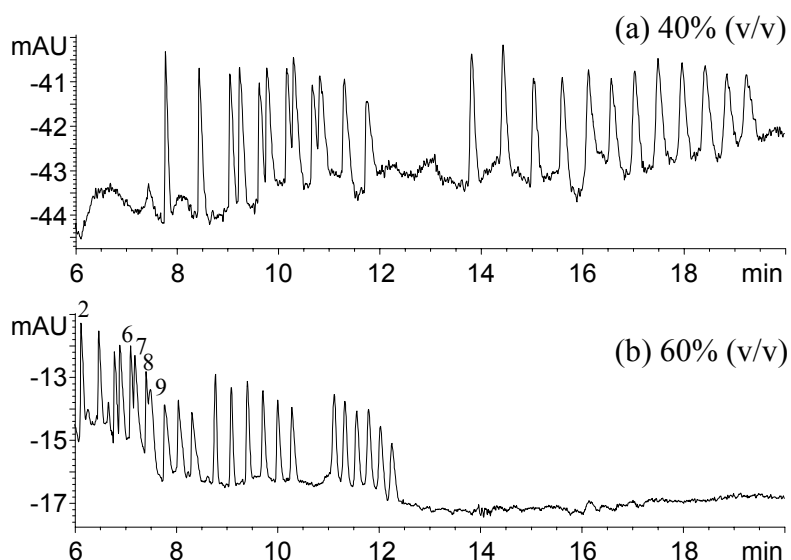

Figure 3-5. Effect of acetonitrile concentration on the CE separation of the amphoterics: (a) 40% and (b) 60%. Other conditions are as in Figure 3-3b.

3-3-2 Effect of organic modifiers

It has been reported that the addition of organic modifiers such as acetonitrile and methanol to the BGS is useful to improve the resolution of cationic surfactants in CE [14,15,29–31].   In this study, THF, methanol, acetone and acetonitrile were selected as organic modifiers.   Figure 3-4 shows the effect of the addition of methanol and acetone on the CE analysis of the amphoteric surfactants at pH 2.0.   When THF (data not shown) and methanol were added to the BGS, time required for the detection of all the analytes was 90 and 60 min, respectively.   This slower migration was mainly due to relatively higher viscosity and low dielectric constant of these modifiers [32,33].   Apparently, the addition of acetone (Figure 3-4b) and acetonitrile (Figure 3-3b) was superior to THF and methanol for the shorter analysis time.   In particular, the complete separation of the 24 analytes was achieved within 17 min in the addition of acetonitrile.   Acetonitrile has the lower viscosity and highest polarity among the four organic modifiers used in this study, which would provide the fastest separation of the amphoteric compounds.

The effect of the acetonitrile concentration was also investigated and the results are shown in Figure 3-5.   When the concentration of acetonitrile was below 30% (v/v), a considerable peak broadening was observed due to the formation of micelles and/or the adsorption onto the inner surface of the capillaries, so that it was necessary to add 40–60% (v/v) acetonitrile for the effective separation.   At the concentration of 40% (v/v), however, the peaks of C_{16}-AO and C_{18}-AO were overlapped with those of C_{12}-APAO and C_{14}-APAO, respectively (Figure 3-5a).   Although the addition of 60% (v/v) acetonitrile provided the faster migration, C_{10}-APAO and C_{12}-APAO were barely resolved from C_{16}-AOand C_{18}-AO, respectively.   Eventually, the baseline separation of

the 24 amphoteric compounds was achieved only at the concentration of 50% (v/v) shown in Figure 3-3b, which is considered to be the optimum acetonitrile concentration.

3-3-3 Effect of chromophores

As mentioned in Section 3-1, alkylbenzylammonium chloride, DBDMAC, BTPAC and BTMAC, were chosen as chromophores.   Prior to the CE–indirect UV analysis of the amphoteric compounds, μ_{ep} of DBDMAC, BTPAC and BTMAC was estimated to be 2.41, 2.83 and 3.80 $\times$ 10^{-4} cm^2 V^{-1} s^{-1}, respectively, whereas the mobility of the amphoteric compounds was ranging from 0.76 to 2.80 $\times$ 10^{-4} cm^2 V^{-1} s^{-1}.   The result indicates that DBDMAC is expected to provide better peak height and shape, especially for the faster migrating species such as the AO homologues, with indirect UV detection.

Figure 3-6 shows the electropherograms of the amphoteric surfactant analytes obtained with DBDMAC and BTPAC.   Although the higher peaks with the better shape were expected to be obtained due to the better mobility matching, the apparent improvement was not observed compared to BTMAC (Figure 3-3b).   In addition, the baseline was disturbed by a system peak [34,35] appeared in the region of the APAO homologues peaks, which caused the lower resolution of the AO and APAO surfactants. As for BTMAC, on the other hand, the reduction of the peak height was concerned according to the mobility mismatching with the analytes.   Actually, 20–40% decreases in the peak height were observed for the fast-migrating species, AO and APAO, compared to DBDMAC, whereas the peak height for the Bt and APB homologues was enhanced, less than 15%.   The improvement of detectability could not be explained by the "mobility matching", so that a specific interaction between the analytes and the chromophores, e.g., hydrophobic interaction, might affect the peak shape and height.

Therefore, the better determination of the amphoterics was accomplished by using BTMAC and it was chosen as the chromophore in the remaining experiments.
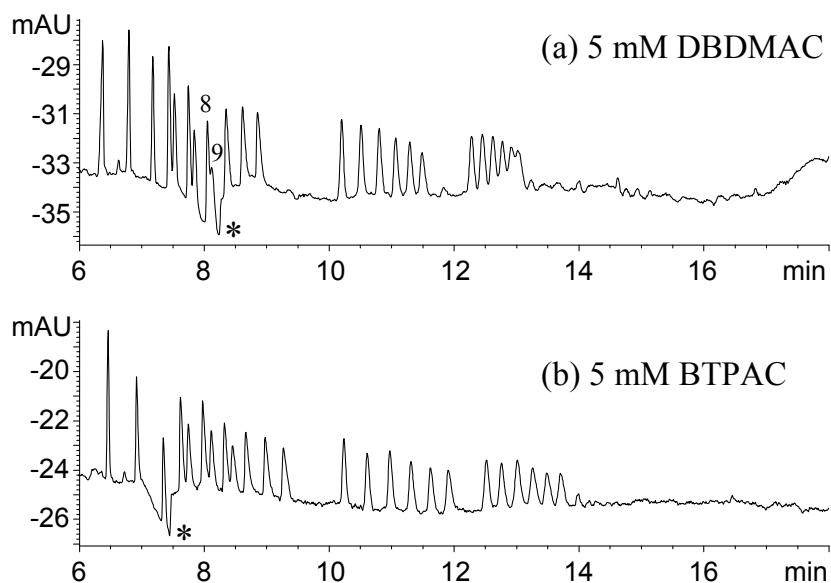

Figure 3-6. Electropherograms of the amphoteric surfactants by using several alkylbenzylammonium compounds as chromophores for indirect UV detection: 5 mM (a) DBDMAC and (b) BTPAC. Asterisk indicates a system peak. Other conditions are as in Figure 3-3b.

3-3-4 Method validation

To validate the developed method, the reproducibility, sensitivity and quantitation were evaluated by using the amphoteric surfactant standards and the results for the C_8 and C_{18} homologues are shown in Table 3-1. The run-to-run repeatability of the migration time was good with the relative standard deviation (RSD) values ranging

from 0.20 to 0.23% (n = 5).   The day-to-day RSD values were acceptable, less than

2.0% (n = 4).   It should be noted that the RSD values for the peak area were almost

comparable with those obtained with the internal standard, which might be according to

the reproducible sample injection and the suppressed surface adsorption of the analytes

and chromophores under the optimal condition.   The limit of detection (LOD) values,

defined as S/N = 3, were in the range of 10–50 μg/mL.   The limit of quantitation

(LOQ) values for the AO, APAO, Bt and APB homologues were estimated to be 50–75,

50–75, 75–100 and 100–150 μg/mL, respectively.   These LOQ values are adequate for

the determination of the amphoterics in real products such as detergents, shampoos and

cosmetics.   The linear range of the peak area was determined to be 50–500 μg/mL for

the AO and APAO homologues, 75–1000 μg/mL for Bt homologues and 100–1000

μg/mL for APB homologues.   The squares of the correlation coefficients (R^2) were

better than 0.9984 for all the analytes.   Since the R^2 values for the Bt and APB

homologues obtained with a conventional HPLC measurement were reported to be

0.9998 and 0.9997, respectively [5], the almost equivalent linearity was achieved by the

developed method.   In this analytical method, therefore, sufficient reproducibility,

sensitivity and quantitativity for the analysis of the amphoteric surfactants were

obtained.   Furthermore, the sufficient separation efficiency was achieved with the

theoretical plate numbers of 49,000–151,000 under the optimum condition, so that the

precise determination of the distribution of alkyl chain length of the amphoterics can be

also conducted.

Comparison with the CE–direct UV analysis was also investigated under the same

condition for indirect UV detection.   For direct UV detection, 5 mM NaCl was added

to the BGS instead of BTMAC to be an equivalent ionic strength with that of the BGS

for indirect UV detection, and the detection was performed at 200 nm.   As a result, the

AO and Bt homologues were not detected and their LOD values were higher than 1000

μg/mL although the separation efficiency and the sensitivity for the APAO and APB

homologues were comparable with indirect UV detection (data not shown).   The result

demonstrates that indirect UV detection is suitable for the determination of the

amphoteric surfactants used in this study.

Table 3-1.   Reproducibility, separation efficiency (N), square of correlation coefficient
(R^2), LOD and LOQ for CE analysis of amphoteric surfactants [a]

Surfactant	%RSD for t_R		N	R^2	LOD [d] (μg/mL)	LOQ (μg/mL)
	run-to-run [b]	day-to-day [c]				
C_8-AO	0.23	1.2	101,000	0.9998	10	50
C_{18}-AO	0.21	1.5	74,000	0.9997	20	75
C_8-APAO	0.23	1.3	88,000	0.9999	20	50
C_{18}-APAO	0.20	1.5	49,000	0.9995	20	75
C_8-Bt	0.20	1.7	151,000	0.9992	20	75
C_{18}-Bt	0.21	1.9	99,000	0.9999	50	100
C_8-APB	0.21	1.8	122,000	0.9999	50	100
C_{18}-APB	0.22	2.0	73,000	0.9986	50	150

[a] Separation conditions are as in Figure 3-3b.

[b] $n = 5$.

[c] $n = 4$.

[d] S/N = 3.

3-3-5 Applications

To demonstrate the utility of the developed method, the determination of the amphoteric surfactants in consumer products containing anionic surfactants (e.g., sodium alkylether sulfate), nonionic surfactants (e.g., alcohol ethoxylate) and other additives (e.g., hydrotropes) was performed. The accuracy and precision were tested using commercially available and chemically defined hand dishwashing detergents and shampoo (the data are supplied from the manufacturer). Figure 3-7 shows the electropherograms of the detergent samples containing known amount of the amphoterics. It should be noted that only the amphoterics peaks were detected and no peak corresponding matrix and other surfactants was observed in the developed method. In this analytical condition, the positively charged amphoteric surfactants migrate toward the cathodic end but anionic and nonionic components cannot migrate toward the detector under the very slower electroosmotic flow condition. As a result, the peaks originating only from the amphoteric surfactants were observed though all the products consist of various raw materials. The quantitative determinations of the amphoteric surfactants in these products are summarized in Table 3-2. The content values of the amphoterics in the products were found to be from 98 to 102% of the expected values. In addition, the alkyl chain distribution of coco-AO (C_8, 8%; C_{10}, 5%; C_{12}, 49%; C_{14}, 22%; C_{16}, 8%; C_{18}, 9%) and coco-Bt (C_8, 5%; C_{10}, 5%; C_{12}, 50%; C_{14}, 19%; C_{16}, 11%; C_{18}, 9%) determined from their peak areas corresponded to that of a typical coconut fatty acid (C_8, ~8%; C_{10}, ~7%; C_{12}, ~50%; C_{14}, ~18%; C_{16}, ~9%; C_{18}, ~8%). It was also found that the migration time of the amphoteric surfactants in real samples agreed with that of the standard amphoterics in spite of the presence of other ionic and nonionic surfactants. Consequently, the detected amphoterics could be

identified by matching their migration time with that of the corresponding standard surfactants. Thus, these results clearly demonstrated that the developed method can provide the high performance and selective analysis of the amphoteric surfactants in real products.
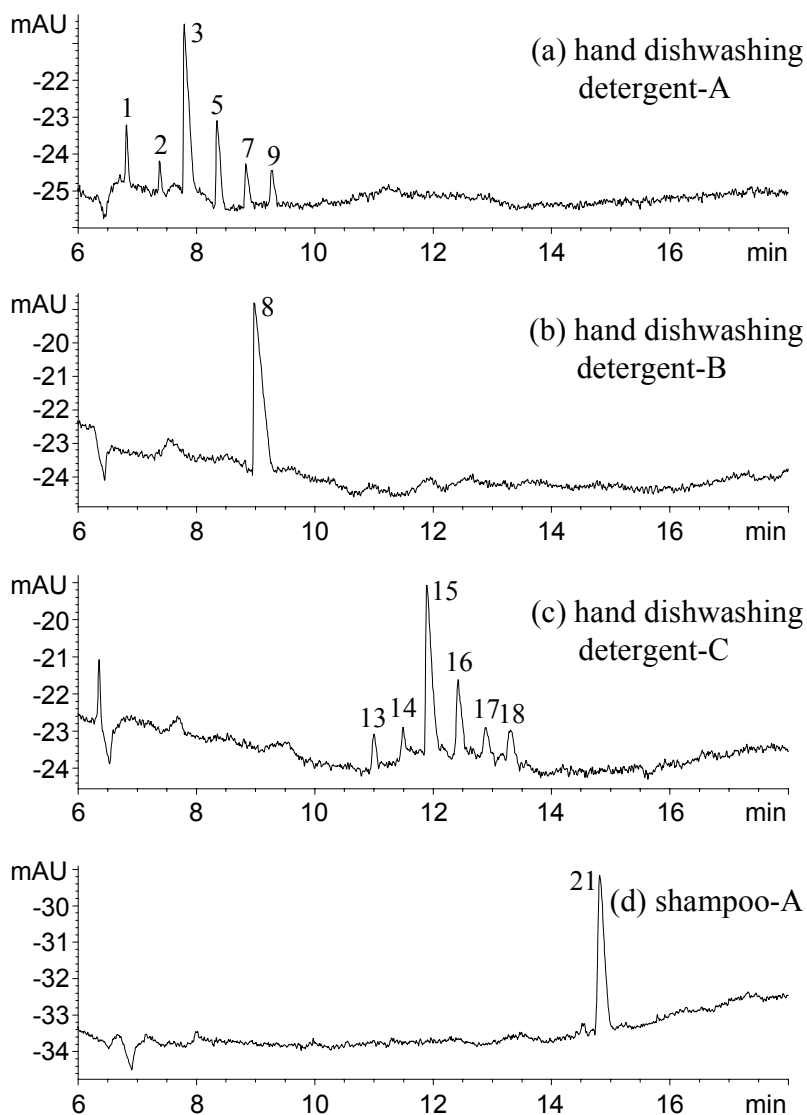

Figure 3-7. CE analysis of the chemically defined detergents: (a) hand dishwashing detergent-A (1:300), (b) hand dishwashing detergent-B (1:250), (c) hand dishwashing detergent-C (1:100) and (d) shampoo-A (1:150). Value in the parenthesis shows a dilution ratio of the sample with methanol. Separation was conducted at pH 2.0 and other conditions are as in Figure 3-3.

Table 3-2. Determination of amphoteric surfactants in commercially available and chemically defined detergents [a]

Sample	Surfactant	Content (%)		RSD [b] (%)	Recovery (%)
		Expected	Found		
Hand dishwashing detergent-A	coco-AO	7.0	6.8	3.1	98
Hand dishwashing detergent-B	C_{12}-APAO	7.0	6.9	3.7	99
Hand dishwashing detergent-C	coco-Bt	5.0	5.1	1.0	102
Shampoo-A	C_{12}-APB	5.0	5.0	1.1	99

[a] Estimated from the CE analysis shown in Figure 3-7.

[b] $n = 5$.

Figure 3-8 shows the typical electropherograms of chemically undefined commercial products. Amphoteric surfactants contained in these products were clearly identified as C_{12}-AO and C_{14}-AO with a trace amount of C_{16}-AO in hand dishwashing detergent-D (Figure 3-8a), C_{12}- and C_{14}-APAO in hand dishwashing detergent-E (Figure 3-8b), or C_{14}-Bt and C_{12}-APB in household cleaner-A (Figure 3-8c). Table 3-3 summarizes the content of the amphoterics in these products determined by using the calibration curves of the standards. The result indicates that the hand dishwashing detergent-B and E have the closely resembled amphoterics contents. Since the information about the content of the amphoteric surfactants and their alkyl chain length distribution were not

given by the manufactures, the validity of this analysis could not be clarified.   Taking

into account of the good recovery of the amphoterics in the chemically defined

detergents shown in Table 3-2, however, the author believes that this determination

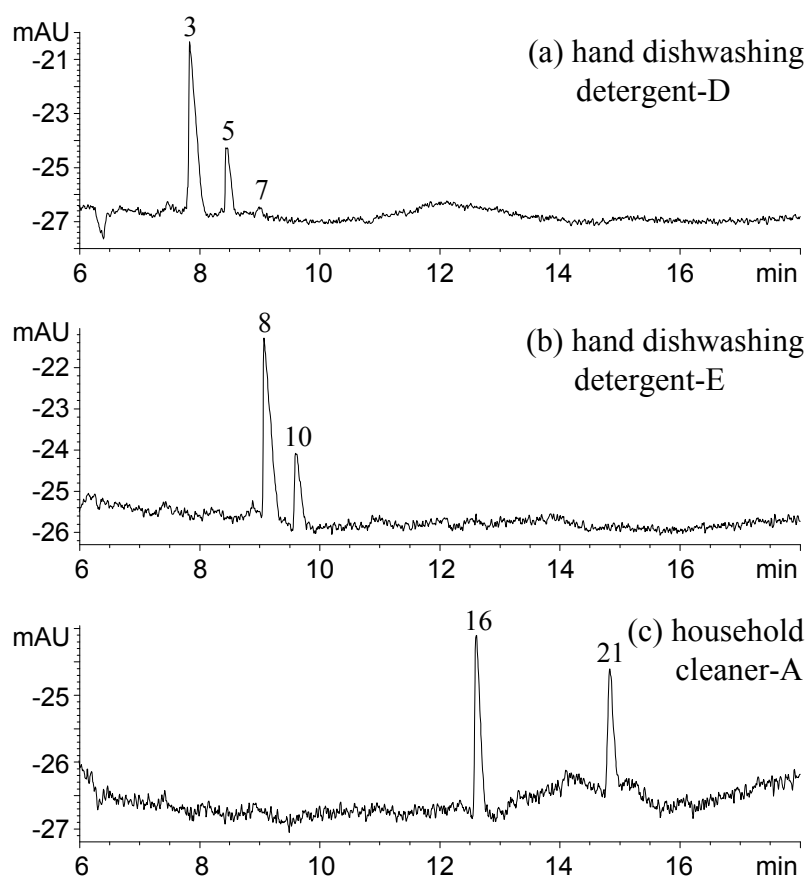based on the developed method was also validated.

Figure 3-8.   Typical electropherograms of the commercial products: (a) hand dishwashing detergent-D (1:200), (b) hand dishwashing detergent-E (1:200) and (c) household cleaner-A (1:100).   Value in the parenthesis represents a dilution ratio of the sample.   Separation conditions are as in Figure 3-3b.

Table 3-3. Determination of amphoteric surfactants in commercial products [a]

Sample	Surfactant	Content (%)	Surfactant	Content (%)
Hand dishwashing detergent-D	C_{12}-AO	7.0	C_{14}-AO	2.1
Hand dishwashing detergent-E	C_{12}-APAO	6.1	C_{14}-APAO	2.3
Household cleaner-A	C_{14}-Bt	1.4	C_{12}-APB	1.2

[a] Estimated from the CE analysis shown in Figure 3-8.

3-4 Conclusions

The simultaneous determination of the amphoteric surfactants was successfully accomplished by CE with indirect UV detection.   Under the optimized condition, four types of amphoterics, C_8–C_{18} homologues of AO, APAO, Bt and APB were completely separated within 17 min without any complicated procedures.   The developed method was satisfactorily validated for the reproducibility, sensitivity and linearity.   It should be noted that the method has a considerable advantage in the selective analysis of the amphoteric surfactants in real samples, i.e., no matrix peak was observed, compared with previous HPLC methods.   These results demonstrated that the CE–indirect UV analysis is very useful for the identification and determination of these amphoteric surfactants in commercial products and can be used as a routine technique in different fields of industrial analyses.

3-5　References

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Chapter 4

Separation of Fatty Alcohol Ethoxylates by Capillary Zone Electrophoresis and Micellar Electrokinetic Chromatography

4-1 Introduction

Fatty alcohol ethoxylates (FAEs), also known as ethoxylated alcohols or polyoxyethylene alkyl ethers, are the most important nonionic surfactants widely used in consumer and industrial products as washing agents, emulsifiers, solubilizers and foaming agents. They are generally produced by solventless addition of ethylene oxide (EO) to fatty alcohols using KOH or magnesium oxide catalyst containing aluminum [1]. Most technical FAE products are complex mixtures of homologues with different numbers of the EO group whose distribution depends on the reaction conditions such as the catalyst. On the other hand, the physical and chemical properties of the FAEs are highly dependent on both the average number and the distribution of the EO groups. Therefore, it is very important to determine the precise EO distribution of the FAEs in support of product development and quality control.

High performance liquid chromatography (HPLC) analysis has been applied to the separation of a wide range of the EO homologues and several separation methods have been developed [1,2]. Efficient separations of ethoxylated nonionic surfactants can be achieved by normal-phase HPLC using unmodified, amino-, cyano- or diol-bonded silica [3–7] and by reversed-phase HPLC [8–16]. Since the FAEs contain no chromophoric groups, they are often derivatized with phenyl isocyanate [8,9],

1-naphthyl isocyanate [10,11], 3,5-dinitrobenzoyl chloride [12–14] and carboxylic anhydrides [15,16] before HPLC analysis employing UV detection.   The use of HPLC coupled with refractive index detection (RID) [17,18] or evaporative light-scattering detection (ELSD) [4,19] is the alternative method; these techniques do not require derivatization.   In ELSD, eluent from the HPLC column is nebulized and the non-volatile components determined by light-scattering [20].   Therefore, ELSD does suffer from the problems associated with RID, such as baseline drift when solvent gradients are used, and this method has been applied to the separation and determination of EO homologues.   However, FAEs with short EO groups are generally too volatile to be quantified by ELSD, which makes it difficult to obtain exact EO distributions [4,21].   HPLC combined with mass spectrometry is very effective for specific and sensitive surfactant analysis.   However, the response factors of the underivatized FAE homologues decrease dramatically below the EO number of 4 and non-ethoxylated alcohols can not detected due to the low retention of the homologues with a low ethoxylation degree to form adducts with protons and other cations [1,22].   Although gas chromatography (GC) employing flame ionization detector (FID) is an excellent analytical technique for volatile compounds, it has been reported that the signal response of the FID decreases with increasing EO groups and serious errors are seen with the lower adducts [23].   Therefore, conventional HPLC–UV is suitable for the full characterization of the EO distribution of the FAEs.

Capillary electrophoresis (CE) has high separation efficiency and is capable of rapid analysis of molecules with similar structures, and the EO distribution of the FAEs can be analyzed by CE.   In capillary zone electrophoresis (CZE), the analytes are separated according to their electrophoretic mobilities which are determined by charge and size of

the ion.   Since nonionic surfactants do not possess a net charge, in simple CZE without sample derivatization all the EO homologues migrate with the same velocity determined by the electroosmotic flow (EOF), resulting in no separation.   However, successful separation of the EO homologues has been reported after derivatization to anionic compounds with phthalic anhydride [24–27], maleic anhydride [26,27], 1,2,4-benzenetricarboxylic anhydride [27,28].   In addition, such derivatization introduces not only a negative charge but also a chromophoric group into the FAEs for UV detection.   Actually, several research groups have reported the separation of alkylphenol ethoxylates by micellar electrokinetic chromatography (MEKC) using anionic surfactants such as sodium dodecyl sulfate (SDS) [27,29–31] and bile salts [32].   Therefore, CE including CZE and MEKC, is also suitable for the separation of the EO homologues of FAEs as well as HPLC.   However, the adequate separation for the detailed analysis of the EO distribution, especially for the homologues with long EO groups, has not been achieved in previous studies.

The purpose of this study was to develop effective separation methods for the EO homologues of FAEs on the basis of the CE technique with UV detection.   To attain this goal, the FAEs were derivatized to cationic compounds with a chromophore group by using 2-fluoro-1-methylpyridinium p-toluenesulfonate (FMPTS) [33,34], and electrophoretic separations by CZE and MEKC with SDS and dodecyltrimethyl ammonium chloride (DTAC) were investigated in detail.

4-2 Experimental Section

4-2-1 Reagents

Tetrahydrofuran (THF), methanol and acetonitrile of HPLC grade were from Kanto Chemical (Tokyo, Japan).   FMPTS and DTAC were obtained from Tokyo Chemical Industry (Tokyo, Japan), triethylamine (TEA) from Wako (Osaka, Japan), SDS from Nacalai Tesque (Kyoto, Japan).   Water was deionized with a Milli-Q purification system (Millipore, Bedford, MA, USA).   All other reagents were of analytical grade.

Technical products of dodecyl alcohol ethoxylate with the general formula $C_{12}H_{25}O(CH_2CH_2O)_xH$, where x is the number of EO groups and the average x of 6 ($C_{12}E_6$), 19 ($C_{12}E_{19}$) and 38 ($C_{12}E_{38}$), were supplied by KAO (Tokyo, Japan). 1-Dodecanol ($C_{12}E_0$), tetraethylene glycol monododecyl ether ($C_{12}E_4$) and octaethylene glycol monododecyl ether ($C_{12}E_8$) were from Wako.   These products were derivatized without any pretreatment.

4-2-2 Derivatization procedure

The derivatization scheme is depicted in Figure 4-1.   To introduce a positive charge and a chromophoric group into the FAEs, sample (0.1–0.5 g) was dissolved in acetonitrile (30 mL) and FMPTS (0.2 g) was added.   TEA (100 μL) was then added and the reaction mixture was stirred gently for 2 h at room temperature.   Finally, the products were evaporated to dryness under nitrogen.   All derivatized samples were diluted to desired concentrations with a background solution (BGS).

Laundry detergent (heavy duty granule) and dried residue (100 °C, 2 h) of fabric softener were dissolved in acetone to extract the FAEs from the products.   After

filtration, the solution containing the FAEs was evaporated to dryness under nitrogen and derivatization with FMPTS was performed as described above.   The acetone extract from the laundry detergent was derivatized by trimethylsilylation reagent (TMSI-H, GL Sciences, Tokyo, Japan) for the GC measurements.
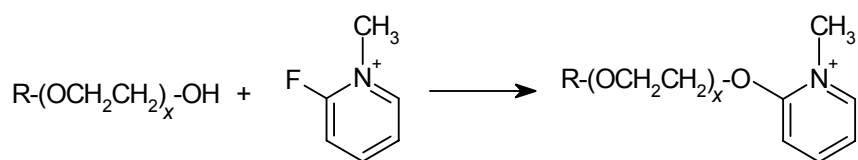
R-(OCH$_2$CH$_2$)$_x$-OH + [2-fluoro-1-methylpyridinium] → R-(OCH$_2$CH$_2$)$_x$-O-[1-methylpyridinium]

Figure 4-1.   Scheme of the derivatization of FAEs with FMPTS.

4-2-3   Apparatus

All CE experiments were performed on a Hewlett-Packard ^{3D}CE system (Waldbronn, Germany) equipped with a diode-array UV detector combined with a ^{3D}CE Chemstation for system control, data collection and data analysis.   Separations were carried out on fused-silica capillaries (Agilent Technologies, Palo Alto, CA, USA) of 56 cm effective length × 50 μm I.D.   Capillaries were thermostated at 25 °C and detection was performed at 280 nm.   To ensure reproducibility, the capillaries were flushed successively with 0.1 M NaOH for 2 min, water for 2 min, 0.1 M HCl for 2 min, water for 2 min, methanol for 2 min and BGS for 4 min prior to each run.   After conditioning the capillary, the derivatized samples were injected hydrodynamically at 50 mbar for 1 s.

The pH was measured with a HORIBA D-51 pH meter (Kyoto, Japan). The BGSs and sample solutions were filtrated through a 0.45 μm pore size membrane syringe filter (Gelman Sciences, Ann Arbor, MI, USA) prior to analyses.

GC analysis of the FAE in the laundry detergent was performed on an Agilent 6890N equipped with an FID. A capillary column DB-1 30 m × 0.25 mm I.D. × 0.25 μm film thickness (Agilent Technologies) was used for the separation. The initial temperature was 100 °C, which was then increased to 320 °C at 10 °C/min and held for 38 min at the maximum temperature. The injected sample volume was 1 μL.

4-3 Results and Discussion

4-3-1 CZE separation of FAEs

Prior to CE analysis, the derivatization reaction was monitored by conventional HPLC. When $C_{12}E_6$ was reacted with FMPTS in acetonitrile, the yield reached approximately 100% in 30 min. Thus, the derivatization of the FAEs with FMPTS would proceed quantitatively at room temperature.

In the CZE analysis of the cationic FMPTS-derivatized FAEs with weakly acidic, neutral and basic BGSs, the separation was poor compared to that with acidic BGSs. Figure 4-2 shows the effect of pH on the separation of derivatized $C_{12}E_6$. Insufficient separations of the FAE homologues obtained above pH 4 would be due to a shorter separation time of the cationic compounds under faster EOF conditions. Actually, the electroosmotic mobilities (μ_{eo}) were evaluated to be 3.8, 1.1 and 0.5 × 10^{-4} cm^2 V^{-1} s^{-1} at pH 7.0, 4.0 and 2.5, respectively. Therefore, the pH value of the BGS for separating the FMPTS-labeled FAEs was selected to be 2.5.
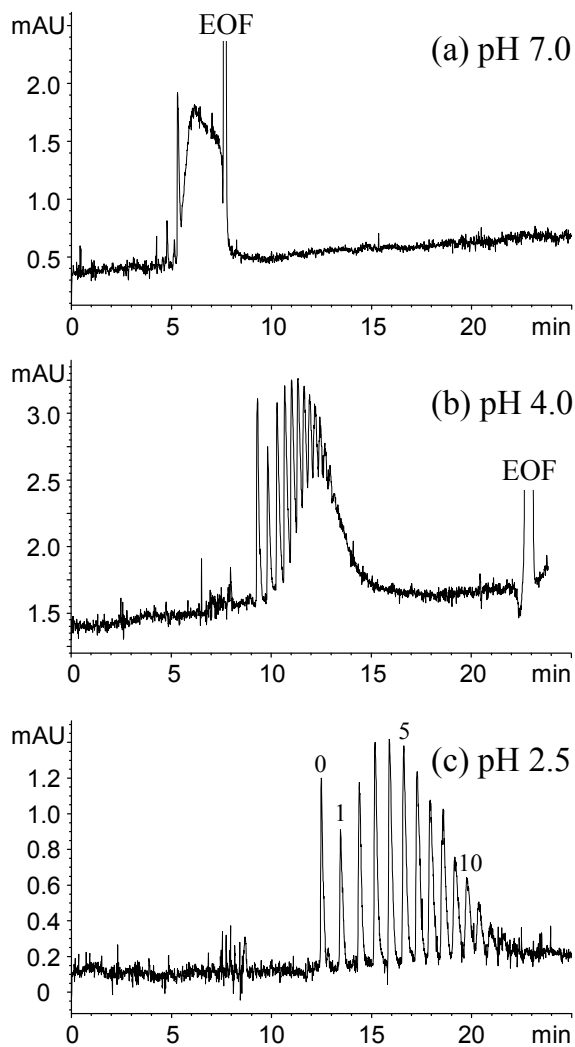
Figure 4-2. CZE separation of the $C_{12}E_6$ derivatives at pH (a) 7.0, (b) 4.0 and (c) 2.5. Capillary, 56.0 cm effective length, 64.5 cm total length, 50 μm I.D.; BGS, 20 mM phosphate buffer; applied voltage, +20 kV; injection, 1 s at 50 mbar; temperature, 25 °C; detection, 280 nm. The numbers above the peaks correspond to the number of the EO groups of the FAEs.

It has been reported that the addition of organic modifiers such as acetonitrile and methanol to the BGS is useful to suppress adsorption onto the capillary wall and micelle formation, leading to peak tailing and insufficient separation efficiency of cationic surfactants in CE [35,36].   Thus, the effect of organic modifiers on the CZE separation of the FAEs was investigated.   Figure 4-3 shows the electropherograms of FMPTS-derivatized $C_{12}E_6$ with the BGSs containing THF, methanol and acetonitrile at pH 2.5.   Separation of the EO homologues was achieved within 25 min without organic modifier.   However, peak tailings were observed and the extent became worse with increasing number of EO groups (Figure 4-3a).   On the other hand, as shown in Figures 4-3b–4-3d, the migration times of the analyte were considerably changed by addition of organic modifiers.   This could result from changes in the aggregation state of the FAEs with the modifiers, resulting in changing their electrophoretic mobility and the viscosity of the BGS.   When THF (Figure 4-3b) and methanol (Figure 4-3c) were added to the BGS, time required for the detection of all the homologues was 50 min. Apparently, the addition of acetonitrile (Figure 4-3d) was more beneficial than that of THF and methanol in terms of analysis time.   Complete separation of all the EO homologues was achieved within 17 min on addition of acetonitrile.   Acetonitrile has the lower viscosity and highest polarity among the three organic modifiers used in this study, and provides the fastest separation of the $C_{12}E_6$ derivatives.

The effect of the acetonitrile concentration was investigated.   At acetonitrile concentrations below 20% (v/v), significant peak broadening was observed due to micelle formation and/or sample adsorption onto the inner surface of the capillaries. Thus, it was necessary to add 30–80% (v/v) acetonitrile for effective separation. Under these conditions, the EO homologues ($x = 17$) were well resolved.   In particular,

at an acetonitrile concentration of 70% (v/v), the best separation of $C_{12}E_6$ regarding analysis time and resolution was achieved (Figure 4-4a). However, a baseline separation of the FAE derivatives with higher average EO groups, i.e., $C_{12}E_{19}$ and $C_{12}E_{38}$, could not be obtained as shown in Figures 4-4b and 4-4c. At the present stage, the resolution of highly ethoxylated homologues is still poor but the developed CZE method will be applied to rapid "fingerprint" analysis of the FAEs with higher average EO number.
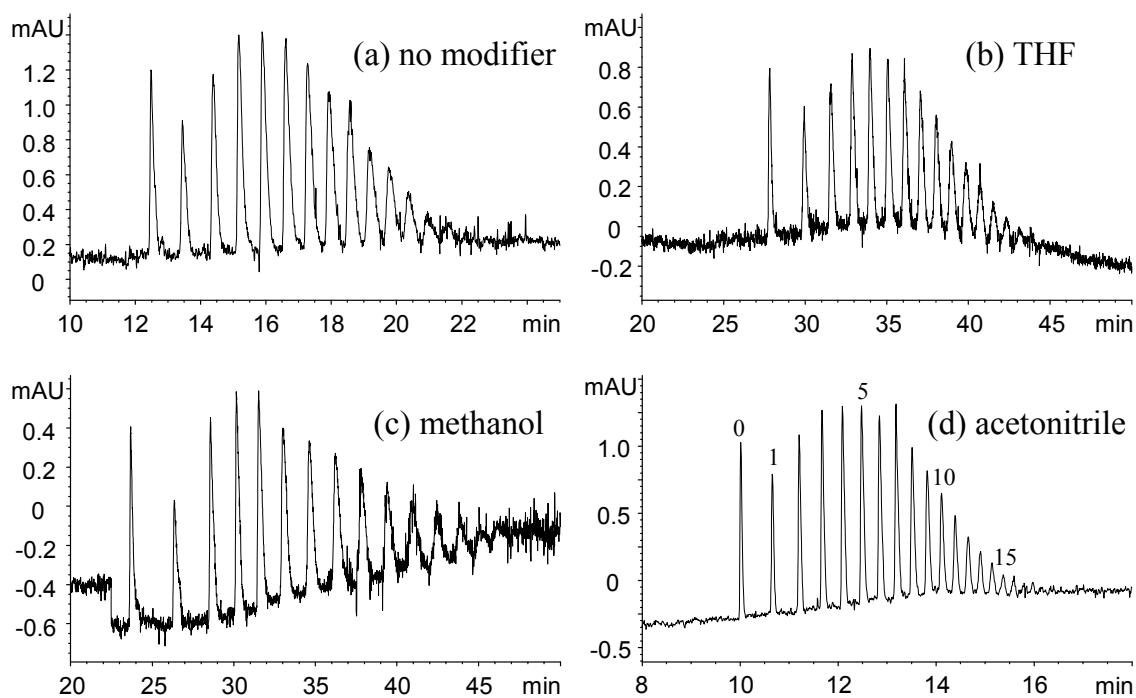
Figure 4-3. Effect of organic modifiers on the CZE separation of the $C_{12}E_6$ derivatives: (a) no modifier, (b) THF, (c) methanol, (d) acetonitrile. BGS, 20 mM phosphate buffer (pH 2.5) / 0 or 30% (v/v) organic modifier. Other conditions and peak identifications are as in Figure 4-2.
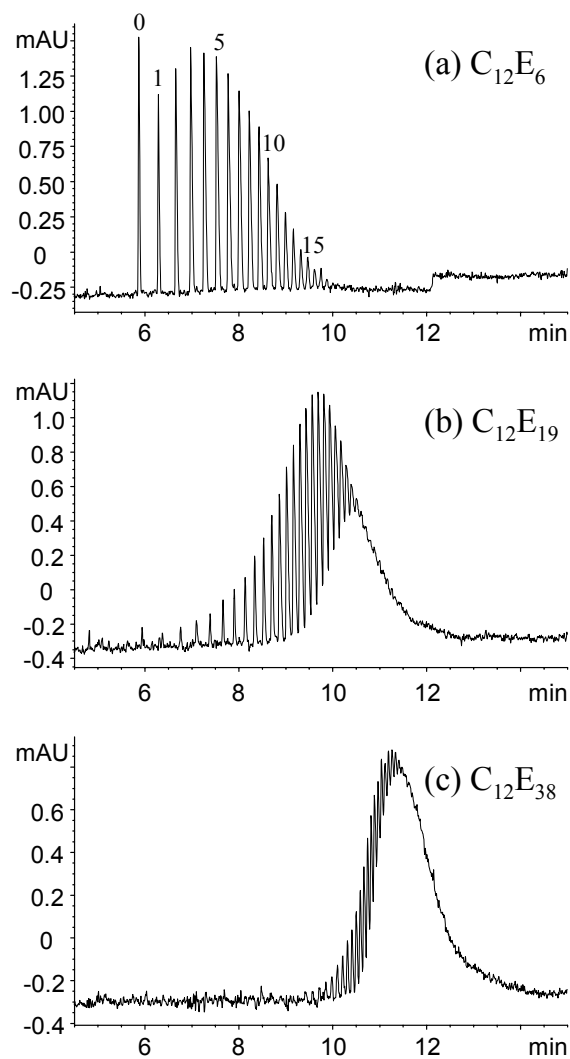
Figure 4-4. Optimal CZE separation of the FAE derivatives: (a) $C_{12}E_6$, (b) $C_{12}E_{19}$, (c) $C_{12}E_{38}$. BGS, 20 mM phosphate buffer (pH 2.5) / 70% (v/v) acetonitrile. Other conditions and peak identifications are as in Figure 4-2.

4-3-2   Effect of SDS

It has been previously reported that the EO homologues of alkylphenol ethoxylates are separated by interaction with anionic surfactant micelles in MEKC [27,29–32]. Furthermore, the addition of ionic surfactants to the BGS can modify the selectivity in the separation of charged analytes via electrostatic and hydrophobic interactions [37]. Thus, the resolution of the higher EO homologues of the FAEs can be improved by addition of surfactant.

The addition of SDS, which is the most widely used surfactant in MEKC, to the BGS was investigated as a means of adjusting the selectivity to increase the resolution.   In the absence of the organic modifier (conventional MEKC), a single peak appeared on injection of $C_{12}E_6$ into the BGS containing 30 mM SDS at pH 2.5 and reversing the polarity of the applied voltage (Figure 4-5a).   This indicates a strong association of the cationic analytes with the anionic SDS micelles.   At optimized concentrations of the organic modifiers such as THF, methanol and acetonitrile, the EO homologues were separated with the BGS containing SDS as shown in Figures 4-5b–4-5d.   It is well known that the SDS micelles are disrupted at concentration above 20–30% of organic modifier, so that the homologues were separated by conventional retention by SDS micelles and/or solvophobic association with the isolated SDS ions at relatively higher concentration of organic modifiers.   The homologues with lower EO groups interacted stronger with SDS than the more polar highly ethoxylated homologues.   Thus, the analytes migrated in order of the number of EO groups.   Although the EO homologues were separated on using SDS and three organic modifiers, the separation performances were still inferior to that of CZE analysis using acetonitrile (Figure 4-3d).
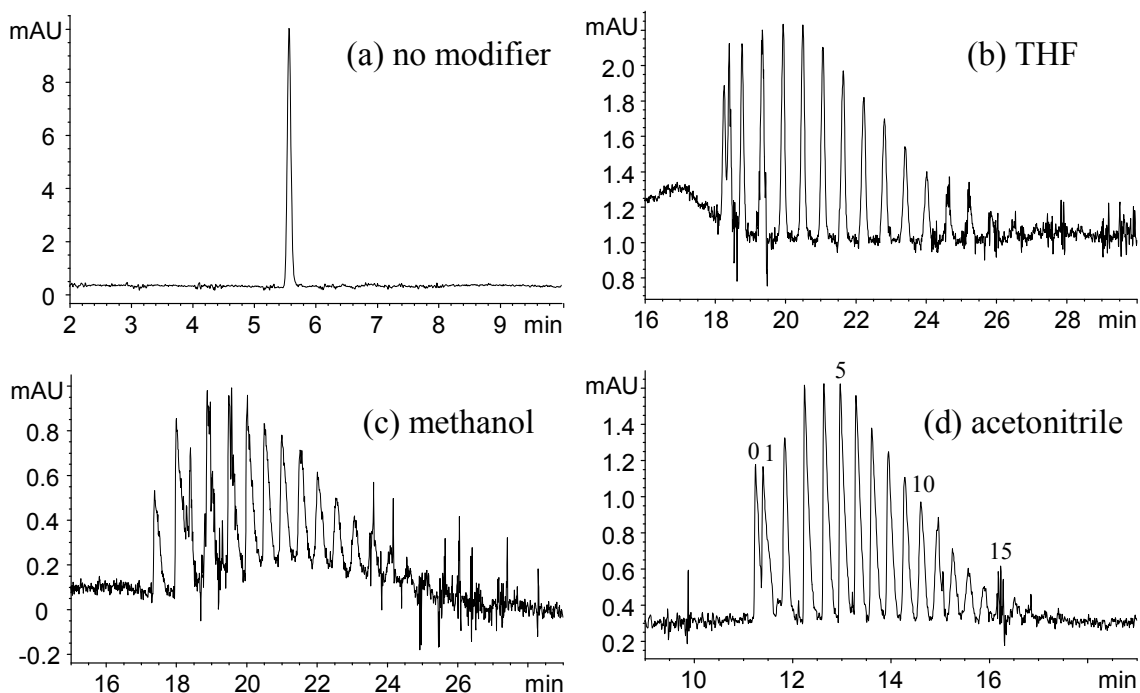
Figure 4-5. Effect of organic modifiers on the separation of the $C_{12}E_6$ derivatives with BGS containing SDS: (a) no modifier, (b) 25% THF, (c) 45% methanol, (d) 30% acetonitrile. BGS, 30 mM SDS in 20 mM phosphate buffer (pH 2.5) containing 0–45% (v/v) organic modifier; applied voltage, –25 kV. Other conditions and peak identifications are as in Figure 4-2.

4-3-3 Effect of DTAC

Further studies to separate the EO homologues of the FAEs were carried out by using the cationic surfactant DTAC. It was considered that the positive analytes can interact, to a greater or lesser extent, with the positive DTAC micelles by hydrophobic interactions and the strength of the interaction is modified in the presence of organic modifier. Hence, addition of organic modifiers to BGS containing DTAC was investigated and the results are shown in Figure 4-6. Detection of the analytes was

performed at the cathodic side since reversal of the EOF by the action of DTAC did not occur the present conditions, and positive ions consequently migrated towards the cathode with their electrophoretic mobilities. At the optimal concentrations of the modifiers, separation of the homologues did not significantly improved relative to that using the SDS solution. The migration times became longer on addition of THF (Figure 4-6b) and methanol (Figure 4-6c). Separation was improved by the addition of acetonitrile although the migration times were slightly longer than those obtained by MEKC using SDS (Figure 4-5d).
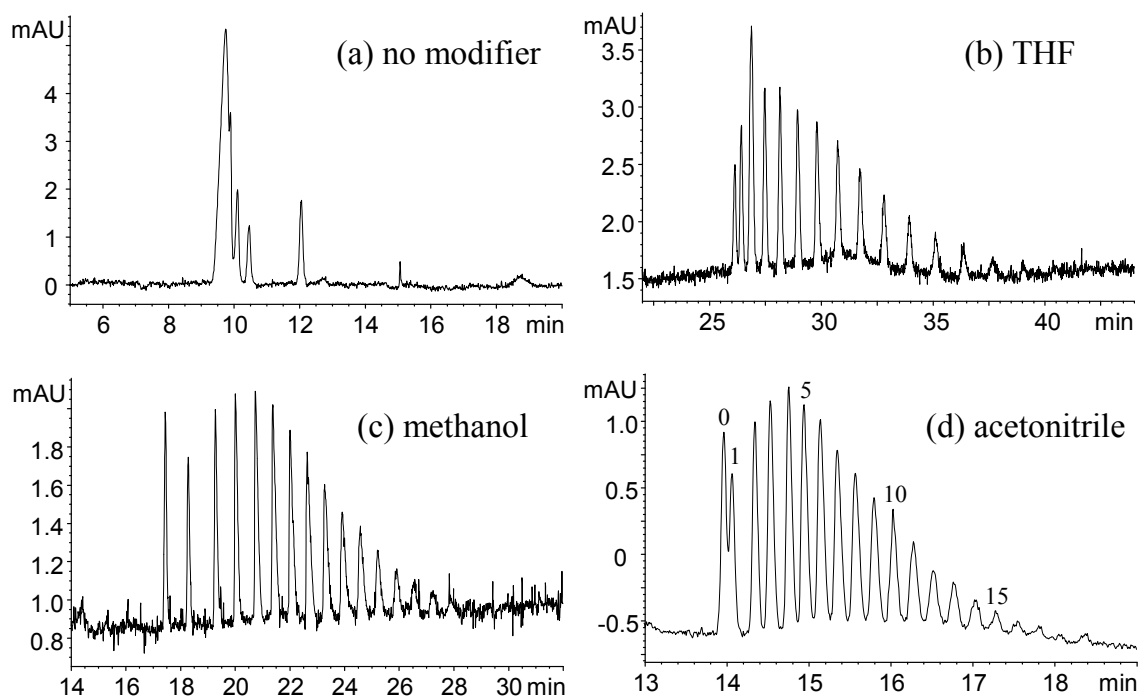

Figure 4-6. Effect of organic modifiers on the MEKC separation of the $C_{12}E_6$ derivatives with BGS containing DTAC: (a) no modifier, (b) 20% THF, (c) 30% methanol, (d) 20% acetonitrile. BGS, 30 mM DTAC in 20 mM phosphate buffer (pH 2.5) containing 0–30% (v/v) organic modifier; applied voltage, +25 kV. Other conditions and peak identifications are as in Figure 4-2.

Next, the effect of acetonitrile concentration on resolution was also investigated.   As shown in Figure 4-6d, sufficient separation of the EO homologues was achieved on addition of 20% (v/v) acetonitrile except for the peak overlapping between $x = 0$ and 1. At the above acetonitrile concentration of 20% (v/v), the analysis time became longer while retaining similar peak resolutions, whereas below 20% (v/v) poor resolutions were obtained.   Therefore, 20% (v/v) acetonitrile in the BGS was used for further investigations due to high peak resolution in short migration times.

At an acetonitrile content of 20% (v/v), the BGS composition was varied with respect to the DTAC concentration in the range of 15–50 mM.   The resolution increased on decreasing the DTAC concentration but a longer separation time was required due to the decrease in the degree of analyte–DTAC association.   On the other hand, the increase in DTAC concentration led to a significant decrease in peak resolution.   Hence, the optimal DTAC concentration of the BGS for the separation of the EO homologues was determined to be 30 mM.   The effect of the buffer concentration on the separation of the EO homologues was also examined in the range of 10–50 mM.   The migration time decreased with decreasing buffer concentration since the μ_{eo} and the electrophoretic mobility of the DTAC micelles and analytes increased due to the increase in zeta potential.   In addition, the peak resolution increased with decreasing buffer concentration and the peaks of the homologues with $x = 0$ and 1 were completely separated when the buffer concentration was altered from 20 (Figure 4-6d) to 10 mM (Figure 4-7a).   This behavior could be explained by the reduced analyte–DTAC association with decreasing ionic strength.   Consequently, the MEKC method using DTAC allowed baseline resolution of the higher EO homologues containing up to 50 EO groups within 30 min under optimal conditions as shown in Figures 4-7b and 4-7c.

The developed method provided successful separation of a wider range of EO number of the FAEs compared to the CZE separation shown in Figure 4-4.
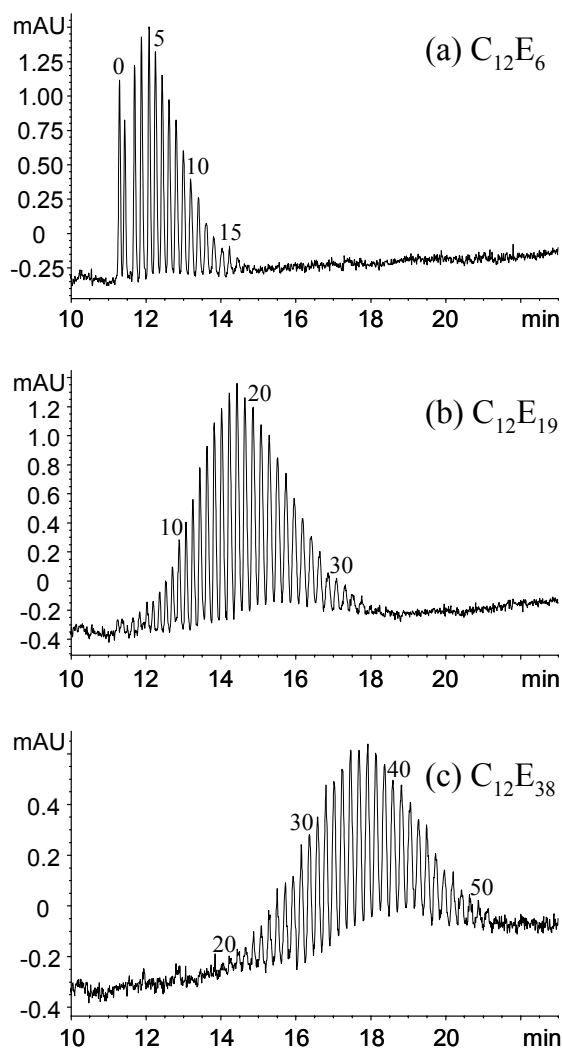

Figure 4-7. Optimal DTAC–MEKC separation of the FAE derivatives: (a) $C_{12}E_6$, (b) $C_{12}E_{19}$, (c) $C_{12}E_{38}$. BGS, 30 mM DTAC in 10 mM phosphate buffer (pH 2.5) containing 20% (v/v) acetonitrile. Other conditions and peak identifications are as in Figure 4-6.

4-3-4 Method validation

To validate the accuracy of the CZE and the MEKC method developed for determination of the EO distribution of the FAEs, the relative molar responses (RMRs) of the EO homologues were determined. The RMRs were evaluated from the peak area of known concentrations of pure mono-disperse EO homologues, $C_{12}E_0$, $C_{12}E_4$ and $C_{12}E_8$. The RMRs of $C_{12}E_4$ and $C_{12}E_8$ normalized to $C_{12}E_0$ are summarized in Table 4-1. All the values obtained with both the CZE and MEKC methods were close to unity. This indicated that the derivatization of the FAEs proceeded quantitatively regardless of the EO number and the molar absorption coefficients of the FMPTS-derivatized FAEs were almost independent of the degree of ethoxylation. Thus, it was confirmed that the accurate and precise determination of the distribution of the EO groups of the FAEs could be realized by the developed method.

Table 4-1. RMRs of the EO homologues

Method	RMRs normarized $C_{12}E_0$ (%RSD)[a]	
	$C_{12}E_4$	$C_{12}E_8$
CZE [b]	0.99 (1.3)	1.00 (1.1)
MEKC [c]	0.95 (1.9)	0.97 (1.6)

[a] $n = 5$.

[b] Separation conditions are as in Figure 4-2.

[c] Separation conditions are as in Figure 4-7.

Table 4-2. Reproducibility and efficiency (N) in CE analysis of FAE derivatives

Analyte	Method	%RSD for t_R [a]		Average EO number (%RSD)[a]	
		run-to-run	day-to-day	run-to-run	day-to-day
$C_{12}E_6$	CZE [b]	0.11	0.25	6.11 (0.77)	6.05 (0.58)
$C_{12}E_6$	MEKC [c]	0.59	1.0	6.12 (0.68)	6.09 (0.14)
$C_{12}E_{19}$	MEKC [c]	0.33	0.50	18.4 (0.16)	18.9 (0.31)
$C_{12}E_{38}$	MEKC [c]	0.44	0.90	37.0 (0.47)	36.9 (0.91)

Analyte	Method	Polydispersity index (%RSD)[a]		N of main component
		run-to-run	day-to-day	
$C_{12}E_6$	CZE [b]	1.142 (0.19)	1.139 (0.21)	142,000 [d]
$C_{12}E_6$	MEKC [c]	1.143 (0.27)	1.144 (0.14)	209,000 [d]
$C_{12}E_{19}$	MEKC [c]	1.073 (0.12)	1.068 (0.12)	182,000 [e]
$C_{12}E_{38}$	MEKC [c]	1.027 (0.09)	1.028 (0.05)	104,000 [f]

[a] $n = 5$.

[b] Separation conditions are as in Figure 4-2.

[c] Separation conditions are as in Figure 4-7.

[d] $x = 4$.

[e] $x = 18$.

[f] $x = 37$.

The reproducibility of the developed MEKC method was estimated using $C_{12}E_6$, $C_{12}E_{19}$ and $C_{12}E_{38}$ as test analytes, and the results are shown in Table 4-2. The run-to-run repeatability of the migration time was good with the relative standard deviation (RSD) values ranging from 0.33 to 0.59% ($n = 5$). The day-to-day RSD values were acceptable, at less than 1.0% ($n = 5$). Regardless of the degree of ethoxylation, the average EO number of the analytes calculated from the peak area showed satisfactory agreement with the theoretical value. The polydispersity index (PDI), which is an important measure indicating the distribution of molecular mass, was also calculated from the weight average molecular weight (M_w) divided by the number average molecular weight (M_n). The values of the average EO number and the PDI for the $C_{12}E_6$ derivative obtained by the MEKC method agreed well with those of CZE. Reproducible determination of the PDI could be attained with RSD values of 0.09–0.27%. These results clearly demonstrated that the developed method performed after derivatizing with FMPTS could provide the accurate EO distribution of the FAEs.

4-3-5 Applications

The developed method was applied to analysis of the FAEs in commercially available laundry detergent and fabric softener containing anionic and cationic surfactants. After a simple preliminary cleanup and derivatization, the samples were analyzed by the MEKC method with DTAC. For comparison, the FAEs in the laundry detergent were characterized by the CZE and GC-FID methods.

Figure 4-8 shows the electropherograms of the FAEs with C_{12} chain in the product sample. Identifications of the EO homologue peaks were made by the standard addition method using $C_{12}E_4$ and $C_{12}E_8$ for the laundry detergent and fabric softener,

respectively. The range of the EO distribution, the average EO number, and the PDI of the FAEs are summarized in Table 4-3. As a result, the laundry detergent sample contained FAEs with EO distribution between $x = 0$ and 15 (Figure 4-8a) and an average EO number of 4.86 was found with a PDI of 1.149. These values agreed with those obtained by the CZE method and with the fact that lower ethoxylated FAEs are often added to laundry detergents [38]. The average EO number and PDI of the FAEs in the detergent determined by the GC-FID measurement with trimethylsylil derivatization were 4.64 and 1.144, respectively. Considering that the FID detection sensitivity of the EO homologues might differ from that of UV detection and higher ethoxylated components ($x = 14$ and 15) could not be detected by GC-FID method, the slight differences of the average EO number in the two methods should be negligible. Thus, the author believes that the EO distribution obtained with the MEKC method is reliable even in the real detergent sample.

In the MEKC analysis of the fabric softener, a higher degree of ethoxylation of the homologues could be recognized, as shown in Figure 4-8b. The FAEs showed the EO distribution between $x = 4$ and 35, and the average EO number and PDI were determined to be 19.8 and 1.067, respectively. It should be noted that only the peaks of the FAEs were observed though the sample products consist of various raw materials. This indicated that sample cleanup process with acetone extraction was effective for eliminating main components of the product such as water-soluble ionic surfactants. In HPLC, the characterization of the FAEs in the real products was difficult due to some ingredients that could not be removed by acetone extraction. Therefore, the developed method using CE combined with acetone extraction is quite useful for the high performance and selective analysis of the FAEs in household formulations.
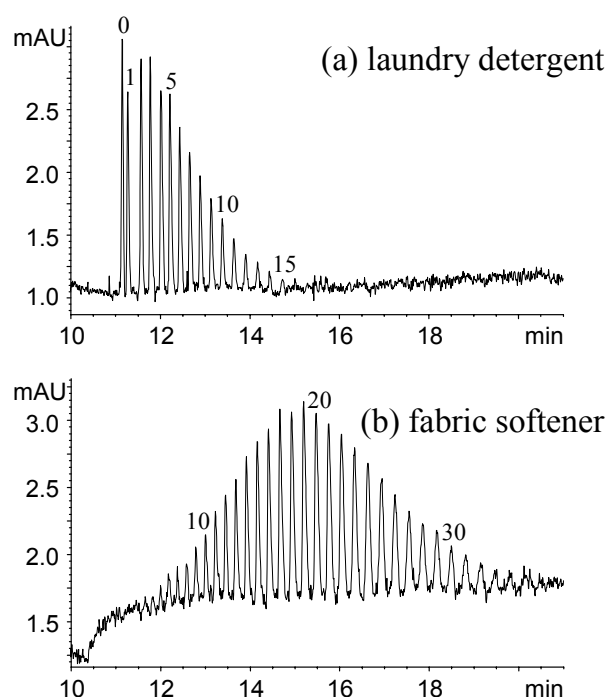
Figure 4-8. Electropherograms of FAEs in commercially available household products: (a) laundry detergent (heavy duty granule), (b) fabric softener. Separation conditions and peak identifications are as in Figure 4-7.

Table 4-3. Characterization of FAEs in household products

Sample	Method	Range of EO groups	Average EO number (%RSD)[a]	Polydispersity index (%RSD)[a]
Laundry detergent	CZE[b]	0–15	4.83 (0.3)	1.146 (0.03)
Laundry detergent	MEKC[c]	0–15	4.86 (1.2)	1.149 (0.32)
Laundry detergent	GC[d]	0–13	4.64 (2.2)	1.144 (0.90)
Fabric softener	MEKC[c]	2–34	19.8 (1.2)	1.067 (0.25)

[a] $n = 3$.

[b] Separation conditions are as in Figure 4-2.

[c] Separation conditions are as in Figure 4-7.

[d] Separation conditions are as in the section 4-2-3.

4-4   Conclusions

Characterization of the EO homologues of FAEs was carried out in a fast and efficient way by MEKC using DTAC after sample derivatization with FMPTS.   A DTAC concentration of 30 mM and acetonitrile content of 20% (v/v) in 10 mM phosphate buffer (pH 2.5) were found to be optimal for separating the FAE derivatives. Oligomer separation was achieved for the FAEs containing up to 50 EO groups within 30 min under the optimal conditions.   The developed method was satisfactorily validated with good reproducibility of the migration time, the average EO number, and the PDI.   It was confirmed that the accurate and precise determination of the distribution of the EO groups of the FAEs could be attained by the developed method. Furthermore, the identification of the FAEs in household products could also be realized by the MEKC method with a simple cleanup of the samples.   These results clearly demonstrate the developed method can be employed as a routine technique to characterize the FAEs in a wide range of industrial products and household formulations for supporting product development and quality control.

4-5　References

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Chapter 5

Separation of Lipophilic Compounds by Micellar Electrokinetic Chromatography with Organic Modifiers

5-1 Introduction

Micellar electrokinetic chromatography (MEKC) [1–4], a mode of capillary electrophoresis (CE) using an ionic micelle as a pseudo-stationary phase, has become a well-known technique to separate small neutral molecules as well as charged solutes. Ordinarily, MEKC is operated with an aqueous solution and hence, highly hydrophobic compounds cannot be separated adequately due to poor solubility in the bulk (aqueous) solution. Although addition of organic modifiers, i.e., methanol [5,6] acetonitrile [6], and 2-propanol [7], to aqueous micellar solutions has been investigated to overcome such limitation, sufficient separations were not attained. In each case, the content of the organic modifier was less than 50% (v/v). Although a high concentration of an organic modifier was considered to prevent the formation of the micelle, Tanaka (personal communication, 1993) has reported that the addition of 80 to almost 100% (v/v) methanol to buffer solutions in MEKC could give good separation of hydrophobic compounds.

Recently, Imasaka and co-workers [8] have reported on MEKC separations of some lipophilic compounds. They used sodium deoxycholate solutions containing 20% (v/v) *N*,*N*-dimethylformamide and laser-induced fluorescence detection. New approaches for the separation of hydrophobic compounds by CE have also been

described, although they are not exactly MEKC. Walbroehl and Jorgenson [9] were the first to investigate the use of solvophobic association in the separation of hydrophobic compounds. Ye and Khaledi [10] used non-aqueous media and Ahuja and Foley [11] reported on hydrophobic interaction electrokinetic chromatography.

In the present investigation, the author focused on the applicability of MEKC with organic modifiers for the separation of hydrophobic compounds using UV detection, which is the most popular detection method in CE. Sodium dodecyl sulfate (SDS), the most popular surfactant in MEKC, was used as a surfactant, and dimethyl sulfoxide (DMSO) and acetone as organic modifiers. By using an SDS solution containing DMSO eight polycyclic aromatic hydrocarbons (PAHs) were separated, whereas 13 compounds were separated with an SDS solution containing acetone. This paper describes these preliminary results. Measurements of critical micelle concentrations (CMCs) of SDS in buffers containing DMSO or acetone are also reported.

5-2 Experimental Section

SDS was obtained from Nacalai Tesque (Kyoto, Japan), and DMSO and acetone from Wako (Osaka, Japan). Separation solutions were prepared by dissolving SDS in borate–phosphate or phosphate buffers (pH 7.0), containing appropriate amount of organic modifiers. Most test solutes, PAHs and others, were purchased from Wako, Tokyo Kasei (Tokyo, Japan), and Nacalai Tesque. Sample solutions were made by dissolving solutes in separation solutions. Although the concentration of each solute was not determined exactly, it was about 0.1–1 mg/mL. All chemicals were of analytical grade and were used as received. Capillary electrophoresis was performed

in the Beckman P/ACE system 2000 (Fullerton, CA, USA), equipped with a UV spectrophotometric detector controlled by a PS/V personal computer system (IBM, Tokyo, Japan). Sample injection was performed by the pressurized method and the injection time was maintained at 1 s. A Shimadzu Chromatopac C–R6A (Kyoto, Japan) was also used for data recording. An untreated fused-silica tube, purchased from Polymicro Technologies (Phoenix, AZ, USA), 52 μm I.D. × 370 mm (300 mm effective length) was used as a separation capillary. Measurement of CMC was carried out by a conductometric titration. The method and apparatus used were the same as in the previous study [12].
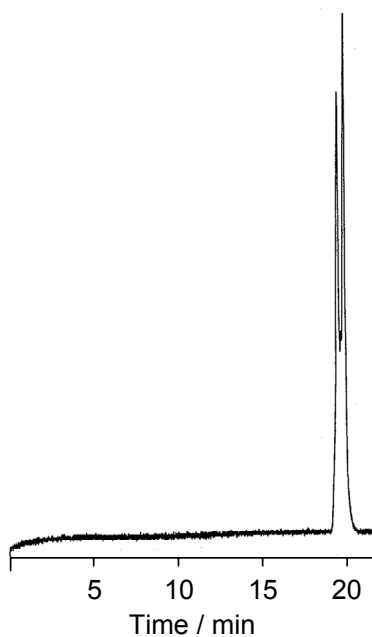
Figure 5-1. MEKC of 5 PAHs: *p*-quinone, quinoline, naphthalene, phenanthrene and pyrene (not assigned). Separation solution: 25 mM SDS in borate–phosphate buffer (pH 7.0), containing 80% (v/v) DMSO. Separation capillary, 52 μm I.D. × 370 mm; effective length, 300 mm; applied voltage, 25 kV (676 V cm^{-1}); detection wavelength, 280 nm; temperature, 25 °C.

5-3　Results and Discussion

5-3-1　SDS–DMSO system

With DMSO as an organic modifier, detection was carried out at 280 nm as DMSO has a strong UV absorption in the region under 270 nm.　Phosphate buffer solutions were preferred because a borate–phosphate buffer always precipitated after mixing with DMSO.　SDS solutions, containing ≤30% (v/v) DMSO, did not offer adequate resolution of PAHs due to poor PAH solubility in aqueous DMSO.　At 50% DMSO, a mixture of *p*-quinone, quinoline, naphthalene, phenanthrene and pyrene was successfully separated in a buffer containing ca. 25 mM SDS (pH 7.0), at an electric field strength of 676 V cm^{-1} and current of 16 μA.　Using a 60% DMSO solution, the results were similar but resolution was impaired.　At even higher DMSO concentrations (up to 80%) resolution was poor and only two peaks were observed (Figure 5-1).　Under these conditions the solutes were present only in the aqueous solution and were not incorporated into the SDS micelle.　Although the author has no evidence the SDS micelles are formed at such high DMSO concentrations, the solute and SDS seem to interact to some extent because the five neutral solutes appeared in two peaks.　Similarly, in SDS solutions (pH 7.0) containing 50% (v/v) DMSO, eight PAHs could be separated with good resolution although benzanthrone and benz(a)anthracene comigrate (Figure 5-2).　At 280 nm used for UV detection in the DMSO system, the sensitivity for some compounds was low compared with 210 nm, and some compounds, e.g., benzene and anthracene, could not be detected.　At a lower SDS concentration, resolution decreased, due to reduced retention factors [3] (Figure 5-3).
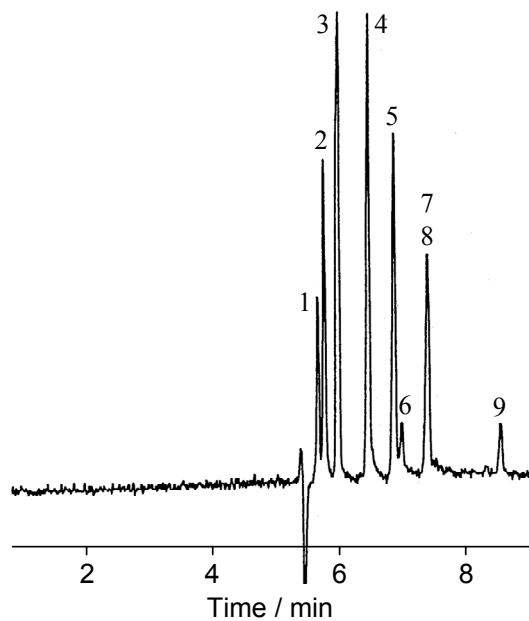
Figure 5-2. MEKC of 9 PAHs: (1) *p*-quinone, (2) quinoline, (3) naphthalene, (4) phenanthrene, (5) pyrene, (6) 2,3-benzofluorene, (7) benzanthrone, (8) benz(a)anthracene, (9) 1,2-benzanthraquinone. Separation solution: 25 mM SDS in phosphate buffer (pH 7.0), containing 50% (v/v) DMSO; temperature, 35 °C. Other conditions are as in Figure 5-1.
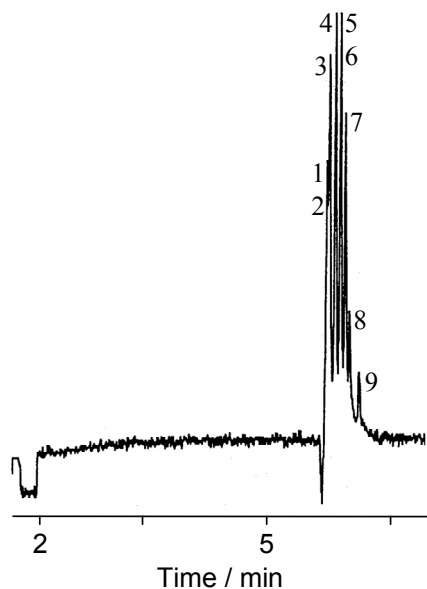

Figure 5-3. MEKC of 9 PAHs: Concentration of SDS: 10 mM; applied voltage, 20 kV (541 V cm^{-1}). Other conditions are as in Figure 5-2.

5-3-2 SDS–acetone system

Acetone is not a popular organic modifier in reversed-phase high performance liquid chromatography because it has a strong UV absorbance around conventionally used wavelength, e.g., 254 nm. The UV absorbance, however, rapidly decreases at 210 nm, and at 200 nm it is almost transparent. Since acetone is a stronger solvent than DMSO for most hydrophobic compounds, a reduced content of acetone was expected to give similar results.
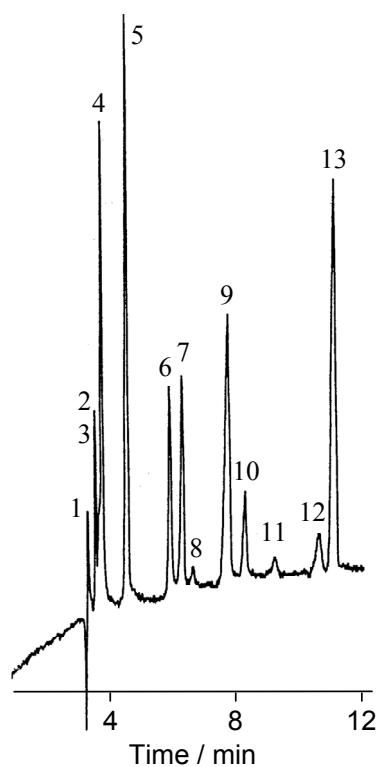

Figure 5-4. MEKC of 13 PAHs in presence of acetone. Solutes: (1) *p*-quinone, (2) quinoline, (3) benzene, (4) benzoin, (5) naphthalene, (6) benzanthrone, (7) phenanthrene, (8) anthracene, (9) pyrene, (10) 1,2-benzanthraquinone, (11) 2,3-benzofluorene, (12) benz(a)anthracene, (13) fluorescein. Separation solution: 25 mM SDS in borate–phosphate buffer (pH 7.0), containing 30% (v/v) acetone; detection wavelength, 200 nm; temperature, 30 °C. Other conditions are as in Figure 5-3.

In Figure 5-4 a successful separation of 13 PAHs is shown, with benzene and anthracene also being detected. In 25 mM SDS–30% acetone, fluorine, diphenylmethane, triptycene, *o*-terphenyl, triphenylene and triphenymethane were partially resolved; fluorine and diphenylmethane comigrated and were not separated well from triptycene (Figure 5-5a) due to small retention factors. The retention factor *k*, depends on net concentration of the micelle, C_{mc}, in MEKC according to the following equation [3],

$$k = Kv^*(C_{sf} - \text{CMC}) = Kv^*C_{mc} \qquad\qquad (5\text{-}1)$$

where K and v^* and C_{sf} are the distribution coefficient, partial specific volume of the micelle and concentration of the surfactant, respectively. Thus, an increase in C_{mc} gives an increase in the retention factor. By using 40 mM SDS–30% acetone, improved separation was obtained, as shown in Figure 5-5b. Under these conditions, *o*-terphenyl and triphenylene comigrated, but fluorine and diphenylmethane were almost completely separated and triptycene was perfectly separated from these two. Since acetone is highly volatile, the composition of the separation solution containing acetone will change within a short time. The acetone solution was freshly prepared before each run and was changed after at least every three or four runs.
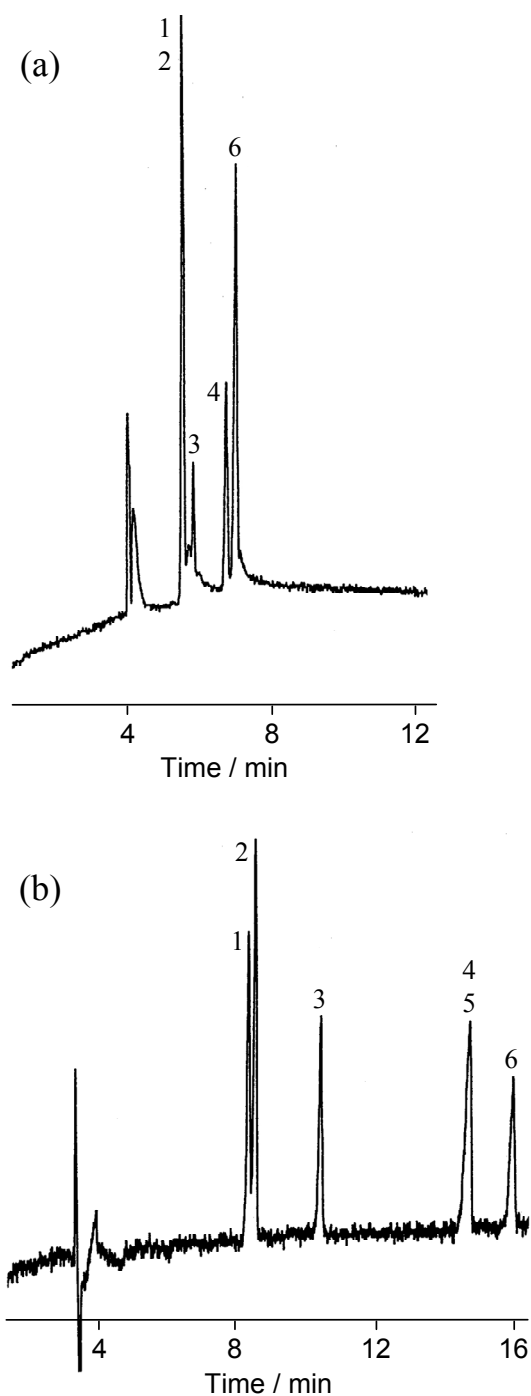
Figure 5-5. MEKC of 6 PAHs in presence of acetone. Solutes: (1) fluorene, (2) diphenylmethane, (3) triptycene, (4) *o*-terphenyl, (5) triphenylene, (6) triphenyl-methane. Concentration of SDS: (a) 25 mM, (b) 40 mM; phosphate buffer (pH 7.0), containing 30% (v/v) acetone; temperature: (a) 30 °C, (b) 25 °C. Other conditions are as in Figure 5-4.

5-3-3 CMC measurement

For the calculation of enthalpy, entropy and Gibbs free energy changes as well as distribution coefficients at different temperatures, CMC must be measured as well as v^*, as shown in Eq. (5-1). In this report the CMC of SDS in buffers containing DMSO or acetone was measured by conductometric titration (Table 5-1). In both systems, similar values were obtained. They are much higher than those in aqueous buffer without any organic modifiers [12].

Table 5-1. CMCs (in mM) of SDS

Temperature/ °C	Buffer		
	DMSO [a]	Acetone [a]	B–P [b]
25	6.0	6.3	2.9
30	6.1	–	2.5
35	6.3	6.3	2.6

[a] The content of the organic modifier was 20% (v/v) in 25 mM $Na_2B_4O_7$–50 mM NaH_2PO_4, pH 7.0.

[b] 100 mM borate–50 mM phosphate buffer, pH 7.0, cited from [12].

5-4 Conclusions

The use of DMSO and acetone as organic modifiers in MEKC is effective for the separation of hydrophobic compounds. Acetone proved more useful because UV detection is also feasible at shorter wavelengths. The results presented in this paper are preliminary. Optimization of the separation will be investigated further. The results of calculations of thermodynamic quantities will be reported elsewhere.

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Chapter 6

Optical Resolution by High Performance Capillary Electrophoresis: Micellar Electrokinetic Chromatography with Sodium *N*-dodecanoyl-L-glutamate and Digitonin

6-1   Introduction

High performance capillary electrophoresis (HPCE) [1–3], which is a highly efficient separation technique, has become popular owing to the development of fully automated instruments.   Among some modes of HPCE, capillary zone electrophoresis (CZE) is the most method because of the case of operation, especially in terms of preparation of capillaries and separation solutions.   Micellar electrokinetic chromatography (MEKC) [4–7], which is a branch of HPCE and uses an ionic micellar solutions, has also a well-known technique to separate small neutral molecules as well as charged solutes.

Optical resolution is one of the major objectives of HPCE, especially in the pharmaceutical field.   Since Zare and co-workers [8,9] first reported enantiomeric separation by CZE using formation of copper (II) complexes, some papers on chiral separation have appeared.   To achieve direct optical resolution by HPCE, the following three modes are usually employed: (1) CZE with chelating reagents or with cyclodextrins (CDs); (2) electrokinetic chromatography (EKC), which includes MEKC with chiral surfactants, cyclodextrin–modified MEKC (CD–MEKC), cyclodextrin EKC (CDEKC) and microemulsion EKC (MEEKC); and (3) capillary gel electrophoresis with immobilized CDs.   The direct enantiomeric separation by CZE using CD

(CD–CZE) was first reported by Fanali [10].   This system can be applied to the optical resolution of charged enantiomers.   Otsuka and Terabe [11] have also demonstrated the enantiomeric separation of *RS*-chlorpheniramine by CD–CZE.   In MEKC, sodium *N*-dodecanoyl-L-valinate (SDVal) [12–16], various bile salts [17–20], digitonin [14] and saponins [21] have been used as chiral surfactants.   Optical resolution by CD–MEKC, in which achiral micelles such as sodium dodecyl sulfate (SDS) are normally used, has also been reported [11,22–24].

In the present investigation, the author first used sodium *N*-dodecanoyl-L-glutamate (SDGlu) instead of SDVal.   Some phenylthiohydantoin (PTH)–DL-amino acids were successfully resolved with a SDGlu–SDS–urea–methanol solution, although the selectivity difference between SDGlu and SDVal was not remarkable.   Then, a digitonin–sodium taurodeoxycholate (STDC) co-micellar system was employed.   In the previous study [14], Otsuka et al. found that a digitonin–SDS system was effective in enantiomeric resolution of PTH–DL-amino acids, but a long separation time was required.   In this study, the author changed some conditions so that a reduced separation time could be achieved.

6-2   Experimental Section

SDGlu was received from Ajinomoto (Tokyo, Japan), SDS and methanol from Nacalai Tesque (Kyoto, Japan), digitonin, urea, PTH–DL-amino acids and benzoin from Wako (Osaka, Japan) and STDC from Sigma (St. Louis, MO, USA).   Separation solutions were prepared by dissolving surfactants and urea in a 50 mM phosphate buffer adjusted to an appropriate pH.   Then, methanol was added to the micellar solutions

when required.   Sample solutions were made by dissolving solutes in a water–acetonitrile (1:1) solution.   All the chemicals were of analytical reagent grade and used as received.

Capillary electrophoresis was performed with a laboratory-built system consisting of a Matsusada HepLL–30P0.08-LS or HCZE–30PN0.25-LDS regulated high-voltage power supply (Shiga, Japan), a Shimadzu SPD-6A UV spectrophotometric detector (Kyoto, Japan) and a Shimadzu Chromatopac C-R6A data processor.   An untreated fused-silica tube purchased from Polymicro Technologies (Phoenix, AZ, USA), 550 mm × 50 μm I.D. (effective length was 350 mm) was used as a separation capillary and on-column UV detection was employed.

Sample injection was carried out by the manual or hydrodynamic method. Separation was performed under the constant voltage and ambient temperature conditions.

6-3   Results and Discussion

6-3-1   MEKC with SDGlu

As reported previously [16], by using a SDVal–SDS mixed micellar solution containing urea and methanol, six PTH–DL-amino acids were successfully separated from each other and each enantiomeric pair was resolved.   Here, urea addition resulted in improved peak shapes compared with those obtained in the absence of urea, probably because of the adsorption of urea to the inside wall of the fused-silica capillary, which might prevent the irreversible adsorption of solutes.

In this study, SDGlu was employed to examine the possibility of optical resolution, instead of SDVal.   As shown in Figure 6-1, the structure of SDGlu is similar to that of SDVal: both have an *N*-dodecanoyl group and consist of an ʟ-amino acid with five carbon atoms.   SDGlu has an *n*-propyl and two carboxyl groups, whereas SDVal has isopropyl and carboxyl groups.   Hence, resolution characteristics were expected to be similar in SDGlu and SDVal micellar systems.   The critical micelle concentrations (CMCs) in an aqueous solution are reported to be 10.6 and 6.4 mM for SDGlu [25] and SDVal [26], respectively, at 40 °C, and were measured by the conductivity method.

(a)

(b)

$$CH_3-(CH_2)_{10}-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-\overset{\overset{COOH}{|}}{\underset{\underset{\underset{COONa}{|}}{\underset{\underset{CH_2}{|}}{CH_2}}}{C}}-H$$

$$CH_3-(CH_2)_{10}-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-\overset{\overset{COONa}{|}}{\underset{\underset{CH_3\quad CH_3}{\diagup\ \diagdown}}{CH}}{C}-H$$
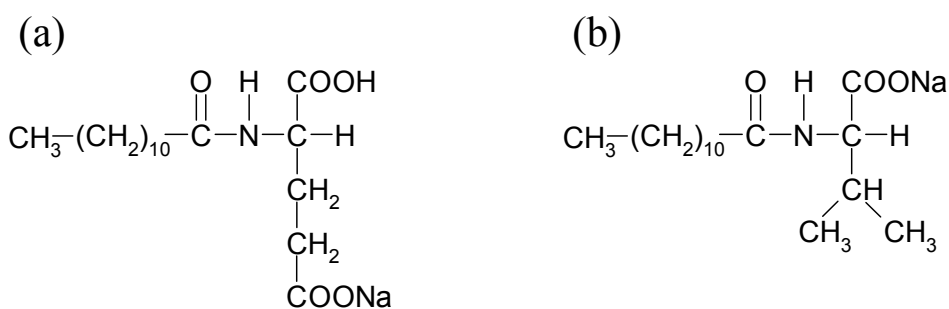
Figure 6-1.   Structure of (a) SDGlu and (b) SDVal.

Similar to the case of SDVal, the author used SDGlu–SDS mixed micellar solutions containing urea and methanol to obtain good peak shapes and enhanced selectivity. Three PTH–ᴅʟ-amino acids, such as Nva, Val and Trp, were separated from each other and each pair was optically resolved, as shown in Figure 6-2.   Here, because the migration time window was not wide enough and also the retention factor of the each pair was small, sufficient resolution could not be achieved.

The retention factor (k) is represented with the concentration of the surfactant (C_{sf}) as [6]:

$$k = Kv^*(C_{sf} - CMC) \qquad (6\text{-}1)$$

where K and v^* are the distribution coefficient and partial specific volume of the micelle, respectively.   The term (C_{sf} − CMC) reveals the net concentration of the micelle. Therefore, an increase in C_{sf} causes an increase in k.   The author then tried to use higher SDGlu or SDS concentrations than in Figure 6-2, keeping the other conditions constant.   However, higher SDGlu or SDS concentrations were not effective in improving resolution, but higher SDS concentrations led to improved results.
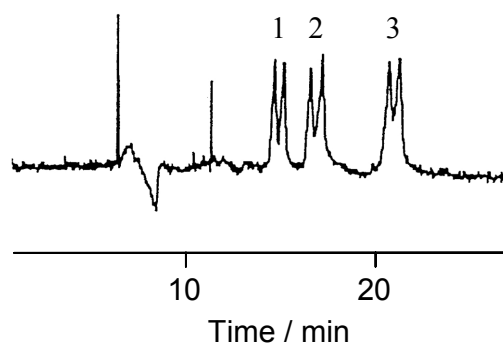
Figure 6-2.   Chiral separation of three PTH–DL-amino acids by MEKC with SDGlu. Corresponding amino acids: 1 = Nva; 2 = Val; 3 = Trp.   Micellar solution, 75 mM SDGlu–30 mM SDS–1 M urea (pH 9.0) containing 10% (v/v) methanol; separation capillary, 550 mm × 50 μm I.D.; effective length, 350 mm; total applied voltage, 12.5 kV (227 V cm^{-1}); current, 38 μA; detection wavelength, 260 nm; temperature, ambient.

The author could obtain improved resolution by using a 75 mM SDGlu–50 mM SDS–1 M urea (pH 9.0) solution containing 10% (v/v) methanol, as shown in Figure 6-3.　In this case, five PTH–DL-amino acids, α-aminobutyric acid (Aba), Met, Nva, Trp and Nle, were successfully separated and each enantiomeric pair was optically resolved. The separation characteristic was similar to that obtained with SDVal [16].
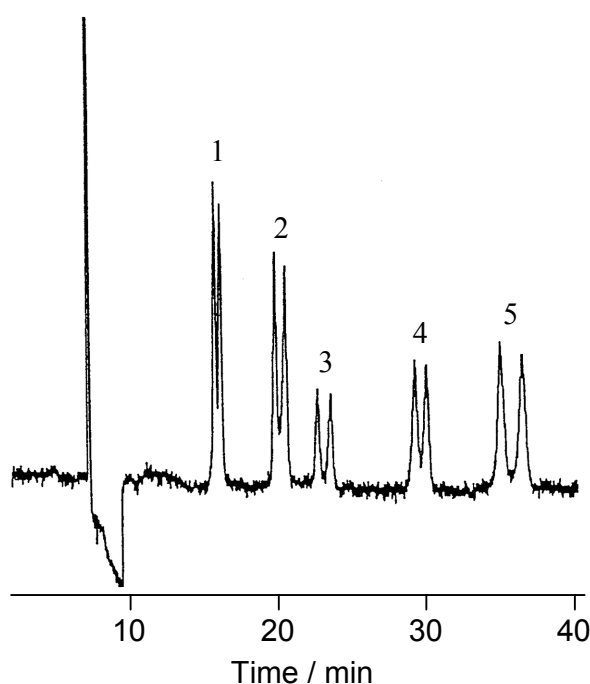

Figure 6-3.　Chiral separation of five PTH–DL-amino acids by MEKC with SDGlu. Corresponding amino acids: 1 = Aba; 2 = Met; 3 = Nva, 4 = Trp, 5 = Nle.　Micellar solution, 75 mM SDGlu–50 mM SDS–1 M urea (pH 9.0) containing 10% (v/v) methanol; current, 32 μA.　Other conditions are as in Figure 6-2.

For each pair of the five PTH–DL-amino acids in Figure 6-3, k and the separation factor (α) are calculated according to the equations reported previously [6]. The results are listed in Table 6-1. Here, the migration times of an unretained solute (t_0) and the micelle (t_{mc}) were measured by acetonitrile and Sudan IV, respectively. Note that Sudan IV was assumed not to exist in the aqueous phase even containing 10% (v/v) methanol. For all the solutes, very similar k values were observed compared with the case in SDVal, regardless of the difference in the micellar concentrations; in the SDVal solution, 50 mM SDVal–30 mM SDS–0.5 M urea (pH 9.0) containing 10% (v/v) methanol was employed. These results imply that SDGlu and SDVal have almost the same characteristics in terms of enantiomeric resolution of PTH–DL-amino acids. In the SDGlu solution, the value of t_0/t_{mc} was 0.12, and this value was smaller than that in the SDVal solution, 0.16, reported previously [16]. This reveals that a wider migration time window can be attained in the SDGlu system than in the SDVal system.

Table 6-1. Retention factors (k) and separation factors (α) of five PTH–DL-amino acids in the SDGlu solution [a]

Solute	k_1	k_2	α
Aba	1.7	1.7	1.06
Met	2.7	2.9	1.04
Nva	3.8	3.9	1.01
Trp	6.2	6.6	1.08
Nle	9.7	11.0	1.14

[a] Conditions are as in Figure 6-3.

By using the same SDGlu–SDS–urea–methanol solution as in Figure 6-3, optical resolution of benzoin was successfully resolved as shown in Figure 6-4.   This was the same result as in the SDVal system [16].   However, warfarin, which could be resolved with SDVal [16], was only partially resolved with the SDGlu solution.

Although the results are preliminary and SDGlu might have no advantage over SDVal, comparison of the resolution characteristics between the SDGlu and SDVal systems will provide useful information to investigate the chiral recognition mechanism of these *N*-acylamino acid micelles.
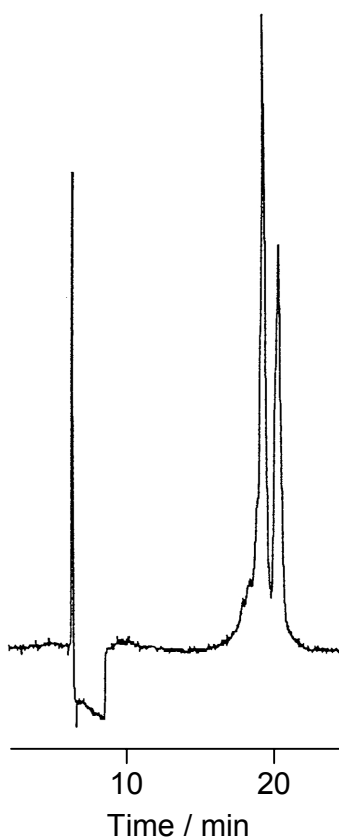
Figure 6-4.   Optical resolution of benzoin by MEKC with SDGlu.   Current, 33 μA. Other conditions are as in Figure 6-3.

6-3-2 MEKC with digitonin

Digitonin is a natural surfactant with optical activity; it is a glycoside of digitogenin and is used for the determination of cholesterol.   Since digitonin is electrically neutral, it is essential to add an ionic micelle to the digitonin solution to form charged mixed micelles that can be used as chiral carriers in MEKC.   As reported previously [14], Otsuka et al. used the digitonin–SDS co-micellar system for optical resolution of some PTH–DL-amino acids.   Although good resolution could be achieved, a long separation time was required, e.g., 90 min for Ala.

In the present investigation, the author used a shorter column than the previous one and changed the co-surfactant from SDS to STDC.   Although STDC alone has found to be capable of enantiomeric separation of dansylated DL-amino acids (Dns–DL-amino acids), it is not effective for optical separation of PTH–DL-amino acids, which is similar to the case in other bile salts [27].   However, by using a 50 mM digitonin–50 mM STDC solution (pH 2.5) containing 1 M urea, three PTH–DL-amino acids such as Nva, Val and Aba were separated from each other and each pair was optically resolved, as shown in Figure 6-5.   In this instance, the electroosmotic velocity was sufficiently suppressed, and the migration direction of the mixed micelle was towards the positive electrode or opposite to the electroosmotic flow, as reported previously [14]: if the solutes are well incorporated into the micelle, they will migrate toward the positive electrode, while the solutes less incorporated into the micelle will migrate toward the negative or the same direction as the electroosmosis [28].   Therefore, as long as the solute migrates toward the positive electrode, that larger the retention factor, the shorter the migration time.

Although this was also a preliminary result and not optimized, a remarkably reduced separation time was achieved, e.g., for Aba *ca.* 22 min in Figure 6-5, compared with *ca.* 43 min with the digitonin–SDS system in the previous study [14].   It should be noted that optical resolution of any Dns–DL-amino acids with the digitonin–STDC solution could not be achieved.   This suggests that digitonin plays a major role in optical resolution in the digitonin–STDC system.
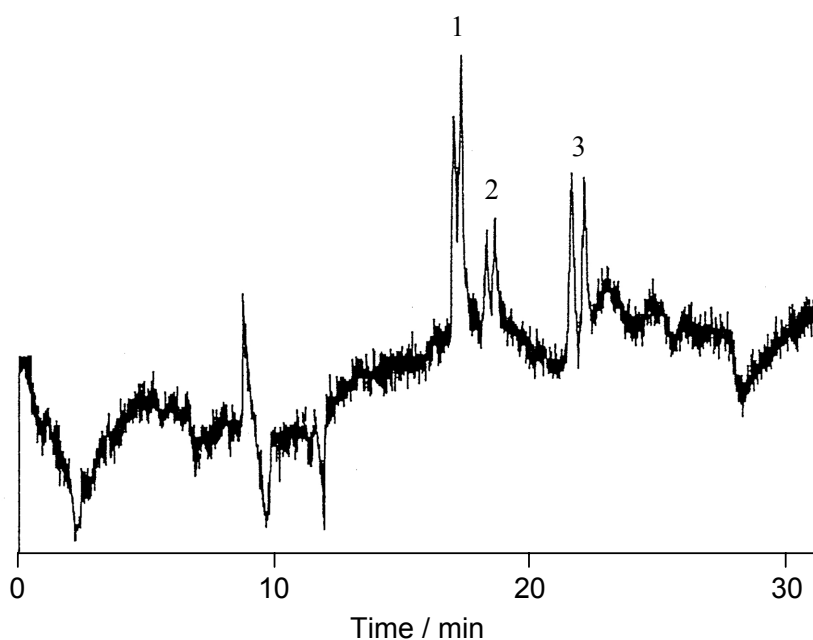

Figure 6-5.   Chiral separation of three PTH–DL-amino acids by MEKC with digitonin. 1 = Nva; 2 = Val; 3 = Aba.   Micellar solution, 50 mM digitonin–50 mM STDC–1 M urea (pH 2.5); capillary, 540 mm × 50 μm I.D.; effective length, 340 mm; total applied voltage, 17.5 kV (324 V cm^{-1}); current, 46 μA.   Other conditions are as in Figure 6-2.

6-4 Conclusions

The use of SDGlu–SDS–urea–methanol solutions could give good results in the optical resolution of PTH–DL-amino acids, although the resolution characteristic was not very different from that in SDVal–SDS micellar solutions.   The digitonin–STDC mixed micellar system was also effective for enantiomeric resolution of PTH–DL-amino acids, even using a shorter capillary than before.   At present, chiral separation by MEKC and by CZE has not been fully investigated, especially compared with HPLC.   Further applications on chiral separations of other compounds are being investigated with some other surfactants and additives.

6-5 References

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General Conclusions

In this thesis, studies on the effect of various additives on the separation to improve the analytical performances of EKC and the development of high performance analytical methods for several surfactants using CZE and MEKC were carried out.

In the Chapter 2, the use of smectite as the PSP in the EKC analysis of the PPEs with the different degree of ethoxylation was investigated. The retention by the smectite depended on the number of the EO groups and the sufficient separation of the homologues was achieved with the plate numbers in the range of 47,000–227,000 under an optimal condition. The proposed method was satisfactorily validated with the reproducibility of the migration time, separation efficiency and peak area. Furthermore, the identification of the PPE homologues in household products could be realized by comparison with the peak patterns of the sample with known composition and thus the average EO number was accurately calculated from their peak areas. On the other hand, under the sweeping condition, a 125-fold sensitivity enhancement for the PPE with a high retention factor was also achieved in smectite–EKC.

In the Chapter 3, a simple, rapid and simultaneous determination of four types of amphoteric surfactants and their C_8–C_{18}-homologues was performed by CE. To optimize the separation condition, effects of pH of BGS, organic modifier and chromophore for indirect UV detection on the CE separation of the amphoterics were investigated. Under an optimal condition, the 24 amphoteric analytes were completely separated in a single run within 17 min, and the developed method was satisfactorily

validated for the reproducibility, sensitivity and quantitativity.　Furthermore, the developed method could also provide a high resolution separation of the amphoteric surfactants in commercially available detergents and shampoo without any sample pretreatments.　These results demonstrated that the method was very useful for the identification and determination of the amphoteric surfactants in commercial products and could be used as a routine technique in different fields of industrial analyses.

In the Chapter 4, the application of CE to the separation of the FAE homologues was investigated.　The FAEs were derivatized with FMPTS to give a cationic charge and chromophore for UV detection prior to the CE analysis.　Under an optimal CZE condition, the FMPTS-derivatized FAEs with the average EO number of 6 were completely separated within 11 min.　In the CZE method, the resolution of highly ethoxylated homologues was poor, while in the MEKC using DTAC a complete separation of the FAEs containing up to 50 EO groups was achieved within 30 min.　It was confirmed that the accurate and precise determination of the distribution of the EO groups of the FAEs could be attained by the developed method.　Furthermore, the identification of the FAEs in household products could be also realized by the MEKC method with a simple sample cleanup.

In the Chapter 5, it was revealed that the use of DMSO and acetone as the organic modifiers in MEKC was effective for the separation of PAHs as well as other organic modifiers such as methanol, acetonitrile, and 2-propanol.　Acetone was more useful than DMSO because UV detection is feasible at shorter wavelengths.　In addition, critical micelle concentrations of SDS in buffers containing DMSO and acetone were

measured to calculate thermodynamic quantities.　They are much higher than those in an aqueous buffer without organic modifiers.

In the Chapter 6, optical resolution by MEKC with SDGlu as a new chiral selector and digitonin–STDC mixed micelles was investigated.　Addition of SDS, urea and methanol to SDGlu micellar solutions could give improved peak shapes and resolution.　Furthermore, five PTH–DL-amino acids were separated from each other and each pair of enantiomers was optically resolved with SDGlu.　On the other hand, three PTH–DL-amino acids were also successfully resolved with a digitonin–STDC–urea solution.

In conclusion, obtained findings throughout the studies will contribute the progress in CE and expand the application area of EKC.　In the present stage, the retention and separation characteristics of the smectite have not been fully clarified.　Therefore, the author is studying on the effects of structural factors and adsorptivity of the smectite to improve the separation efficiency and detectability in smectite–EKC and will apply the technique to the separation of various uncharged polar analytes.　Furthermore, the on-line sample preconcentration of the derivatized FAEs by cation-selective exhaustive injection and sweeping is currently under study to apply the developed method to environmental analysis.　More than 10,000-fold sensitivity enhancement for the FAEs has been already achieved.　Finally, the author believes that this thesis will become a milestone for further advances and spreading of CE, and contribute to advance of industry in the near future.

Future Perspectives

The obtained results throughout the studies should improve the analytical performances in CE.   In particular, the smectite–EKC technique is the most remarkable development since the smectite–EKC can achieve the highly effective separation of nonionic compounds with the characteristic retention mechanism which is different from conventional PSPs.   Furthermore, it should be noted that the highly effective on-line sample preconcentration of the PPEs was achieved in smectite–EKC.   Since the clay minerals interact with various polar compounds, alcohols and amines will be separated by using the smectite as the PSP.   To widen the applicability of EKC, therefore, other inorganic layered compounds such as mica, kaolin, and vermiculite can be applied to the EKC analysis.

Meanwhile, when an organizing reagent, e.g., quaternary ammonium salt, phosphonium salt, and imidazolium salt, is inserted into the layers of the clay minerals by their cation exchange properties, they can be dispersed in an organic solvent. Therefore, it is expected that these pro-organic clay minerals can allow to use a wide range of organic modifier content in the BGS, which will realize non-aqueous CE analysis.   Furthermore, the use of an intercalation complex with a chiral selector may provide a novel method for the optical resolution in CE.   In addition, when a clay mineral is dispersed in water at high concentrations, a house-of-cards structure will be formed due to the electrostatic platelets bonding and the solution becomes highly viscous or gel.   Hence, the clay minerals are also attractive materials for CGE and CEC as well as the EKC analyses.   Furthermore, the application of other detection methods such as fluorescence and MS detections will help to perform continuous EKC

studies using clay minerals in the future. This will lead to improved retention and resolution of target analytes. These specific characteristics of clay minerals should further expand the application area of CE and improve the analytical performances in CE. Therefore, the author considers that the use of clay minerals will be a noteworthy technique in CE.

Furthermore, the author will attempt the development of the technique for integrating in-capillary chemical derivatization using FMPTS with on-line sample preconcentration of the FAEs by the CE–UV analysis. Coupling of these techniques is effective for improving the method reliability, reducing total analysis time and facilitating high-throughput analyses. Although on-line sample preconcentration is not always necessary, this technique is useful for environmental samples containing the FAEs in ppb levels. Take into account the derivatization of hydroxyl groups in the FAEs, the application of non-aqueous CE using acetonitrile or tetrahydrofuran will be suitable for this strategy. The author considers that the realization of those proposed techniques should enhance the applicabilities in CE.

List of Publications

Chapter 2. "Separation of Nonionic Compounds by Electrokinetic Chromatography Using an Inorganic Layered Compound as a Pseudostationary Phase"
Ryo Koike; Fumihiko Kitagawa; Koji Otsuka
Journal of Separation Science **2008**, *31*, 829–836.

Chapter 3. "Simultaneous Determination of Amphoteric Surfactants in Detergents by Capillary Electrophoresis with Indirect UV Detection"
Ryo Koike; Fumihiko Kitagawa; Koji Otsuka
Journal of Chromatography A **2007**, *1139*, 136–142.

Chapter 4. "Separation of Fatty Alcohol Ethoxylates by Capillary Zone Electrophoresis and Micellar Electrokinetic Chromatography"
Ryo Koike; Fumihiko Kitagawa; Koji Otsuka
Journal of Separation Science **2009**, *32*, 399–407.

Chapter 5. "Separation of Lipophilic Compounds by Micellar Electrokinetic Chromatography with Organic Modifiers"
Koji Otsuka; Mitsuo Higashimori; Ryo Koike; Kaoru Karuhaka; Yukihiro Okada; Shigeru Terabe
Electrophoresis **1994**, *15*, 1280–1283.

Chapter 6. "Optical Resolution by High Performance Capillary Electrophoresis: Micellar Electrokinetic Chromatography with Sodium *N*-dodecanoyl-ʟ-glutamate and Digitonin"
Koji Otsuka; Masanori Kashihara; Yasushi Kawaguchi; Ryo Koike; Toshio Hisamitsu; Shigeru Terabe
Journal of Chromatography A **1993**, *652*, 253–257.

Acknowledgments

Ryo Koike

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