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Micellar electrokinetic chromatography–mass spectrometry using a high-molecular-mass surfactant On-line coupling with an electrospray ionization interface

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Abstract

On-line coupling between micellar electrokinetic chromatography (MEKC) and mass spectrometry (MS) was studied with a high-molecular-mass surfactant and an electrospray ionization interface (ESI). A high-molecular-mass surfactant, butyl acrylate–butyl methacrylate–methacrylic acid copolymer sodium salt (BBMA), was employed as a pseudo-stationary phase for an on-line MEKC–MS system. BBMA and a minor component separated by size-exclusion chromatography were determined by ESI-MS. No major ion from the BBMA polymer was detected. The BBMA micelle functioned successfully as the pseudo-stationary phase in a 10 m*M* ammonium formate buffer containing 10% methanol. Five standard compounds, phenyltrimethylammonium chloride, 1-naphthylamine, quinine sulfate, tetraphenylphosphonium chloride and octaoxyethylenedodecanol, were separated by MEKC and detected by MS. The effects of the concentrations of BBMA on the separation and sensitivity in MEKC–MS were studied. MEKC–MS with BBMA was applied to the separation and detection of a standard mixture of sulfamides.

1. Introduction

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

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[6–9]. Ionspray interfaces, a type of ESI using a high-velocity gas flow at the tip of the capillary, have been studied by Henion and co-workers [10,11] and Pleasance et al. [12]. A fast atom bombardment (FAB) ionization interface with a coaxial continuous flow was developed by Tomer and co-workers [13,14]. The latest work involved a coupling of CE with laser-desorption mass spectrometry, which was 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, the direct coupling of MEKC with MS has not yet been developed, probably because of the presence of the surfactant in the running solution. On-line coupling of MEKC with 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 the signal for an analyte from a separated fraction owing to the existence of surfactant. Smith et al. [6] studied the ESI mass spectrum of sodium dodecyl sulfate (SDS), which is the surfactant most frequently employed as the pseudo-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 yet been reported, probably owing to the low ionization efficiency caused by a relatively high concentration of SDS. Varghese and Cole [22] used a cationic surfactant, cetyltrimethylammonium 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, below the critical micelle concentration (CMC) 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 the atmospheric pressure ionization inlet. Mass spectra obtained by ESI are simple because ESI is a soft ionization technique and yields only intact molecular ions 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 that it is not suitable for non-polar compounds because of the low ionization and information on molecular structure is limited to molecular mass only, hence a more advanced technique such a MS–MS is required for more information.

The choice of the surfactant added to the running solution in MEKC is also highly significant because surfactants are non-volatile and in many cases cause strong background ions in the mass spectra, as has been observed with SDS [6]. High-molecular-mass surfactants are oligomers of monomeric surfactants [24,25] or polymers which show surface-active properties as a whole [26,27], and they are expected to be useful as pseudo-stationary phases for MEKC–MS. Fig. 1 is a schematic illustration of an electrospray interface and the expected behaviour of micelles in the interface. In Fig. 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. 1B shows that low-molecular-mass surfactant micelles produce abundant gas-phase surfactant ions. On the other hand, as shown in Fig. 1C, high-molecular-mass surfactant micelles will be stable in the ESI system because the micelle is a single covalently bonded molecule. Therefore, the high-molecular-mass surfactant micelle will not generate high levels of low-molecular-mass background ions.

A high-molecular-mass surfactant, which is an undecylenate oligomer synthesized by polymeriz-

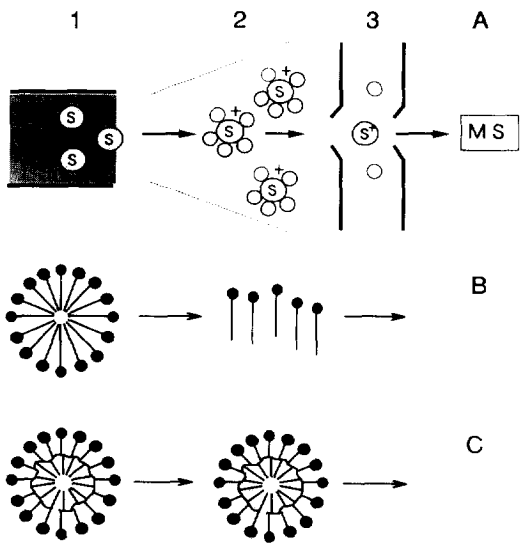

Fig. 1. (A) Schematic illustration of an electrospray interface and expected behaviour of (B) low-molecular-mass and (C) high-molecular-mass surfactant micelles. 1 = Capillary; 2 = electrospray; 3 = mass spectrometer; S = solute molecule; S^+ = solute molecular ion; O = solvent.

ing micellized sodium 10-undecylenate, was employed as a pseudo-stationary phase for MEKC by Palmer and co-workers [24,25]. We have reported MEKC with a high-molecular-mass surfactant, butyl acrylate–butyl methacrylate–methacrylic acid copolymer sodium salt (BBMA) [26,27]. The molecular mass of the BBMA was measured to be about 40 000 by size-exclusion chromatography (SEC) using standard polyoxyethylene 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]. The CMC of BBMA was found to be effectively zero [26]. BBMA is therefore 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 separations.

In this paper, we describe an on-line MEKC–ESI-MS system with BBMA as the pseudo-stationary phase.

2. Experimental

2.1. Apparatus

A schematic diagram of the MEKC–ESI-MS system is presented in Fig. 2. The structure is almost the same as for 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 Matsusada Precision Devices HCZE30PN0.25-LDSW high-voltage power supply (Kusatsu, Shiga, Japan) and a fused-silica capillary (50 cm × 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. and 350 μm O.D. (G 28), inside which the 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 the sheath flow.

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

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

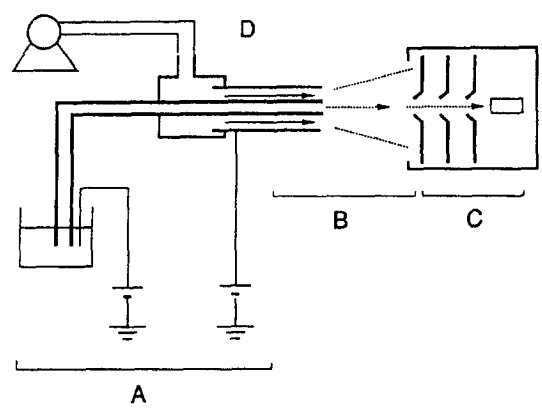

Fig. 2. Schematic illustration of experimental framework of the MEKC–ESI-MS system. A = MEKC; B = ESI; C = mass spectrometer; D = sheath liquid flow.

SEC of BBMA was performed with a Shimadzu (Kyoto, Japan) LC-9A liquid-delivery pump, a Shodex (Tokyo, Japan) RI SE-51 refractive index detector and Tosoh (Tokyo, Japan) TSK-gel G3000SW and G2000SW columns (both 60 cm × 8 mm I.D.) with a series connection.

2.2. Reagents

BBMA was provided as a 23% aqueous solution having a viscosity of 170 cP at 25°C by Dai-ichi Kogyo Seiyaku (Kyoto, Japan). As 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 obtained from Tokyo Kasei. 3-[(3-Cholamidopropyl)dimethylammonio]propanesulfonic acid (CHAPS) was supplied by Wako (Osaka, Japan). All other reagents were of analytical-reagent grade and water was purified with a Milli-Q system (Millipore). All sample compounds, phenyltrimethylammonium chloride, tetraphenylphosphonium chloride, quinine sulfate dihydrate, octaoxyethylenedodecanol and pentaoxyethylenedodecanol obtained from Wako, 1-naphthylamine from Merck (Darmstadt, Germany) and sulfamethazine, sulfisomidine, sulfadiazine and sulfisoxazole from Aldrich (St. Louis, MO, USA), were of analytical-reagent grade and used as received. Sample solutes were dissolved in about 50% aqueous methanol.

2.3. Procedure

On-line MEKC–ESI-MS work was performed with the system described above using BBMA solutions in 10 m*M* ammonium formate buffer (pH 7) containing 10% methanol. Samples were injected by the hydrostatic injection method (10–30 s 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 the ESI interface). The sheath liquid was water–methanol–formic acid (50:50:1, v/v/v) and was delivered by a Hitachi L6300 HPLC pump at ca. 5 μl/min. Electrospray was performed using a 3 kV gradient between the capillary end and the first MS sampling orifice. The MS system was operated in the positive-ion mode. Almost all MS detection was obtained in the scanning mode, from m/z 1 to 1000, at 4 s per scan. The drift voltage was 70 V, the focusing voltage 140–150 V and the resolution 50–55. The calibration of m/z for ESI-MS was effected with *p*-nitroaniline, 1-naphthylamine and 4,4′-diaminodiphenylmethane. Therefore, in the high-mass region, the m/z value was not exact because this calibration was effective in the lower mass region only. All work described was performed with the MEKC–ESI-MS system mentioned above at the Himeji Institute of Technology, except for the data in Fig. 10B, which were 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 with a microsyringe. The other conditions were the same as in 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 m*M* ammonium carbonate (pH 8)–acetonitrile (20:80, v/v) at a mobile phase flow-rate of 0.8 ml/min. Each fraction was introduced into the mass spectrometer after adding 100 μl of 20% formic acid to 2 ml of the fractionated solution.

3. Results and discussion

3.1. Optimization of the ESI interface

As the mass spectrometer employed in this study was operated in 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 must be

acidic, generally of pH 3–5, as reported for CE–ESI-MS [9,16]. To meet this 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, an acidic buffer was not used because BBMA is insoluble in water at low pH or it precipitates below pH 4 [26], and normal MEKC conditions in which the electroosmotic flow is strong were to be employed to take advantage of MEKC to separate non-ionic analytes. BBMA solutions in ammonium formate (pH 7) were employed and aqueous methanol (1:1) containing 1% formic acid was used as a sheath liquid to acidify the total liquid sprayed at the ESI interface. A similar procedure has also been employed for 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 signal intensity of analytes because of the conical distribution of aerosol generated by the electrospray. It has been reported that the distributions of compositions were different among ions generated by ESI [28]. In this work, the capillary tip was positioned at a separation distance of 20 mm, offset 5 mm horizontally and 5 mm vertically from the centre of the orifice to maximize the signal intensity of ions generated from standard compounds. This fixed position may not have been optimum for all analytes, but repositioning of the capillary tip was not tried throughout this work.

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

3.2. *Background ions from BBMA*

Fig. 3 shows examples of the mass spectra of MEKC separation solutions: (A) ammonium formate buffer only, (B) buffer containing 2% BBMA and (C) buffer containing 50 m*M* SDS.

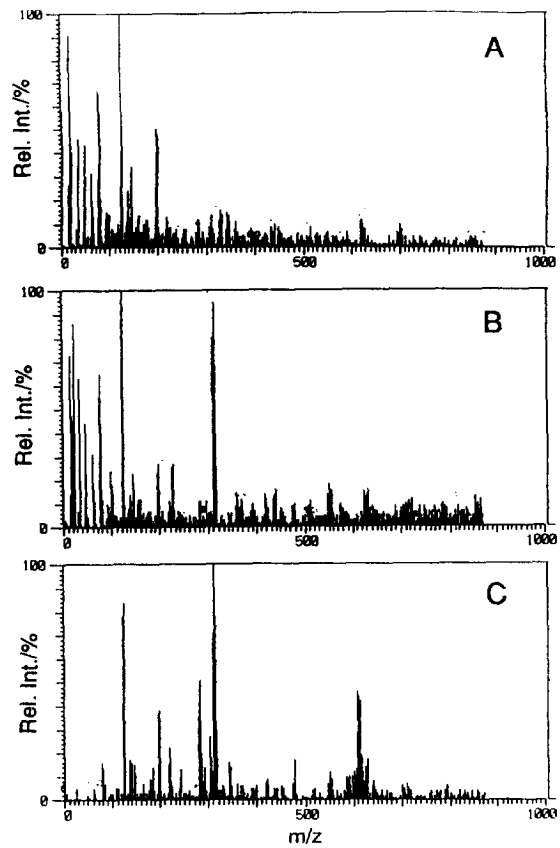

Fig. 3. ESI mass spectra of MEKC separation solutions: (A) 10 m*M* ammonium formate buffer (pH 7); (B) (A) + 2% BBMA; (C) (A) + 50 m*M* SDS. Conditions: electrospray voltage, 3 kV; MS scanning, from *m/z* 1 to 1000 at 4 per scan; drift voltage, 70 V; focusing voltage, 140 V; resolution, 55; sheath liquid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

In the spectrum of 10 m*M* ammonium formate buffer (pH 7), many peaks below *m/z* 200 were observed. The strongest peak was at ca. *m/z* 120. It should have come from the buffer system, but could not be identified. In the spectrum with 2% BBMA added to the buffer, strong peaks were recorded at ca. *m/z* 310, which was characteristic of the BBMA solution. In the spectrum of buffer containing 50 m*M* SDS, two very strong peaks were observed at ca. *m/z* 310 and 610. The former was assigned to the molecular ion of SDS due to sodium attachment and the latter to the singly charged dimer. Both peaks showed a stronger intensity than those from the buffer system. Observation of the molecular ion of SDS due to sodium attachment in ESI-MS has been reported by Smith et al. [6]. To identify the *m/z* 310 ion generated from BBMA solution, BBMA was fractionated by SEC. Fig. 4 shows the SEC of BBMA and the ESI mass spectrum of each fraction. From fractions A and B, consisting of the polymer and oligomer of BBMA, no strong signals were observed. From fraction C, a strong *m/z* 310 peak was recorded. It should be noted that signal intensities cannot be compared among different spectra, because the spectra show only signal intensities relative to the strongest peak. Using analytical SEC, it was clarified that an additive of BBMA appeared in this region, but no components of BBMA, butyl acrylate, butyl methacrylate or methacrylic acid sodium salt. Therefore, this ion was proba-

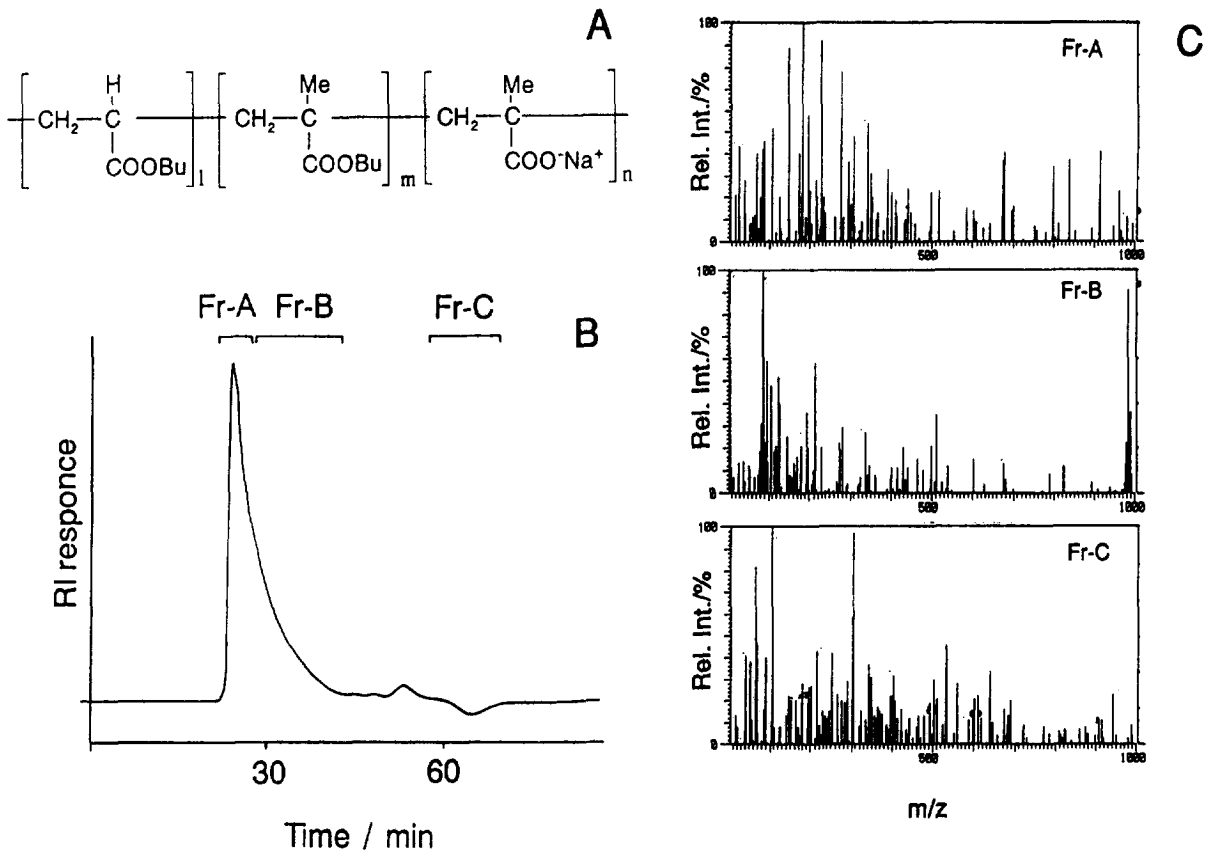

Fig. 4. (A) Molecular structure of BBMA; (B) SEC for fractionation of BBMA; (C) ESI mass spectra of fractions. SEC conditions (B): column, Tosoh TSK-gel G3000SW and G2000SW (both 60 cm × 8 mm I.D.) with a series connection; separation solution, 50 m*M* ammonium carbonate (pH 8)–acetonitrile (20:80, v/v) at 0.8 ml/min; detection, refractive index. MS conditions (C): sheath liquid flow, water–methanol (50:50, v/v) at ca. 5 μl/min; drift voltage, 80 V; other MS conditions as in Fig. 3.

bly from the additive, the amount of which in BBMA was very small. The results shown in Figs. 3 and 4 strongly suggest that BBMA does not disturb the on-line measurement of the mass spectra of analytes separated by MEKC with BBMA as a pseudo-stationary phase.

Some ionic low-molecular-mass surfactants other than SDS were introduced into the ESI-MS system. Sodium cholate, sodium dodecane sulfonate and CHAPS produced strong signals of molecular ions due to cation attachment. Sodium laurate gave no strong ion.

3.3. ESI-MS of analytes

Fig. 5 shows an ESI mass spectrum of a standard mixture and the 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, higher mass ion was selected as the monitored ion of the single-ion chromatogram for the on-line MEKC–ESI-MS described below. On the other hand, no MS signals were observed from 1-naphthol and 1-naphthalenemethanol, which are usually used as MEKC standard compounds, or from phenanthrene and Sudan IV, which are used as micelle markers in MEKC. These compounds seemed not to be ionized by ESI-MS.

3.4. On-line MEKC–ESI-MS

Fig. 6 shows the single-ion chromatograms obtained by (A) MEKC–ESI-MS and (B) CE–ESI-MS. The solutes are the same as in Fig. 5. All the solutes were separated and detected successfully under the conditions of MEKC with 2% BBMA, as shown in Fig. 6A. The separation and sensitivity were practically reproducible in several successive runs. The migration times of all solutes in Fig. 6A were longer than the

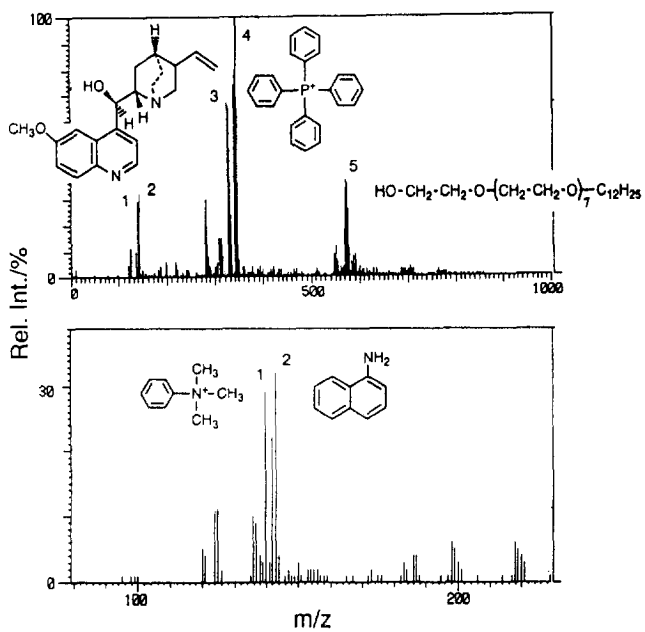

Fig. 5. Example of an ESI mass spectrum of standard mixture and molecular structures of the analytes. Peaks: 1 = phenyltrimethylammonium chloride; 2 = 1-naphthylamine; 3 = quinine sulfate; 4 = tetraphenylphosphonium chloride; 5 = octaoxyethylenedodecanol. Conditions as in Fig. 3.

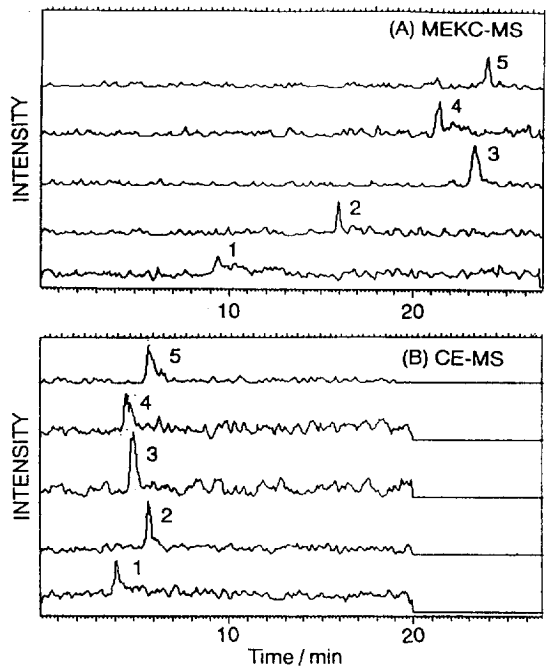

Fig. 6. Single-ion chromatograms obtained by (A) MEKC–ESI-MS and (B) CE–ESI-MS. Solutes as in Fig. 5. MEKC conditions: capillary, 50 cm × 50 μm I.D.; separation solution, (A) 2% BBMA in 10% methanol and 10 m*M* ammonium formate buffer (pH 7) and (B) 10 m*M* ammonium formate buffer (pH 7); applied voltage, 13 kV. MS conditions as in Fig. 3.

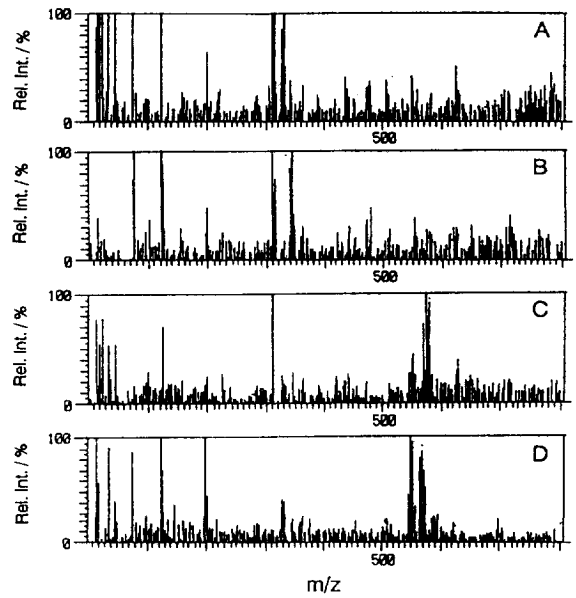

Fig. 7. Mass spectra acquired from (A) peak 3, (B) peak 4 and (C) peak 5 in MEKC–ESI-MS shown in Fig. 6A and (D) peak 5 in CE–ESI-MS shown in Fig. 6B. The MS intensity was normalized by the signal observed over *m/z* 310.

corresponding times in Fig. 6B, where no micelle was used, and the solutes were separated more widely according to the MEKC mechanism; octaoxyethylenedodecanol migrated more slowly than 1-naphthylamine, although both are neutral and unresolved in Fig. 6B.

Fig. 7 shows mass spectra acquired from the peaks shown in Fig. 6A and B. Under the MEKC conditions with 2% BBMA, abundant intact molecular ions of quinine sulfate and tetraphenylphosphonium chloride and abundant intense octaoxyethylenedodecanol molecular ion due to cation attachment were were observed, as shown in Fig. 7A, B and C. The signal at *m/z* 310 in Fig. 7A, B and C was generated from the additive of BBMA as shown in Fig. 3B. Some peaks below *m/z* 200 recorded in all spectra in Fig. 7 were from the buffer system as shown in Fig. 3A.

Fig. 8 shows the dependence of the migration time on the concentration of BBMA. The migration times of all solutes increased with increase in the concentration of BBMA. This result indicates that the separation is based on the differential partitioning of the solutes between

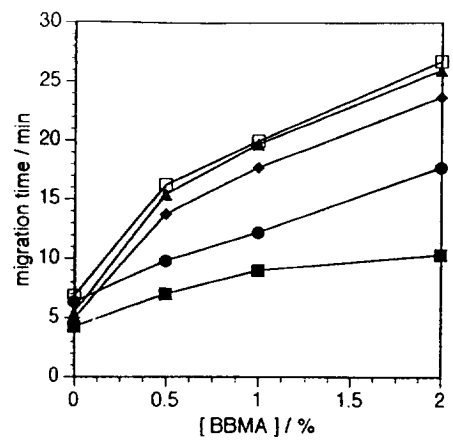

Fig. 8. Dependence of the migration time on the concentration of BBMA. Solutes: ■ = phenyltrimethylammonium chloride; ● = 1-naphthylamine; ▲ = quinine sulfate; ◆ = tetraphenylphosphonium chloride; □ = octaoxyethylenedodecanol. Conditions as in Fig. 6.

the slower migrating BBMA micelle and the faster migrating surrounding aqueous phase. Octaoxyethylenedodecanol, a non-ionic surfactant, migrated last. In MEKC using octaoxyethylenedodecanol added to the BBMA solution, the capacity factors of solutes increase with increase in the concentration of octaoxyethylenedodecanol [27], which means that BBMA forms mixed micelles with octaoxyethylenedodecanol. Octaoxyethylenedodecanol was therefore more suitable as a tracer of the BBMA micelle than quinine sulfate, which is used as a tracer of the SDS micelle.

Fig. 9 shows the dependence of the MS intensities of phenyltrimethylammonium chloride and octaoxyethylenedodecanol on the concentration of BBMA. The signal intensity of phenyltrimethylammonium chloride decreased with increase in the concentration of BBMA. The intensity at 2% BBMA was ca. 20% of that in the absence of BBMA. The signal intensities of the other solutes except for octaoxyethylenedodecanol tended to decrease with increase in the concentration of BBMA. The signal intensity of octaoxyethylenedodecanol, however, was maximum at 1% BBMA. Further details of the mass spectrum of octaoxyethylenedodecanol were investigated at each concentration of BBMA. Fig. 7C and D indicate

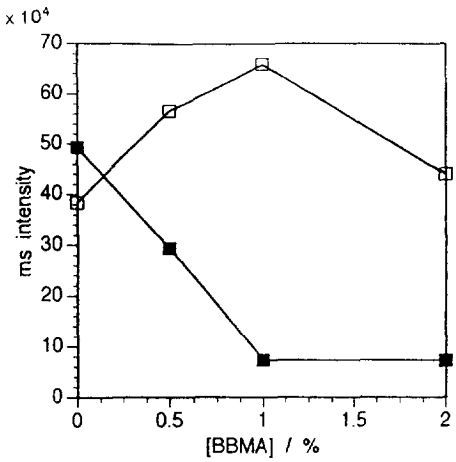

Fig. 9. Dependence of intensities on the concentration of BBMA. ■ = Phenyltrimethylammonium chloride; □ = octaoxyethylenedodecanol. Conditions as in Fig. 6.

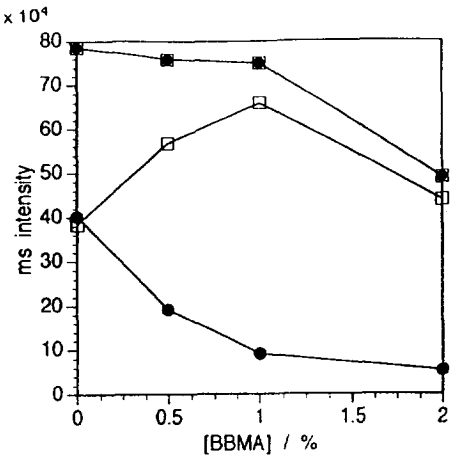

Fig. 10. Dependence of intensities on the concentration of BBMA. □ = Octaoxyethylenedodecanol high-mass peak, $[M + NH_4]^+$; ● = octaoxyethylenedodecanol low-mass peak, $[M + H]^+$; ■ = □ + ●. Conditions as in Fig. 6.

the difference in the mass spectra of octaoxyethylenedodecanol in the absence and presence of BBMA. Fig. 10 shows the dependence of the intensities of the two molecular ions of octaoxyethylenedodecanol, which are assigned due to proton attachment, $[M + H]^+$, and ammonium attachement, $[M + NH_4]^+$, on the concentration of BBMA. Fig. 9 shows the intensity of $[M + NH_4]^+$. However, the intensify of $[M + H]^+$ decreased with increase in the concentration of BBMA, as shown in Fig. 10. A homologous non-ionic surfactant, pentaoxyethylenedodecanol, also showed the same tendency as octaoxyethylenedodecanol, producing the molecular ion due to ammonium attachment more than that due to proton attachment under MEKC–ESI-MS conditions with 2% BBMA. The sum of the intensities of $[M + H]^+$ and $[M + NH_4]^+$ for octaoxyethylenedodecanol decreased with increase in the concentration of BBMA, as shown in Fig. 10. It is generally noted that the MS intensity of the solute decreases with increase in the concentration of BBMA. This result is consistent with that observed as an effect on salt concentration on the ionization efficiency of ESI [29–31].

Fig. 11 shows (A) MEKC separation with a UV detector and (B) MEKC–ESI-MS of sulfamides. The single-ion chromatograms in Fig.

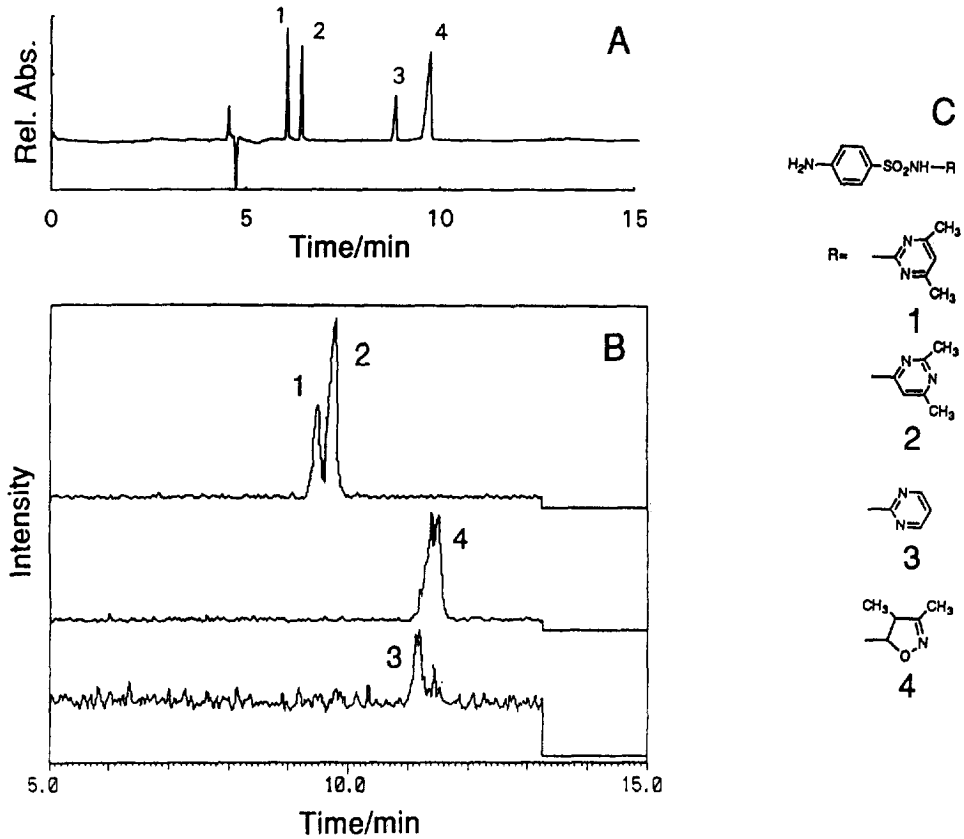

Fig. 11. (A) MEKC with a UV detector and (B) MEKC–ESI-MS of sulfamides and (C) their molecular structures. Solutes: 1 = sulfamethazine; 2 = sulfisomidine; 3 = sulfadiazine; 4 = sulfisoxazole. Conditions: (A) separation solution, 1% BBMA in 10% methanol and 100 m*M* borate–50 m*M* phosphate buffer (pH 7); capillary, 48 cm (40 cm to the detector) × 50 μm I.D. fused silica; applied voltage, 20 kV; detection wavelength, 210 nm. (B) Other conditions as in Fig. 6 except for the interface; the MS instrument was operated in the SIM mode.

11B were recorded in the SIM mode without scanning. From Fig. 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. 11A. The separation efficiency in MEKC–ESI-MS was far less than in 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.

4. Conclusions

On-line MEKC–ESI-MS using BBMA, a high-molecular-mass surfactant, was achieved. This system was employed successfully under the conditions of MEKC with 2% BBMA. This concentration of BBMA is high enough for most purposes. Because the MS signal intensities of solutes decrease with increase in the concentration of BBMA, a separation solution containing a low concentration BBMA should be employed, especially for the detection of analytes for which the ionization efficiency is low. This work is preliminary and the performance of this system, in terms of both separation and

sensitivity, is not yet satisfactory. A more advanced ESI interface and optimization of MEKC, sheath flow and MS detection conditions will produce better performance in future work.

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