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Studies on

Micellar Electrokinetic Chromatography

Using Polymer Surfactants

and

Its Combination with Mass Spectrometry

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Contents

Chapter 1
Introduction

1. 1 MEKC

Capillary electrophoresis (CE) is a general name for the microscale separation techniques that employ narrow-bore capillaries and high electric fields. Since the first papers on CE [1-3] between 1979 to 1983, the technique has advanced rapidly and has become popular. The basic instrumental setup for CE is simple: it consists of a fused-silica capillary, a dc high voltage power supply, two buffer reservoirs with electrode assemblies and a detector as shown in Fig.1.1. After filling the capillary with a buffer, the sample is introduced from the inlet end of the capillary. The electrophoretic separation is performed by applying a high electric field and the separated compounds are detected by the detector placed near the outlet end of the capillary. Electroosmotic flow (EOF) caused by the negative charge on the wall of the fused silica capillary plays an important part of the mass transport in separation solution. In high performance liquid chromatography (HPLC), a liquid delivery pump is employed and hence the laminar flow causes dispersion of sample zone. In contrast, EOF employed in CE is close to the plug flow, which rarely contributes to the sample dispersion. Jorgenson et al. [2] demonstrated the potential of capillary electrophoresis as analytical technique with 75 μm i.d. capillaries, applied voltages up to 30kV and on-column fluorescence detection.

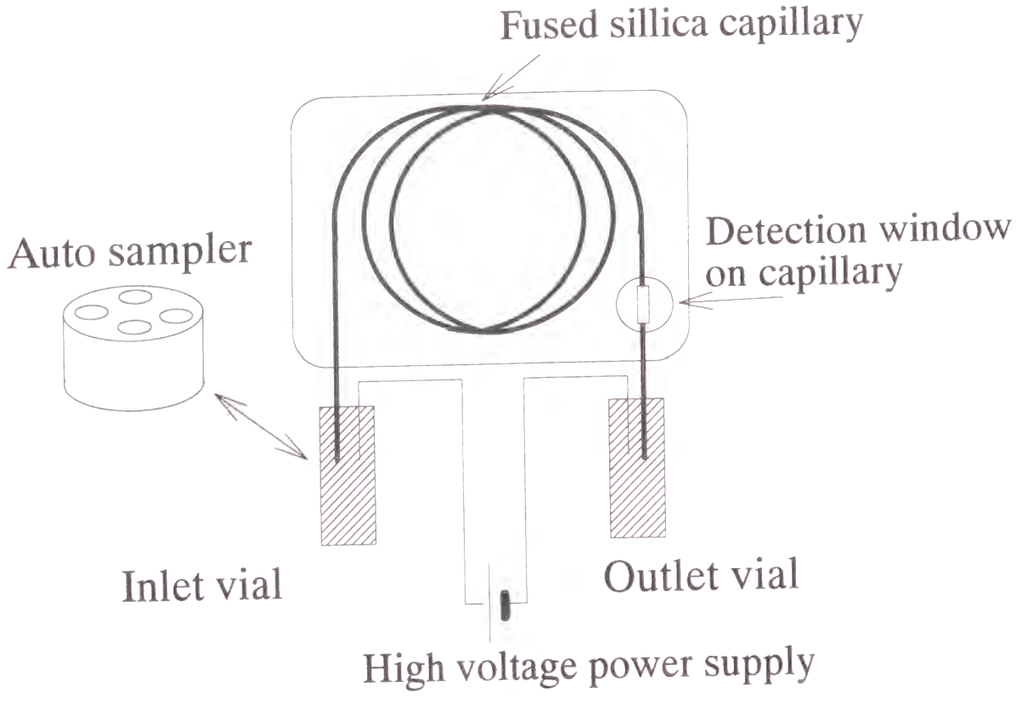


Fig. 1.1 Schematic illustration of CE

CE has various advantages in comparison with HPLC: (1) high separation efficiency, (2) high speed, and (3) economy of the separation system and sample size. CE is an instrumental technique different from the conventional electrophoresis and easy to automate. Several different separation modes have been developed: capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), capillary isotachophoresis (CITP), capillary isoelectric focusing (CIEF), capillary gel electrophoresis (CGE) and capillary electrochromatography (CEC). CE has some disadvantages in comparison with HPLC: (1) low detection sensitivity in terms of concentrations, (2) relatively poor reproducibility in quantitation. To solve the problems in low sensitivity many techniques have been advanced including on-column

concentration, the use of a long optical pass detection cells, new detection methods of high sensitivity such as laser excited fluorometry and mass spectrometry. Analytical reproducibility of CE has been improving in the automated instruments. The great potential for CE lies in the area of analytical biotechnology and pharmaceutical science, because there are real needs for micro-scale separation methods of proteins, nucleic acids, peptides and drugs. CZE, CGE and CIEF are applicable to the biopolymers and MEKC and CEC to organic small molecules.

MEKC [4-7] is a mode of CE and unique with respect to the capability of separating both non-ionic and ionic analytes. Fig.2 shows schematically the separation principle of MEKC for the separation of non-ionic analytes by using anionic surfactant micelles which are the case for most MEKC separations. The ionic surfactant added to the separation solution forms ionic micelles above the critical micelle concentration (CMC). When a high voltage is applied across the whole capillary length, the separation solution in the capillary is transported toward the cathode by EOF. The anionic micelle migrates toward the anode with its electrophoretic mobility. However, EOF is much stronger than the electrophoretic migration of the micelle, causing a retarded migration of the micelle also toward the cathode. When neutral analytes are injected from the anodic end, fractions of the analytes are incorporated by the micelle and migrate at the same velocity as that of the micelle. The remaining fractions are in the aqueous phase and migrate at the velocity of EOF. The distribution equilibria are quickly established and the neutral analytes are separated by the difference in the distribution coefficients between the micellar phase and the aqueous phase. The migration time (t_R) is limited between the migration time of the EOF (t_0) and that of the micelle (t_{mc}). The retention factor k of the analyte can be calculated by the equation [4]

$$k = (t_R - t_0)/t_0\ (1 - t_R/t_{mc})$$

On the other hand, the micelle works as the pseudo-stationary phase in MEKC and the volume of the micelle (V_{mc}) is directly related to the k through

$$k = K(V_{mc}/V_{aq})$$

where K is the distribution coefficient and V_{aq} is the volume of the aqueous phase excluding the micelle. V_{mc} is related to the concentration of surfactant (C_{surf}) through

$$V_{mc} = v(C_{surf} - \mathrm{CMC})$$

where v is the partial specific volume of the surfactant.

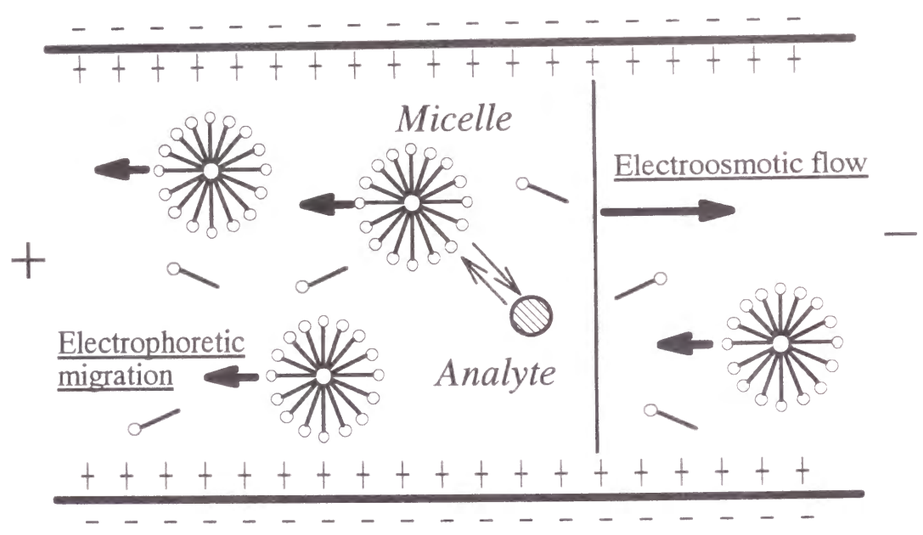


Fig. 1.2 Schematic illustration of separation principle of MEKC

MEKC has been successfully used and popular as a powerful tool for the separation and analysis since the first work of Terabe et al in 1984 [4]. As described above, MEKC is a hybrid of electrophoresis and chromatography. Almost all advantages of CE apply to MEKC as well: 1) MEKC is a high efficiency separation method and more

than 100,000 plates can be easily obtained. 2) MEKC is an ultramicroscale analytical method. In general, a few nL of the sample solution or a few pg the analyte is injected and about a few mL of the running solution is sufficient for successive runs. 3) MEKC is an instrumental technique and easy to automate. The great potential for MEKC lies in the area of pharmaceutical analyses involving acidic, basic and neutral drugs. Many applications of MEKC have been studied including purity testing, analysis of drugs in biomedical matrices and separation of enantiomers. MEKC is especially powerful for the separation of complex mixtures such as natural products, hence it is also suitable for food analyses. Whereas the other modes of CE are the separation techniques of ionic analytes only, MEKC and CEC are capable of separating both ionic and non-ionic analytes. CEC is a chromatographic method using a capillary column packed with fine packing material and EOF for the delivery of the mobile phase through the capillary column. Therefor, CEC holds a position between reversed phase (RP)-HPLC and MEKC. MEKC is advantageous over CEC because of easy changing of separation conditions, low cost of columns and easy reconditioning of columns by rinsing.

MEKC has similar disadvantages as described above on CE. Moreover, there are some limitations associated with conventional micelles used as pseudo-stationary phases on MEKC: (1) effect of temperature on reproducibility, (2) separation of highly hydrophobic analytes (3) MS detection. In particular, the problem of the relatively low reproducibility is enhanced by the change in the concentration of the micelle which is in equilibrium with the free surfactant molecules in the aqueous phase. As described above, changes in CMC will cause changes in k. A change in temperature will cause a change in CMC and K. Therefore, in comparison with CE, temperature effects will be more critical in MEKC using the micelle in dynamic equilibrium. To optimize the separation

of hydrophobic analytes by MEKC, some additives to the separation solution are needed. In many cases, organic solvents miscible with water are used. However, a high concentration of the organic solvent generally breaks down the micelle. In contrast to HPLC, the adjustment of the separations by the addition of the organic solvent is not always easy in MEKC. Difficulty of on-line coupling with the MS detection due to the presence of a significant amount of the surfactant diminishes the value of MEKC because in the other CE modes including CZE and CITP, on-line coupling with MS is achieved by using volatile buffer system in the separation solution.

1.2 Pseudo-stationary phase of MEKC

Selection of the surfactant micelle as the pseudo-stationary phase is important in MEKC because the separation is achieved by the chromatographic separation principle combined with the capillary electrophoretic technique. Long-alkyl-chain surfactants, such as sodium dodecyl sulfate (SDS), have been employed in most MEKC. In general, these surfactants form spherical micelles having ionic groups on the surface and the hydrophobic core. In the case of SDS micelle, the CMC is about 8 mM and the aggregation number is about 60 in water. For the pseudo-stationary phase of MEKC, SDS micelle has been successfully used and widely accepted because of several advantages: 1) relatively low CMC, 2) weak absorption in the UV region, 3) good solubility in water, 4) reasonable cost in high purity, 5) a low Krafft point (15 ℃ in water). Effect of the surfactant structure on selectivity in MEKC has been described in many papers including several reviews [5-7]. In the case of the long alkyl chain surfactants such as SDS, the hydrophilic group or polar group is generally important in determining selectivity. For example, the distribution coefficients of some test solutes were significantly different

between SDS and sodium dodecanesulfonate [8]. Bile salts, such as sodium cholate, sodium deoxycholate, sodium taurocholate, are supposed to form helical micelles and show selectivity significantly different from the long-alkyl-chain surfactants [9, 10]. These conventional surfactants used as pseudo-stationary phases in MEKC are low molecular mass surfactants and form the micelles in dynamic equilibria.

Polymer surfactant is a general term for oligomers of surfactant monomers or polymers that show surface active properties as a whole. The former is named polysoaps or micelle polymers because they are synthesized from reactive low molecular mass surfactant in micellar forms. The latter is amphiphilic copolymers synthesized from hydrophilic and hydrophobic monomers. Polymer surfactant has been shown to be useful for a variety of practical applications in recent studies, as protective colloids, emulsifiers, drug delivery media and separation media [11]. Polymer surfactant is considered to form the micelle from a single molecule, which may be called a molecular micelle. The CMC of the molecular micelle can be zero or meaningless. Although the micelle formed from low molecular mass surfactant exists in a dynamic equilibrium, the molecular micelle is stable as shown in Fig.1.3. Therefore, the polymer surfactant is expected to show superior characteristics as a pseudo-stationary phase in MEKC. Compared to low molecular mass surfactant, polymer surfactant is relatively unknown as the pseudo-stationary phase yet. Palmer et al. utilized the micelle polymer synthesized by polymerization of sodium 10-undecylenate [12] and sodium 10-undecenyl sulfate [13] for MEKC. As described in this thesis, the author utilized some amphiphilic copolymers: butyl acrylate-butyl methacrylate-methacrylic acid copolymer sodium salts (BBMA) [14], butyl methacrylate-methacryloyloxyethyltrimethylammonium chloride copolymer (BMAC) [15], and diisobutylene-maleic acid copolymer sodium salt (DMA) [16]. The

other micelle polymers [17, 18] and amphiphilic copolymers [19] were also utilized in MEKC by other groups. Tanaka and co-workers [20] and others [21] synthesized several starburst dendrimers and used as pseudo-stationary phases in electrokinetic chromatography.

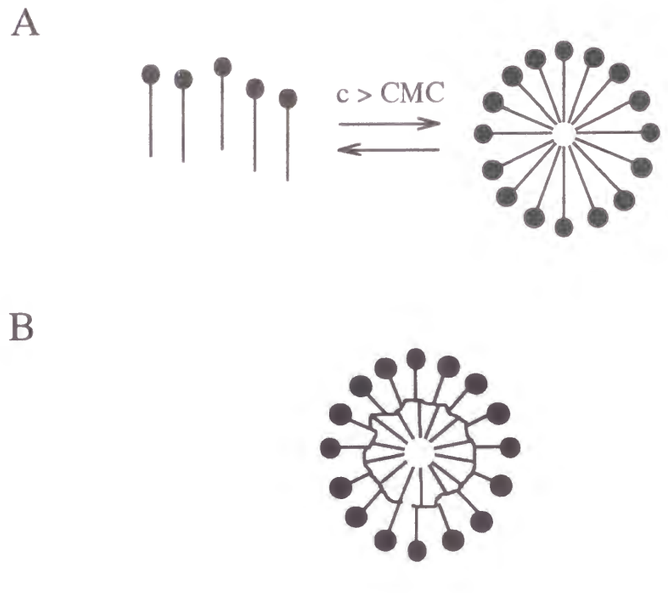


Fig. 1.3 Micellar models of low molecular mass surfactant (A) and polymer surfactant (B)

1.3 MS detection in MEKC

Same as in HPLC, the most popular detection method in MEKC is UV photometry. In general, commercial automated CE instruments are equipped with a UV or UV diode-array detector. However, the UV detector does not have enough capability as a sensitive and specific detector to solve problems of increasing sample complexity or decreasing sample quantities in recent analytical demands. MS is one of the most powerful detection methods of CE because of the high sensitivity, high selectivity and structural information on the analytes. The combination of CE and MS needs an interface transferring analytes from the CE capillary to the mass spectrometer without

sacrificing separation efficiency and detectability. Several new ionization methods have been developed for MS: including fast atom bombardment (FAB), thermospray (TSP), electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and matrix-assisted laser desorption ionization (MALDI). All of them except for TSP are capable of producing ions from the condensed phase without high temperature and applicable to the polar compounds readily ionizable in solution.

On-line coupling of CE with MS using ESI was first reported in 1987 [22]. ESI is suitable for the on-line coupling of MS with liquid phase separation techniques such as HPLC and CE, because gas-phase ions of the analyte are produced efficiently under the atmospheric pressure. Recently, studies of the CE-ESI-MS system have been reported by many groups [23-27] and this technique has become popular. However, the ESI-MS detection for MEKC was reported in only one paper before 1995 [28], in which a special separation capillary system for MEKC with SDS was employed to avoid introducing surfactant molecules to the ESI-MS detector. The conventional MEKC system with SDS can not be coupled with ESI-MS because the surfactant molecules introduced into the ESI-MS system cause a decrease of sensitivity, generation of the surfactant related spectra and contamination of the interface. SDS generates stable sodium attached ions in the positive ionization mode ESI-MS or sulfate ion in the negative mode ESI-MS. The decrease of the detection sensitivity caused by SDS micelle is probably due to easier ionization of excessive free SDS molecule than the analyte molecule. To make MEKC-MS possible, the polymer surfactant was employed as a pseudo-stationary phase in this study. Polymer surfactant is expected to be an ideal pseudo-stationary phase of MEKC coupled with ESI-MS because of the stability and the high molecular mass. In contrast to low molecular mass surfactant, polymer surfactant micelles will be stable even in ESI-MS

system because of a single covalently bonded molecule as shown in Fig.1.3. As described in this thesis, BBMA [29] and DMA [16] were successfully employed as pseudo-stationary phases and several standard compounds were separated by MEKC and detected by ESI-MS. However, a decrease in detectability was observed with an increase of the concentrations of polymer surfactants [16, 30]. Details of the ionization mechanism of ESI are complex and theoretical studies have been developed. According to Kebarle et al. [31], there are four major processes as shown in Fig. 1.4: formation of charged droplets at the capillary tip by an ion separation mechanism, reduction in size of the charged droplets by solvent evaporation, repeated droplet disintegration and formation of gas-phase ions from the extremely small and highly charged droplets.

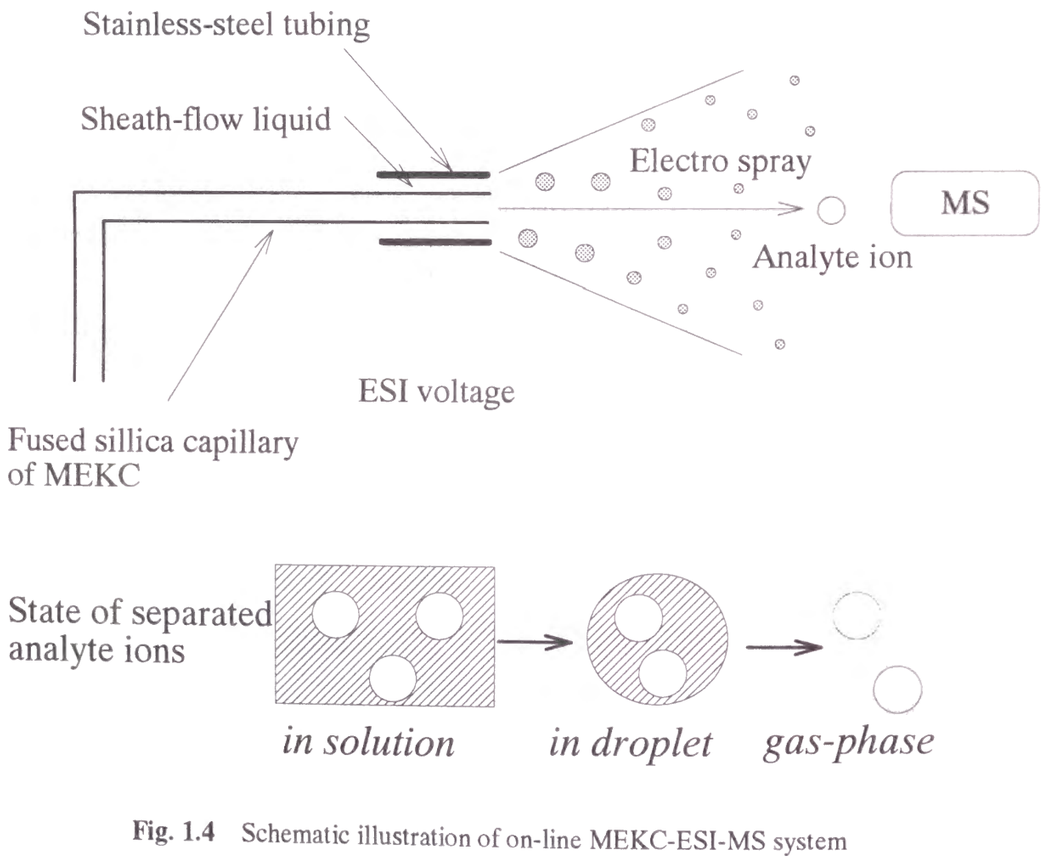


Fig. 1.4 Schematic illustration of on-line MEKC-ESI-MS system

Not only the effects of surfactants but also those of the other factors of separation solution including salt concentration, pH and the content of the organic solvent are not clarified yet. Effects of surfactants on ESI were reported [23, 30, 32], but they were still elementary. The difference in ESI mechanisms between polymer surfactant and low molecular mass surfactant was proposed [33], but it was not verified experimentally. The details of the ESI mechanism will be clarified by future advances.

For the on-line coupling of MEKC and ESI-MS, the other several techniques have been developed. Lamoree et al. [28] employed a heart-cut technique, where neutral analytes were separated by MEKC in the first capillary filled with a micellar solution and the separated zones were transferred to the second capillary through a liquid junction. The second capillary was filled with a buffer solution containing only volatile electrolytes and used to transfer the analyte zone to the ESI interface. We have employed a partial filling technique to prevent the micelle from entering the MS interface. Fig.1.5 shows the schematic illustration of the partial filling method of MEKC (PF-MEKC). The capillary is filled with buffer, followed by the introduction of a micellar zone and finally by the sample injection. By applying a high electric field, the MEKC separation is performed while the sample passes through the micellar zone and the separated analytes migrate into the buffer zone free from the surfactant. PF-MEKC was first introduced by the author as a potential technique for on-line coupling with ESI-MS [34]. The group of the author [30, 35] and Nelson et al. [36, 37] discussed the capability and mechanism of PF-MEKC.

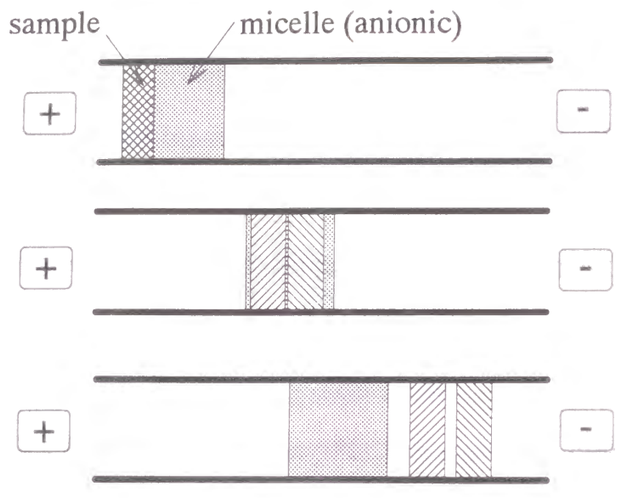


Fig. 1.5 Schematic illustration of PF-MEKC

An APCI interfacing for coupling with CE has been developed by Takada et al. [38, 39]. The interface between CE and MS consists of an electrospray-type nebulizer, a vaporizer, and an atmospheric pressure chemical ionization source using needle electrode for the corona discharge, and this system is called electrospray-chemical ionization (ES-CI) interface. In the APCI mode, molecules are ionized by the corona discharge followed by ion/molecule reactions. With the make up solvent such as methanol, methanol is first ionized and analyte molecules having stronger proton affinity than methanol will be easily protonated by the ion/molecule reactions. APCI is expected to be a useful interfacing technique for the coupling with CE including MEKC because ionization processes are not significantly affected by the presence of salt in CE buffers. Takada et al. reported on-line coupling of APCI-MS with the CE system with sodium phosphate buffer [38] and the MEKC system with SDS[39]. With the same interfacing device, the authors utilized BBMA as pseudo-stationary phase and successfully demonstrated MEKC-APCI-MS in preliminary work [40]. As described in this thesis,

the authors utilized some conventional surfactants including SDS and sodium cholates as pseudo-stationary phases in MEKC-APCI-MS [16].

On-line coupling between CE and FAB-MS have been reported with a liquid junction [41] or a coaxial sheath flow [42]. The FAB method generally requires a viscous liquid component in the solvent system which is introduced from the liquid junction or the coaxial sheath flow interface. Off-line coupling between MEKC and FAB-MS was tried, but SDS in separated fraction caused the decrease of sensitivity [43].

1.4 Purpose of the work

In this thesis, the author reports further developed techniques of MEKC; use of polymer surfactants as pseudo-stationary phases and direct coupling between MEKC and MS. In comparison with RP-HPLC or CEC, the most striking characteristic of MEKC is that no solid packing material is necessary as the separation media. MEKC is composed of an open tubular capillary, a homogeneous solution of the mobile phase and the pseudo-stationary phase and is easy to perform and powerful. If we can develop new pseudo-stationary phases having advantages over conventional low molecular mass surfactants, MEKC will be a more useful and powerful separation technique. As an alternative pseudo-stationary phase, polymer surfactant is expected to be advantageous as described in section 1.2: zero CMC, stability and MS detection. However, only one type of polymer surfactant, micelle polymer synthesized from reactive low molecular mass surfactant in the micellar form, was reported as a pseudo-stationary phase by Palmer et al. before 1994 [12]. The other type of polymer surfactant, amphiphilic polymer synthesized by co-polymerization of hydrophilic and hydrophobic monomers, was not reported previously. The amphiphilic copolymers have some advantages as the

pseudo-stationary phase: 1) Some of them are commercially available because of the industrial uses. 2) Monomers are also available and most of them are inexpensive. 3) Studies on the synthesis of them have been developed well. Therefore, utilization of amphiphilic copolymers as pseudo-stationary phases is interesting and important. One of the purpose of this thesis is the basic study on the amphiphilic copolymers as pseudo-stationary phases in MEKC.

As the other separation analytical techniques, MS will be the most powerful detection method of MEKC because of high sensitivity, high selectivity and amount of information on analytes. As the ionization technique for the coupling of liquid phase separation techniques such as HPLC and CE, ESI has been successfully used and popular. However, only one study on the on-line MEKC-MS with ESI was reported by Lamoree et al. before 1995 [28]. To solve the problems of low detectability of ESI caused by SDS, they employed the heart-cut technique described in 1.3. This technique is unique and ingenious taking the advantage of MEKC separation. However, this technique has limited applicability, because the special device is needed, analytes are limited to UV detectable species and the continuous detection of multiple peaks is difficult. An ideal MEKC-MS system should be able to detect continuously all analytes including non-UV-detectable analytes. To generalize a new method, conventional instruments should be used without special devices. Polymer surfactants are promising pseudo-stationary phases for the direct coupling of the conventional MEKC with ESI-MS without special devices. Another purpose of this thesis is the basic study on the on-line coupling of the conventional MEKC with ESI-MS using polymer surfactants. In addition to this study, a partial filling method and a coupling with APCI-MS are studied. The partial filling of the micellar zone in the capillary is used to avoid introducing micelles to ESI-MS without

special devices. APCI-MS can be used for the on-line coupling with MEKC using conventional low molecular mass surfactants.

As results of this work, several advantages of polymer surfactants including zero CMC, unique selectivity, and MS detection were verified and polymer surfactants were given a place in the practical and useful pseudo-stationary phase for MEKC. In near future, the MEKC should be indispensable as a separation technique with wide applications. The MS detection coupled with liquid phase separation techniques should be one of the key techniques of analytical chemistry. In the case of coupling with HPLC, the potential of MS detection is clear because the biologically important compounds and pharmaceuticals are suitable for the separation by HPLC and for the detection by MS or MS/MS with higher sensitivity and better selectivity than the UV detector. Therefore, the on-line coupling between MEKC and MS should be needed as a separation method both with high separation efficiency and high amount of information on analytes. Several techniques presented in this thesis for MEKC-MS includes ESI using polymer surfactant as pseudo-stationary phase, ESI combined with the partial filling technique and APCI. Each techniques gave promising results on this field.

1.5 Abstract

BBMA is a synthetic polymer surfactant whose hydrophilic site is methacrylic acid and hydrophobic site is butyl acrylate-butyl methacrylate. BBMA was utilized successfully as a negatively charged pseudo-stationary phase in MEKC. Fundamental study of utilization of BBMA as a pseudo-stationary phase for MEKC is presented in Chapter 2.

Advanced MEKC techniques with polymer surfactants are presented in Chapter 3.

As BBMA, BMAC was successfully employed as a pseudo-stationary phase. Effects of the additives were studied in MEKC with BBMA. It should be emphasized that BBMA is advantageous in chiral separation by cyclodextrin (CD) modified MEKC. Enantiomers of dansylated-DL-amino acids were separated with BBMA and β-CD system, giving larger separation factors than those obtained with SDS and β-CD system because of the lack of interaction between surfactant molecules and β-CD.

In Chapter 4, the first demonstration of the on-line coupling of conventional MEKC with MS is presented. Conventional MEKC system with SDS can not be coupled with ESI-MS because the surfactant molecules introduced into the ESI-MS system cause a decrease of sensitivity, generation of surfactant related spectra and the contamination of the interface. In contrast to low molecular mass surfactant micelles, polymer surfactant micelles will be stable in ESI-MS system because of the micellar structure of a single covalently bonded molecule. In practice, no major ion from the BBMA polymer was detected by ESI-MS. The on-line ESI-MS detection system was consisted of a modified Hitachi M-1000 LC-API-MS system and a laboratory built ESI interface. Details of the optimization of the ESI interface coupled with MEKC separation system using BBMA are discussed in Chapter 4. The BBMA micelle functioned successfully as a pseudo-stationary phase and some standard compounds were separated by MEKC and detected by MS.

In Chapter 5, the effect of the concentration of surfactants on sensitivity was studied with the direct injection to the ESI-MS. The high BBMA concentration degraded significantly ESI-MS signal intensity. Therefore, PF-MEKC with BBMA was investigated. PF-MEKC was applied to the ESI-MS detection of caffeine and its metabolites.

In Chapter 6, MEKC-ESI-MS using the other polymer surfactant was employed.

DMA functioned successfully as a pseudo-stationary phase and showed significantly different selectivity in comparison with BBMA. Furthermore, the APCI interface was applied to the on-line MS detection. APCI should be suitable as ESI because gas-phase ions of analytes are produced efficiently under the atmospheric pressure. On-line MEKC-APCI-MS using a conventional pseudo-stationary phase was demonstrated.

Many applications reported in Chapters 4 to 6 show the usefulness of the on-line MEKC-MS system. The mixture composed of a quaternary ammonium salt, an aromatic amine, alkaloid, a quaternary phosphonium salt and non-ionic surfactant and the mixture of four sulfonamides were studied in chapter 4. Seven pharmaceuticals and six industrial surfactants were separated and detected with conventional MEKC system and the mixture of caffeine and its metabolites were studied with PF-MEKC system in Chapter 5. Some pharmaceuticals were studied with APCI-MS system in Chapter 6.

References

1 F. Mikkers, F. Everaerts and T. Verheggen, *J. Chromatogr.*, **169** 11-20 (1979).

2 J. Jorgenson and K. Lukas, *Anal. Chem.*, **53**1298-1302 (1981)

3 S. Hjerten, *J. Chromatogr.*, **270** 1-6 (1983).

4 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal Chem.*, **56** 111-113 (1984)

5 J. Vindevogel, P. Sandra, *Introduction to Micellar Electrokinetitic Chromatography*, Huthing, Heidelberg, 1992.

6 S. Terabe, in N. Guzman (Editor) *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, pp. 65-87.

7 S. Terabe, N. Chen and K. Otsuka, *Adv. Electrophoresis*, 787-153 (1994).

8 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, **57** 834 (1985).

9 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, **513** 279 (1990).

10 R. O. Cole, M. J. Sepaniak, W. C. Hinze, J. Gorse and K. Oldiges, *J. Chromatogr.*, **557** 113 (1991).

11 A. Laschewsky, *Adv Polym Sci*, **124** 3-86 (1995)

12 C. P. Palmer, M. Y. Khaled and H. M. McNair, *J. High Resolut. Chromatogr.*, **15** 756-762 (1992).

13 C. P. Palmer and S. Terabe, *Anal Chem.*, **69** 1852-1860 (1997).

14 H. Ozaki, A. Ichihara and S. Terabe, *J. Chromatogr.*, **680** 117-123 (1994).

15 H. Ozaki, A. Ichihara and S. Terabe, *J. Chromatogr.*, **709** 3-10 (1995).

16 H. Ozaki and S. Terabe, *Bunseki Kagaku*, **46** 421-427 (1997).

17 J. Wang and I. M. Warner, *Anal. Chem.*, **66** 3773-3776 (1994).

18 A. Dobashi, M. Hamada and Y. Dobashi, *Anal. Chem.*, **67** 3011-3017 (1995).

19 S. Y. Yang, J. G. Bumgarner and M. G. Khaledi, *J. High Resolut. Chromatogr.*, **18** 443-445 (1995).

20 N. Tanaka, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki and S. Terabe, *Chem.Lett.* 959-962 (1992).

21 S. A. Kuzdzal, C. A. Monning, G. R. Newkome and C. N. Moorefield, *J. Chem. Soc., Chem. Commun.*, 2139-2140 (1994).

22 J. A. Olivares, N. T. Nguyen, C. R. Yonker and R. D. Smith, *Anal. Chem.*, **59** 1230-1232 (1987).

23 R. D. Smith, C. J. Barinaga and H. R. Udseth, *Anal. Chem.*, **60** 1948-1952 (1988).

24 H. R. Udseth, J. A. Loo and R. D. Smith, *Anal. Chem.*, **61** 1989 (1989).

25 R. D. Smith, J. H. Whal, D. R. Goodlett and S. A. Hofstadler, *Anal. Chem.*, **65** 574 (1993).

26 J. H. Wahl and R. D. Smith, J. Cap. Elec., 1(1994)62.

27 I. M. Johasson, R. Pavelka and J. D. Henion, *J. Chromatogr.*, **559** 515-528 (1991).

28 M. H. Lamoree, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, **712** 219 (1995).

29 H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi and H. Koizumi, *J. Chromatogr.*, **716** 69-79 (1995).

30 H.Ozaki, S. Terabe, *J. Chromatogr.*, in press.

31 P. Kebarle and L. Tang, *Anal. Chem.*, **65** 972A-986A (1993).

32 J. Varghese and R. B. Cole, *J. Chromatogr.*, **652** 369-3728 (1993).

33 K. L. Rundlett and D. W. Armstrong, *Anal. Chem.*, **68** 3493-3497 (1996).

34 S. Terabe, H. Ozaki, Y. Takada, M. Sakairi and H. Koizumi, *Proceedings for the seventh International Symposium on High Performance Capillary Electrophoresis* (1995) p53.

35 K. Koezuka, H. Ozaki, N. Matsubara and S. Terabe, *J. Chromatogr., B*, **689** 3-11 (1997).

36 W. Nelson, Q. Tang, A. Harrata and C. Lee, *J. Chromatogr.*, **749** 219-226 (1996).

37 L. Yang, A. Harrata and C. Lee, *Anal. Chem.*, **69** 1820-1826 (1997).

38 Y. Takada, M. Sakairi and H. Koizumi, *Anal. Chem.*, **67** 1474-1476 (1995).

39 Y. Takada, M. Sakairi and H. Koizumi, *Rapid Commun. Mass Spectrom.*, **9** 488-490 (1995).

40 H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi and H. Koizumi, *Bunsekikagaku-Toronkai- Koenyoshishu,* **56** 181-182 (1995).

41 R. Caprioli, W. Moore, M. Martin and B. Dague, *J. Chromatogr.*, **480** 247 (1989).

42 J. DeWit, L. Deterding, M. Moseley, K. Tomer and J. Jorgenson, *Rapid Commun. Mass Spectrom.*, **2** 100 (1988).

43 A. Lecoq, S. Biase and L. Montanarella, *J. Chromatogr.*, **638** 363-373 (1993).

Chapter 2
MEKC using butyl acrylate-butyl methacrylate-methacrylic acid copolymers sodium salts as pseudo-stationary phases

2.1. Introduction

Micellar electrokinetic chromatography (MEKC) [1-4], which uses an ionic micellar solution as a separation solution, is a mode of capillary electrophoresis (CE). CE is a separation technique of ionic analytes only, whereas MEKC is capable of separating both ionic and non-ionic analytes. Almost all advantages of CE apply to MEKC as well and many applications of MEKC separations have been reported [3,4]. The MEKC separation is based on the differential partitioning of an analyte between the micelle, which is a pseudo-stationary phase, and the surrounding aqueous phase, and therefore the choice of surfactants and modifiers of the aqueous phase is important for manipulating separation selectivity [5]. The effect of surfactant structure on selectivity has been discussed elsewhere [5]. It is generally recognized that different surfactants show different selectivity. In particular, the polar group of the surfactant affects selectivity more significantly than the hydrophobic group. Most surfactants have a long alkyl chain as a hydrophobic group but some have different structures: semiplanar structures such as bile salts or multiple chain structures such as lecithins or some synthetic surfactants [6]. These surfactants are known to have some advantages over the single alkyl-chain surfactants: bile salts have low solubilizing capability [7-9] and can recognize chirality [10-14]; a double-chain surfactant has shown significantly different selectivity [6]. The micelle is in

equilibrium with the monomeric surfactant, whose concentration is called critical micelle concentration (CMC) and is constant irrespective of the concentration of the surfactant. Since the micelle works as the pseudo-stationary phase in MEKC, the volume of the micelle, *V*mc, is directly related to the retention factor, *k*, through

$$k = K(V\text{mc}/V\text{aq}) \quad (1)$$

where *K* is the distribution coefficient and *V*aq is the volume of the aqueous phase excluding the volume of the micelle. The volume of the micelle is given as

$$V\text{mc} = \text{v}(C\text{srf} - \text{CMC}) \quad (2)$$

where v is the partial specific volume of the surfactant forming the micelle and *C*srf is the concetration of the surfactant. CMC depends on experimental conditions such as temperature, salt concentration and other additives. When a high voltage is applied across the capillary length, the temperature inside the capillary will rise due to Joule heating even with a thermostated capillary [15-18]. The temperature rise of the running solution inside the capillary probably causes a change in CMC, the distribution coefficient and hence the retention factor in addition to the viscosity. Therefore, the effect of the temperature rise on the migration time will be more serious in MEKC that in capillary zone electrophoresis (CZE).

Polymer surfactants called oligo-soaps or poly-soaps are oligomers of monomeric surfactants or polymers that show surface active properties as a whole. The polymer surfactant is considered to form the micelle from a single molecule, which may be called a molecular micelle. The CMC can be zero or meaningless. Therefore, we can expect a constant concentration of the micelle for the polymer surfactant irrespective of the experimental conditions. Although the micelle formed from low molecular mass

surfactants exists in a dynamic equilibrium and has a limited life time less than 1 s, the molecular micelle is stable. Therefore, the polymer surfactant is expected to show different characteristics for the use in MEKC. The size of the micelle has a distribution, which contributes to the band broadening in MEKC [19]. The effect of the size distribution on efficiency is significant only for analytes having a large retention factor [19]. The effect of the size distribution can be leveled out by the dynamic exchange of the micellar size in the case of low-molecular surfactants. The distribution of the micellar size of the polymer surfactant will be wider than that of the low-molecular surfactant and may adversely affect efficiency of MEKC. However, the polymer surfactant will have the other advantages over the low molecular surfactant: a high content of organic solvent will not break down the micelle; very low concentration of the micelle will be available; no monomeric surfactant that does not contribute to the separation is present. So far, only one polymer surfactant has been reported for MEKC; Palmer et al. [20, 21] synthesized undecylenate oligomer by polymerizing micellized sodium 10-undecylenate in aqueous solution. The oligomer was successful for the separation of hydrophobic compounds with relatively high concentrations of acetonitrile. Butyl acrylate-butyl methacrylate-methacrylic acid copolymers sodium salts (BBMA) are a group of polymer surfactants, whose molecular structure is shown in Fig. 2.1. We tried to utilize BBMA as a pseudo-stationary phase for MEKC [22].

This chapter describes some characteristics of BBMA as the pseudo-stationary phase in MEKC. Some other natural or synthetic polymer surfactants were also examined for use as pseudo-stationary phases in MEKC.

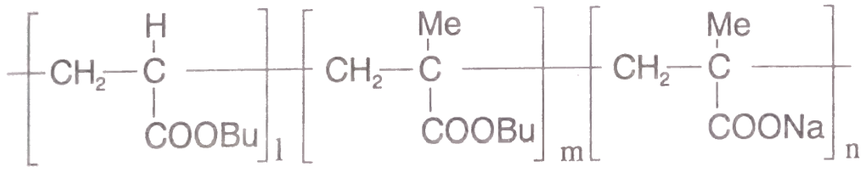


Fig. 2.1 Molecular structure of BBMA: Me, methyl; Bu, butyl.

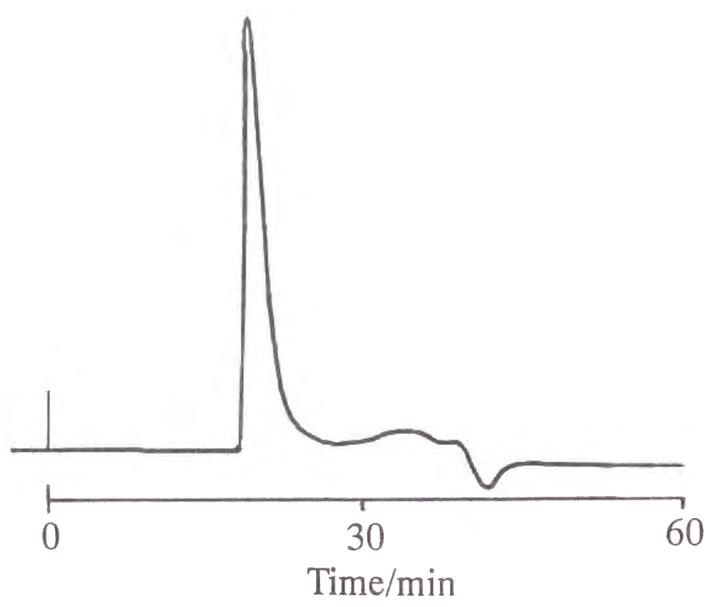


Fig. 2.2 Size exclusion chromatogram of BBMA. Column, TSK-gel G3000SW + G2000SW; mobile phase, 50 mM NaCl containing 20% acetonitrile; flow rate, 1 mL min^{-1}; temperature, ambient; detector, refractometer.

2.2 Experimental

2.2.1 Reagent

BBMAs were supplied by Dai-ichi Kogyo Seiyaku (Kyoto, Japan) as aqueous solutions. The molecular mass of the BBMA was about 40000 from size exclusion chromatography (SEC) using standard polyethylene glycols. Since BBMA contained a minor amount of low molecular mass components, it was purified by a reprecipitation method as follows: a portion of the BBMA solution was mixed with 50 portions of acetone; a precipitated polymer was separated by decantation and dried in vacuo at room temperature. Thus purified BBMA was used in this work if it is not mentioned otherwise. Three grades of BBMA having different content of methacrylic acid (MAA) were used: 50%, 40%, and 30% of MAA with the same composition of butyl acrylate/butyl methacrylate. Alginic acid, carboxymethylcellulose sodium salt, and poly (N-vinyl-2-pyrrolidone) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). SDS and Chitosan were from Nacalai Tesque (Kyoto, Japan). Other reagents were of analytical grade and water was purified with a Milli-Q system. Phenanthrene was used as a tracer of the micelle. Sample solutes were dissolved in 25% aqueous methanol, which was also a marker of the electroosmotic flow.

2.2.2 Apparatus

MEKC was performed with a Bio-Rad BioFocus 3000 (Hercules, CA, U.S.A.) using a fused silica capillary of 36.5 cm (32 cm to the detector) x 50 μm i.d. obtained from Polymicro Technologies (Phoenix, AZ, U.S.A.). The temperature of the capillary was thermostated at 30°C. Samples were injected by the pressurization method and detected at

210, 250, and 280 nm simultaneously under the multi-wavelength mode. The electropherograms were recorded at 210nm. SEC was carried out with a Shimadzu LC-9A liquid delivery pump (Kyoto, Japan) and a Shodex RI SE-51 refractive index detector (Tokyo, Japan) using a Tosoh TSK-gel G3000SW (60 cm x 8mm i.d.) and G2000SW (60 cm x 8 mm i.d.) (Tokyo, Japan) at room temperature. A sodium chloride solution (50 mM) containing 20% acetonitrile was employed as a mobile phase.

2.3 Results and discussion

2.3.1 SEC of BBMA

A size exclusion chromatogram of BBMA is shown in Fig. 2.2. The main peak was eluted early and assigned to BBMA. The peak is relatively sharp and hence the molecular mass will not be widely distributed. The weak negative peak was due to the sample solvent, water. The low and broad peak between the two peaks was considered to show a low molecular compound. Almost the same chromatogram was observed for the purified BBMA as described in Experimental Section.

2.3.2 Separation by MEKC with BBMA and other polymer surfactants

MEKC separations of three test mixtures, benzene derivatives, cold medicines, and naphthalene derivatives, are shown in Fig. 2.3, obtained with unpurified BBMA. The benzene derivatives were successfully separated, as shown in Fig. 2.3A, and the migration order was the same as that obtained with SDS [2]. The separation of the cold medicines was not very successful, as shown in Fig. 2.3B. The retention factors were too small for the cold medicines but the migration order was the same as that observed with SDS. The

naphthalene derivatives were well resolved, as shown in Fig. 2.3C. The migration order of the naphthalene derivatives was significantly different from that obtained with SDS. Efficiency was slightly lower than that usually obtained with low molecular surfactants, but it was still high enough for most purposes. The high efficiency shown in Fig. 2.3 suggests that the distribution of the molecular mass of BBMA does not cause a serious loss of efficiency. The separation selectivity was extremely different especially for naphthalene derivatives in comparison with that observed using SDS. In particular, it is interesting that 1-naphthol migrated much slower than 1-naphthalenemethanol or 1-naphthaleneethanol. This has also been observed with a double-chain surfactant, 5,12-bis(dodecylmethyl)-4,7,10,13-tetraoxa-1,16-hexadecanedisulfonate (DBTD) [6]. The cold medicines had lower retention factors than those with SDS, which is probably due to the difference in the polar group of the surfactants: a carboxyl group in BBMA, whereas a sulfate group in SDS. A similar difference in selectivity has also been observed between SDS and sodium trioxyethylene alkyl ether acetate (ECT), which has a carboxyl group [23]. Timepidium bromide, which is often used as a good tracer of the SDS micelle [24], migrated faster than Sudan IV and phenanthrene, which had the same migration times. Therefore, timepidium bromide cannot be used as a tracer of the BBMA micelle.

Solutions (2%) of alginic acid and carboxymethylcellulose in the phosphate-borate buffer (pH 8.0) were viscous and not suitable for use in MEKC.

A 0.5% solution of chitosan in 1 N phosphoric acid was employed in MEKC. No resolution was obtained for the test mixtures but only a broad single peak was observed by applying -5 kV.

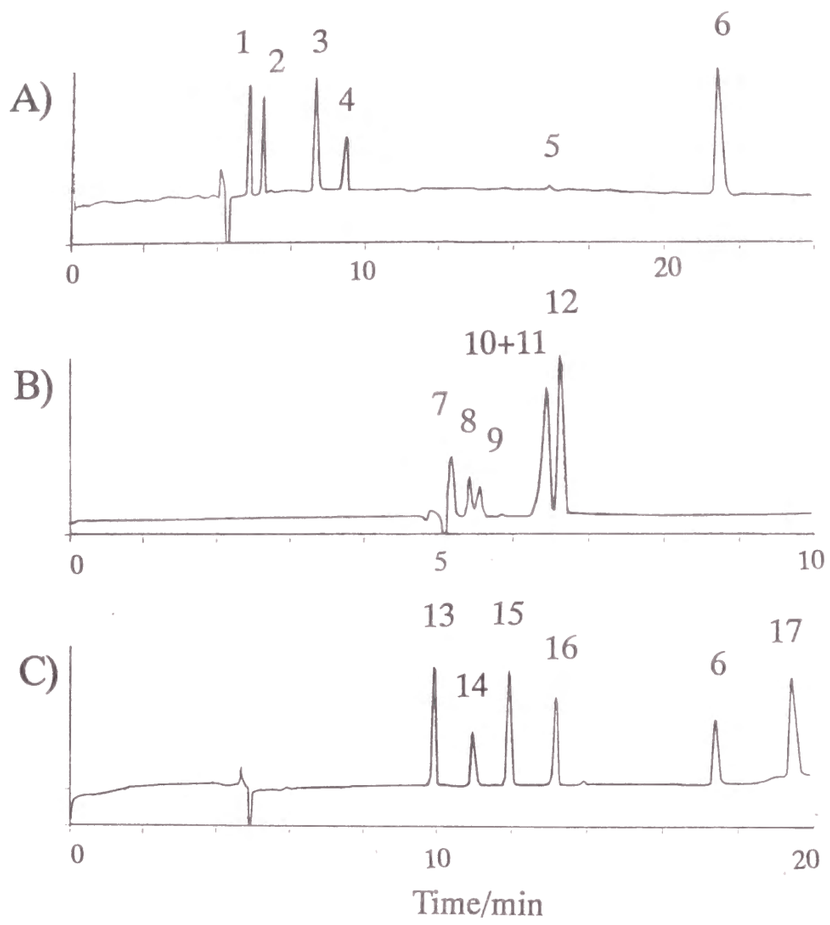


Fig. 2.3 MEKC separations of benzene derivatives (A), cold medicines (B), and naphthalene derivatives (C) using BBMA: 1, resorcinol; 2, phenol; 3, *p*-nitroaniline; 4, nitrobenzene; 5, toluene; 6, 2-naphthol; 7, acetaminophen; 8, caffeine; 9, guaifenesin; 10, ethenzamide; 11, isopropylantipyrine; 12, trimetoquinol; 13, 1-naphthalenemethanol; 14, 1,6-dihydroxynaphthalene; 15, 1-naphthylamine; 16, 1-naphthaleneethanol; 17, 1-naphthol. Conditions: capillary, 36.5 cm (32cm to the detector) x 50 μm; running solution, 2% unpurified BBMA in 50 mM phosphate-100 mM borate buffer (pH 8.0); applied voltage, 20kV; detection wavelength, 210nm.

Polyvinylpyrrolidone solution (2%) in the buffer (pH 7.0) was not effective for the separation of the test mixtures. Only a single peak was observed at about 10 min by applying 10 kV (40 mA).

2.3.3 Effects of concentration of BBMA and pH on migration time.

The dependence of the retention factors of naphthalene derivatives on the concentration of BBMA is shown in Fig. 2.4. The retention factors were proportional to the BBMA concentration and the plotted line for each analyte passed the origin closely when it was extrapolated. The results clearly demonstrate that the CMC of BBMA is virtually zero, as deduced from eqn. 2. Thus, the micelle of BBMA can be assumed to be formed from one molecule by considering the molecular mass of BBMA.

The dependence of the migration-time window (t_{mc}/t_0, where t_{mc} and t_0 are migration times of the micelle and the aqueous phase) on pH is given in Fig. 2.5. The concentration of BBMA was 0.17%, because the solubility of BBMA was low at low pH. The migration-time window became wider with increasing pH values and it was almost constant between pH 7 and 9, although the results were not shown in Fig. 2.5. BBMA precipitated below pH 4 probably due to the decrease of the surface charge. The retention factors of the naphthalene derivatives decreased with an increase in pH as shown in Fig. 2.6, which means that the solubilizing power of BBMA is reduced probably due to the increased surface charge or ionization of the carboxyl group. The results strongly suggest that although the solutes are neutral, the surface charge of the micelle significantly affects the distribution coefficient.

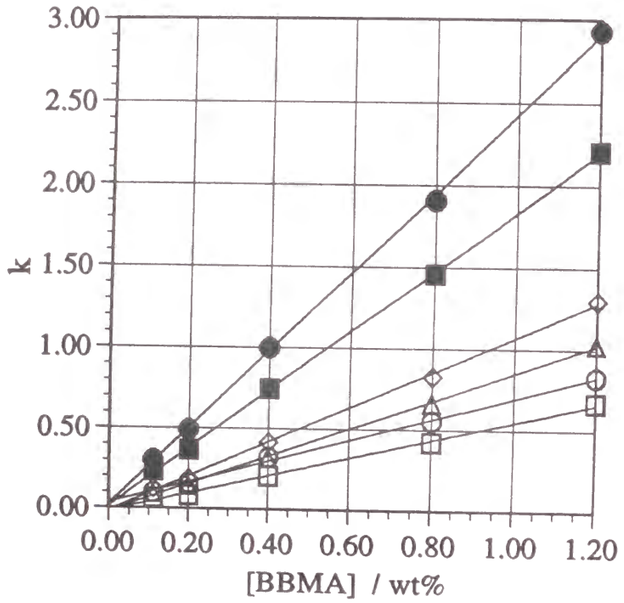


Fig. 2.4 Dependence of the retention factor (k) on the concentration of BBMA: solutes, □ = 1-naphthalenemethanol; ○ = 1,6-dihydroxynaphthalene; △ = 1-naphthylamine; ◇ = 1-naphthaleneethanol; ■ = 2-naphthol; ● = 1-naphthol. The conditions were the same as in Fig. 2.3 except for the concentration of BBMA.

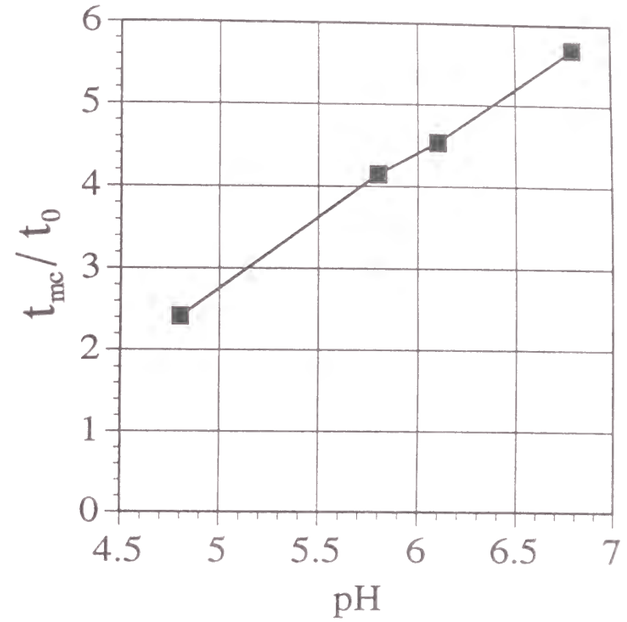


Fig. 2.5 Dependence of the separation window (t_{mc}/t_0) on the pH. Running solution was 0.17% BBMA in 50 mM phosphate buffer. Other conditions are the same as in Fig. 2.3.

Fig. 2.6 Dependence of the retention factors (k) of naphthalene derivatives on the pH. The solutes and conditions are the same as given in Fig. 2.4.

2.3.4 Effects of the composition and molecular mass of BBMA on the separation

Three BBMAs having different contents of MAA were employed to study the effects of the composition on separation. Fig. 2.7 shows the separations of the naphthalene derivatives at pH 8.0 and Fig. 2.8 gives the dependence of their retention factors on the content of MAA. The migration-time window increased with an increase in the MAA content, as clearly seen from Fig. 2.7, which was ascribed to an increase of the surface charge owing to the increased number of carboxyl groups. The retention factors decreased with an increase of the MAA content. The dependence of the retention factor on the content of MAA was very similar to that on the pH described above. Both dependencies can be superficially explained in terms of surface charge.

However, it should be mentioned that the electroosmotic flow is independent of the MAA content, whereas it is significantly dependent on the pH in the acidic region. Therefore, the use of BBMA having a different MAA content is more advantageous over the choice of the pH to manipulate the migration-time window or retention factor. One more disadvantage of the pH change is that BBMA tends to precipitate below pH 5. Three BBMAs with different viscosities but same composition were employed to see the effect of molecular mass on the separation of naphthalene derivatives. Three BBMAs gave almost the same chromatograms for the naphthalene derivatives, which suggests that the solubilizing power and the electrophoretic mobilities of the BBMAs are independent of the molecular mass provided the composition is unchanged. Therefore, we can conclude that the molecular mass distribution will not be critical for the reproducibility of the migration time and selectivity, although a wider distribution of molecular mass values may cause lower efficiency.

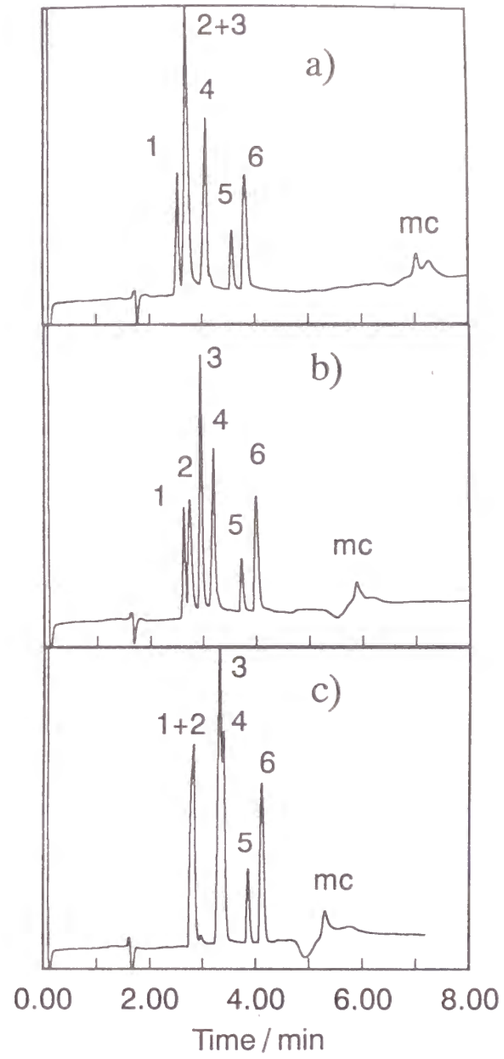


Fig. 2.7 Separation of naphthalene derivatives using BBMAs having different contents of MAA (a, 50%; b, 40%, c, 30%): 1, 1-naphthalenemethanol; 2, 1,6-dihydroxynaphthalene; 3, 1-naphthylamine; 4, 1-naphthaleneethanol; 5, 2-naphthol; 6, 1-naphthol. Running solution, 2% BBMA in a 100 mM borate-50 mM phosphate buffer (pH 8.0). Other conditions are the same as in Fig. 2.3.

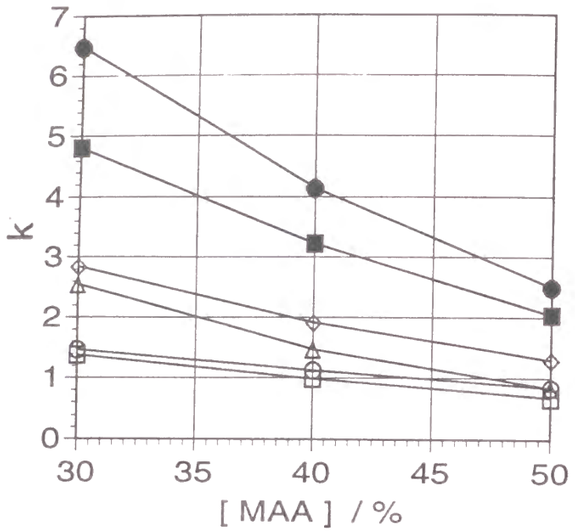


Fig. 2.8 Dependence of the retention factor (k) of naphthalene derivatives on the content of MAA in BBMA. Solutes are the same as in Fig. 2.4. Conditions are the same as in Fig. 2.7.

2.4 Conclusions

BBMA has been found to be a useful polymer surfactant for the use in MEKC and has some advantages over low-molecular surfactants: zero CMC or molecular micelle; different selectivity; and possible manipulation of the migration-time window and retention factor by changing the pH or content of MAA. The molecular micelle is characteristic of polymer surfactants and ensures the constant concentration of the micelle irrespective of the conditions. Although its constant concentration was not confirmed in this study, the advantage of constant concentration will be taken to produce highly reproducible migration time data in a further work.

The molecular micelle will be stable in a running solution containing a high concentration of an organic solvent. Since BBMA has carboxyl groups, it does not dissolve in acidic solution below pH 4. However, other polymer surfactants having

phosphate or ammonium groups are expected to be usable in a wider pH range, and the study using such polymer surfactants are under investigation.

References

1. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, Anal.Chem., 56(1984)111-113.
2. S. Terabe, K. Otsuka and T. Ando, Anal, Chem., 57(1985)834-841.
3. J. Vindevogel and P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Huthig, Heidelberg, 1992.
4. S. Terabe, in N. Guzman (Editor) Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993, pp. 65-87
5. S. Terabe, J. Pharm. Biomed. Anal., 10(1992)705-715.
6. M. Tanaka, T. Ishida, T. Araki, A. Masuyama, Y. Nakatsuji, M. Okahara and S. Terabe, J. Chromatogr., 648(1993)468-473.
7. H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 513(1990)279-295.
8. R. O. Cole, M. J. Sepaniak, W. L. Hinze, J. Gorse and K. Oldiges, J. Chromatogr., 557(1991)113-123.
9. R. D. Holland and M. J. Sepaniak, Anal. Chem., 65(1993)1140-1146.
10. S. Terabe, M. Shibata and Y. Miyashita, J. Chromatogr., 480(1989)403-411.
11. H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Microcol. Sep., 1(1989)234-241.

12. H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Chromatogr., 515(1990)233-241.

13. H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, Anal. Chim. Acta, 236(1990)281-286.

14. R. O. Cole, M. J. Sepaniak and W. L. Hinze, J. High Resolut. Chromatogr., 13(1990)579-582.

15. H. Watzig, Chromatographia, 33(1992)445-448.

16. M. S. Bello, M. Chiari, M. Nesi, P. G. Righetti and M. Saracchi, J. Chromatogr., 625(1992)323-330.

17. S. Terabe, T. Katsura, Y. Okada, Y. Ishihama and K. Otsuka, J. Microcol. Sep., 5(1993)23-33.

18. J. H. Knox and K. A. McCormack, Chromatographia, 38(1994)207-214.

19. S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 61(1989)251-260.

20. C. P. Palmer, M. Y. Khaled and H. M. McNair, J. High Resolut. Chromatogr., 15(1992)756-762.

21. C. P. Palmer and H. M. McNair, J. Microcol. Sep., 4(1992)509-514.

22. S. Terabe, H. Ozaki and Y. Tanaka, J. Chin. Chem. Soc., in press.

23. H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, J. Pharm. Sci., 79(1990)519-523.

24. H. Nishi, N. Tsumagari and S. Terabe, Anal. Chem., 61(1989)2434-2349.

Chapter 3
Effect of modifiers and comparison between anionic and cationic polymer surfactants.

3.1 Introduction

Micellar electrokinetic chromatography (MEKC) [1-5], is a mode of capillary electrophoresis, where ionic micelles are used as pseudo-stationary phases. The separation by MEKC is based on the differential partitioning of analytes between the micelle and the surrounding aqueous phase. Therefore, it is important for manipulating resolution and selectivity in MEKC to select surfactants and modifiers [6]. Different surfactants generally show different selectivity. Bile salts [7-9] and a double-chain surfactant [10], which have significantly different molecular structures from that of sodium dodecyl sulfate (SDS), have shown remarkably different selectivity in comparison with SDS.

In MEKC, the retention factor, k, which is defined as the ratio of the number of the analyte molecules incorporated into the micelle to that in the aqueous phase, is related to the volume of the micelle, Vmc, through

$$k = K(V\text{mc}/V\text{aq}) \qquad (1)$$

where K is the distribution coefficient and Vaq is the volume of the aqueous phase. The volume of the micelle is given as

$$V\text{mc} = v(C\text{srf-CMC}) \qquad (2)$$

where v is the partial specific volume of the surfactant forming the micelle, Csrf is the concentration of the surfactant, and CMC is the critical micelle concentration. CMC

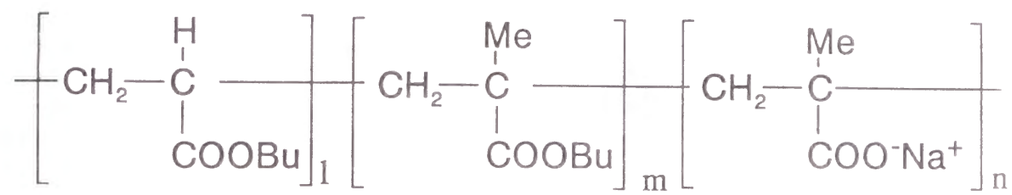
depends on conditions: temperature, salt concentration, and additives.

Polymer surfactants are oligomers of monomeric surfactants or the polymers which show surface active properties as a whole. Palmer et al. [11,12] reported MEKC with an oligomer synthesized from undecylenate. We reported MEKC with butyl acrylate-butyl methacrylate-methacrylic acid copolymer sodium salt (BBMA), whose structure is shown in Fig. 3.1 (A) [13,14]. BBMA is not an oligomer of the surfactant but a polymer which shows surface active properties. BBMA showed significantly different selectivity for naphthalene derivatives in comparison with SDS [14]. The CMC of BBMA was found to be effectively zero [14]. It was suggested that BBMA should be superior to the other surfactants in respect of the stability of the micelle concentration.

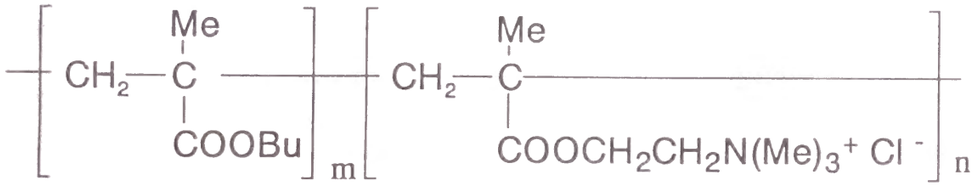
Cyclodextrin (CD) is a chiral compound and capable of recognizing the molecular chirality. Therefore, CD modified MEKC (CD-MEKC) is useful for separating enantiomers [15-18]. Chiral separation of Dns-DL-AAs by CD-MEKC with SDS was described previously [19]. SDS or low molecular mass surfactant molecules are considered to be co-included into the cavity of CD with the analyte enantiomers. BBMA is expected to be too large to be included into the cavity of CD.

In this chapter, the author describes the use of a cationic polymer surfactant, effects of modifiers in MEKC with BBMA and chiral separation by CD-MEKC. Butyl methacrylate-methacryloyloxyethyltrimethylammonium chloride (BMAC), as shown in Fig. 3.1 (B), is a cationic polymer surfactant whose main chain is similar to that of BBMA. In MEKC with BBMA, methanol was used as a modifier of the aqueous phase and octaoxyethylene dodecanol [$(EO)_8R_{12}$], which is expected to form a mixed micelle, as a modifier of the micelle. CD-MEKC with BBMA was applied to separation

of enantiomers of dansylated-DL-amino acids (Dns-DL-AAs). Results of CD-MEKC with BBMA were compared with those using SDS.



(A)



(B)

Fig. 3.1 Molecular structure of BBMA (A) and BMAC (B). Me= methyl; Bu= butyl.

3.2 Experimental

3.2.2 Reagent

BBMA and BMAC were supplied by Dai-ichi Kogyo Seiyaku (Kyoto, Japan). BBMA was provided as a 23% aqueous solution. Since BBMA contains a minor amount of low molecular mass components, it was purified by the reprecipitation method with acetone [13,14]. BMAC was obtained as a 50% solution in water containing 40% 2-propanol and 10% methanol, and used without further purification. SDS, $(EO)_8R_{12}$, α-CD, β-CD and γ-CD were purchased from Nacalai Tesque (Kyoto, Japan). All other reagents were of analytical grade and water was purified with a Milli-Q system. All sample compounds, naphthalene derivatives and Dns-DL-AAs, were of analytical grade and used as received. Sample solutes were dissolved in about 25% aqueous methanol, which served also as a marker of the electroosmotic flow. Phenanthrene was used as a tracer of the micelle.

3.2.2 Apparatus

MEKC was performed with a Bio-Rad BioFocus 3000 CE System (Hercules, CA, U.S.A.) using a fused silica capillary of 50 μm i.d. obtained from Polymicro Technologies (Phoenix, AZ, U.S.A.). The length of the capillary used in MEKC was 36.5 cm (32 cm to the detector) or 54.5 cm (50 cm to the detector). The capillary was thermostated at 30°C and sample vials were at 15°C. Samples were injected by the pressurization method (2-5s at 0.07 atm). Separated zones were detected at 210, 250 and 280 nm simultaneously under the multi-wavelength mode and the electropherograms shown in this paper were recorded at 210 nm.

3.3 Results and discussion

3.3.1 Separation by MEKC with BMAC

A mixture of naphthalene derivatives was separated by MEKC with 2% BMAC and 20% 2-propanol in 100 mM borate-50 mM phosphate buffer (pH 7.0). BMAC was not soluble in an aqueous buffer without 2-propanol. Since the direction of electroosmotic flow was reversed, all solutes were injected at the negative end and migrated toward the positive electrode. Fig. 3.2 shows separations of naphthalene derivatives by MEKC with three different surfactants, BBMA, BMAC, and SDS. The migration order with BMAC was similar to that with BBMA, except for 1-naphthylamine and 1-naphthaleneethanol. However, it was significantly different from that with SDS. In Fig. 3.2 (A) and (B), the peaks with BMAC were just as sharp as with BBMA, suggesting that the adsorption of BMAC on capillary wall did not cause a serious loss of efficiency.

Fig.3.3 shows the dependence of retention factors of the naphthalene derivatives on the concentration of BMAC. The retention factors were proportional to the BMAC concentration and all the plotted lines passed through or nearly through the origin. The CMC of BMAC was nearly zero as deduced from eqn. 2, therefore the micelle of BMAC was formed from one molecule.

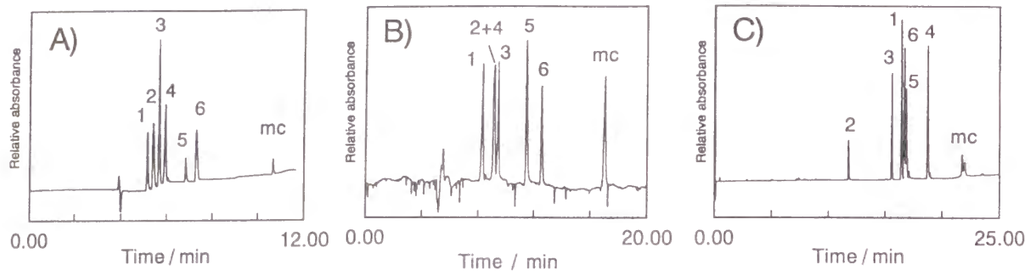


Fig. 3.2 MEKC separations of naphthalene derivatives with BBMA (A), BMAC (B), and SDS (C). Peaks: 1 = 1-naphthalenemethanol; 2 = 1,6-dihydroxynaphthalene; 3 = 1-naphthylamine; 4 = 1-naphthaleneethanol; 5 = 2-naphthol; 6 = 1-naphthol; mc = phenanthrene. Conditions: capillary, 50 um x 36.5cm (32 cm to the detector); separation solution, (A) 2% BBMA in 50 mM phosphate-100 mM borate buffer (pH 8.0), (B) 2% BMAC and 20% 2-propanol in 50 mM phosphate-100 mM borate buffer (pH 7.0), (C) 50 mM SDS in 50 mM phosphate-100 mM borate buffer (pH 7.0); applied voltage, (A) 15 kV, (B) 20 kV, (C) 20 kV; detection 210 nm.

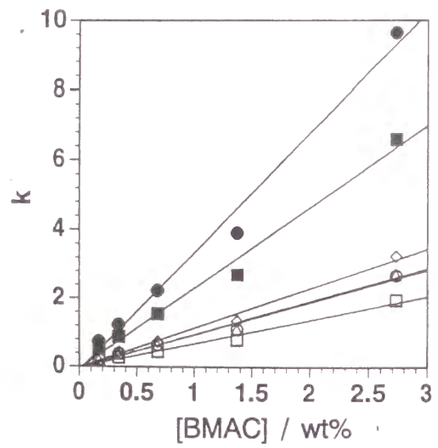


Fig. 3.3 Dependence of the retention factor (k) on the concentration of BMAC. Solutes: □ = 1-naphthalenemethanol; ○ = 1,6-dihydroxynaphthalene; △ = 1-naphthylamine; ◇ = 1-naphthaleneethanol; ■ = 2-naphthol; ● = 1-naphthol. The conditions were the same as in Fig. 3.2(B) except for the concentration of BMAC.

3.3.2 Effects of additions of methanol and a non-ionic surfactant on the MEKC separation with BBMA

Effects of the pH, the composition and the molecular mass of BBMA on the MEKC separation were reported previously [13,14]. Effects of modifiers of the aqueous phase on separation were investigated in this study. Fig. 3.4 shows the dependence of retention factors of the naphthalene derivatives on the concentration of methanol. The retention factors decreased with an increase in the methanol concentration. Fig. 3.5 shows the effect of methanol on the separation using BBMA in comparison with that using SDS. The migration order of 1,6-dihydroxynaphthalene was altered by the methanol addition to the BBMA system as shown in Fig. 3.5 (B) and that of 1-naphthol changed in the SDS system as in Fig. 3.5 (D). The retention factors decreased by the methanol addition both to the BBMA and SDS systems.

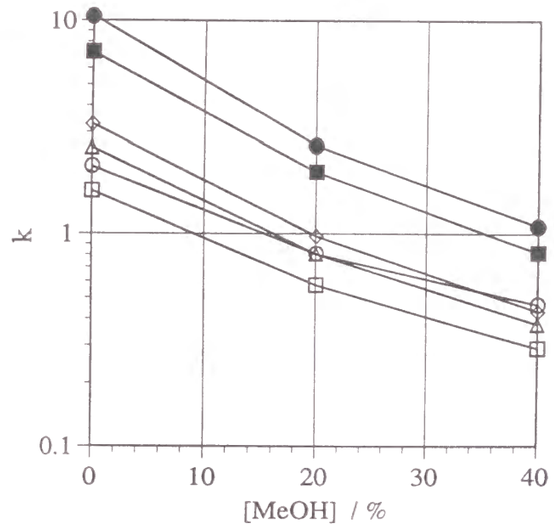


Fig. 3.4 Dependence of the retention factors (k) of the naphthalene derivatives on the concentration of methanol. The solutes are the same as in Fig. 3.3. Separation solution, 2% BBMA and methanol in 50 mM phosphate-100 mM borate buffer (pH 8.0); applied voltage, 10 kV. The other conditions are the same as in Fig. 3.2.

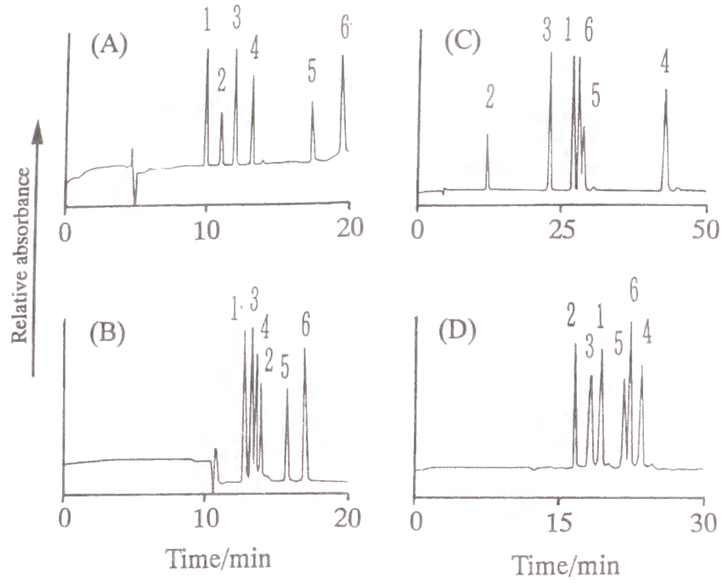


Fig. 3.5 Separation of the naphthalene derivatives with BBMA (A), BBMA and methanol (B), SDS (C), and SDS and methanol (D). The solutes are the same as in Fig. 2. Separation solution, (A) 2% BBMA in 50 mM phosphate-100 mM borate buffer (pH 8.0), (B) 40% methanol in the same BBMA solution as used in (A), (C) 50 mM SDS in 50 mM phosphate-100 mM borate buffer (pH 7.0), (D) 40% methanol in the same SDS solution as used in (C); applied voltage, 10 kV. The other conditions are the same as in Fig. 3.2.

Fig. 3.6 shows the dependence of the retention factors on the concentration of $(EO)_8R_{12}$ added to the BBMA solution. The retention factors increased with an increase in the amount of $(EO)_8R_{12}$ probably because of the increase in the micellar volume by the formation of a mixed micelle [6, 21]. In the case that mixed micelle is not formed and two kind of micelles are formed, the retention factors should decrease with an increase in the ammount of the non-ionic surfactant. Therefore, the results strongly suggest that $(EO)_8R_{12}$ formed a mixed micelle with BBMA. The migration order of 1,6-dihydroxynaphthalene was changed more significantly than the others with an increase in the concentration of $(EO)_8R_{12}$. The migration-time window became narrower with an increase of the non-ionic surfactant concentration because of the decrease in the surface

charge density.

Fig.3.7 shows the separation of the naphthalene derivatives by MEKC with three different mixed micelles containing $(EO)_8R_{12}$. It should be noted that the mixed micelle with BBMA show different selectivity from either the mixed micelle with SDS or that with sodium laurate, which like BBMA has a carboxylate group as the polar group. The addition of a non-ionic surfactant to SDS solutions resulted on the alteration of selectivity [5, 21, 22] as indicated in Fig. 3.5 (C) and Fig. 3.7 (B).

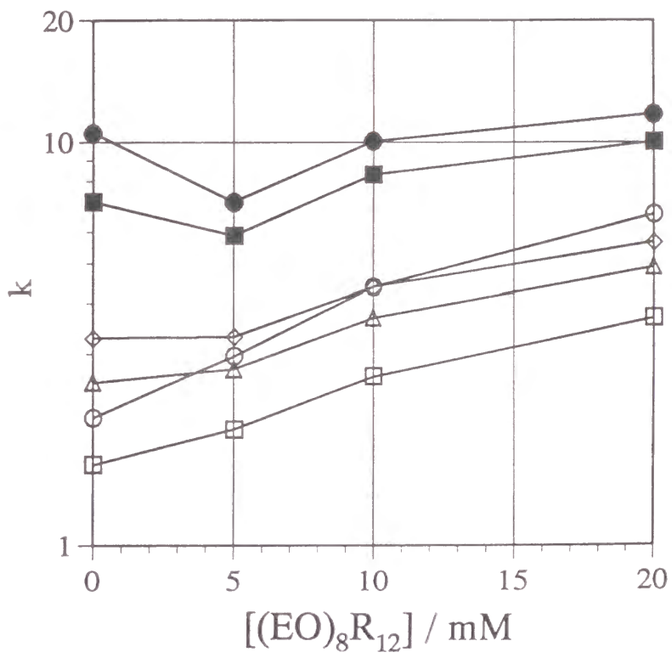


Fig. 3.6 Dependence of the retention factors (k) of the naphthalene derivatives on the concentration of $(EO)_8R_{12}$. The solutes are the same as in Fig. 3.3. Separation solution, 2% BBMA and $(EO)_8R_{12}$ in 50 mM phosphate-100 mM borate buffer (pH 8.0); applied voltage, 10 kV. The other conditions are the same as in Fig. 3.2.

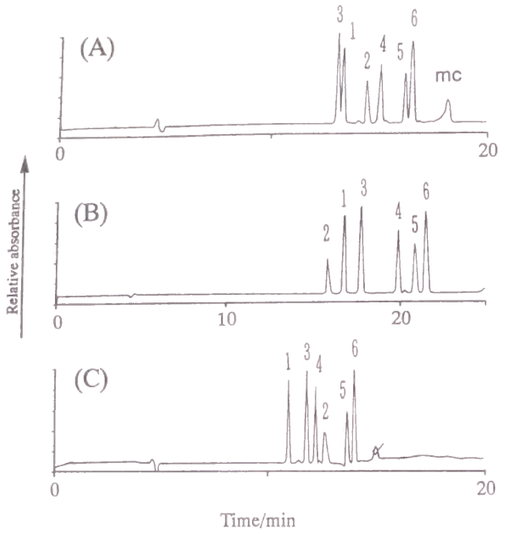


Fig. 3.7 Separations of the naphthalene derivatives using $(EO)_8R_{12}$ with sodium laurate (A), SDS (B), and BBMA (C). The solutes are the same as in Fig. 3.2. Separation solution, (A) 50 mM sodium laurate and 20 mM $(EO)_8R_{12}$ in 50 mM phosphate-100 mM borate buffer (pH 7.0), (B) 50 mM SDS and 20 mM $(EO)_8R_{12}$ in the same buffer as used in (A), (C) 2% BBMA and 20 mM $(EO)_8R_{12}$ in the same buffer as used in (A); applied voltage, 20 kV. Other conditions are the same as in Fig. 3.2.

3.3.3 Chiral separation of DNS-DL-AAs by CD-MEKC with BBMA

Table 3.1 lists the results of the separation of enantiomers of ten Dns-DL-AAs by CD-MEKC with 10 mM β-CD and 2% BBMA. The results with 60 mM β-CD and 100 mM SDS [19] are also given in Table 3.1. The migration time of the BBMA micelle in the CD-BBMA system was assumed to be equal to that of phenanthrene observed with 2% BBMA in the absence of β-CD. BBMA was used without purification in these experiments. The separation solution used was clear at first, but a white precipitate was observed in few hours. In Table 3.1, nine pairs of the ten enantiomeric pairs were succesfully separated with the BBMA system and the separation factors of eight pairs of the nine were larger than those with the SDS system.

Table 3.1

Separation of Dns-DL-Aas by CD-MEKC

Dns-DL-AAs	2% BBMA-10 mM β-CD			100 mM SDS-60 mM β-CD
	t_1 / min	t_2 / min	α^a	α^b
Phe	10.00	10.00	1.00	1.04
Leu	10.61	10.73	1.05	1.03
Nle	10.67	10.78	1.04	1.02
Trp	10.54	10.60	1.02	1.04
Met	10.67	10.76	1.03	1.00
Nva	10.70	10.87	1.07	1.02
Val	10.61	10.75	1.05	1.03
Ser	10.92	11.01	1.03	1.00
Thr	10.76	10.91	1.05	1.00
Asp	15.47	15.97	1.10	1.03

Conditions: capillary, 54.5 cm (50 cm to the detector) x 50 μm i.d.; separation solution, 2% unpurified BBMA and 10 mM β-CD in 50 mM phosphate-100 mM borate buffer (pH 8.0); applied voltage, 20 kV; detection wavelength, 210 nm.

a The migration time of the micelle was assumed to be equal to that of phenanthrene observed with 2% BBMA solution without β-CD.

b From Ref. [19].

Table 3.2

Separation of Dns-DL-Aas by CD-MEKC with methanol

Dns-DL-AAs	2% unpurified BBMA 10 mM β-CD			2% purified BBMA 10 mM β-CD			100 mM SDS 60 mM β-CD
	t_1 / min	t_2 / min	α^a	t_1 / min	t_2 / min	α^b	α^c
Phe	13.03	13.25	1.05	10.46	10.61	1.06	1.03
Leu	13.52	13.80	1.07	10.87	11.08	1.08	1.03
Nle	13.57	13.80	1.05	10.84	11.01	1.06	1.02
Trp	13.77	13.84	1.02	11.31	11.36	1.02	1.01
Met	13.91	14.08	1.04	11.15	11.31	1.06	1.02
Nva	13.93	14.12	1.04	11.15	11.31	1.06	1.02
Val	14.09	14.35	1.06	11.59	11.79	1.07	1.03
Ser	14.35	14.53	1.04	11.73	11.89	1.24	1.02
Thr	14.68	14.98	1.07	11.90	12.14	1.08	1.04
Asp	25.67	27.08	1.30	21.49	22.50	2.15	1.05

Conditions are the same as shown in Table 1 except for the addition of 20% methanol to the separation solution.

a, b See the footnote a and b in Table 3.1

c From Ref. [19].

The migration times of different DNS-AAs were close each other in the BBMA system; from 10 to 11min, expect for those of Dns-DL-Asp in comparison with the results of SDS system, where the migration times of DNS-AAs were widely spread [19]. The migration order with the BBMA system was significantly different from that with the SDS system. It should be noted that Dns-DL-Phe migrated fastest of all and Dns-DL-Nle migrated faster than Dns-DL-Nva. This order is not consistent with that of hydrophobicity of the analyte. The results suggest that Dns-DL-AAs are solubilized by the BBMA micelle mainly with the incorporation of the Dns-group into the hydrophobic core of the micelle.

Table 3.2 summarizes the results obtained with 2% unpurified or purified BBMA together with 10mM β-CD and 20% methanol and those with 60mM β-CD, 100mM SDS and 20% methanol from a published paper [19]. The separation solution prepared with unpurified BBMA generated a white precipitate in a day, but that with purified BBMA did not show any changes. In Table 3.2, all enantiomers were separated with both the BBMA systems and all separation factors were larger than those with the SDS system. The purified BBMA clearly showed better resolutin than with unpurified BBMA.

BBMA forms a molecular micelle which consists of one molecule, whereas SDS forms a micelle in the presence of the monomeric molecule whose concentration is equal to CMC. The white precipitate mentioned above was probably a complex formed from β-CD and a minor component of BBMA which was removed by the purification, because the BBMA molecule should not form a complex with β-CD. The results of Table 3.1 show that BBMA was superior to SDS for the separation of enantiomeric Dns-DL-AAs owing to the absence of the monomeric surfactant molecules which might be

co-included with the enantiomer into the cavity of β-CD. The results of Table 3.2, furthermore, show that the purified BBMA was superior to the unpurified BBMA because of the absence of the minor component which gave the precipitate. From Table 3.1 and 3.2, the addition of methanol to the BBMA system increased the resolution except for Dns-DL-Nva in comparison with the results with SDS system [19]. Fig. 3.8 shows the example of the separation of enantiomers by CD-MEKC with purified BBMA. Enantiomers of timepidium bromide were successfully resolved under the same condition as in Fig. 3.8.

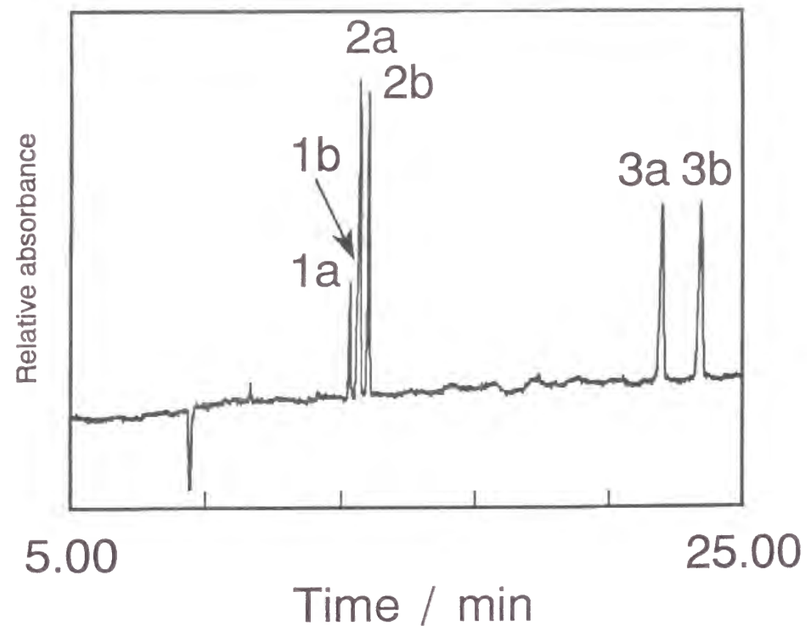


Fig. 3.8 Separations of Dns-DL-AAs by CD-MEKC with BBMA: 1a, 1b, Dns-DL-Phe; 2a, 2b, Dns-DL-Thr; 3a, 3b, Dns-DL-Asp. Separation solution, 10 mM β-CD, 2% BBMA and 20% methanol. Other conditions are the same as in Table 3.1.

The other CDs were also employed together with purified BBMA. No enantiomer of the ten Ds-DL-AAs were separated with 10 mM α-CD and 2% BBMA. The separation solution did not generate any precipitate. Seven of the ten Dns-DL-AAs, Leu, Nle, Met, Nva, Val, Thr and Asp, were successfully separated with 10 mM γ-CD and 2% BBMA. The separation factors of three of the seven, Val, Thr and Asp, were larger, but those of the other four were less than those obtained with 60 mM γ-CD and 100 mM SDS [19]. The separation solution gave a white precipitate in few hours. It should be noted that γ-CD tends to generate the precipitate more than β-CD. These results suggest that in the BBMA system β-CD is the most suitable for CD-MEKC separation of Dns-DL-AAs among the three CDs. In the SDS system γ-CD is more suitable than β-CD except for Trp, Ser and Asp [19]. With the more purified BBMA which will not generate precipitate with γ-CD, Dns-DL-AAs will be separated with the BBMA and γ-CD system better than with the SDS and γ-CD system.

3.4 Conclusions

BMAC, like BBMA, is found to be a useful polymer surfactant for the use in MEKC. BMAC gave zero CMC, reversed direction of electroosmotic flow, and different selectivity. Additions of methanol or $(EO)_8R_{12}$ changed separation and selectivity in MEKC with BBMA. For the chiral separation of Dns-DL-AAs by CD-MEKC, BBMA was superior to SDS because of the absence of monomeric surfactant.

References

1 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, Anal. Chem., 56(1984) 111-113.

2 S. Terabe, K. Otsuka and T. Ando, Anal. Chem., 57(1985)834-841.

3 J. Vindevogel, P. Sandra, Introduction to Micellar Electrokinetic Chromatography, Huthig, Heidelberg, 1992.

4 S. Terabe, in N. Guzman (Editor) Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993, pp. 65-87.

5 S. Terabe, N. Chen and K. Otsuka, Adv. Electrophoresis, 7(1994)87-153.

6 S. Terabe, J. Pharm. Biomed. Anal., 10(1992)705-715.

7 H. Nishi, T. Fukuyama, M.Matsuo and S. Terabe, J. Chromatogr., 513(1990)279-295.

8 R. O. Cole, M. J. Sepaniak, W. L. Hinze, J. Gorse and K. Oldiges, J. Chromatogr., 557(1991)113-123.

9 R.D.Holland and M. J. Sepaniak, Anal. Chem., 65(1993)1140-1146.

10 M. Tanaka, T. Ishida, T. Araki, A. Masuyama, Y. Nakatsuji, M. Okahara and S. Terabe, J. Chromatogr., 648(1993)468-473.

11 C. P. Palmer, M. Y. Khaled and H. M. McNair, J. High Resolut. Chroma-togr., 15(1992)756-762.

12 C. P. Palmer and H. M. McNair, J. Microcol. Sep., 4(1992)509-514.

13 S. Terabe, H. Ozaki and Y. Tanaka, J. Chin. Chem. Soc., 41(1994)251-257.

14 H. Ozaki, S. Terabe and A. Ichihara, J. Chromatogr., 680(1994)117-123.

15 H. Nishi, T. Fukuyama and S. Terabe, J. Chromatogr., 553(1991)503

16 T. Ueda, F. Kitamura, R. Mitchel, T. Metcalf, T. Kuwana and A. Nakamoto,

Anal. Chem., 63(1991)2979-2981.

17 K. Otsuka and S. Terabe, J. Liq. Chromatogr., 16(1993)945-953.

18 K. Otsuka and S.Terabe, in N.Guzman (Editor) Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993, pp. 617-629.

19 S. Terabe, Y. Miyashita, Y. Ishihama and O. Shibata, J. Chromatogr., 636(1993)47-55.

20 K. Otsuka, S. Terabe and T. Ando, J. Chromatogr., 332(1985)219-226.

21 S. Terabe, H. Ozaki and Y. Ishihama, Bunseki Kagaku, 42(1993)859-866.

22 E. L. Little and J. P. Foley, J. Microcol, Sep., 4(1992)145-154.

Chapter 4
MEKC-Electrospray ionization mass spectrometry using the polymer surfactants

4.1 Introduction

Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis (CE), where ionic micelles are used as pseudo-stationary phases. Capillary zone electrophoresis (CZE) is a separation technique of ionic analytes only, whereas MEKC is capable of separating both ionic and non-ionic analytes. Almost all advantages of CZE apply to MEKC as well and many applications of MEKC separations have been reported [1-5]. The most common detector is UV absorbance detectors both in CZE and MEKC. However, information on the analyte structure obtained from UV spectra is limited. Coupling of CZE or MEKC with general spectroscopic detection methods must be useful. Mass spectrometry (MS) is a compatible with CE in respect of the sample amount and one of the most powerful detection method for obtaining structural information on separated analytes.

On-line coupling techniques for CE-MS have been studied by several groups [6-18]. Electrospray ionization (ESI) interfaces for CE-MS have been developed by Smith et al. [6-9]. Ion spray interfaces, a type of ESI using a high-velocity gas flow at the tip of the capillary, have been studied by Henion et al. [10, 11] and Thibault et al. [12]. A fast atom bombardment (FAB) ionization interface with a coaxial continuous flow was developed by Tomer et al. [13, 14]. A latest work described a coupling of CE with laser-desorption mass spectrometry, which was yet an off-line technique [15]. Several reviews

on on-line CE-MS have been published [16-18]. Some other separation modes of CE such as capillary gel electrophoresis [10] and capillary isotachophoresis [19] have been studied for coupling with MS.

On the other hand, a combination of MEKC with MS has not been developed yet probably because of the presence of the surfactant in the running solution. An On-line coupling of MEKC to ESI-MS was reported only with a coupled capillary set-up and on-line heart-cutting of the MEKC separation zones [20]. An off-line coupling of MEKC with MS was designed with a FAB interface [21], but it was difficult to obtain signal of analyte from a separated fraction due to the existence of surfactant. Smith et al. [6] studied ESI spectrum of sodium dodecyl sulfate (SDS), which is a surfactant most frequently employed as a pseud-stationary phase in MEKC. They observed both positive and negative ESI spectra of SDS and suggested the possibility of MEKC-MS with SDS. In practice, however, MEKC-MS using SDS has not reported yet, probably due to a low ionization efficiency caused by a relatively high concentration of SDS. Varghese and Cole [22] used a cationic surfactant, cethyltrimethylammonium chloride, as an additive to the running solution to reverse the electroosmotic flow for CE-ESI-MS of cationic compounds. The separation mode described was not MEKC owing to the low concentration of the surfactant.

For on-line MEKC-MS, selection of an interface is important. ESI is one of the on-line interfacing methods which has been successfully used for CE-MS. The use of ESI for MEKC-MS is expected to have the same advantages as in CE-MS. Operation and maintenance of the ESI interface are easy owing to an atmospheric pressure ionization inlet. Mass spectra obtained by ESI are simple because ESI is a soft ionization technique and yields intact molecular ions only without fragmentation. Spectra of biopolymers,

peptides and proteins, can be easily measured by CE-MS [16-18], because ESI yields stable multiple ions from high molecular mass analytes exceeding the hardware MS range. An analytical condition for peptide mapping was studied by CE-MS [23]. However, disadvantages of ESI are: it is not suitable for non-polar compounds because of low ionization and information on molecular structure is limited to molecular mass only and hence a more advanced technique such as MS/MS is required for more information.

Choice of a surfactant added to the running solution in MEKC is most significant because surfactants are non-volative and in many cases cause strong background ions on mass spectra as observed with SDS [6]. Polymer surfactants are oligomers of monomeric surfactants [24, 25] or the polymers which show surface active properties as a whole [26,27] and they are expected to be useful as a pseudo-stationary phase of MEKC-MS. Fig. 4.1 is a schematic illustration of an electrospray interface and expected behavior of micelles in the interface. In Fig. 4.1A, two main stages in the generation of gas-phase ions of analytes are shown: the first stage is the production of charged droplets (S with O), and the second the production of gas-phase ions from the charged droplets (S^+). Fig. 4.1B shows that low molecular mass surfactant micelles produce abundant gas-phase surfactant ions. On the other hand, shown as in Fig. 4.1C, polymer surfactant micelles will be stable in the ESI system because the micelle is a covalently bonded one molecule. Therefore, the polymer surfactant micelle will not generate low-molecular background ions very much.

A polymer surfactant, which is an undecylenate oligomer synthesized by polymerizing micellized sodium 10-undecylenate was employed as a pseudo-stationary phase for MEKC by Palmer et al. [24, 25]. We reported MEKC with a polymer surfactant, butyl acrylate-butyl methacrylate-methacrylic acid copolymer sodium salt

(BBMA) [26, 27]. The molecular mass of the BBMA was measured to be about 40000 by size exclusion chromatography (SEC) using standard polyoxethylene glycols. BBMA showed high efficiency and significantly different selectivity in MEKC for naphthalene derivatives in comparison with SDS [26]. The CMC of BBMA was found to be effectively zero [26]. BBMA are, thus, expected to be suitable for MEKC-MS because of the formation of the micelle at low surfactant concentrations, a higher molecular mass beyond the hardware mass range, and high efficiency in separations.

In this chapter, the author describes an on-line MEKC-ESI-MS system with BBMA as the pseud-stationary phase.

4.2 Experimental

4.2.1 Apparatus

An experimental framework of the MEKC-ESI-MS system is schematically represented in Fig. 4.2. The structure is almost the same as a CE-ESI-MS system using a coaxial sheath liquid flow [6-9, 23]. MEKC was performed with laboratory-built instruments which consisted of a Mastheads Precision Devices HCZE30PN0.25-LDSW high-voltage powersupply (Kusatsu, Shiga, Japan) and a fused silica capillary of 50 cm long, 50 μm i.d., 150 μm o.d. obtained from Tokyo Kasei (Tokyo, Japan). An ESI interface was laboratory-built and consisted of a stainless steel tube of 190 μm i.d. 350 μm o.d. (G 28), inside which the capillary was coaxially inserted, a polytetrafluoroethylene (PTFE) tee union, which holded the stainless steel tube, the fused silica capillary and a PTFE tube for the delivery of a sheath flow.

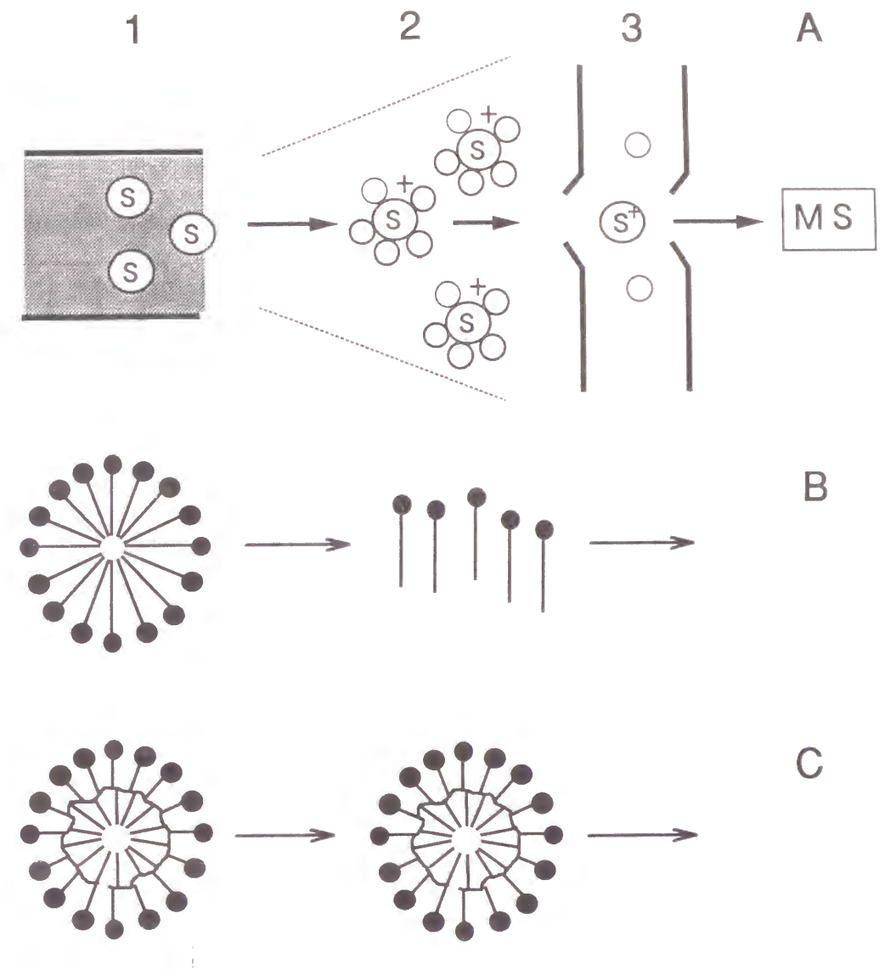


Fig. 4.1 Schematic illustration of an electrospray interface (A) and expected behavior of low molecular mass surfactant micelles (B) and polymer surfactant micelles (C). 1 = capillary; 2 = electrospray; 3 = MS; S = solute molecule; S^+ = solute molecular ion; O = solvent.

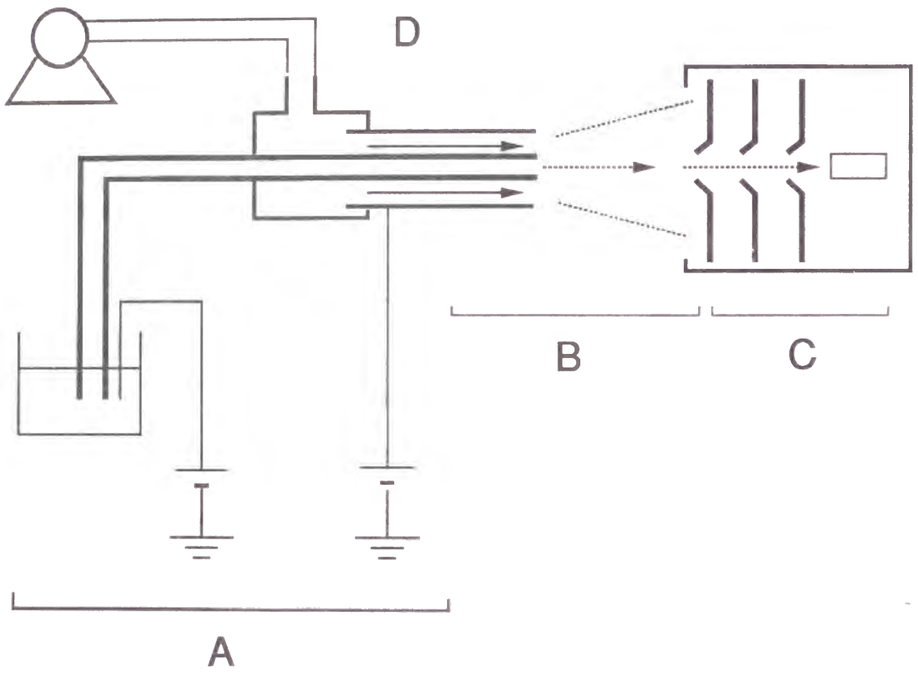


Fig. 4.2 Schematic illustration of an experimental framework of the MEKC-ESI-MS system. A = MEKC; B = ESI; C = MS; D = Sheath liquid flow.

A MS system consisted of a modified Hitachi M-1000 LC-APCI-MS (Tokyo, Japan) which was constructed of a quadrupole mass spectrometer and a differential pumping region.

MEKC using UV detection was performed on a Hewlett Packard HP3DCE system (Waldbronn, Germany) and a Bio Rad BioFocus 3000 system (Hercules, CA, USA) to establish the MEKC separation conditions.

SEC of BBMA was performed with a Shimazu LC-9A liquid-delivery pump (Kyoto, Japan), a Shodex RI SE-51 refractive index detector (Tokyo, Japan) and Tosoh TSK-gel G3000SW and G2000SW (both 60 cm, 8 mm i.d.) (Tokyo, Japan).

4.2.2 Procedure

On-line MEKC-ESI-MS work was performed by the system described above using BBMA solutions in 10 mM ammonium formate buffer (pH 7) containing 10% methanol.

All sample compounds were dissolved in water containing 25-50% methanol to make a 0.2-1 mg/ml solution. Samples were injected by the hydrostatic injection method (10-30s at 15 cm).

The MEKC applied voltage was 10 kV (13 kV at the capillary inlet and 3 kV at the end of the capillary located in ESI interface). The sheath liquid was consist of water, methanol and formic acid (50:50:1, v/v/v) and was delivered by a Hitachi HPLC pump L6300 at approximately 5 μl/min. Electrospray was performed using a 3 kV gradient between the capillary end and the first MS sampling orifice.

MS system was operated in the positive ion mode. Almost all MS detection was

obtained in the scanning mode, m/z 1 to 1000, at 4 sec/scan. Drift voltage was 70 V, focusing voltage 140-150 V and resolution 50-55. All work described was performed with the MEKC-ESI-MS system mentioned above at Himeji Institute of Technology except for the data given in Fig. 4.10B which was obtained with a fundamentally similar system at Hitachi Central Research Laboratory.

Off-line MS work was performed with another laboratory-built interface equipped with another fused-silica capillary using a sheath liquid flow. Sample solutions were introduced by a micro-syringe. The other conditions were same as the on-line work.

MEKC using the UV detector was performed as described previously [26,27]. Fractionation of BBMA by SEC was performed with 50 mM ammonium carbonate (pH 8) and acetonitrile (20:80, v/v) at a mobile phase flowrate of 0.8 ml/min. Each fraction was introduced to the MS spectrometer after adding 100 μl 20% formic acid to the 2 ml fractionated solution.

4.2.3 REAGENT

BBMA was supplied by Dai-ichi Kogyo Seiyaku (Kyoto, Japan). BBMA was provided as a 23% aqueous. Since BBMA contains a minor amount of low molecular mass components, it was purified by the reprecipitation method with acetone [26, 27]. Low molecular mass surfactants, SDS and cholic acid sodium salt were purchased from Nacalai tesque (Kyoto, Japan). Sodium laurate was from Tokyo Kasei. All other reagents were of analytical grade and water was purified with a Milli-Q system. All sample compounds, phenyltrimethylammonium chloride, tetraphenyl-phosphonium chloride, quinine sulfate dihydrate, octaoxyethylenedodecanol and pentaoxyethylenedodecanol

obtained from Wako Pure Chemical (Osaka, Japan), 1-naphthylamine from Merck (Darmstadt, Germany), sulfamethazine, sulfisomidine, sulfadiazine and sulfisoxazole from Aldrich (St. Louis, MO, USA), were of analytical grade and used as received.

4.3 Results and discussion

4.3.1 Optimization of the ESI interface

Since the mass spectrometer employed in this study was operated under the positive-ion mode only, the ESI interface had to be optimized to produce positive ions efficiently. To generate positive ions by ESI, the liquid sprayed was required to be acidic, generally pH 3-5, as reported for CE-ESI-MS [9,16]. To meet the requirement, acidic buffers consisting of ammonium acetate or formate were employed in CE-ESI-MS or an acidic sheath flow was added to the running solution at the end of the separation capillary [9, 16].

In this work, however, acidic buffer was not used for the following reasons: BBMA was insoluble in water under low pH or it precipitated at pH below 4 [26]; normal MEKC conditions in which the electroosmotic flow was considerably strong were to be employed to take advantages of MEKC to separate non-ionic analytes. BBMA solutions in ammonium formate (pH 7) were employed and an aqueous methanol (1:1) containing 1% formic acid was used as a sheath liquid to acidify the total liquid sprayed at the ESI interface. Such procedure was employed also on peptide mapping by CE-ESI-MS using a basic electrophoretic buffer and an acidic sheath liquid [23].

Optimization of positioning of the capillary tip in the ESI interface against the first MS sampling orifice is critical to the MS intensity of analyte because of the conical

distribution of aerosol generated by the electrospray. It was reported that distributions of compositions were different among ions generated by ESI [28]. Therefore, the fixed position of the capillary tip would not be always optimal, but repositioning of the capillary tip was not tried throughout this work. The capillary tip was positioned at the separation distance of 20 mm, offset horizontally of 5 mm and offset vertically of 5 mm from the center of the orifice to maximize the signal intensity of ions generated from standard compounds.

The ESI interface system employed was evaluated under the MEKC condition using BBMA as a pseudo-stationary phase. Even a 2% BBMA solution introduced successively to the ESI-MS system generated a stable electrospray and did not impair the MS detection significantly. It was required to rinse the capillary with 1M sodium hydroxide after each run to obtain reproducible migration times. Therefore, the effect of an introduction the sodium hydroxide solution to the ESI-MS system was investigated. When the sodium hydroxide solution was introduced, the electrospray became unstable and the signal intensity was deteriorated. Thus, when the sodium hydroxide solution was introduced to rince the capillary, the electrospary was interrupted whereas the sheath flow was delivered.

4.3.2 Background ions from BBMA

Fig.4.3 shows examples of the mass spectra of MEKC separation solutions: an ammonium formate buffer only (A) , the buffer containing 2% BBMA (B) and that containing 50 mM SDS (C). In the spectrum of 10mM ammonium formate buffer (pH 7), many peaks below m/z 200 were observed. The most strong peak was at approximately

m/z 120. It should have come from the buffer system, but could not be identified. In the spectrum of 2% BBMA added to the buffer, strong peaks were recorded at approximately m/z 310 which was characteristic of the BBMA solution. In the spectrum of the 50 mM SDS, two very strong peaks were recognized at approximately m/z 310 and 610. The former would be assigned to the molecular ion of SDS due to sodium attachment, and the latter to the singly charged dimmer. Both peaks showed stronger intensity than the those from the buffer system. Observation of the molecular ion of SDS due to sodium attachment in ESI-MS was reported by Smith et al [6]. To identify the m/z 310 ion generated from BBMA solution, BBMA was fractionated by SEC. Fig.4.4 shows a chromatogram of BBMA and ESI mass spectra of each fractions. From Fr-A and B, polymer and oligomer region of BBMA, any strong signals were observed. From Fr-C, a strong m/z 310 peak was recorded. According to analytical SEC, it was clarified that an additive of BBMA appeared in this region and any components of BBMA, butyl acrylate, butyl methacrylate and methacrylic acid sodium salt, did not appear in this region. Therefore this ion was probably from the additive of BBMA. The results shown on Fig.4.3 and 4.4 strongly suggest that BBMA will not disturb the on-line measurement of the mass spectra of analytes separated by MEKC with BBMA.

Some ionic low molecular mass surfactants other than SDS were introduced to the ESI-MS system. All surfactants were dissolved to make a 50-100 mM solution. Sodium cholate, sodium dodecane sulfonate and CHAPS produced strong signals of molecular ions due to cation attachment. Sodium laurate gave no strong ions.

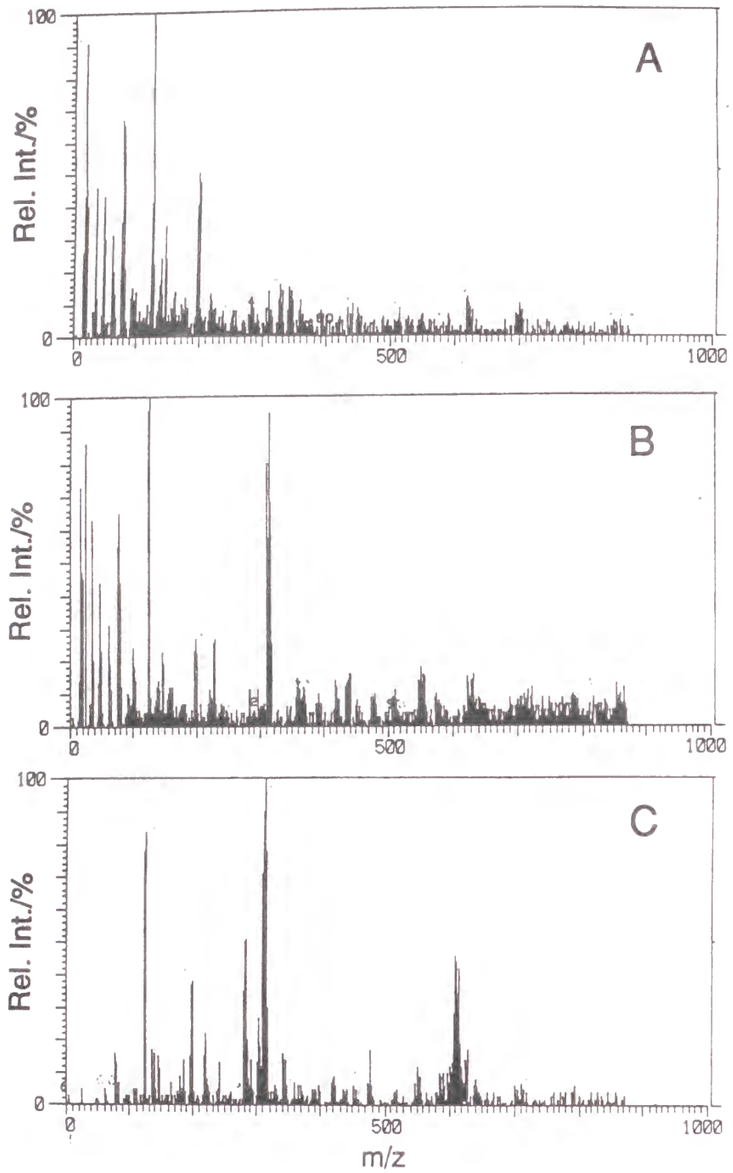


Fig. 4.3 ESI mass spectra of MEKC separation solutions: (A) 10 mM ammonium formate buffer (pH 7), (B) (A) + 2% BBMA, (C) (A) + 50 mM SDS. Conditions: electrospray voltage, 3 kV; MS scanning, m/z 1 to 1000 at 4 sec/scan; drift voltage, 70 V; focusing voltage 140 V; resolution 55. sheath liquid flow, water/methanol/formic acid (50:50:1, v/v/v) at approximately 5 μl/min; sample solutions were introduced by syringe injection.

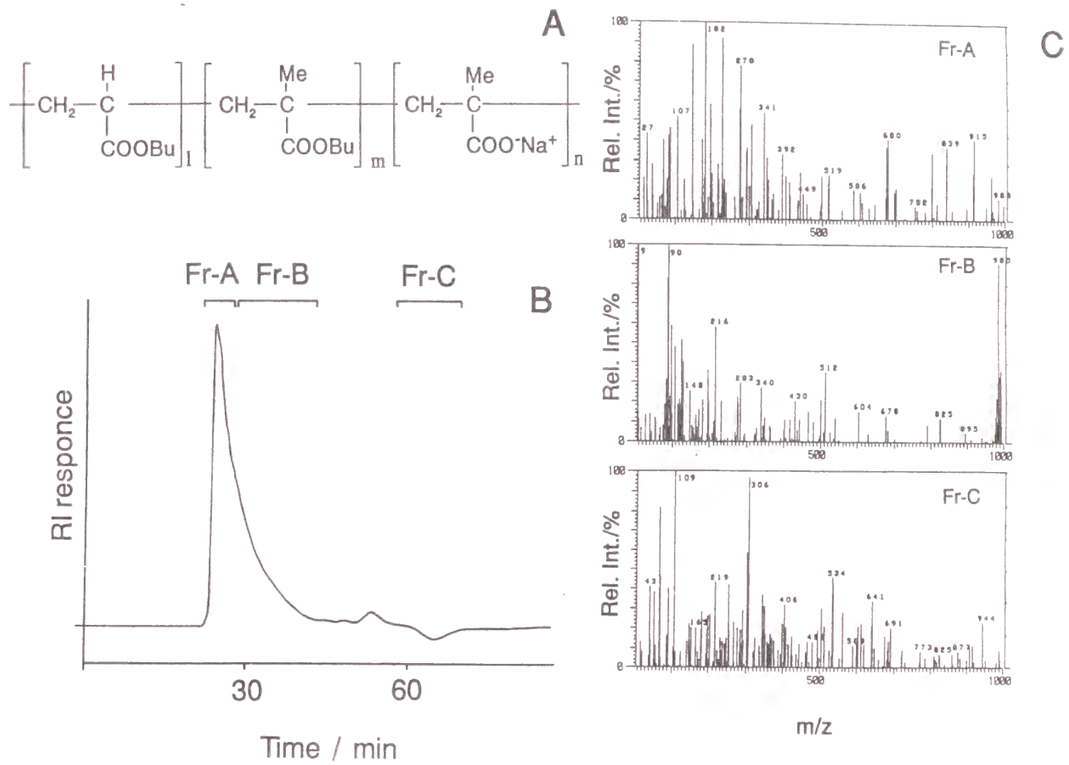


Fig. 4.4 Molecular structure of BBMA (A), SEC chromatogram for fractionation of BBMA (B) and ESI mass spectra of fractions (C). SEC conditions (B): column, Tosoh TSK-gel G3000SW and G2000SW (both 60 cm, 8 mm i.d.); separation solution, 50 mM ammonium carbonate (pH 8) and acetonitrile (20:80, v/v) at 0.8 ml/min; detection, refractive index. MS conditions (C): sheath liquid flow, water/methanol (50:50, v/v) at approximately 5 μl/min; drift voltage, 80 V. The other MS conditions are same as in Fig.4.3.

4.3.3 ESI-MS of analytes

Fig. 4.5 shows an ESI mass spectrum of a standard mixture and molecular structures of the analytes. The mixture was composed of a quaternary ammonium salt, an aromatic amine, an alkaloid, a quaternary phosphonium salt and a non-ionic surfactant having a polyoxyethylene group. Phenyltrimethylammonium chloride, 1-naphthylamine, Quinine sulfate dihydrate and tetraphenylphosphonium chloride showed abundant intact molecular ions. Octaoxyethylenedodecanol generated two intense molecular ions due to cation attachment. The more intense one, higher-mass ion, was selected as a monitored ion of the single ion chromatogram by the on-line MEKC-ESI-MS described below. On the other hand, no MS signals were observed from 1-naphthol and 1-naphtharenemethanol, which are usually used as MEKC standard compounds, phenanthrene and Sudan 4, which are used as micelle markers in MEKC. These compounds seemed not to be ionized by ESI-MS.

4.3.4 On-line MEKC-ESI-MS

Fig. 4.6 shows the single-ion chromatograms by MEKC-ESI-MS (A) and CE-ESI-MS (B). Solutes are the same as in Fig. 4.5. All the solutes were separated and detected successfully under the condition of MEKC with 2% BBMA as shown in Fig. 4.6A. Separation and sensitivity were practically reproducible in several successive runs. Migration times of all solutes in Fig. 4.6A were longer than those corresponding in Fig. 4.6B, where no micelle was used, and the solutes were separated more widely according to the MEKC mechanism: octaoxyethyledodecanol migrated slower than 1-naphthylamine although both are neutral and unresolved in Fig. 4.6B.

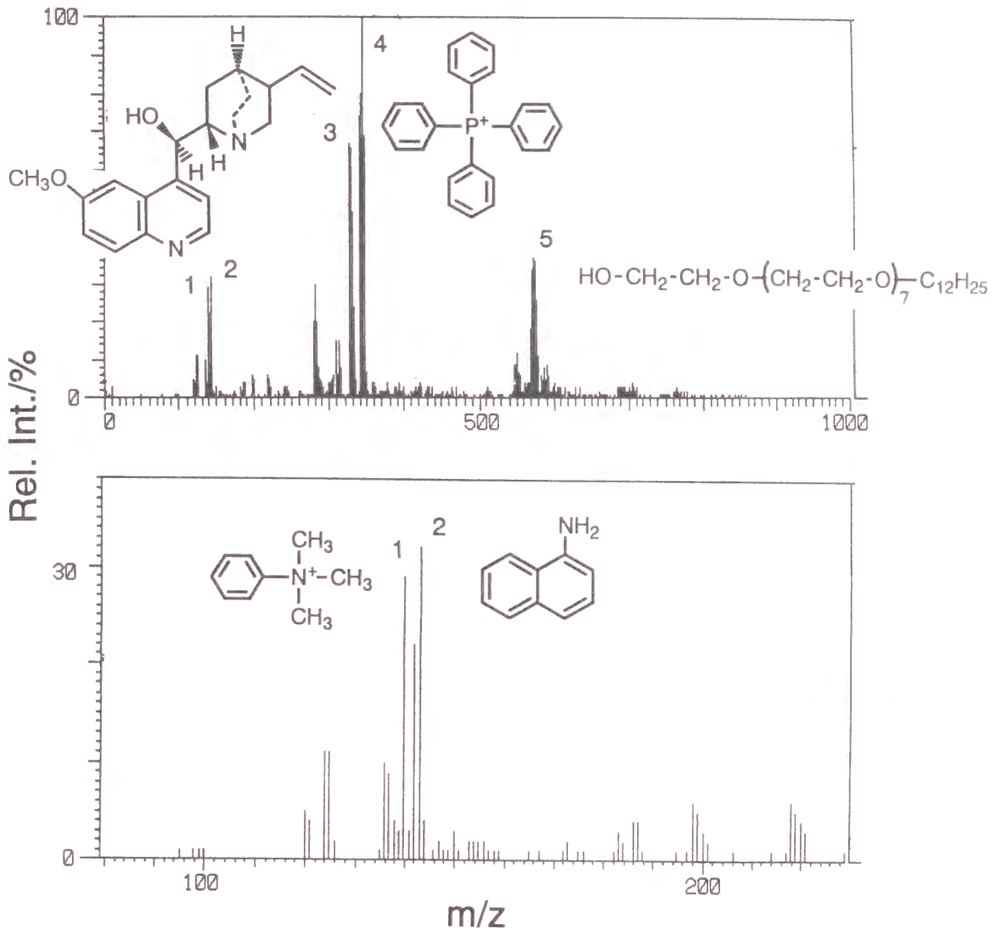


Fig. 4.5 Example of an ESI mass spectrum of standard mixture and molecular structures of them. Peaks: 1 = phenyltrimethylammonium chloride; 2 = 1-naphthylamine; 3 = Quinine sulfate; 4 = tetraphenylphosphonium chloride; 5 = octaoxyethylenedodecanol. Conditions are same as in Fig.4.3.

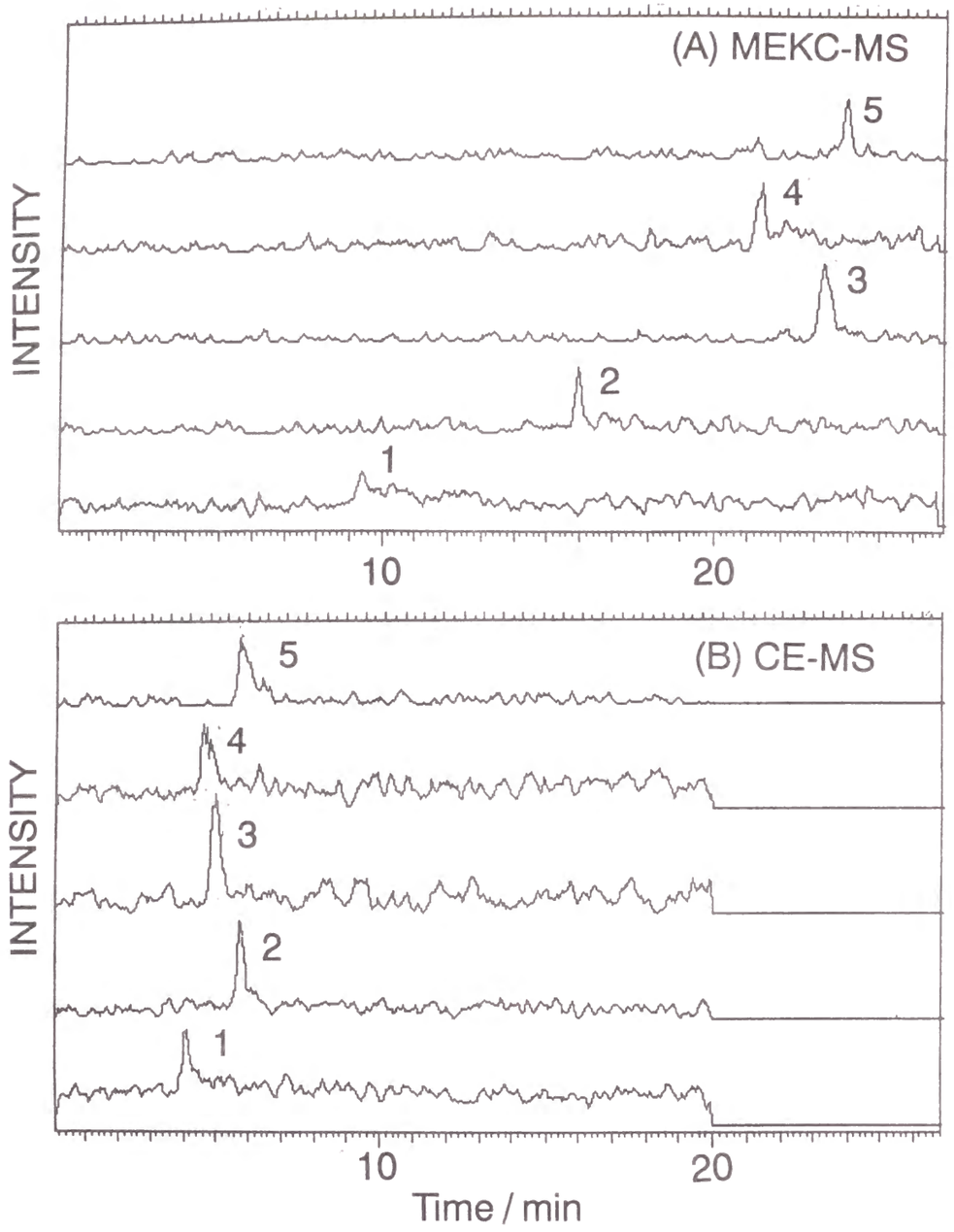


Fig. 4.6 Single-ion chromatograms by MEKC-ESI-MS (A) and CE-ESI-MS (B). Solutes are same as in Fig.5. MEKC conditions; capillary, 50 μm i.d. x 50 cm; separation solution, (A) 2% BBMA in 10% methanol and 10 mM ammonium formate buffer (pH 7), (B) 10 mM ammonium formate buffer (pH 7); applied voltage, 13 kV. MS conditions are same as in Fig.4.3.

Fig. 4.7 shows mass spectra acquired from the separation peaks shown in Fig. 4.6A and B. Under the condition of MEKC with 2% BBMA, abundant intact molecular ions of quinine sulfate and tetraphenylphosphonium chloride and abundant intense molecular ion due to cation attachment of octaoxyethylenedodecanol were observed as shown in Fig. 4.7A, B and C. Signals of m/z 310 in Fig. 4.7A, B and C was generated from the additive of BBMA as shown in Fig. 4.3B. Some peaks below m/z 200 recorded in all spectra of Fig. 4.7 were from the buffer system as shown in Fig. 4.3A.

Fig. 4.8 shows the dependence of the migration time on the concentration of BBMA. The migration times of all solutes increased with an increase in the concentration of BBMA. This results indicates that the separation is based on the differential partitioning of the solutes between the slower migrating BBMA micelle and the faster migrating surrounding aqueous phase. It is noted that octaoxyethylenedodecanol, a non-ionic surfactant, migrated last. In MEKC using octaoxyethylenedodecanol added to the BBMA solution, retention factors of solutes increase with an increase in the concentration of octaoxyethylenedodecanol [27], which means that BBMA forms mixed micelle with octaoxyethylenedodecanol. It notes that octaoxyethylenedodecanol was fittable as a tracer of BBMA micelle more than quinine sulfate, which is used as a tracer of SDS micelle.

Fig. 4.9 shows the dependence of the observed ion signal intensities of phenyltrimethylammonium chloride and octaoxyethylenedodecanol on the concentration of BBMA. The signal intensity of phenyltrimethylammonium chloride decreased with an increase in the concentration of BBMA. The intensity at 2% BBMA was approximately less than 20% of that in the absence of BBMA. The signal intensities of the other solutes except for octaoxyethylenedodecanol, tended to decrease with an increase in the

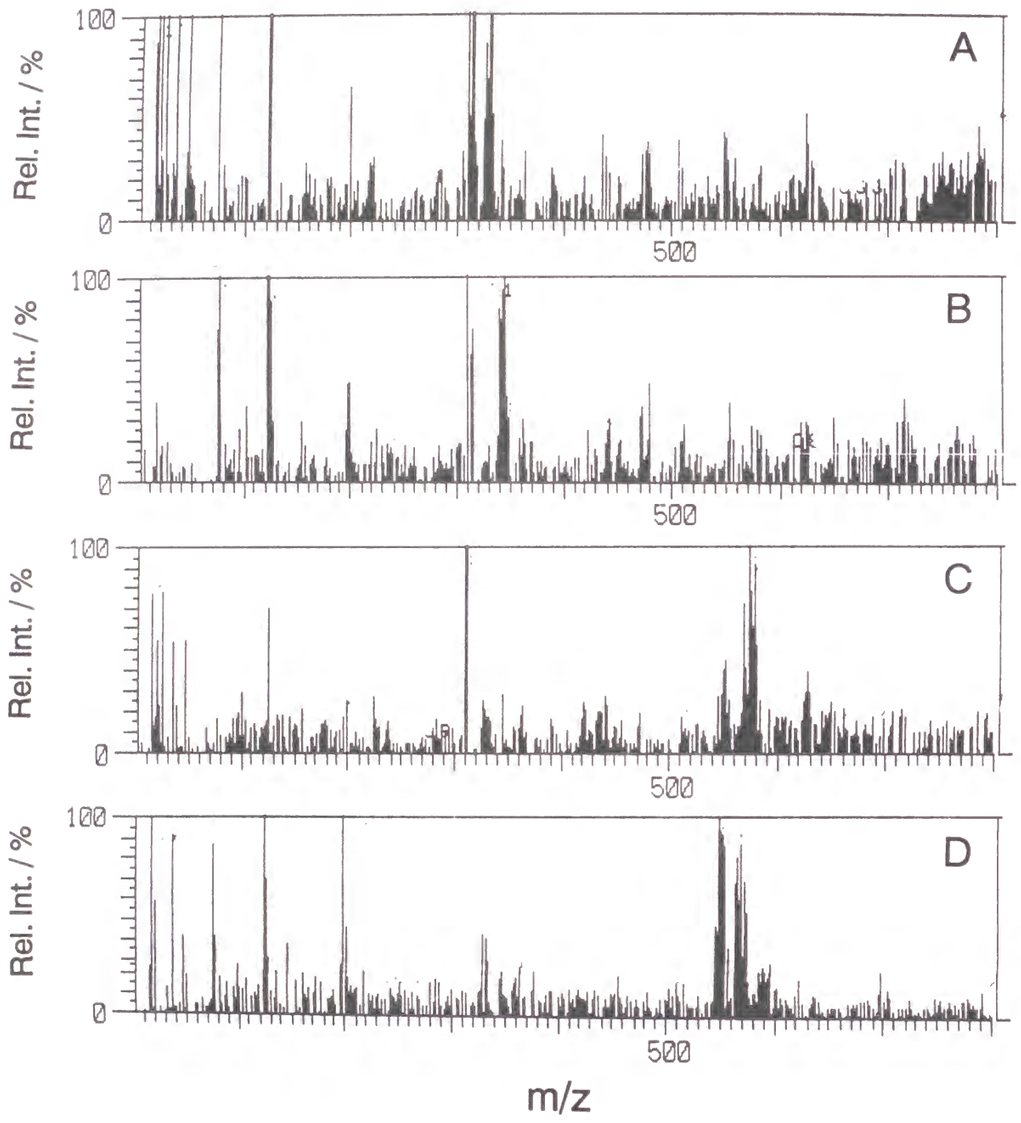


Fig. 4.7 Mass spectra acquired from the peak 3 (A), peak 4 (B) and peak 5 (C) in MEKC-ESI- MS shown in Fig. 6A and the peak 5 (D) in CE-ESI-MS shown in Fig. 6B. MS intensity was normalized by the signal observed over m/z 310.

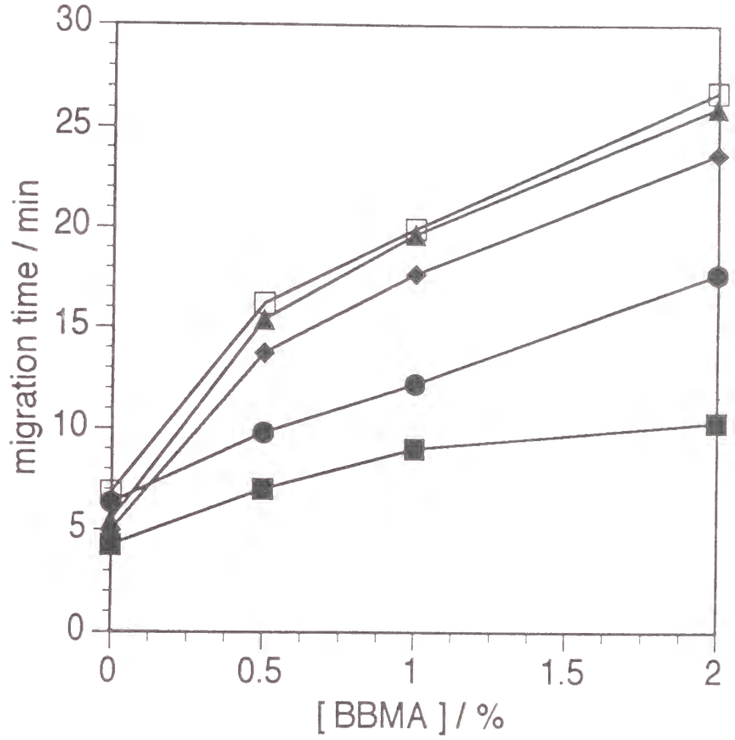


Fig. 4.8 Dependence of the migration time on the concentration of BBMA. Solutes: ■ = phenyltrimethylammonium chloride; ● = 1-naphthylamine; ▲ = Quinine sulfate; ◆ = tetraphenylphosphonium chloride; □ = octaoxyethylenedodecanol. Conditions are same as in Fig.4.6.

concentration of BBMA. The signal intensity of octaoxyethylenedodecanol, however, was maximal at 1% BBMA. Further details of the mass spectrum of octaoxyethylenedodecanol were investigated at each concentration of BBMA. Fig. 4.7C and D indicates the change of the mass spectra of octaoxyethylenedodecanol.

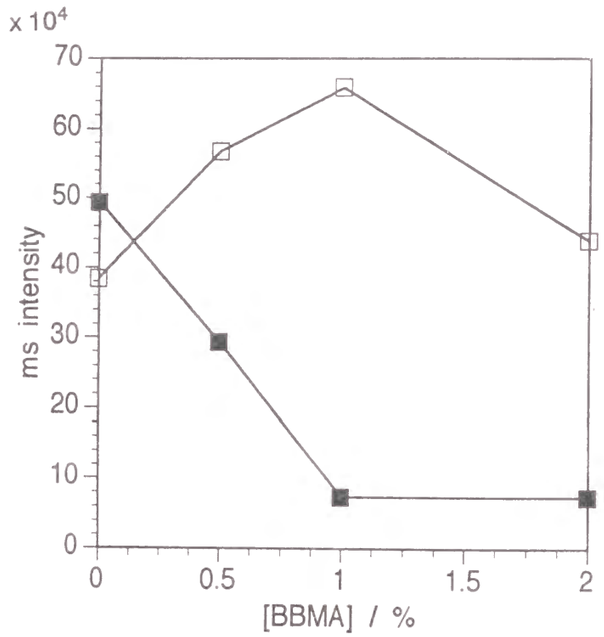


Fig. 4.9 Dependence of the intensities on the concentration of BBMA. Symbol: ■ = phenyltrimethylammonium chloride; □ = octaoxyethylenedodecanol. Conditions are same as in Fig.4.6.

Fig. 4.10 shows the dependence of the intensities of the two molecular ions of octaoxyethylenedodecanol, which are assigned to proton attachment, $[M+H]^+$, and ammonium attachment, $[M+NH_4]^+$, on the concentration of BBMA. Fig. 4.9 shows the intensity of $[M+NH_4]^+$. However, the intensify of $[M+H]^+$ decreased with an increase in the concentration of BBMA as shown in Fig. 4.10. A homologous non-ionic surfactant, pentaoxyethylenedodecanol, also showed the same tendency as octaoxyethylenedodecanol. Pentaoxyethylenedodecanol produced the molecular ion due to ammonium attachment stronger than due to proton attachment under MEKC-ESI-MS conditions with 2% BBMA. The sum of the intensities of $[M+H]^+$ and $[M+NH_4]^+$ decreased with an increase in the concentration of BBMA as shown in Fig. 4.10. It is generally noted that the MS intensity of the solute decreases with an increase in the concentration of BBMA. This result is consistent with that observed as an effect of the salt concentration on the ionization efficiency of ESI [29-31]

Fig. 4.11 shows MEKC separation with a UV detector (A) and MEKC-ESI-MS (B) of sulfamides. The single-ion chromatograms in Fig. 4.11B were recorded in the SIM mode without scanning. From Fig. 4.11B, all four sulfamides were separated and detected by MEKC-ESI-MS with 1% BBMA. All four sulfamides were easily separated by MEKC as shown in Fig. 4.11A. Separation efficiency in MEKC-ESI-MS was far less than conventional MEKC as easily judged from the separation between peaks 1 and 2. The significant deterioration of separation efficiency was probably caused by the ESI interface. No optimization was tried to improve the efficiency in this study. To take advantage of the high efficiency separation of MEKC, more work is needed to increase the separation efficiency in MEKC-ESI-MS.

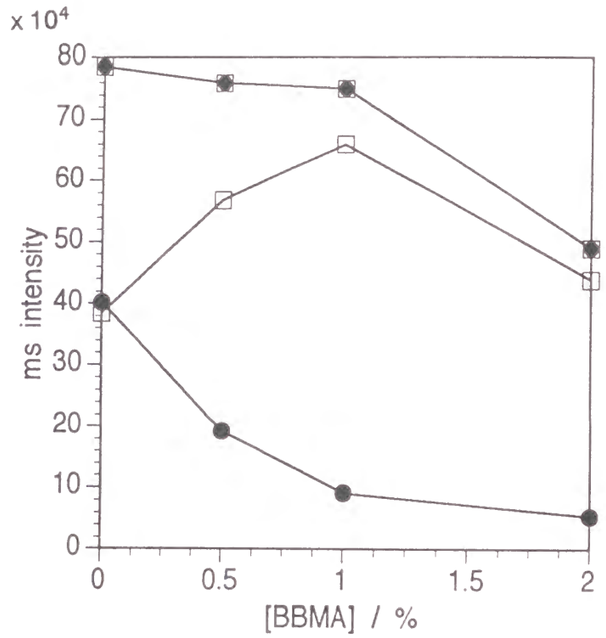


Fig. 4.10 Dependence of the intensities on the concentration of BBMA. Symbol: □ = octaoxyethylenedodecanol high mass peak, $[M+NH_4]^+$; ● = octaoxyethylenedodecanol low mass peak, $[M+H]^+$; ■ = □ + ●. Conditions are same as in Fig.4.6.

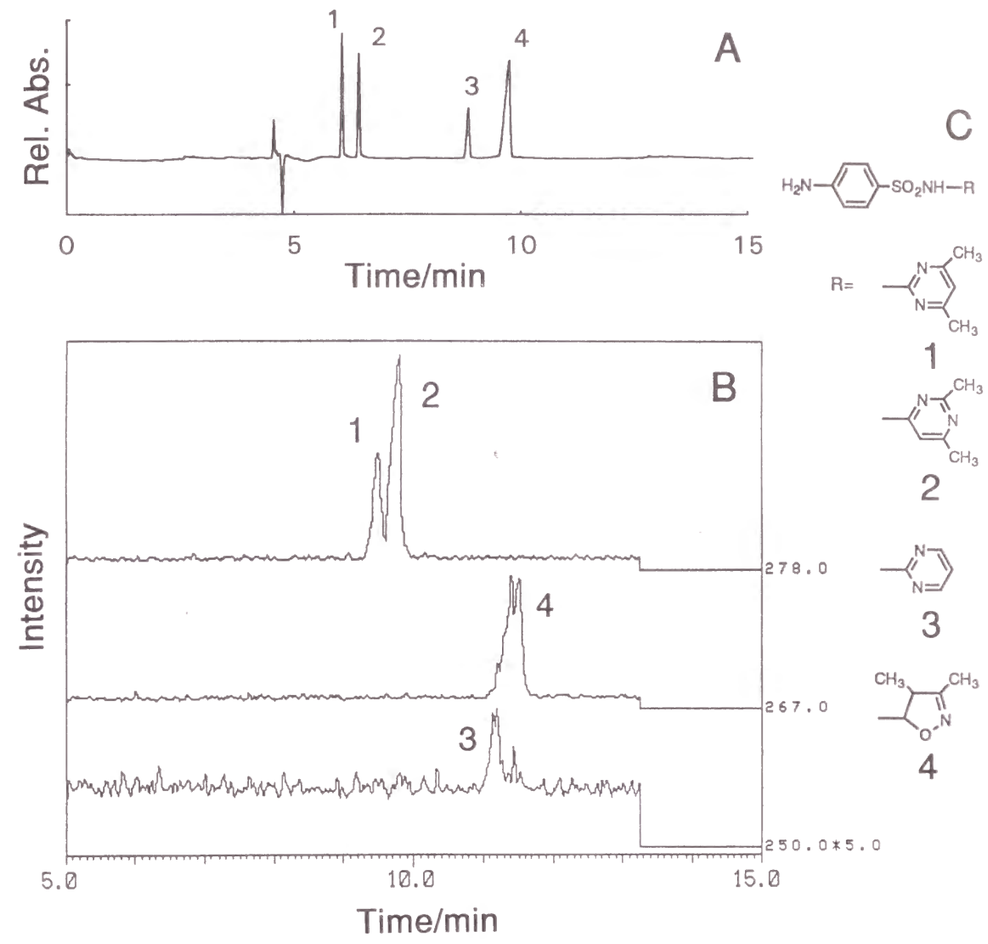


Fig. 4.11 MEKC with a UV detector (A) and MEKC-ESI-MS (B) of sulfamides and molecular structures of them (C). Solute: 1 = sulfamethazine; 2 = sulfisomidine; 3 = sulfadiazine; 4 = sulfisoxazole. Conditions: (A) separation solution, 1% BBMA in 10% methanol and 100 mM borate-50 mM phosphate buffer (pH 7); capillary, 50 μm i.d., 48cm (40 cm to the detector) fused-silica; applied voltage, 20kV; detection wavelength, 210 nm. (B) same as in Fig.4.6 except for the interface, MS instrument and operation on the SIM mode.

4.4 Conclusions

On-line MEKC-ESI-MS using BBMA, a polymer surfactant, was achieved. This system was employed successfully under the condition of MEKC with 2% BBMA. The concentration is high enough for most purposes. The results that intensities of solutes decrease with an increase in the concentration of BBMA suggest to use a separation solution including low concentration BBMA for the detection to the analytes which ionization efficiency is low. This work is fundamental and performance of this system, separation and sensitivity, is not enough high yet. More advanced ESI interface and optimization of conditions, MEKC, sheath flow and MS detection, will produce highly performance in a further work

References

1 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, **56** 111-113 (1984).

2 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, **57** 834-841 (1985).

3 J. Vindevogel, P. Sandra, *Introduction to Micellar Electrokinetitic Chromatography*, Huthing, Heidelberg, 1992.

4 S. Terabe, in N. Guzman (Editor) *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, pp. 65-87.

5 S. Terabe, N. Chen and K. Otsuka, *Adv. Electrophoresis*, **7** 87-153 (1994).

6 R. D. Smith, C. J. Barinaga and H. R. Udseth, *Anal. Chem.*, **60** 1948 (1988).

7 H. R. Udseth, J. A. Loo and R. D. Smith, *Anal. Chem.*, **61** 1989 (1989).

8 R. D. Smith, J. H. Whal, D. R. Goodlett and S. A. Hofstadler, *Anal. Chem.*, **65** 574 (1993).

9 J. H. Wahl and R. D. Smith, *J. Cap. Elec.*, **1** 62 (1994).

10 F. Garcia and J. D. Henion, *Anal. Chem.*, **64** 985-990 (1992).

11 I. M. Johasson, R. Pavelka and J. D. Henion, *J. Chromatogr.*, **559** 515-528 (1991).

12 S. Pleasance, P. Thibault and J. Kelly, *J. Chromatogr.*, **591** 325-339 (1992).

13 M. A. Moseley, L. J. Deterding, K. B. Tomer, J. W. Jorgenson, *Anal. Chem.*, **63** 109-114 (1991).

14 L. J. Deterding, C. E. Parker, J. R. Perkins, M. A. Moseley, J. W. Jorgenson and K. B. Tomer, *J. Chromatogr.*, **554** 329-338 (1991).

15 W. Weinmann, C. E. Parker, L. J. Deterding, D. I. Papac, J. Hoyes, M. Przybylski, and K. B.Tomer, *J. Chromatogr.*, **680** 353-361 (1994).

16 R. D. Smith and H. R. Udseth, in N. Guzman (Editor) *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, pp. 525-567.

17 K. B. Tomer, in N. Guzman (Editor) *Capillary Electrophoresis TeTechnology*, Marcel Dekker, New York, 1993, pp. 569-586.

18 W. M. A. Niessen, U. R. Tiaden and J. van der Greef, *J. Chromatogr.*, **636** 3-19 (1993).

19 R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *Anal Chem.*, **62** 882-899 (1990).

20 M. H. Lamoree, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, **712** 219 (1995).

21 A. F. Lecoq, S. D. Biase, L. Montanarella, *J. Chromatogr.*, **638** 363-373 (1993).

22 J. Varghese and R. B. Cole, *J. Chromatogr.*, **652** 369-376 (1993).

23 Y. Takada, K. Nakayama, M. Yoshida and M. Sakairi, *Analytical Sciences*, **10** 713-717 (1994).

24 C. P. Palmer, M. Y. Khaled and H. M. McNair, *J. High Resolut. Chromatogr.*, **15** 756-762 (1992).

25 C. P. Palmer and H. M. McNair, *J. Microcol. Sep.*, **4** 509-514 (1992).

26 H. Ozaki, A. Ichihara and S. Terabe, *J. Chromatogr.*, **680** 117-123 (1994).

27 H. Ozaki, A. Ichihara and S. Terabe, *J. Chromatogr.*, **709** 3-10 (1995).

28 K. Hiraoka, *Rapid Commun. Mass Spectrom.*, **6** 463 (1992).

29 L. Tang and P. Kebarle, *Anal. Chem.*, **63** 2709-2715 (1991).

30 L. Tang and P. Kebarle, *Anal. Chem.*, **65** 3654-3668 (1993).

31 L. Tang and P. Kebarle, *Anal. Chem.*, **65** 972A-986A (1993).

Chapter 5
Exploration of the Electrospray ionization mass detection for MEKC using the polymer surfactant and study of the partial filling method.

5.1 Introduction

Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis (CE), where ionic micelles are used as pseudo-stationary phases [1-5]. Almost all advantages of CZE apply to MEKC as well and many applications of MEKC separations have been reported. Coupling of CZE or MEKC with general spectroscopic detection methods must be useful. On-line coupling techniques for CE-MS have been studied by several groups [6-19]. Electrospray ionization (ESI) interfaces developed by Smith et al. [6-9] is useful for CE-MS. On the other hand, on-line coupling techniques for MEKC-MS has not been well developed yet, because of the presence of the surfactant in the running solution. Several methods have been reported for on-line MEKC-MS. Using a coupled capillary set-up and on-line heart-cutting of the MEKC separation zones was reported [20]. We reported on-line MEKC-MS system with a polymer surfactant, butyl acrylate-butyl methacrylate-methacrylic acid copolymer sodium salt (BBMA) [21]. Partial filling technique in MEKC for MS detection was reported [22,23]. Although Varghese and Cole used a cationic surfactant, cethyltrimethylammonium chloride [24], and a anionic surfactant, sodium dodecyl sulfate (SDS) [25], as an additive to the running solution for CE-ESI-MS, the separation mode described was not MEKC because the surfactant concentrations were below critical micelle concentration (CMC).

Advantage of using a polymer surfactant is a direct coupling of conventional

MEKC to MS detector without any special interfacing devices and separation methods. We reported conventional MEKC with BBMA [26, 27]. The molecular mass of the BBMA was measured to be about 40000 by size exclusion chromatography (SEC) using standard polyethylene glycol (PEG). BBMA showed high efficiency and significantly different selectivity in MEKC for naphthalene derivatives in comparison with SDS [26]. The CMC of BBMA was found to be effectively zero [26]. Thus, BBMA is suitable for MEKC-MS because of the formation of the micelle at low surfactant concentrations, a higher molecular mass beyond the hardware mass range, and high efficiency in separations. Mechanism of signal suppression by anionic surfactants including BBMA in ESI-MS detection was discussed elsewhere [28].

In this chapter, we describe some applications of the on-line MEKC-ESI-MS system and study of the partial filling technique with BBMA. Partial filling technique in MEKC (PF-MEKC) was first presented by us [29].

5.2 Experimental

5.2.1 Apparatus

The experimental framework of the MEKC-ESI-MS system was the same as in a previous report [21]. MEKC was performed with laboratory-built instruments which consisted of a Matsusada Precision Devices HCZE30PN0.25-LDSW high-voltage power supply (Kusatsu, Shiga, Japan) and a fused silica capillary of 50 cm long, 50 μm i.d., 150 μm o.d. obtained from Tokyo Kasei (Tokyo, Japan). The partial filling MEKC was performed with a Jasco CE-910 CE system (Tokyo, Japan) and a fused silica capillary of 1 m long, 50 μm i.d., 150 μm o.d. An ESI interface was laboratory-built and consisted of a stainless steel tube of 190 μm i.d. 350 μm o.d. (G 28), inside which the fused silica

capillary was coaxially inserted, a polytetrafluoroethylene (PTFE) tee union, which holded the stainless steel tube, the capillary and a PTFE tube for the delivery of a sheath flow. The MS system consisted of a modified Hitachi M-1000 LC-APCI-MS (Tokyo, Japan) which was constructed with a quadrupole mass spectrometer and a differential pumping region.

MEKC using UV detection was performed on a Hewlett Packard HP3DCE system (Waldbronn, Germany).

5.2.2 Procedure

On-line MEKC-ESI-MS work was performed with the system described above using BBMA solutions in 10 mM ammonium formate buffer (pH 7) containing 10% methanol. Samples were injected by the hydrostatic injection method (10-30s at 15 cm). The MEKC applied voltage was 10 kV (13 kV at the capillary inlet and 3 kV at the end of the capillary located in ESI interface). The sheath liquid was consist of water, methanol and formic acid (50:50:1, v/v/v) and was delivered by a Hitachi HPLC pump L6300 at approximately 5 μl/min or a Harvard Apparatus Model 44 syringe pump (Natick, MA, USA) at 1 μl/min. Electrospray was performed using a 3 kV gradient between the capillary end and the first MS sampling orifice. MS system was operated in the positive ion mode. All MS detection was performed in the scanning mode, m/z 1 to 1000, at 4 s/scan. Drift voltage was 70 V, focusing voltage 140-150 V and resolution 50-55. The m/z value was not always accurate because the calibration was not performed frequently and the room temperature was not constant. And so, in order to see if the analyte ion is produced and also to find the m/z value for the major peaks, the mass spectra of all analytes were obtained by the direct injection to the detector before the on-line MEKC-

MS work.

Conventional MEKC using a UV detector was performed as described previously [26,27]. In partial filling MEKC, micellar zone was introduced in a part of the capillary by pressurization with the instruments described above.

5.2.3 Reagent

BBMA was supplied as a 23% aqueous solution by Dai-ichi Kogyo Seiyaku (Kyoto, Japan). Since BBMA contains a minor amount of low molecular mass components, it was purified by the reprecipitation method with acetone [26, 27]. SDS was purchased from Nacalai Tesque (Kyoto, Japan). All other reagents were of analytical grade and water was purified with a Milli-Q system.

Sample compounds, pyridoxine, nicotinamide, octyltrimethylammonium bromide, decyltrimethylammonium bromide, docecyltrimethylammonium bromide, cethltrimethyl-ammonium bromide and PEG 1000 obtained from Nacalai Tesque, caffeine, phenyltrimethylammonium chloride, tetraphenylphosphonium chloride, and octaoxyethylenedodecanol obtained from Wako Pure Chemical (Osaka, Japan), 1,7-dimethylxanthine, 1-methylxanthine, 7-methylxanthine, 1-methyluric acid and 7-methyluric acid from Aldrich (St. Louis, MO, USA), were of analytical grade and used as received. Other pharmaceuticals were supplied commercially and used as received. Sample solutes were dissolved in about 50% aqueous methanol.

5.3 Results and discussion

5.3.1 MEKC-ESI-MS with BBMA

For the pseudo-stationary phase in on-line MEKC-ESI-MS, BBMA is expected to

be suitable because of the formation of the micelle at low surfactant concentrations. This advantage was verified by the separation of non-ionic naphthalene derivatives, 1-naphthalenemethanol, 1-naphthol and 2-naphthol, with 0.025% BBMA using UV detection. This BBMA concentration is practically lower than the CMC of SDS expressed by weight %. BBMA has not been frequently employed in applications of MEKC, compared with SDS. BBMA showed significantly different selectivity in contrast to SDS [26]. Therefore, it is important to investigate characteristics of separation and selectivity in BBMA system. Sulfamides, sulfamethazine, sulfisomidine, sulfadiadine and sulfisoxazole, were separeted and detected by ESI-MS with 2% BBMA previously [21].

Fig. 5.1 shows single-ion chromatograms of some pharmaceuticals by MEKC-ESI-MS with BBMA. All the solutes were used as hydrochloride. The mass spectra were scanned from m/z 5 to 800 for 4 s/scan. All the solutes were successfully detected both by single-ion chromatograms and by total-ion chromatogram under the condition of 1% BBMA. However, Azelastine, Dibucaine and Eperisone were not separated. Significantly broad peaks were probably ascribed to the ESI interface, because MEKC-UV gave much narrower peaks. Poor S/N ratios were also due to the poor transfer efficiency of our ESI system and poor sensitivity of our MS system. If a modern MS system is employed, S/N ratio will be much higher.

Fig. 5.2 shows single-ion chromatograms of another pharmaceuticals with 1% BBMA. Isopropylantipyrine was well detected. Some other cold medicines, acetaminophen, ethenzamide and guaifenesin, were not detected under the same condition as in Fig.5.2.

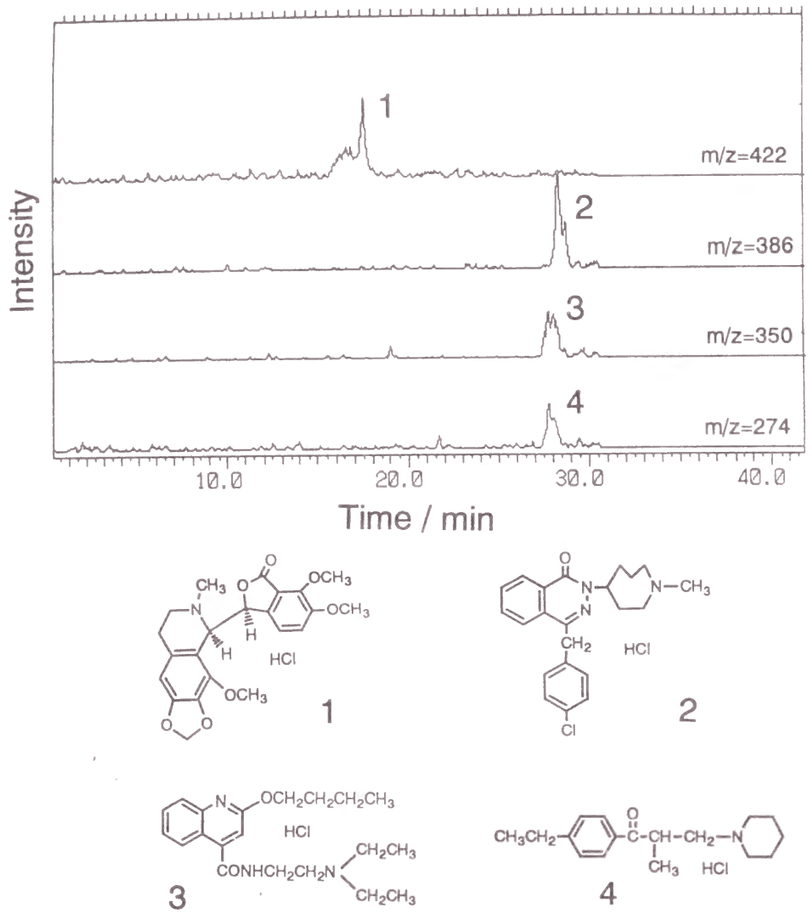


Fig. 5.1 MEKC-ESI-MS of pharmaceuticals (A) and their molecular structures (B). Solute: 1 = noscapine; 2 = azelastine; 3 = dibucaine; 4 = eperisone. MEKC conditions: capillary, 50 μm i.d., 50cm fused-silica; separation solution, 1% BBMA in 10 mM ammonium formate (pH 7); applied voltage, 13kV. ESI-MS conditions: electrospray voltage, 3kV; MS scanning, from m/z 1 to 800 at 4 per scan; mode, positive; drift voltage, 70V; focusing voltage, 140V; resolution, 55; sheath liquid flow, water-methanol-formic acid (50:50:1, v/v/v) at ca. 5 μl/min.

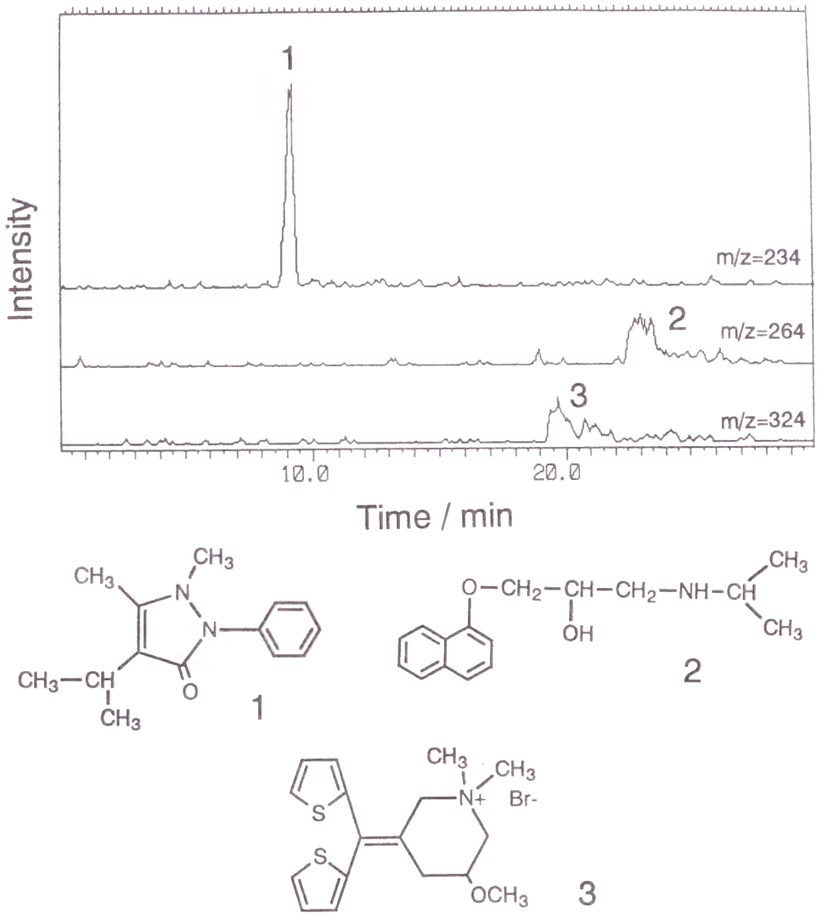


Fig. 5.2 MEKC-ESI-MS of pharmaceuticals (A) and molecular structures of them (B). Solute: 1 = isopropylantipyrine; 2 = propranolol; 3 = timepidium bromide. Conditions as in Fig.5.1.

Water soluble vitamins, caffeine, pyridoxine and nicotinamide, were detected, but all of them were migrated fast and not separated enough under the same condition. MEKC is suitable for analyses of pharmaceuticals involving cationic, anionic and neutral analytes, but it is important to optimize the MEKC separation conditions for the on-line ESI-MS detection of pharmaceuticals. In this work, however, no optimization was tried because the purpose of the study was to investigate the possibility of MEKC-MS using the polymer surfactant. The MEKC separation with BBMA may be improved by the addition of organic solvent, organic modifiers and changing pH. The effect of the addition of methanol to the BBMA system has been studied previously [27] and methanol will not cause a problem in ESI-MS detection. The effect of pH with BBMA system has also been studied [26]. Although the lower pH condition was suitable to ESI-MS detection in positive ion mode, BBMA precipitated below pH4 [26].

Fig. 5.3A shows the single-ion chromatograms of cationic (1 to 4) and non-ionic surfactants (5 and 6) with 2% BBMA. PEG 1000 was a mixture and the molecular masses were widely distributed. The single-ion chromatogram of PEG 1000 was detected by the strongest peak below m/z 1000. Although the intensity of the peak 6 in Fig.3A was weak, the mass spectrum of this peak was the same as that of PEG 1000 observed by the direct injection as shown in Fig.5.3B. It should be noted that PEG 1000 migrated close to t_0 owing to the weak interaction with BBMA micelle. All the cationic surfactants were successfully separated and the migration order was the same as carbon numbers of the alkyl chain. Increase of the spectrum background caused by separation solution was observed in the chromatogram of the peak 3. Under the same condition as Fig. 5.3, non-ionic surfactants having polyoxyethylene group, octa-, penta-, and di-oxyethylenedodecanol, were well detected, but not separated.

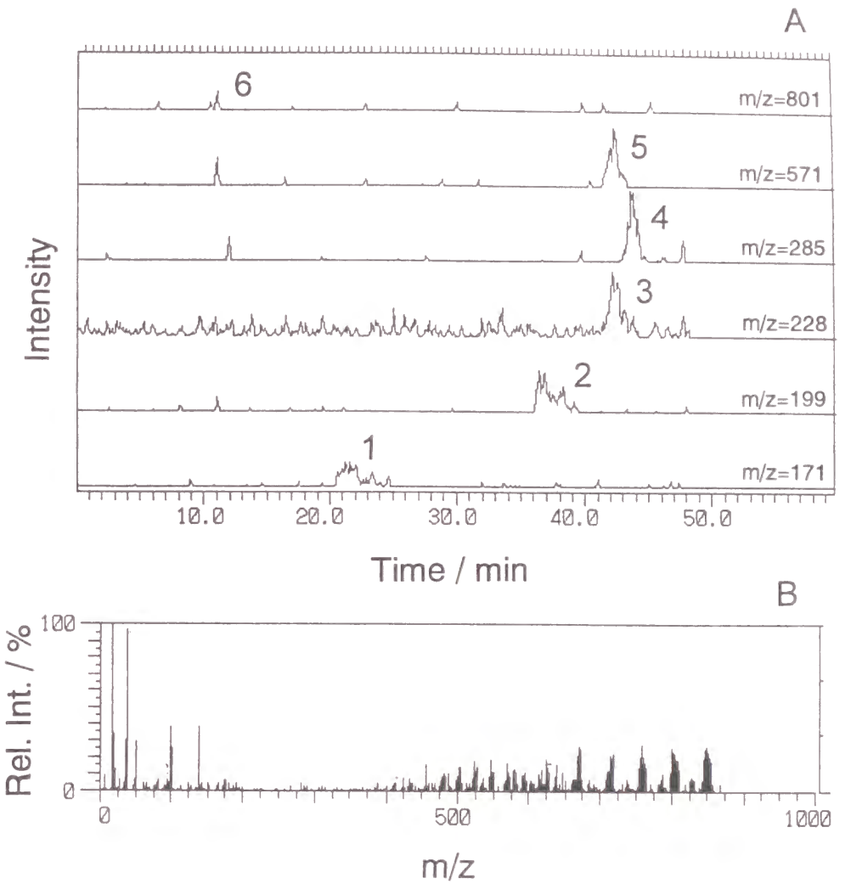


Fig. 5.3 MEKC-ESI-MS of cationic and non-ionic surfactants (A) and mass spectrum of the peak 6 (B). Solute: 1 = octyltrimethylammonium bromide; 2 = decyltrimethylammonium bromide; 3 = dodecyltrimethylammonium bromide; 4 = cetyltrimethylammonium bromide; 5 = octaoxyethylenedocecanol; 6 = PEG1000. Separation solution; 2% BBMA in 10% methanol and 10 mM ammonium formate (pH 7). Other conditions as in Fig.5.1.

With 0.5% BBMA, all the cationic surfactants showed significant tailing owing to the strong interaction with the negatively charged capillary wall.

5.3.2 Dependence of ESI signal intensities on the concentration of surfactant

Dependence of ESI signal intensities of caffeine on the concentrations of surfactants, BBMA and SDS, were studied with direct introduction of the sample solution to the ESI system. The direct introduction of the caffeine solution containing BBMA or SDS was performed with the same sheath liquid flow as the on-line work. The concentration of caffeine was 10 mM and the solution was introduced by pressurization. Fig. 5.4. shows that the signal intensity of the proton attached ion of caffeine decreased with the increase of either BBMA or SDS concentration. A rapid and steady decrease was observed with SDS rather than with BBMA. It should be noted that the decrease in signal intensity was slight with an increase in BBMA concentration from 0 to 0.5% compared with SDS. Fig. 5.5 shows examples of ESI-MS spectra of caffeine in the presence of BBMA (A and B) or SDS (C and D). In Fig. 5.5C and 5.5D, the molecular ion of SDS due to sodium attachment (marked as $[SDS+Na]^+$) and the singly charged dimer ion (marked as $[2SDS+Na]^+$) were observed. Weak sodium attached ions of caffeine (marked as $[M+Na]^+$) were observed with either BBMA or SDS system. The sodium attached ions of caffeine increased with increases of the concentrations of BBMA or SDS. When the drift voltage was reduced down to 30 V, the sodium attached ion of caffeine was observed stronger than the proton attached ion in the presence of 0.5% BBMA.

Cole et al [25] studied the decrease of ESI-MS signal of tamoxifen and its metabolites with the SDS system in methanol media. In this case, the abundance of the

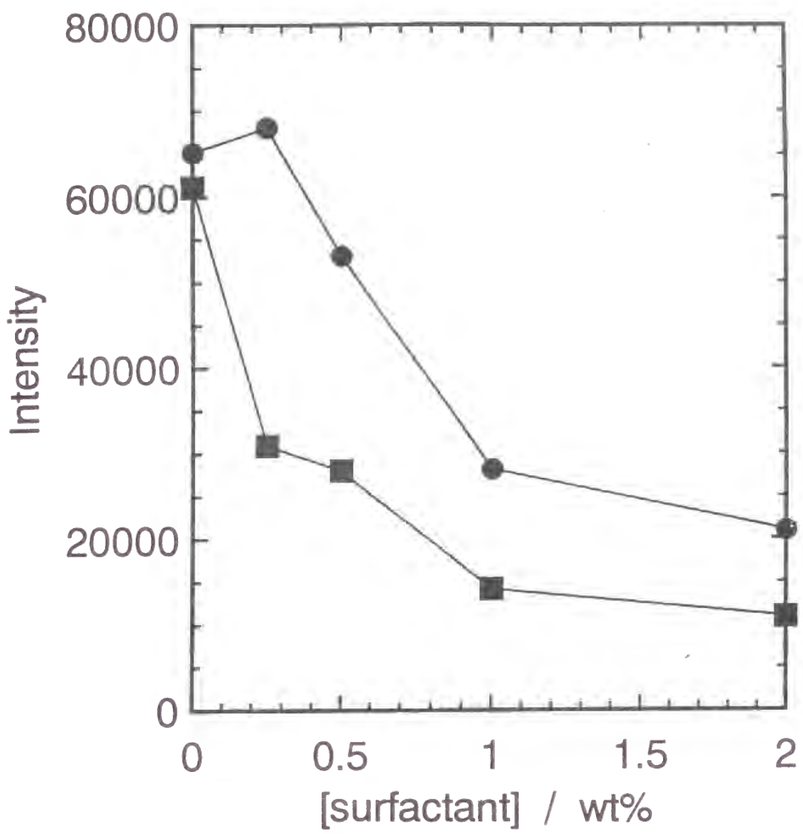


Fig. 5.4 Dependence of the ESI-MS intensities of caffeine on the concentration of surfactants by liquid injection. Symbol: ● = BBMA; ■ = SDS.

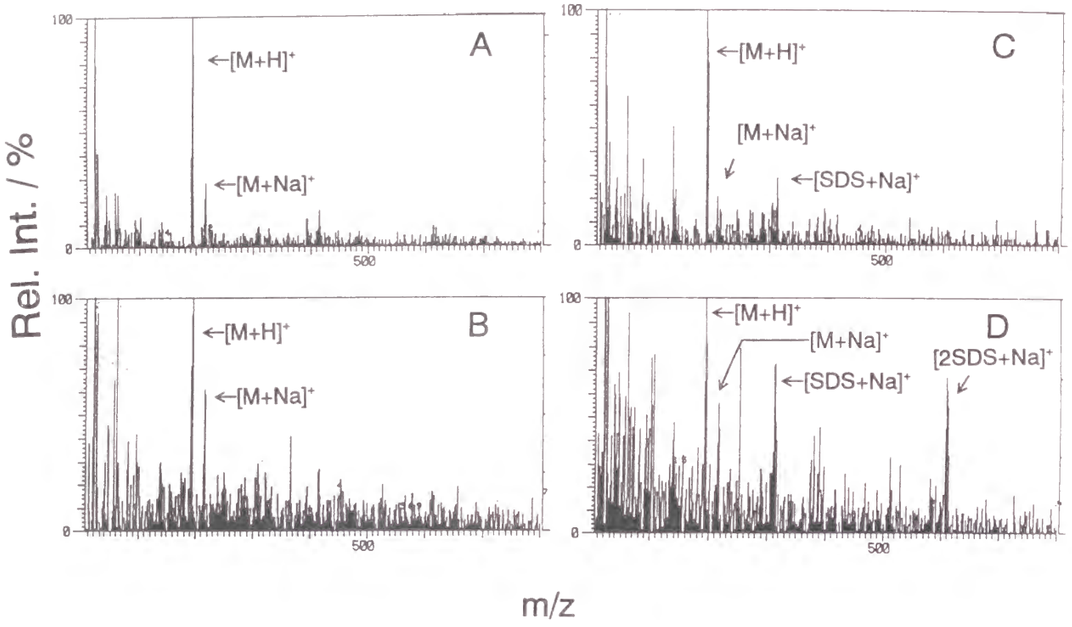


Fig. 5.5 ESI mass spectra of caffeine with BBMA (A and B) and SDS (C and D). Surfactant concentration; 0.5%(A and C) and 2% (B and D).

protonated tamoxifen analogs decreased steadily as the SDS concentration increased from 0 to 10 mM (from 0 to 0.29 %) and leveled off between 10 and 30 mM (between 0.29 and 0.87 %). In Fig. 5.4, it can be seen that the intensity leveled off over 1 % SDS.

The detailed mechanism of the signal suppression by the surfactants in MEKC-ESI-MS should be complex because there are two main steps, the production of charged droplets and the production of gas-phase ions from the charged droplets, in the generation of gas-phase ions of the analyte by ESI and both steps should be affected by the pH, buffer ions and surfactant ions of the MEKC separation solution. Rundlett et al. [28] presented a mechanism of the signal suppression by anionic surfactants in ESI-MS. They proposed the ESI-produced offspring droplets model caused by Coulombic interaction between oppositely charged solute and surfactant ions. For the detailed study of the mechanism in ESI-MS with surfactants, it is interesting that the the relatively strong signal of the SDS dimer ion was observed as shown in Fig. 5.5. Furthermore, the observation of sodium attached caffeine ions as shown in Fig. 5.5 indicate that the presence of sodium, the counter ion of the anionic surfactant, should cause the signal suppression of proton attached ions of analyte in MEKC-ESI-MS.

On the basis of the study with direct introduction of the caffeine to the ESI-MS, the on-line MEKC-ESI-MS of caffeine with BBMA was performed under the condition of relatively slight signal suppression. Fig. 5.6 shows the single-ion chromatogram(A) and spectrum of the peak (B) of caffeine by MEKC-ESI-MS with 0.5% BBMA. Sheath liquid flow rate was 1 ml/min. Other conditions were the same as in Fig.5.1. The amount of the injected sample was 180 ng (concentration was 5 mg/ml and 37nl injected). The detectability in MEKC-ESI-MS was far less than that in conventional LC-MS. The lower

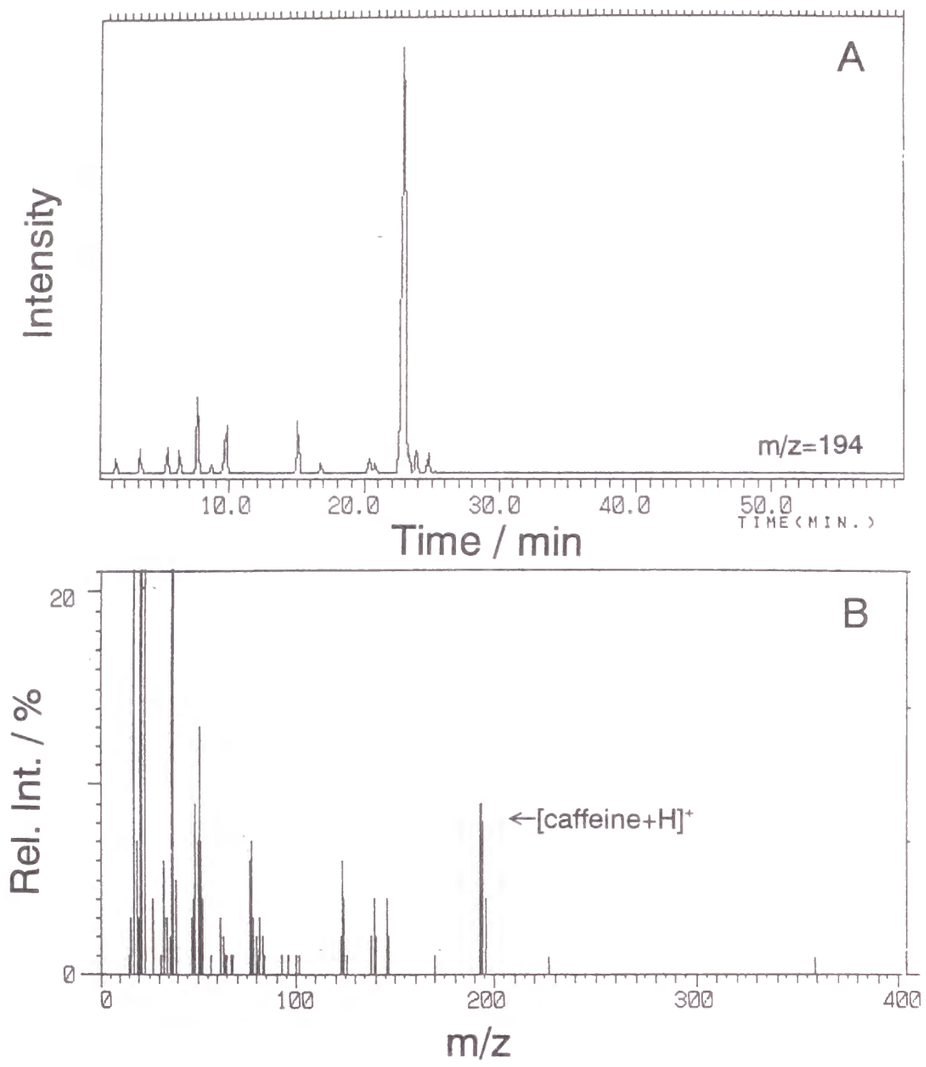


Fig. 5.6 MEKC-ESI-MS of caffeine (A) and mass spectrum of the peak (B). Conditions; capillary, 50 μm i.d., 100cm fused-silica; separation solution, 0.5% BBMA in 20 mM ammonium formate (pH 7); sheath liquid flow, water-methanol-formic acid (50:50:1, v/v/v) at 1 μl/min; the amount of the injected sample, 180 ng. Other conditions as in Fig.5.1.

detectability was probably caused by the ESI-interface which was laboratory-built and not fully optimized.

5.3.3 Partial filling technique with BBMA

The study described above indicates that the intensity of analyte ion decreases with the increase of the BBMA concentration. Partial filling technique (PF) of the pseudo-stationary phase is expected to solve the problem of signal suppression in ESI-MS detection on-line coupled with MEKC [22,23,29]. Fig. 5.7 shows the separation of naphthalene derivatives by the PF-MEKC with UV. Before the BBMA zone (marked with mc in Fig.5.7A) reached the detector, six naphthalene derivatives were separated successfully and detected with 5% BBMA zone introduced at 0.05 atm for 30 s as shown in Fig.5.7B. Effect of the buffer system on the PF-MEKC was studied. Borate, which is a nonvolatile buffer component, and formic acid, acetic acid, and propionic acid ammonium salts, which are volatile components, were compared. In the borate buffer, BBMA zone was relatively sharp as shown in Fig. 5.7. With other buffers, however, the BBMA zone became broader. The BBMA zone showed remarkable fronting and overlapped with analyte peaks in the ammonium formate, acetate and propionate buffers. It was clearly shown that choice of the buffer system is critical for on-line coupling of PF-MEKC with ESI-MS, and more work is needed. BBMA should be useful to study PF-MEKC because the surfactant zone peak is directly detected by UV. In the study with SDS [22], a micellar marker, quinine, was used to detect the SDS zone.

Fig. 5.8 shows the PF-MEKC separation of caffeine and its metabolite compounds in the borate buffer system. Before the BBMA zone passed through the detector, five analytes were separated successfully with 5% BBMA zone introduced at

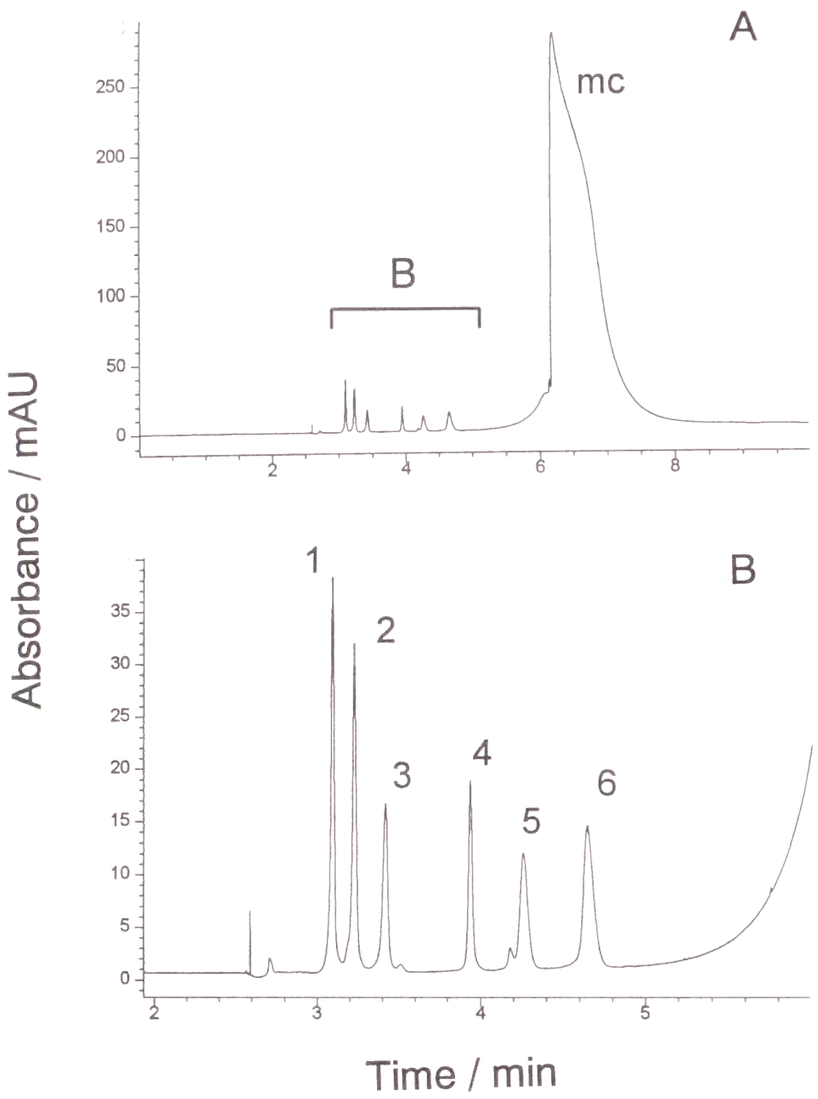


Fig. 5.7 PF-MEKC separation of naphthalene derivatives using BBMA. Solute: 1 = 1-naphthalenemethanol; 2 = 1,6-dihydroxynaphthalene; 3 = 1-naphthylamine; 4 = 1-naphthaleneethanol; 5 = 2-naphthol, 6 = 1-naphthol. BBMA zone peak marked with mc. Conditions: capillary, 50 μm i.d., 48 cm (40 cm to the detector) fused-silica; separation solution, 5% BBMA zone introduced at 0.05 atm for 20 s in 100 mM borate buffer (pH 9); applied voltage, 20kV; detection wavelength, 210 nm.

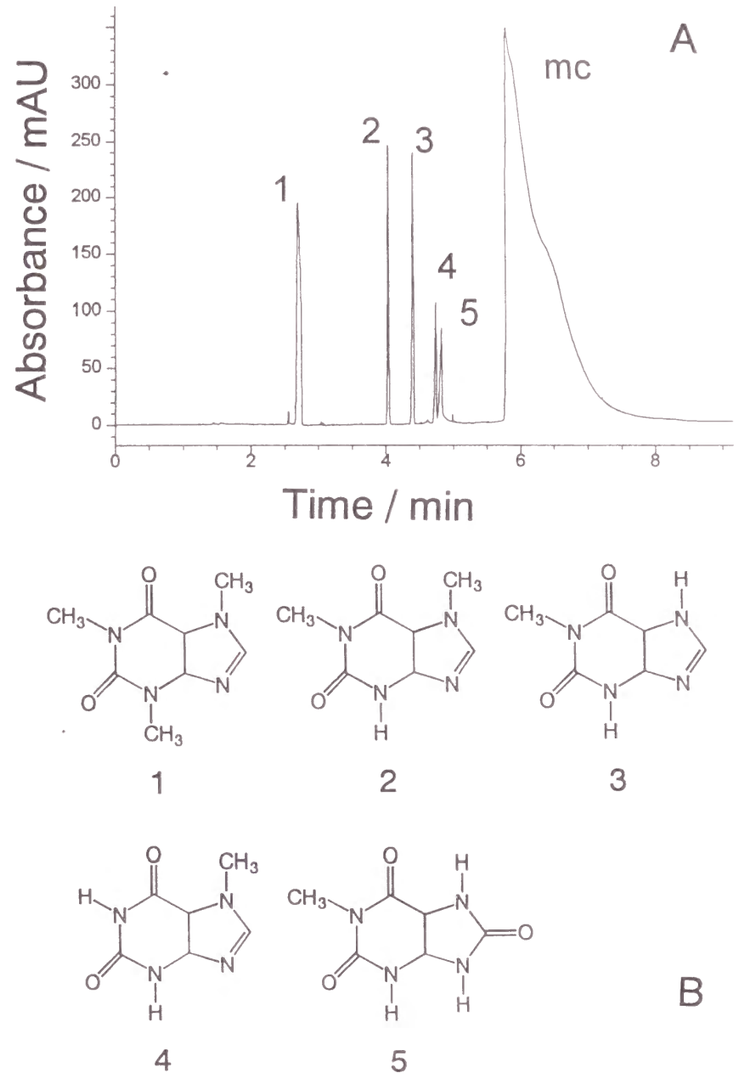


Fig. 5.8 PF-MEKC separation of caffeine and its metabolites using BBMA (A) and molecular structure of them (B). Solute: 1 = caffeine; 2 = 1,7-dimethylxanthine; 3 =1-methylxanthine; 4 = 7-methylxanthine; 5 = 1-methyluric acid. BBMA zone peak marked with mc. Conditions as in Fig.5.7.

0.05 atm for 20s. Migration order was the same as by conventional MEKC with BBMA.

Fig. 5.9A shows the total-ion and single-ion chromatograms of the same analytes as in Fig. 5.8 by PF-MEKC-ESI-MS in the ammonium formate system. Fig.5.9B shows the mass spectra acquired from peaks in Fig. 5.9A. Peaks of Caffeine , 1,7-dimethylxanthine, 1-methylxanthine and 7-methylxanthine were detected both by the single-ion chromatograms and total-ion chromatogram. 1-methyluric acid was not detected owing to the decrease of detectability brought about by the overlap of the partially filled BBMA zone in ammonium formate buffer system.

5.4 Conclutions

On-line MEKC-ESI-MS using the diluted BBMA solutions was explored. Although the resolution was not enough high, some standard mixtures, the pharmaceuticals and industrial surfactants, were separated and detected successfully by the direct coupling of the conventional MEKC to the ESI-MS detector. In order to improve the resolution, the higher BBMA concentration was required. However, by the direct injection study, the high BBMA concentration degraded significantly ESI-MS intensity. Therefore, PF-MEKC with BBMA was investigated and applied to the on-line MS detection of caffeine and its metabolites. The performance of the on-line MS detection system in this work was not very high yet. The more detailed study about signal suppression by surfactants in MEKC-ESI-MS will be necessary for further development.

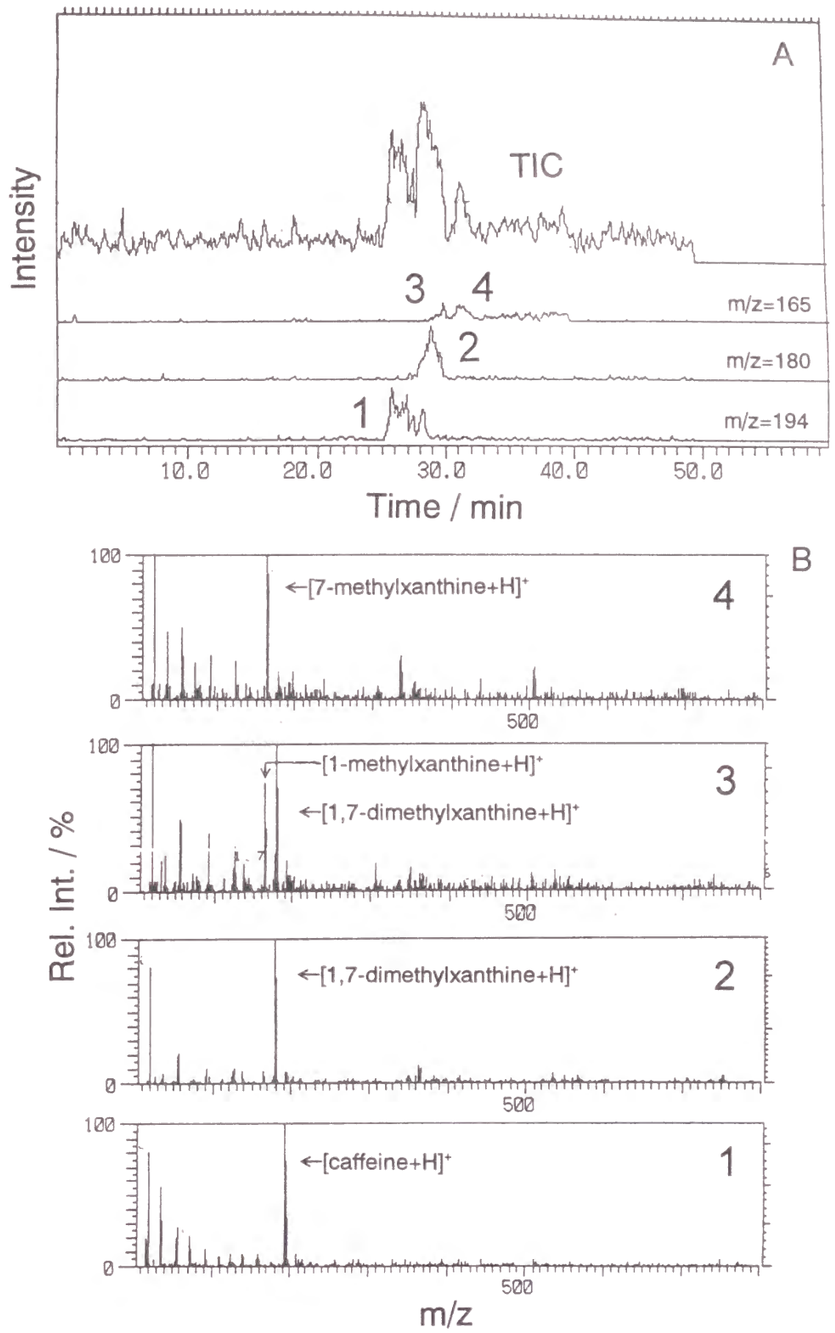


Fig. 5.9 MEKC-ESI-MS of caffeine and its metabolites using BBMA (A) and mass spectra of the peaks (B). Solute as in Fig.5.8. Conditions: capillary, 50 μm i.d., 100 cm fused-silica; separation solution, 2% BBMA introduced at 0.1 atm for 120 s in 20 mM ammonium formate (pH 7); applied voltage, 20 kV. Other conditions as in Fig.5.1.

References

1 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal Chem.*, **56** 111-113 (1984).

2 S. Terabe, K. Otsuka and T. Ando, *Anal, Chem.*, **57** 834-841 (1985).

3 J. Vindevogel, P. Sandra, *Introduction to Micellar Electrokinetitic Chromatography*, Huthing, Heidelberg, 1992.

4 S. Terabe, in N. Guzman (Editor) *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, pp. 65-87.

5 S. Terabe, N. Chen and K. Otsuka, *Adv. Electrophoresis*, **7** 87-153 (1994).

6 R. D. Smith, C. J. Barinaga and H. R. Udseth, *Anal. Chem.*, **60** 1948 (1988).

7 H. R. Udseth, J. A. Loo and R. D. Smith, *Anal. Chem.*, **61** 1989 (1989).

8 R. D. Smith, J. H. Whal, D. R. Goodlett and S. A. Hofstadler, *Anal. Chem.*, **65** 574 (1993).

9 J. H. Wahl and R. D. Smith, *J. Cap. Elec.*, **1** 62 (1994).

10 F. Garcia and J. D. Henion, *Anal. Chem.*, **64** 985-990 (1992).

11 I. M. Johasson, R. Pavelka and J. D. Henion, *J. Chromatogr.*, **559** 515-528 (1991).

12 S. Pleasance, P. Thibault and J. Kelly, *J. Chromatogr.*, **591** 325-339 (1992).

13 M. A. Moseley, L. J. Deterding, K. B. Tomer, J. W. Jorgenson, *Anal. Chem.*, **63** 109-114 (1991).

14 L. J. Deterding, C. E. Parker, J. R. Perkins, M. A. Moseley, J. W. Jorgenson and K. B. Tomer, *J. Chromatogr.*, **554** 329-338 (1991).

15 W. Weinmann, C. E. Parker, L. J. Deterding, D. I. Papac, J. Hoyes, M. Przybylski, and K. B.Tomer, *J. Chromatogr.*, **680** 353-361 (1994).

16 R. D. Smith and H. R. Udseth, in N. Guzman (Editor) *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, pp. 525-567.

17 K. B. Tomer, in N. Guzman (Editor) *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, pp. 569-586.

18 W. M. A. Niessen, U. R. Tiaden and J. van der Greef, *J. Chromatogr.*, **636** 3-19 (1993).

19 R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *Anal. Chem.*, **62** 882-899 (1990).

20 M. H. Lamoree, U. R. Tjaden and J. van der Greef, *J. Chromatogr.*, **712** 19-25 (1995).

21 H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi and H. Koizumi, *J. Chromatogr.*, **716** 69-79 (1995).

22 W. M. Nelson, Q. Tang, A. K. Harrata and C. S. Lee, *J. Chromatogr.*, **749** 219-226 (1996).

23 S. Terabe, K. Koezuka, N. Matsubara and H. Ozaki, *J. Chromatogr. B*, **689** 3-11 (1997).

24 J. Varghese and R. B. Cole, *J. Chromatogr.*, **652** 369-376 (1993).

25 W. Lu, G. K. Poon, P. L. Carmichael and R. B. Cole, *Anal. Chem.*, **68** 668-674 (1996).

26 H. Ozaki, A. Ichihara and S. Terabe, *J. Chromatogr.*, **680** 117-123 (1994).

27 H. Ozaki, A. Ichihara and S. Terabe, *J. Chromatogr.*, **709** 3-10 (1995).

28 K. L. Rundlett and D. W. Armstrong, *Anal. Chem.*, **68** 3493-3497 (1996).

29 S. Terabe, H. Ozaki, Y. Takada, M. Sakairi and H. Koizumi, *Proceedings of the Sevence International Symposium on High Performance Capillary*

Electrophoresis, 1995, pp.53.

Chapter 6
Electrospray ionization MS detection using a new polymer surfactant and atmospheric pressure chemical ionization MS detection

6.1 Introduction

Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis (CE) [1-3], where ionic micelles are used as pseudo-stationary phases, and almost all advantages of CE apply to MEKC as well and many applications of MEKC separations have been reported [4-6]. Coupling of CE or MEKC with general spectroscopic detection methods must be useful. On-line coupling techniques for CE-MS have been studied by several groups and electrospray ionization (ESI) interfaces have been successfully used and widely accepted [7-12]. For the on-line coupling between CE and ESI, a separation solution must be composed of volatile electrolytes. Non-volatile electrolytes introduced into the ESI-MS cause the some problems: 1) the decrease of sensitivity, 2) contamination of the interface, 3) high spectral backgrounds. The decrease of sensitivity caused by the non-volatile electrolytes is a serious disadvantage on ESI-MS [13], and volatile electrolytes such as ammonium formate or acetate are used for CE-MS with ESI. Non-volatile surfactants introduced into the ESI-MS also cause the same problems on MEKC-MS with ESI. To solve the problems, we reported on-line MEKC-ESI-MS system with a polymer surfactant, butyl acrylate/butyl methacrylate/ methacrylic acid copolymer sodium salt (BBMA) [14]. The BBMA micelle functioned successfully as a pseudo-stationary phase in an ammonium formate buffer and some non-ionic standard compounds were separated by MEKC and detected by ESI-MS with 0-2% BBMA. The

ESI interface used was similar to the commercial system. Takada et al. reported on-line coupling between the MEKC system with SDS and atmospheric pressure chemical ionization (APCI) -MS system [15]. The APCI interface is advantageous for CE-MS because even non-volatile sodium phosphate does not significantly degrade sensitivity. However, the APCI interface for on-line coupling of CE or MEKC is not similar to the commercial system for LC owing to a low flow rate on CE. The eluent from the capillary is electrosprayed and solvent molecules are ionized by the corona discharge followed by ion/molecule reaction which produces analyte ions as in the conventional APCI. To avoid the introduction of surfactants to the MS instruments, the partial filling technique was reported in MEKC [16,17]. ESI and APCI are suitable for the on-line coupling of MS with liquid phase separation techniques such as HPLC and CE, because gas-phase ions of the analyte are produced efficiently under the atmospheric pressure. Most commercial LC-MS systems are equipped with ESI and APCI interfaces. Therefore, these powerful ionization methods will be useful in on-line coupling of MS with CE or MEKC. In this chapter, the author describes the on-line MEKC-ESI-MS system with a polymer surfactant, diisobutylene-maleic acid copolymer sodium salt (DMA) and the MEKC-APCI-MS with some conventional surfactants, sodium dodecyl sulfate (SDS) and sodium cholate.

6.2. Experimental

6.2.1. Reagent

BBMA was supplied as aqueous solution by Dai-ichi Kogyo Seiyaku (Kyoto, Japan) and it was purified by the reprecipitation method with acetone. DMA was supplied as aqueous solution by Kao (Tokyo, Japan) and it was purified by the reprecipitation with formic acid.

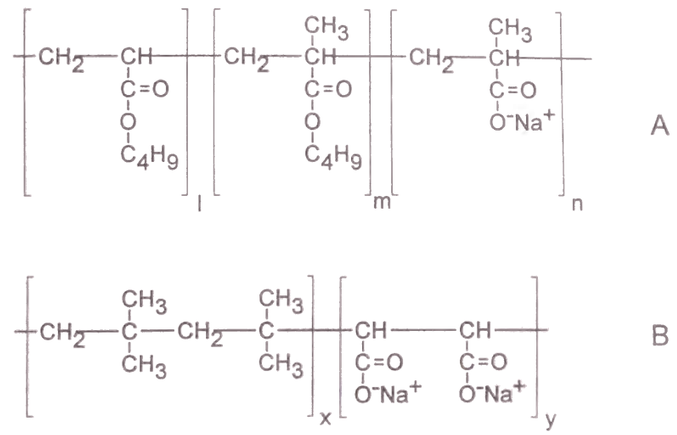


Fig. 6.1 Molecular structures of BBMA (A) and DMA (B).

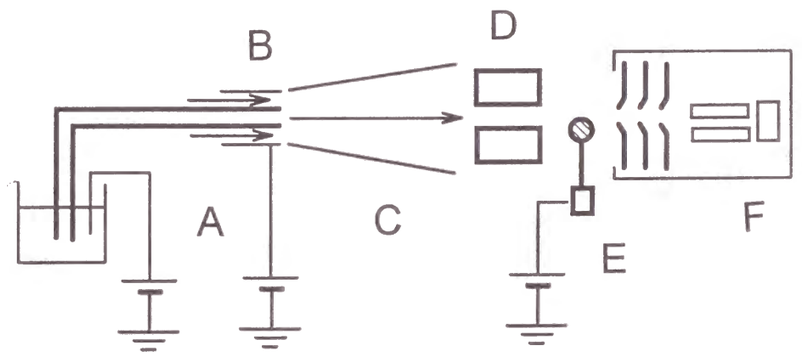


Fig. 6.2 Schematic illustration of an experimental framework of the MEKC-APCI-MS system. A = MEKC; B =Sheath liquid flow; C=Electrospray; D =Heating block; E= APCI; F=MS.

Fig.6.1 shows the molecular structures of BBMA and DMA.

SDS, sodium cholate, sodium deoxycholate and sodium taurocholate were purchased from Nacalai Tesque (Kyoto, Japan). All other reagents were of analytical grade and water was purified with a Milli-Q system. Sample compounds were supplied commercially and used as received.

6.2.2. Apparatus

Fig. 6.2 shows the schematic illustration of an experimental framework of the MEKC-MS system with APCI. Except for the ion source, it was similar to the ESI interface [14]. MEKC was performed with a laboratory-built instrument which consisted of a Matsusada Precision Devices HCZE30PN0.25-LDSW high-voltage power supply (Kusatsu, Shiga, Japan) and a fused silica capillary of 50 cm long, 50 μm i.d., 150 μm o.d. obtained from Tokyo Kasei (Tokyo, Japan). The laboratory-built nozzle, which was used for ESI or APCI, consisted of a stainless steel tube of 190 μm i.d. 350 μm o.d. (G 28), inside which the fused silica capillary was coaxially inserted, a polytetrafluoroethylene (PTFE) tee union, which held the stainless steel tube, the capillary and a PTFE tube for the delivery of a sheath flow. The MS system consisted of a modified Hitachi M-1000 LC-APCI-MS (Tokyo, Japan) which was constructed with a quadrupole mass spectrometer and a differential pumping system.

6.3. Results and discussion

6.3.1 MEKC with DMA

SEC chromatograms of BBMA and DMA are shown in Fig. 6.3. The main peak

of BBMA was eluted early and the peak was sharp. However, the main peak of DMA was eluted late and the molecular mass was widely distributed.

As a pseudo-stationary phase for MEKC, DMA was utilized by UV detection. Fig.6.4 shows the MEKC separation of non-ionic test solutes, naphthalene derivatives. All solutes migrated fast and the separation efficiency was lower than that of the same solutes by MEKC with BBMA [18]. It should be noted that selectivity was different between BBMA and DMA, that is, the migration orders of 1,6-dihydroxynaphtalene and 1-naphthylamine were changed. As shown in Fig.6.1, the molecular structures of these two polymer surfactants are significantly different. The hydrophobic group of BBMA is acrylate ester and that of DMA is diisobutylene. The hydrophilic group of BBMA is methacrylic acid and that of DMA is maleic acid. The change of the selectivity is probably due to the difference in the molecular structures.

6.3.2. On-line MEKC-ESI-MS with DMA

For the pseudo-stationary phase in on-line MEKC-ESI-MS, DMA is expected to be suitable because of the formation of the molecular-micelle as BBMA. Fig.6.5 shows the single-ion chromatograms obtained by on-line MEKC-ESI-MS. All the solutes and the conditions were the same as in the previous study with BBMA [14]. 1-Naphthylamine, quinine, tetraphenylphosphonium and octaoxyethylenedodecanol were separated and detected successfully. Phenyltrimethylammonium was separated but the S/N ratio was poor. It should be noted that the selectivity was changed as compared with the BBMA system, that is, quinine was migrated faster than tetraphenylphosphonium.

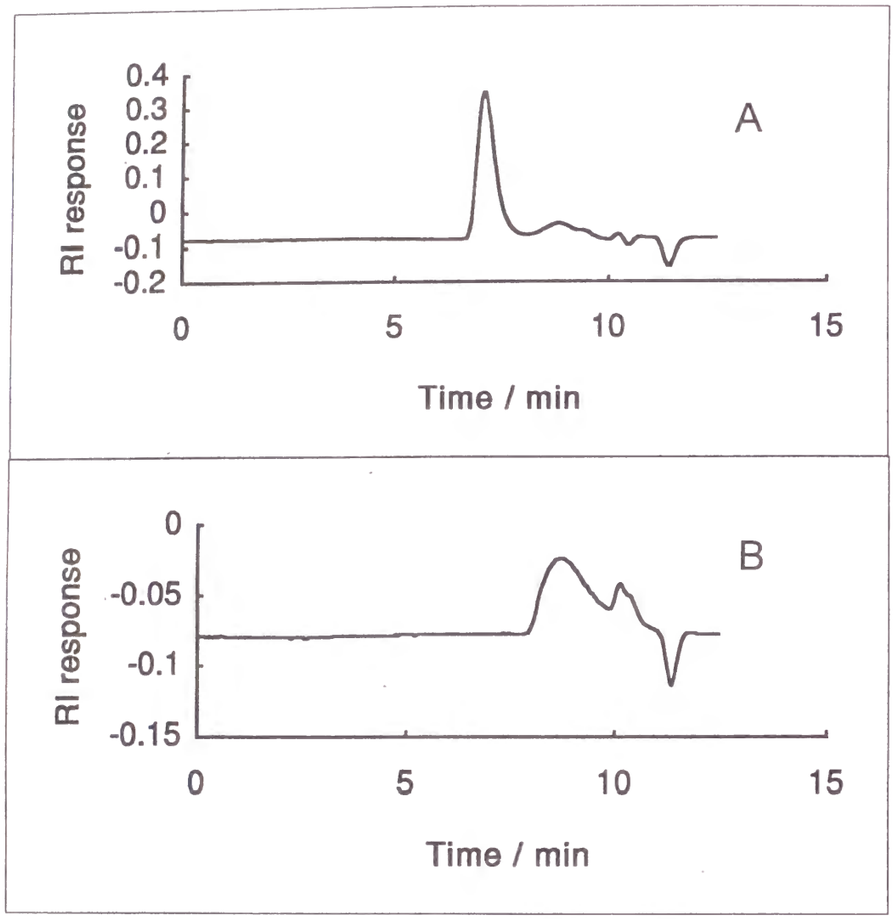


Fig. 6.3 Size exclusion chromatograms of BBMA (A) and DMA (B). Conditions: column, Waters ultrahydrogel linear (30 cm x 5 mm i.d.); mobile phase, 0.4 M $NaNO_3$ containing 20% acetonitrile at 1 ml/min; temperature, 40°C; detection, differential refractive index.

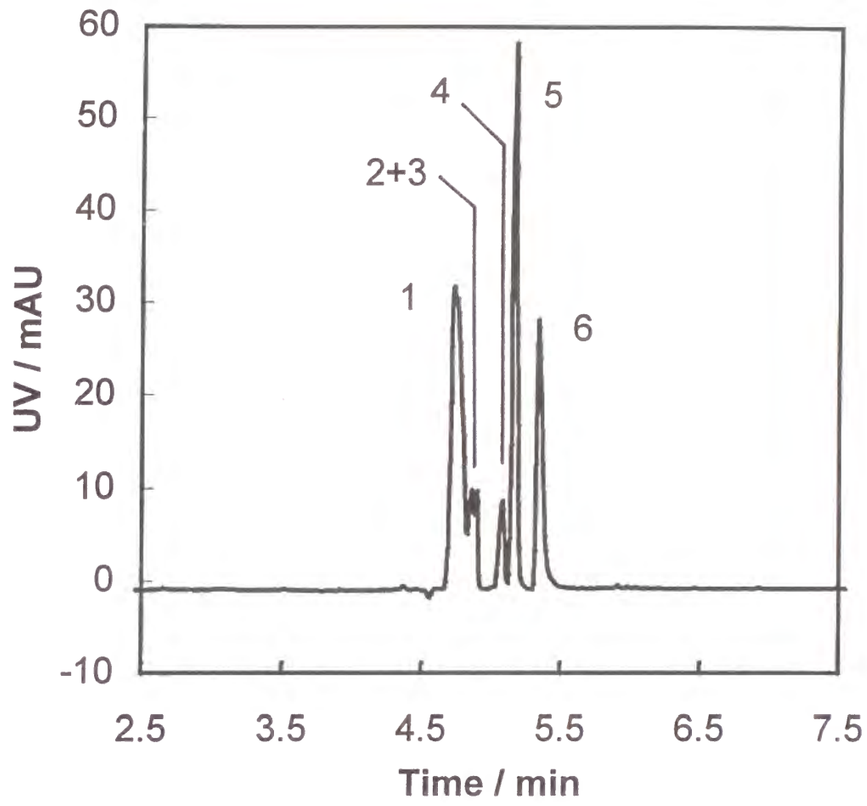


Fig. 6.4 MEKC separation of naphthalene derivatives with DMA. Solute: 1 = 1-naphthylamine; 2 = 1-naphthalenemethanol; 3 = 1-naphthaleneethanol; 4 = 2-naphthol; 5 = 1-naphthol; 6 = 1,6-dihydroxynaphthalene. Conditions: separation solution, 2% DMA in 50 mM phosphate buffer (pH 7); capillary, 50 μm i.d. x 48cm (40 cm to the detector) fused-silica; applied voltage, 20kV; detection wavelength, 300 nm.

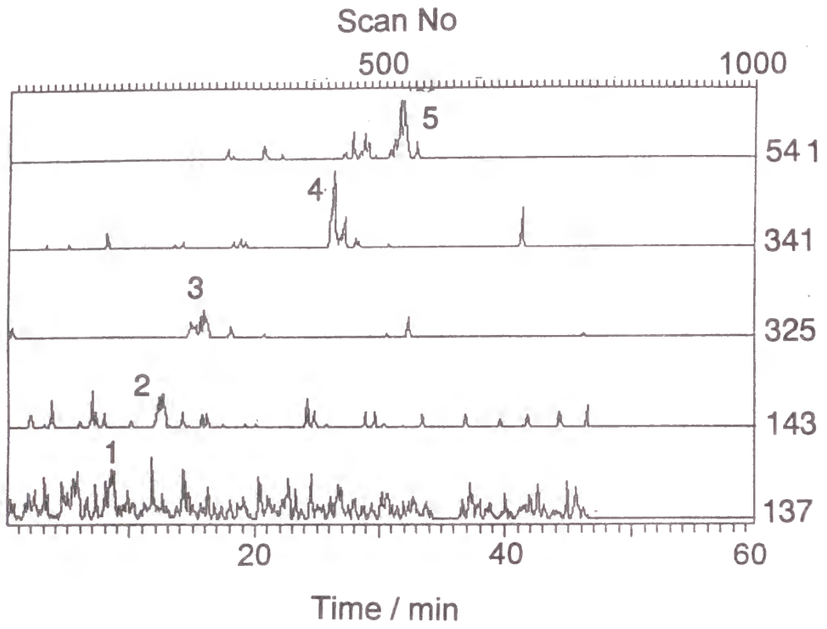


Fig. 6.5 Single-ion chromatograms by MEKC-ESI-MS with DMA. Solutes, 1 = phenyltrimethylammonium chloride; 2 = 1-naphthylamine; 3 = quinine sulfate; 4 = tetraphenylphosphonium chloride; 5 = octaoxyethylenedodecanol. MEKC conditions; capillary, 50 μm i.d. x 50 cm; separation solution, 2.5% DMA in 10 mM ammonium formate buffer (pH 7) containing 10% methanol; applied voltage, 13 kV. electrospray voltage, 3 kV; MS scanning, m/z 5 to 800 at 4 sec/scan; drift voltage, 70 V; focusing voltage 140 V; resolution 55, sheath liquid, water/methanol/formic acid (50:50:1, v/v/v) at approximately 5 μl/min.

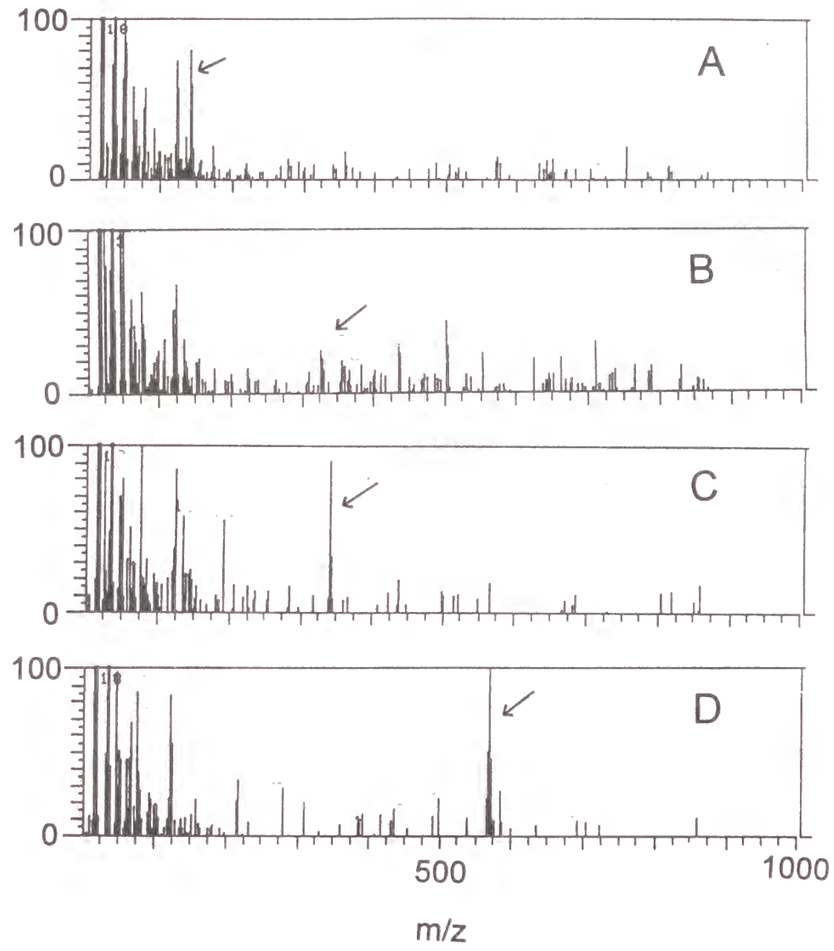


Fig. 6.6 Mass spectra acquired from peak 2(A), peak 3 (B), peak 4 (C) and peak 5 (D) in MEKC-ESI-MS shown in Fig. 5. MS intensity was normalized by the strongest signal observed above m/z 50. The signal of the analyte ion was marked with an arrow.

Fig. 6.6 shows mass spectra acquired from the separated peaks shown in Fig. 6.5. Under the condition of MEKC with 2.5% DMA, abundant intact molecular ions of 1-naphthylamine, quinine, tetraphenylphosphonium chloride and abundant intense molecular ion due to a cation attachment of octaoxyethylenedodecanol were observed as shown in Fig. 6.6.A, B, C and D.

Dependence of ESI signal intensities of caffeine on the concentrations of DMA and BBMA were studied with direct introduction of the sample solution to the ESI system. The direct introduction was performed with the same sheath liquid flow as the on-line study. Fig. 6.7. shows that the signal intensity of the proton attached ion of caffeine decreased with the increase of either DMA or BBMA concentration. A steady decrease was observed with DMA rather than with BBMA. Rundlett et al. [19] proposed a mechanism of the signal suppression in ESI-MS by anionic surfactants including SDS and BBMA.. The detailed mechanism of the signal suppression by the surfactants in MEKC-ESI-MS must be complex because there are two main steps, the production of charged droplets and the production of gas-phase ions from the charged droplets, in the generation of gas-phase ions of the analyte by ESI and both steps should be affected by the pH, buffer ions and surfactant ions of the MEKC separation solution. However, a detailed study of the mechanism in ESI-MS with surfactants is needed. The steady decrease observed with DMA is probably ascribed to the sodium ion contained on the lower molecular-mass components of DMA.

These results suggests that DMA is useful as the pseudo-stationary phase for on-line MEKC-ESI-MS due to the unique selectivity, but the concentration of DMA must be kept low.

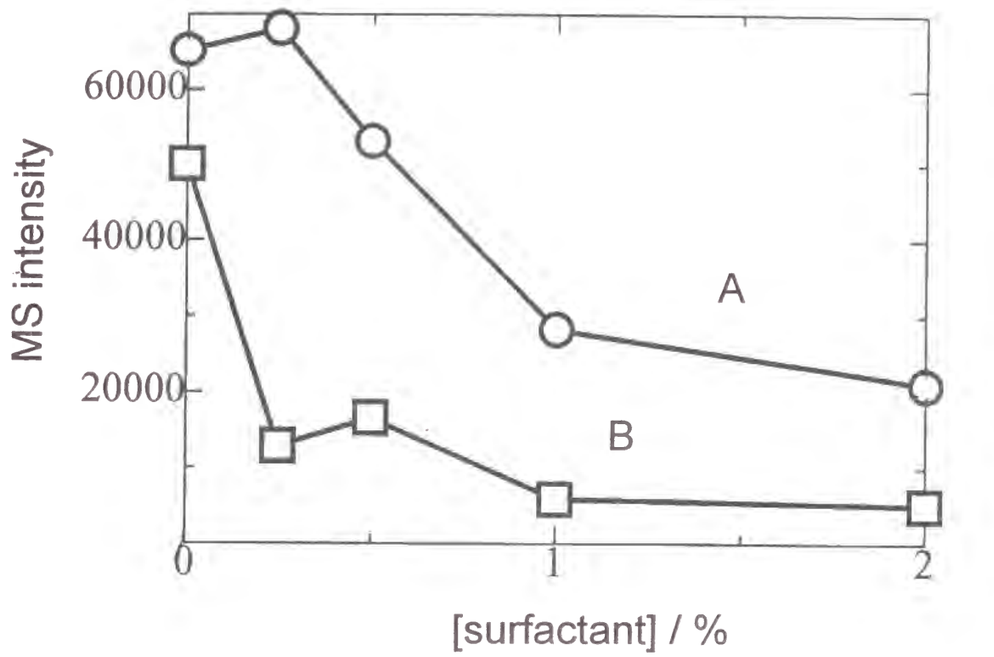


Fig.6.7 Dependence of the intensities of the proton attached ion of caffeine on the concentration of BBMA (A) and DMA (B).

6.3.3 On-line MEKC-APCI-MS

In the study about APCI-MS detection by Takada et al.[15], the MS detection mode was fixed in the SIM mode and there was no information about the mass spectra of observed peaks. In this study with the same interfacing device as reported, the scanning mode was used and the spectrum background caused by the surfactants was examined.

Fig. 6.8 shows single-ion chromatograms of caffeine, isopropylantipyrine and quinine sulfate by MEKC-APCI-MS with SDS. Fig. 6.9 shows the mass spectra acquired from the separated peaks shown in Fig. 6.8. MS intensity was normalized by the strongest signal observed above m/z 5 and magnified by 20 times above m/z 180. In the case of MEKC-ESI-MS with SDS [14], the very strong peaks which assigned to the molecular ion of SDS due to sodium attachment, $(M+Na)^+$, and the singly charged dimmer, $(2M+Na)^+$, were observed. In contrast to the ESI interface, Fig.6.9 shows that the APCI interface did not generate strong ions due to SDS.

As the pseudo-stationary phase for MEKC-APCI-MS, sodium cholate, sodium deoxycholate and sodium taurocholate were investigated using the same analytes as in SDS system described above. The running solution containing each surfactant at 50 mM was used and the other conditions were the same as in Fig.6.8. With each surfactant, all analytes were successfully detected without strong background due to the surfactant, but, the separations were not enough. Fig. 6.10 and 6.11 shows the single-ion chromatograms by MEKC-APCI-MS with sodium cholate and the mass spectra acquired from the peaks.

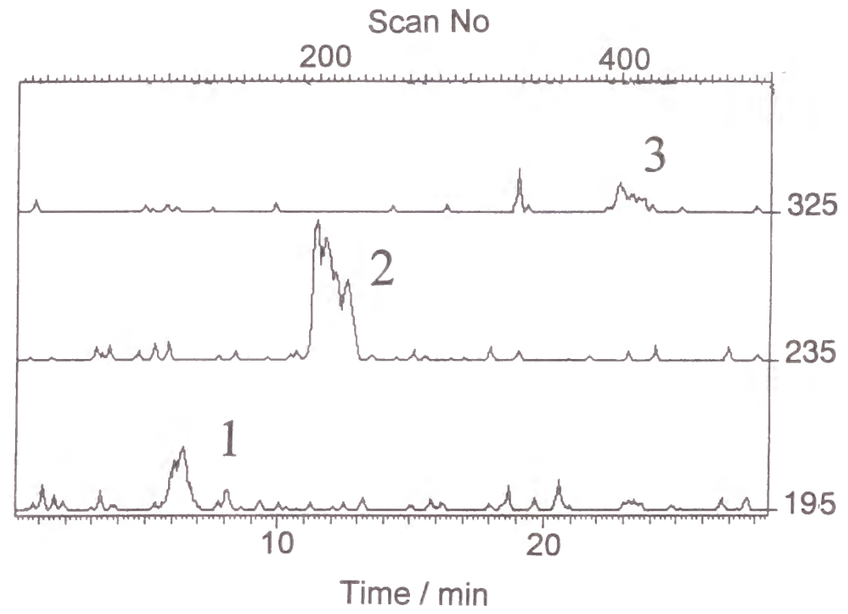


Fig. 6.8 Single-ion chromatograms by MEKC-APCI-MS with SDS. Solutes, 1 = caffeine; 2 = isopropylantipyrine; 3 = quinine sulfate. MEKC conditions; capillary, 50 μm i.d. x 40 cm; separation solution, 50 mM SDS in 20 mM borate- 10 mM phosphate buffer (pH 7) containing 5% methanol; applied voltage, 18 kV. electrospray voltage, 3 kV; APCI voltage, 3 kV; MS scanning, m/z 5 to 800 at 4 sec/scan; drift voltage, 70 V; focusing voltage 140 V; resolution 58, sheath liquid flow, methanol at 20 μl/min.

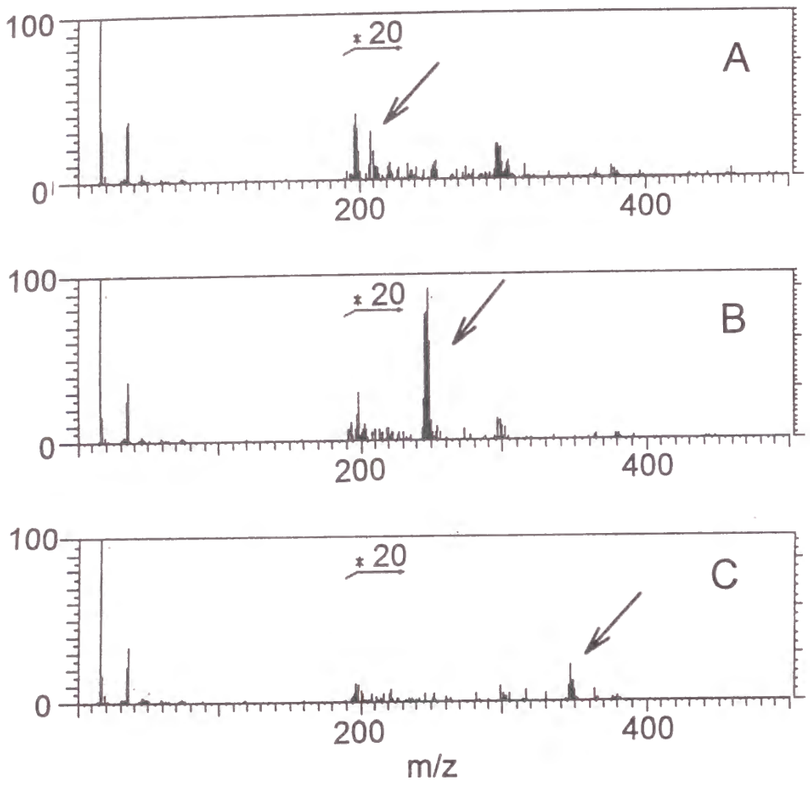


Fig. 6.9 Mass spectra acquired from the peak 1 (A), peak 2 (B), and peak 3 (C) in MEKC-APCI-MS shown in Fig. 6.8. MS intensity was normalized by the strongest signal observed above m/z 5 and magnified by 20 times above m/z 180. The signal of the analyte ion was marked with an arrow..

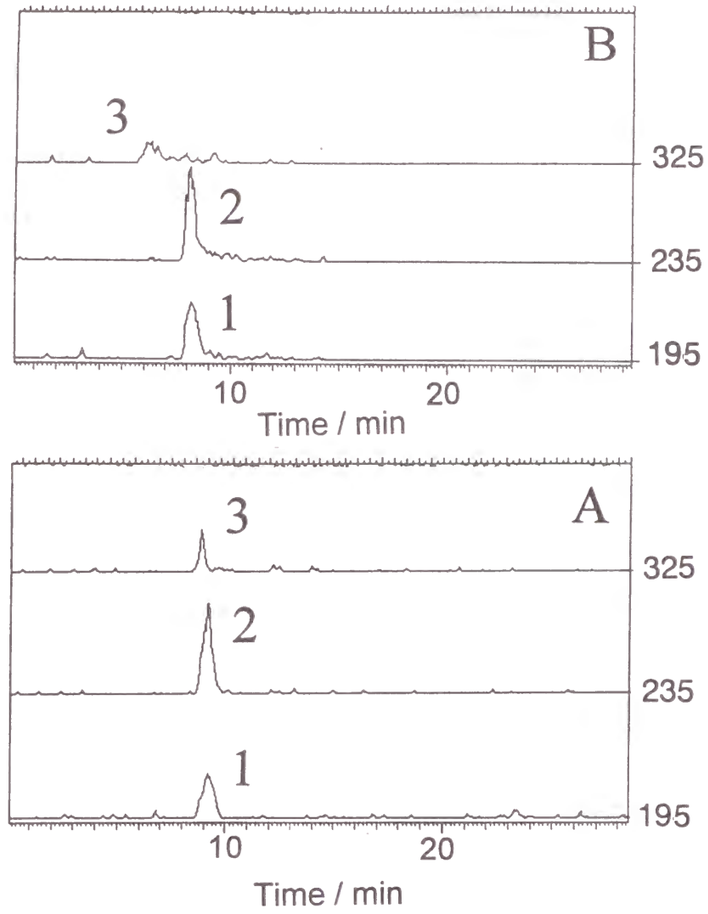


Fig. 6.10 Single-ion chromatograms by MEKC-APCI-MS with SDS. Solutes are the same as in Fig.8. Separation solution, 20 mM (A) and 100 mM (B) sodium cholate in 20 mM borate-10 mM phosphate buffer (pH 7). Other conditions are the same as in Fig.8.

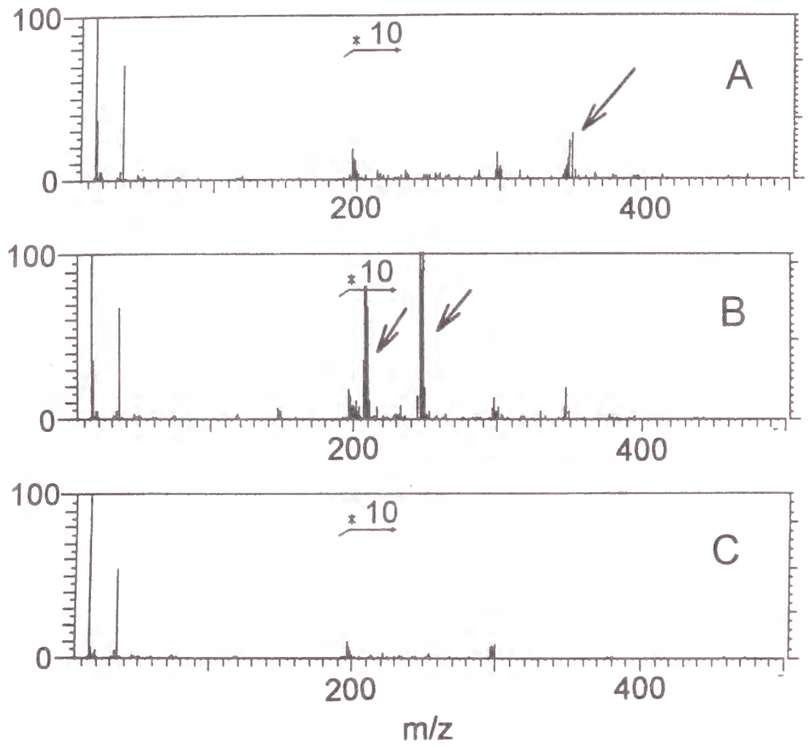


Fig. 6.11 Mass spectra acquired from the peak 3 (A), peak 1 and 2 (B), and back ground (C) in MEKC-APCI- MS shown in Fig. 10. MS intensity was normalized by the strongest signal observed above m/z 5 and magnified by 10 times over m/z 180. The signal of the analyte ion was marked with an arrow.

6.4. Conclusions

On-line MEKC-ESI-MS using the DMA was explored. The DMA micelle functioned successfully as the pseudo-stationary phase and showed significantly different selectivity in comparison with BBMA. The APCI interface was used for a conventional MEKC system using sodium dodecyl sulfate and sodium cholates.

As the on-line coupling technique between MEKC and MS, the use of polymer surfactants including BBMA and DMA should be advantageous for the optimization of the separation of the analytes which are well detected by ESI-MS but not separated by CZE. The APCI interface is expected to be a useful interfacing technique for the coupling with MEKC because ionization processes are not significantly deteriorated by the presence of surfactants. As the performance of the on-line MS detection system with APCI in this work has not been studied well yet, the more detailed study including conditions of ionization will be necessary for further development.

References

1. J. W. Jorgenson, K. D. Lukacs, *Anal. Chem.*, **53**, 1298 (1981).

2. S. Terabe, *Bunseki* 599(1991).

3. S. F. Y. Li: "*Capillary Electrophoresis: Principles, Practice and Applications*"; Journal of Chromatography Library, p.52 (1992), (Elsevier, Amsterdam).

4. S. Terabe, K. Otsuka, K. Ichikawa, A.Tsuchiya, T. Ando, *Anal. Chem.*, **56**, 111(1984).

5. J. Vindevogel, P. Sandra: "*Introduction to Micellar Electrokinetic Chromatography*", (1992) (Huthig, Heidelberg).

6. H. Nishi, S. Terabe : *J. Chromatogr.*, **735**, 3(1996).

7. R. D. Smith, C. J. Barinaga, H. R. Udseth, *Anal. Chem.*, **60**,1948(1988).

8. J. H. Wahl,R. D. Smith, *J. Cap. Elec.*, **1**,62(1994).

9. F. Garcia, J. D. Henion, *Anal. Chem.*, **64**, 985(1992).

10. I. M. Johasson, R. Pavelka, J. D. Henion, *J. Chromatogr.*, **559**, 515(1991).

11. S. Pleasance, P. Thibault. J. Kelly, *J. Chromatogr.*,**591**, 325(1992).

12. R. D. Smith, H. R. Udseth: "*Capillary Electrophoresis Technology*", Edited by N. Guzman, p.525(1993), (Marcel Dekker, New York).

13. K. Hiraoka, *Rapid Commun. Mass Spectrom.*, **6**,463(1992).

14. H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi, H. Koizumi: *J. Chromatogr.*,**716**, 69(1995).

15. Y. Takada, M. Sakairi, H. Koizumi, *Rapid Commun. Mass Spectrom.*, **9**,488(1995).

16. K. Koezuka, H. Ozaki, N. Matsubara, S. Terabe, *J. Chromatogr. B*, **689**,3(1997).

17. W. M. Nelson, Q. Tang, A. K. Harrata, C. S. Lee, *J. Chromatogr.*,**749**,219(1996).

18. H. Ozaki, A. Ichihara, S. Terabe, *J. Chromatogr.*, **680**, 117(1994).

19. K. L. Rundlett, D. W. Armstrong, *Anal. Chem.*,**68**,3493(1996).

Publication List

I . Parts of this thesis have been, or are to be published in the following journals.

Chapter 2. Micellar electrokinetic chromatography using high-molecular surfactants: use of butyl acrylate-butyl methacrylate-methacrylic acid copolymers sodium salts as pseudo-stationary phases.
Hiroto Ozaki, Akinobu Ichihara and Shigeru Terabe,
J. Chromatogr. A, **680**, 117-123 (1994).

Chapter 3. Micellar electrokinetic chromatography using high-molecular-mass surfactants: comparison between anionic and cationic surfactants and effects of modifiers.
Hiroto Ozaki, Akinobu Ichihara and Shigeru Terabe,
J. Chromatogr. A, **709**, 3-10 (1995).

Chapter 4. Micellar electrokinetic chromatography-mass spectrometry using high-molecular-mass surfactant: on-line coupling with an electrospray ionization interface.
Hiroto Ozaki, Noritaka Itou, Shigeru Terabe, Yasuaki Takada, Minoru Sakairi and Hideaki Koizumi,
J. Chromatogr. A, **716**, 69-79 (1995).

Chapter 5. On-line micellar electrokinetic chromatography-mass spectrometry with a high-molecular-mass surfactant.
Hiroto Ozaki and Shigeru Terabe ,
J. Chromatogr. A, in press.

Chapter 6. On-line micellar electrokinetic chromatography/MS with electrospray ionization and atmospheric pressure chemical ionization interfaces.
Hiroto Ozaki and Shigeru Terabe,
Bunseki Kagaku, **46**, 421-427 (1997).

II. Other publications not included in this thesis.

1. Electrokinetic chromatography with 2-*o*-carboxymethyl-β-cyclodextrin as a moving "stationary" phase.
Shigeru Terabe, Hiroto Ozaki, Koji Otsuka and Teiichi Ando,
J. Chromatogr. , **332**, 211-217 (1985).

2. Effect of nonionic surfactants on the resolution and selectivity in micellar electrokinetic chromatography.
Shigeru Terabe, Hiroto Ozaki and Yasushi Ishihama,
Bunseki Kagaku, **42**, 859-866 (1993).

3. New pseudostationary phases for electrokinetic chromatography: a high-molecular surfactant and proteins.
Shigeru Terabe, Hiroto Ozaki and Yoshihide Tanaka,
J. Chin. Chem. Soc., **41**, 251-257 (1994).

4. Separation and detection of closely related peptides by micellar electrokinetic chromatography using the partial filling technique.
Kiyohiro Koezuka, Hiroto Ozaki, Norio Matsubara and Shigeru Terabe,
J. Chromatogr. B, **689**, 3-11 (1997).

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