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Review

Micellar electrokinetic chromatography–mass spectrometry

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Abstract

The combination of micellar electrokinetic chromatography (MEKC) with mass spectrometry (MS) is very attractive for the direct identification of analyte molecules, for the possibility of selectivity enhancement, and for the structure confirmation and analysis in a MS–MS mode. The direct coupling of MEKC with MS can be hazardous due to the effect of nonvolatile MEKC surfactants on MS performance, including the loss of analyte sensitivity and ion source contamination. The possibility of off-line coupling between MEKC and matrix-assisted laser desorption/ionization (MALDI)-MS remains to be investigated. Various approaches for on-line coupling MEKC with electrospray ionization (ESI)-MS, including the use of high-molecular-mass surfactant, an electrospray-chemical ionization (ES-CI) interface, a voltage switching and buffer renewal system, partial-filling micellar plug and anodically migrating micelles, are reviewed and evaluated. The use of an ES-CI interface is most promising for routine operation of on-line MEKC–MS under the influence of nonvolatile salts and surfactants. The use of a high-molecular-mass surfactant allows the formation of a micellar phase at very low surfactant concentrations and avoids the generation of a high level of background ions in the low m/z region. Alternatively, the application of a partial-filling micellar plug and anodically migrating micelles eliminate the introduction of MEKC micelles into the ESI-MS system. It is possible to directly transfer the conventional MEKC separations to partial-filling MEKC–ESI-MS and MEKC–ESI-MS using anodically migrating micelles without any instrument modifications. © 1997 Elsevier Science B.V.

Keywords: Reviews; Micellar electrokinetic chromatography–mass spectrometry; Detection, electrophoresis

Contents

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1. Introduction

Capillary electrophoresis (CE), an alternative or complementary separation method to high-performance liquid chromatography, is capable of rapid analysis and excellent separation efficiency and resolving power. Even though the UV absorbance is most commonly employed for analyte detection, only limited structure information is available from UV spectra. Thus, on-line coupling of CE with mass spectrometry (MS) is very attractive for mass determination of analyte molecules and for structure confirmation and analysis in a MS–MS mode.

Various interface designs for the integration of CE with electrospray ionization (ESI)-MS have been developed, including the use of metallized capillary terminus, sheath (coaxial) interface, liquid junction and electrical contact through use of a gold wire [1–6]. The comparison between sheath interface and liquid junction was made by Pleasance et al. [7] and the coaxial sheath flow appeared to have several advantages with regard to ruggedness, ease of use, better sensitivity and electrospray performance. The so-called ionspray interface, the addition of nebulization gas at the tip of electrospray capillary, was introduced and studied by Henion and co-workers [8,9] and Pleasance et al. [7]

The multiple charging phenomenon in ESI makes the detection of macromolecules possible using a quadrupole mass spectrometer with limited m/z range. Several applications of on-line capillary zone electrophoresis (CZE)–ESI-MS for peptide and protein analyses have been reported [4,10–13]. Examples of CZE–ESI-MS in the pharmaceutical area included the metabolic studies of neuroleptic drugs and the identification of non-steroidal anti-inflammatory drugs in human urine [14,15]. Furthermore, CZE–ESI-MS has been applied for the analysis of compounds of environmental concern, such as agrochemicals, pesticides, inorganic compounds and dyes [7,16–19].

However, the applications of CZE–ESI-MS for various biological and biomedical studies are limited due to the nonvolatile buffer components and organic additives used to achieve a wide variety of CZE separations. Buffer additives including cetyltrimethylammonium chloride (CTAC) and sodium dodecyl sulfate (SDS) were employed in CZE for resolving closely related tripeptides and tamoxifen metabolites, respectively [20,21]. Although the concentrations of added CTAC and SDS were below their critical micelle concentrations (CMCs), the ESI-MS signal responses for tripeptides and tamoxifens were significantly reduced.

Micellar electrokinetic chromatography (MEKC), one of the separation modes in CE, was first introduced by Terabe and coworkers [22,23] for the analysis of neutral and charged analytes. In MEKC, ionic micelles are used as pseudo-stationary phase and the separation is based on the differential partition of neutral and charged compounds between the micellar phase and the surrounding aqueous solution. The possibility of on-line integration of MEKC with ESI-MS was suggested because both positive and negative ESI mass spectra of SDS were reported by Smith et al. [2] In practice, the direct coupling of MEKC to ESI-MS is hazardous due to the effect of nonvolatile MEKC surfactants on ESI-MS performance. The continuous introduction of nonvolatile surfactants into the ESI-MS system at a relatively high concentration results in a significant loss of electrospray efficiency and ion source contamination.

In this paper, we describe and evaluate various approaches reported in the literature for off- and on-line coupling MEKC with MS. The possibility of off-line combination of MEKC with either fast atom bombardment (FAB)-MS or matrix-assisted laser desorption/ionization (MALDI)-MS is discussed. The significance of surfactant choice, the elimination of surfactant introduction into MS, and the modification of ESI interface are three main considerations for achieving on-line mass detection of analyte molecules eluted and resolved in the MEKC capillary.

2. Off-line micellar electrokinetic chromatography–mass spectrometry

Various fraction collection procedures for CE, including the use of membranes, drums, porous glass connections and an on-column frit structure, have been developed [24–26]. These approaches have the advantage that all zones can be continuously collected without the need to interrupt the electric

current. However, the bulk electroosmotic flow is required to transport the analytes on the solid surfaces or into the collection vials. Recently, the use of a coaxial sheath flow interface, similar to that employed for coupling CE with ESI-MS, was studied for fraction collection in CE [27–29]. As the analyte zones exit the capillary, the sheath flow delivers the analytes into appropriate collection vials or capillaries.

2.1. Fast atom bombardment mass spectrometry

The possible usage of MEKC with FAB-MS for the characterization of deoxyribonucleic acid (DNA) adducts was investigated by Lecoq et al. [30]. Multiple MEKC fraction collection runs were performed using fraction vials which contained the electrophoresis buffer without SDS. Parameters such as the maximum mass loadings and the reproducibility of migration times and sample injection were addressed. The fractions were reanalyzed by MEKC to demonstrate the recovery of analyte molecules. The remaining fractions were then added into pure glycerol for obtaining FAB mass spectra of resolved DNA adducts.

In comparison to the analyte concentration, the extremely high SDS concentration in the fraction, however, produced a very intense background signal with strong chemical noise at the low mass region. A possible ion suppression effect of SDS against the collected nucleosides and nucleotides in the glycerol matrix was further suggested for their failure in obtaining FAB mass spectra [30]. The more hydrophilic DNA adducts migrate into the interior of the glycerol droplet at the FAB interface. In contrast, the SDS surfactants tend to concentrate near the surface layer of the droplet and suppress completely the ionization of other compounds. Additional steps to drastically reduce the concentration of SDS in the fractions are required for off-line analysis of MEKC eluents using FAB-MS.

2.2. Matrix-assisted laser desorption/ionization mass spectrometry

Off-line combinations of CZE and capillary isoelectric focusing (CIEF) with MALDI-MS have been developed and demonstrated for the structure characterization of peptides and proteins [27,31–33]. Mass assignments corresponding to the protonated molecular ions were in good agreement with those predicted from molecular sequences. By utilizing a miniaturized fraction collection interface, good quality mass spectra were obtained from CZE separations of as little as 25 fmol of protein [32]. Selective chemical or enzymatic degradation such as N-terminal sequencing and peptide mapping procedure were combined with molecular mass measurements for achieving additional structure confirmation [31].

MALDI-MS is a powerful analytical technique capable of excellent sensitivity and tolerant of diverse analysis conditions. As demonstrated by Foret et al., direct mass analysis of protein fractions eluted from the cIEF capillary was accomplished using MALDI-MS in the presence of carrier ampholytes [33]. Carrier ampholytes for the creation of a pH gradient in the cIEF capillary exhibit their molecular masses of less than 10 000, away from the mass region of most protein analytes. In MALDI, the SDS surfactants in the MEKC factions will again concentrate near the surface layer of the matrix droplet. The negative effects of high SDS concentration on the MALDI-MS analysis include a high level of low-molecular-mass background ions and an ion suppression or competition with MEKC analytes. The possibility of off-line combination of MEKC with MALDI-MS for the analysis of small neutral and charged molecules remains to be investigated.

3. On-line micellar electrokinetic chromatography–mass spectrometry

The need for on-line coupling MEKC with ESI-MS has led to several new approaches in the recent years [34–40]. Among these approaches, the use of high-molecular-mass surfactant [34] is aimed in the reduction of low-molecular-mass background ions. The introduction of electrospray-chemical ionization (ES-CI) interface [35,36] is directed toward the generation of gas phase ions under the influence of nonvolatile salts and surfactants. Other approaches, including the applications of voltage switching and buffer renewal system [37], partial-filling MEKC [38,39] and anodically migrating micelles [40], are

all based on the elimination of surfactant introduction into ESI-MS.

3.1. High-molecular-mass surfactant

A high-molecular-mass surfactant, butyl acrylate–butyl methacrylate–methacrylic acid copolymer sodium salt (BBMA), was employed by Ozaki et al. [34] as a pseudo-stationary phase for the studies of on-line MEKC–ESI-MS. The molecular mass of BBMA measured by size-exclusion chromatography was about 40 000. The CMC of BBMA was found to be effective zero [41]. Thus, BBMA was able to form a micellar phase at very low surfactant concentrations and did not generate a high level of background ions in the low m/z region.

BBMA precipitates when the solution pH is lower than 4 [41]. MEKC separation of sulfamides was therefore performed in a solution containing 2% BBMA, 10% methanol and 10 mM ammonium formate buffer at pH 7. An water–methanol (1:1) solution containing 1% formic acid was used as a sheath liquid to acidify the MEKC eluents in the ESI interface. The precipitation of BBMA at the ESI interface apparently did not impair the MS detection of sulfamides resolved in the MEKC capillary. As shown in Fig. 1, all four sulfamides were separated and detected by MEKC–ESI-MS in the selected ion monitoring (SIM) mode.

The separation efficiency in MEKC–ESI-MS was far less than in MEKC–UV. The significant deterioration of separation efficiency might be caused by

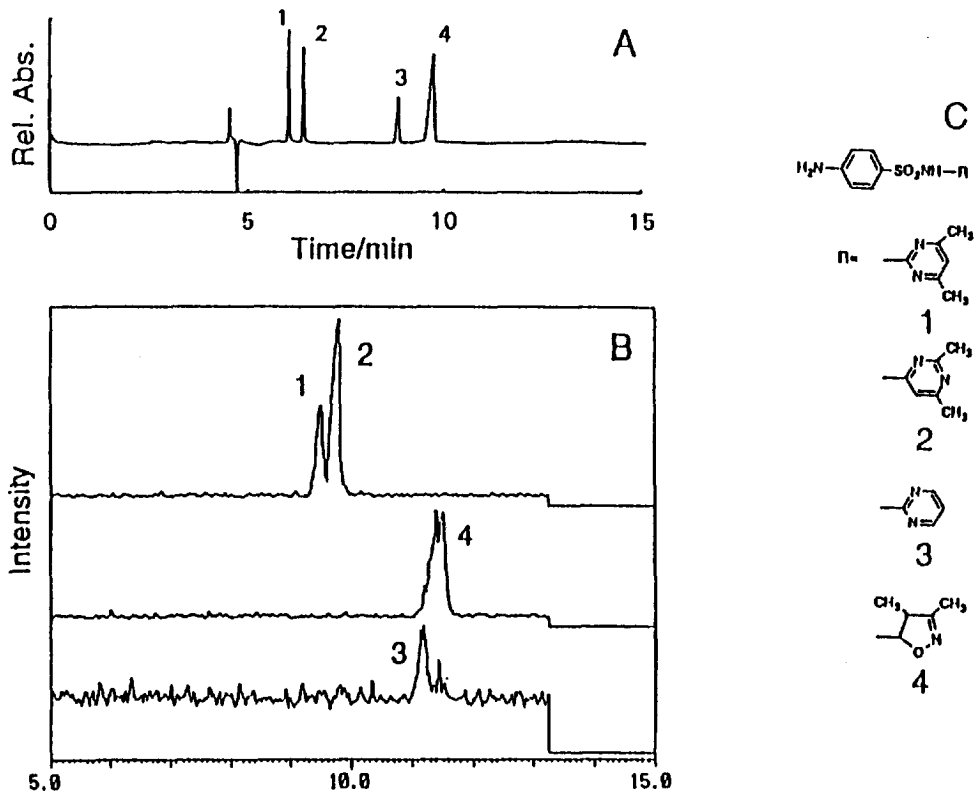

Fig. 1. (A) MEKC with a UV detector, (B) MEKC–ESI-MS and (C) molecular structures of sulfamides. Sulfamide solutes: 1= sulfamethazine; 2=sulfisomidine; 3=sulfadiazine; 4=sulfisoxazole. MEKC–UV conditions: 1% BBMA in 10% methanol and 100 mM borate–50 mM phosphate buffer at pH 7; capillary, 48 cm (40 cm to detector)×50 μm I.D. fused-silica; applied voltage, 20 kV; detection wavelength, 210 nm. MEKC–ESI-MS conditions: 2% BBMA in 10% methanol and 10 mM ammonium formate buffer at pH 7; capillary, 50 cm×50 μm I.D. fused-silica; applied voltages, 13 kV at the capillary inlet, 3 kV at the ESI interface; sheath liquid, water–methanol–formic acid (50:50:1, v/v/v), 5 μl/min. (From Ref. [34]. With permission).

the mixing between the capillary eluent and the sheath liquid at the capillary tip. The difference in the electric field strengths employed during the MEKC–UV and MEKC–ESI-MS studies resulted in further reduction in separation efficiency and the increase in migration times of sulfamide molecules. Additionally, the ion intensity of MEKC analytes decreased with increasing BBMA concentration. Thus, the surfactant ions similar to simple electrolyte ions led to higher solution conductivity and contributed to the establishment of the charge excess necessary for the electrospray process [42–44].

3.2. Electrospray-chemical ionization interface

An ES-CI interface shown in Fig. 2 was introduced and studied by Takada et al. [35,36] for on-line integration of CE with MS under the influence of nonvolatile salts and surfactants. In this interface, the solution was electrosprayed and the droplets were vaporized using a vaporizer. Vaporized samples were ionized by corona discharge followed by ion–molecule reactions under atmospheric pressure. Based on their preliminary results, the ion intensity of protonated caffeine molecules in ES-CI-MS was not heavily affected by the presence of sodium phosphate. In contrast, no protonated caffeine molecule was observed in ESI-MS when a 20 m*M* phosphate buffer was employed for CZE [35].

The observed ion intensity of aromatic amines during the direct infusion studies was not strongly affected by SDS concentrations up to 50 m*M*. Selected ion electropherogram of aromatic amines resolved in MEKC is shown in Fig. 3 with the

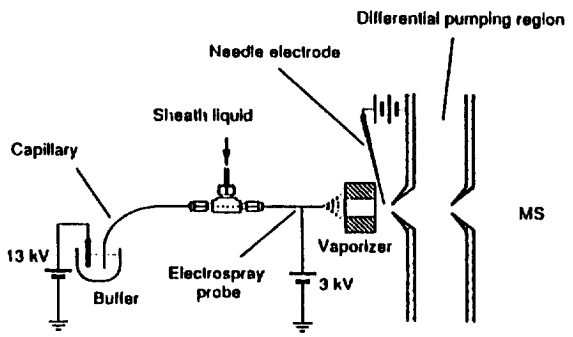

Fig. 2. Schematic diagram of an electrospray–chemical ionization interface. (From Ref. [36]. With permission).

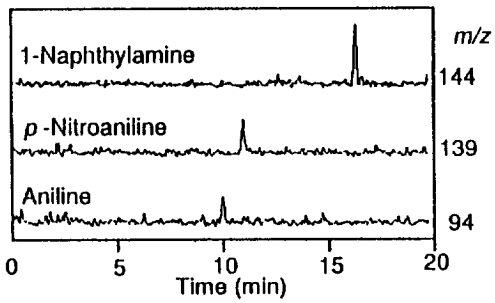

Fig. 3. Selected ion electropherogram of aromatic amines using MEKC–ES-CI-MS. Separation conditions: 20 m*M* SDS and 20 m*M* phosphate buffer at pH 6.6; capillary, 50 cm×50 μm I.D. fused-silica; applied voltages, 13 kV at the capillary inlet, 3 kV at the ESI probe, 3 kV at the CI electrode; sheath liquid, pure methanol, 10 μl/min. (From Ref. [36]. With permission).

elution order of aniline, *p*-nitroaniline and 1-naphthylamine. Due to corona discharge and ion–molecule interaction, a sheath liquid of pure methanol (instead of a solution mixture of water, methanol and acid) was employed at the ES-CI interface. The ES-CI interface was used all day for about ten runs without any clogging problems due to nonvolatile salts and SDS surfactants.

3.3. Voltage-switching and buffer renewal

A coupled-capillary setup with the possibilities of voltage switching and buffer renewal has been developed by Lamoree et al. to avoid the introduction of MEKC surfactants into ESI-MS [37]. As shown in Fig. 4, the setup allowed heart-cutting of the zones of interest in the MEKC capillary with subsequent transfer via a second CZE capillary to ESI-MS. Thus, the MEKC–ESI-MS studies of mepenzolate and pipenzolate consisted of three separate stages. The actual MEKC separation of mepenzolate and pipenzolate in the first capillary was followed by a transfer of analyte zones to the second capillary. Finally, the analytes were separated from the SDS micelles by CZE in the second capillary before they were eluted into ESI-MS.

Applied voltages at the inlet electrode of MEKC capillary and the electrode in the connection vial were varied during the MEKC separation, the transfer of analyte molecules and the CZE separation. The connection vial was initially filled with the MEKC

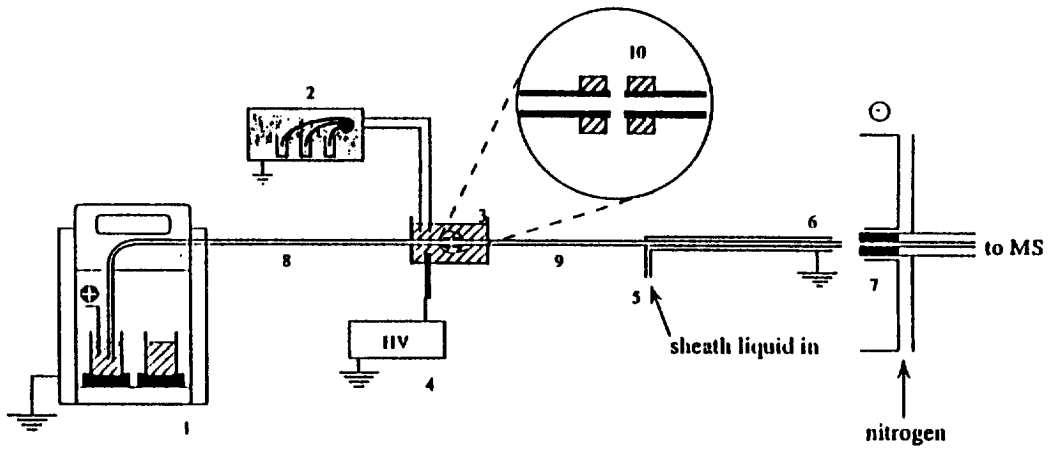

Fig. 4. Schematic representation of a coupled-capillary setup for on-line MEKC–ESI-MS: (1) programmable injector with internal high-voltage power supply; (2) outlet buffer exchange unit; (3) custom-made connection vial with electrode connection; (4) external high-voltage power supply; (5) inlet for the sheath liquid; (6) grounded electrospray needle; (7) electrospray sampling capillary; (8) MEKC capillary; (9) CZE capillary; (10) capillary coupling device. (From Ref. [37]. With permission).

buffer during the MEKC separation and was later emptied and replaced with the CZE buffer for analyte transfer. Additionally, the timing of separation events in this coupled-capillary setup was very critical for on-line integration of MEKC with ESI-MS. The analytes could migrate into the connection vial if the MEKC separation was carried out for a long period of time. When the MEKC separation was ended too early, a large plug of MEKC buffer could enter the CZE capillary before the analytes of interest were transferred. Clearly, only a small separation window containing limited number of analytes could be transferred from the MEKC capillary to the CZE capillary.

The selected ion electropherogram of 200 pg of quaternary amines is shown in Fig. 5 with the elution order of mepenzolate, followed by pipenzolate. Both the separation efficiency and resolution of analyte molecules in a coupled-capillary setup were lower than those obtained from a single capillary MEKC–UV separation (data not shown). In comparison to an electric potential of 25 kV employed for MEKC–ESI-MS, a higher electric potential of 30 kV applied during the MEKC–UV studies partially accounted for the differences in the separation efficiency and resolution. Additional band broadening of analyte molecules in a coupled-capillary setup was the result of analyte transfer across the connection vial and analyte diffusion during the time needed to empty and replace the MEKC buffer with the CZE buffer in the connection vial.

3.4. Partial-filling micellar plug

In comparison with conventional MEKC, partial-filling MEKC (see Fig. 6) involved filling a small portion of the capillary with a micellar solution to achieve a separation. The capillary in partial-filling MEKC mode was filled with electrophoresis buffer, followed by an introduction of micellar solution and finally sample injection. Analytes first migrated into the micellar plug where the separation occurred and then into the electrophoresis buffer, which was free of surfactants. The analytes in the electrophoresis buffer sequentially eluted out the MEKC capillary and were subsequently introduced into ESI-MS, while the surfactant plug remained behind in the capillary. Once the analytes were detected by ESI-MS, electrophoresis was terminated to avoid the surfactant plug eluting into the detector [39].

The mechanistic studies of partial-filling MEKC and the comparisons with conventional MEKC in terms of separation efficiency and resolution of triazine herbicides were investigated by Nelson and Lee [38]. A theoretical model was proposed for predicting the separation behavior of triazine her-

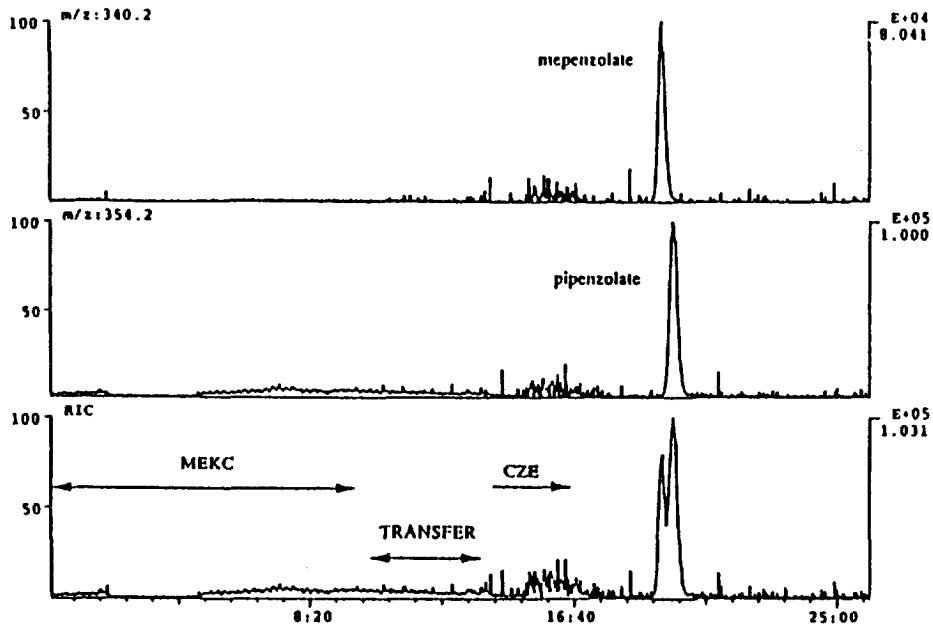

Fig. 5. Selected ion electropherogram of mepenzolate and pipenzolate using MEKC–ESI-MS in a coupled-capillary setup. MEKC conditions: 25 m*M* SDS in a mixture of ammonium acetate buffer (10 m*M*, pH 4.5)–methanol (3:2, v/v); capillary, 57.5 cm×75 μm I.D. fused-silica; applied voltages, 15 kV at the capillary inlet, −10 kV at the connection vial, 9 min. The analyte zones were transferred across the connection vial for 3 min. CZE conditions: a mixture of ammonium acetate buffer (50 m*M*, pH 4.5)–methanol (3:2, v/v); capillary, 32.5 cm×75 μm I.D. fused-silica; applied voltage, 15 kV at the connection vial; sheath liquid, a mixture of ammonium acetate buffer (100 m*M*, pH 4.5)–methanol (1:4, v/v), 5 ml/min. (From Ref. [37]. With permission).

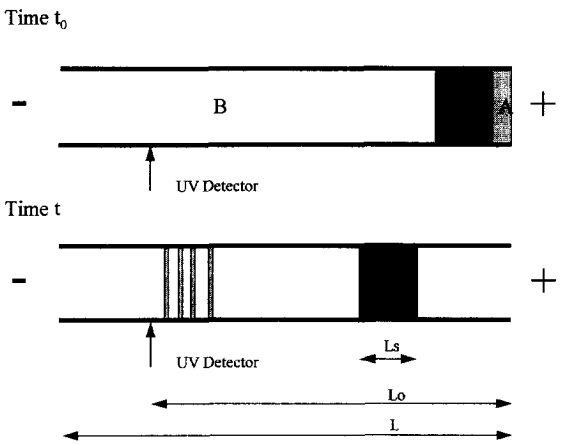

Fig. 6. Schematic diagram of partial-filling MEKC: B, background electrophoresis buffer; S, SDS micelle plug; A, sample analytes; L_S, SDS micelle plug length; L_0, distance between the injection point and the UV detector; L, total capillary length. (From Ref. [38]. With permission).

bicides in partial-filling MEKC. Since the electric field strength in the micelle plug was significantly different from the electric field strength in the electrophoresis buffer, additional band broadening occurred in the analyte band. More specifically, the analyte band in the micelle plug migrated slower due to its partitioning with the micelle and the presence of a lower electric field. Thus, all analyte molecules that diffused past the micelle front on the left (see Fig. 6) encountered a higher electric field, and therefore, migration speed of neutral analytes driven by electroosmosis increased. In comparison to conventional MEKC, the presence of an additional band-broadening phenomenon at the interface between the micelle plug and the leading electrophoresis buffer contributed to the generally lower separation efficiencies of triazine herbicides in partial-filling MEKC.

The electroosmotic velocities in partial-filling MEKC were different between the micelle plug and the electrophoresis buffer region due to the differ-

ence in their electric field strengths. In addition to differences in electric field strengths, differences in ionic strength of the micelle plug and the buffer led to different zeta potentials and consequently to different electroosmotic mobilities or velocities. However, the bulk velocity of fluid at each cross section in the capillary had to be the same for a noncompressible fluid. The difference between the local electroosmotic velocities and the bulk velocity generated hydrostatic pressure and additional laminar flows in both the micelle plug and the electrophoresis region [45]. In general, laminar flows display a characteristic parabolic flow pattern which gives rise to additional band broadening of analytes in partial-filling MEKC.

As shown in Fig. 7, the triazine herbicides including atrazine, propazine, ametryne and prometryne were sequentially eluted and monitored by ESI-MS in the SIM mode. All four triazine herbicides were baseline resolved in partial-filling MEKC–UV studies (data not shown). However, prometryne appeared as a shoulder and overlapped with ametryne in the reconstructed ion electropherogram. The mixing between the capillary eluent and the sheath liquid at the capillary tip contributed to the decrease in the separation efficiency and resolution of triazine herbicides in partial-filling MEKC–ESI-MS. Due to the absence of SDS surfactant in the electrospray process, long-term stable operation of partial-filling MEKC–ESI-MS was ensured [39].

3.5. Anodically migrating micelles

The micellar velocity in MEKC is determined by the sum of its electrophoretic velocity and the opposing electroosmotic flow. Thus, the micellar velocity can be directly manipulated by the adjustment of electroosmosis rather than the electrophoretic velocity of the micelle. Reported efforts for affecting the electroosmotic flow included the use of

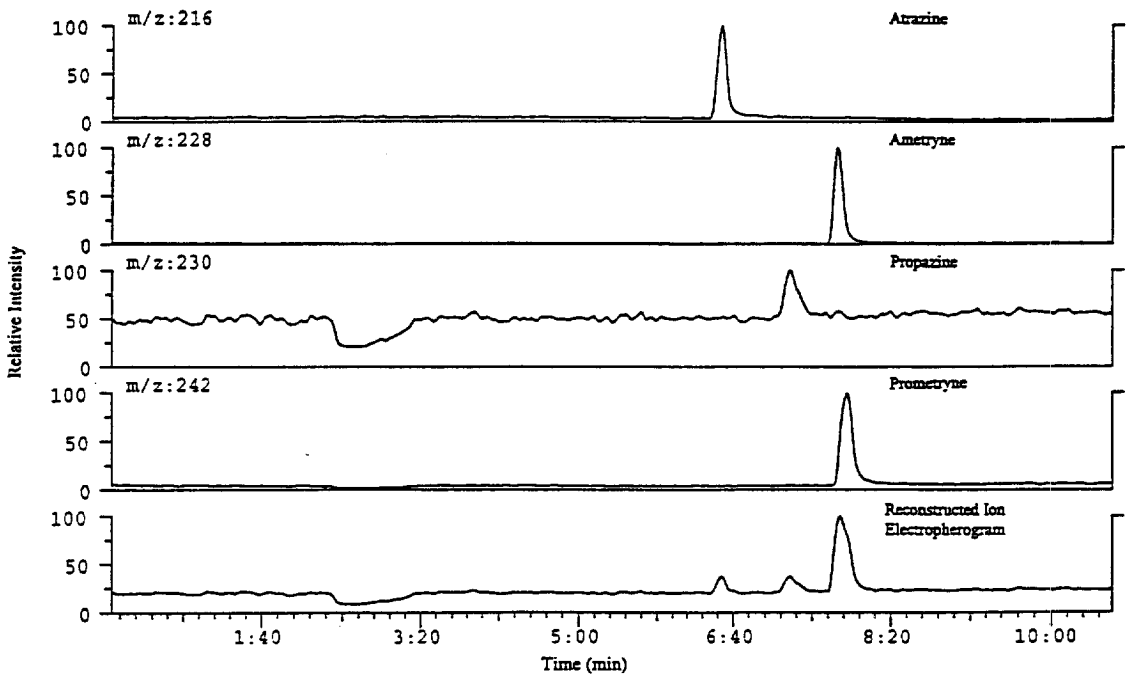

Fig. 7. Selected ion electropherogram of triazine herbicides in partial-filling MEKC–ESI-MS. Electrophoresis buffer, 20 m*M* ammonium acetate, pH 6.8; SDS micelle plug, 25 m*M* SDS in 20 m*M* ammonium acetate, pH 6.8; capillary, 45 cm×50 μm I.D. fused-silica; applied voltages, 14 kV at the capillary inlet, 5 kV at the ESI probe; sheath liquid, water–methanol–acetic acid (50:49:1, v/v/v), 5 μl/min. (From Ref. [39]. With permission).

surface active agents, buffer pH, buffer composition, temperature, chemical derivatization of the surface and a radial electric potential gradient across the capillary [46–50]. In this particular approach for on-line coupling of MEKC with ESI-MS, the electroosmotic flow was adjusted against the electrophoretic velocity of the SDS micelle by changing the solution pH in MEKC [40]. The elimination of SDS surfactant introduction into ESI-MS was achieved using anodically migrating micelles, moving away from ESI-MS.

By adjusting the MEKC buffer pH to 5.9, the SDS micelles migrated toward the inlet reservoir with a net migration mobility of $-1.00 \cdot 10^{-4}$ $cm^2/V/s$ and avoided the introduction of nonvolatile surfactants into ESI-MS. On-line MEKC–ESI-MS was demonstrated for the analysis of chlorotriazine herbicides (see Fig. 8) and barbiturates (see Fig. 9) using the positive and negative electrospray modes, respectively. The additional m/z 174 ion together with the molecular ion of atrazine at m/z 216 (see Fig. 8) was due to the loss of isopropyl group of atrazine during the electrospray process.

In comparison to MEKC–UV studies at pH 5.9 (data not shown), the migration time of triazine herbicides in MEKC–ESI-MS was slightly shorter under the same separation distance. For example, the migration times of atrazine in MEKC–UV and MEKC–ESI-MS were 10.8 min and 9.30 min, respectively. The decrease in the migration time of triazine herbicides in MEKC–ESI-MS was attributed to the migration of SDS micelles toward the inlet reservoir, the anodic end. In MEKC–ESI-MS, the cathodic end of the electrospray probe contained a sheath liquid of acetic acid solution without any SDS surfactant. Thus, the continuous elution of SDS micelles into the inlet reservoir resulted in a moving boundary of SDS surfactants in MEKC–ESI-MS.

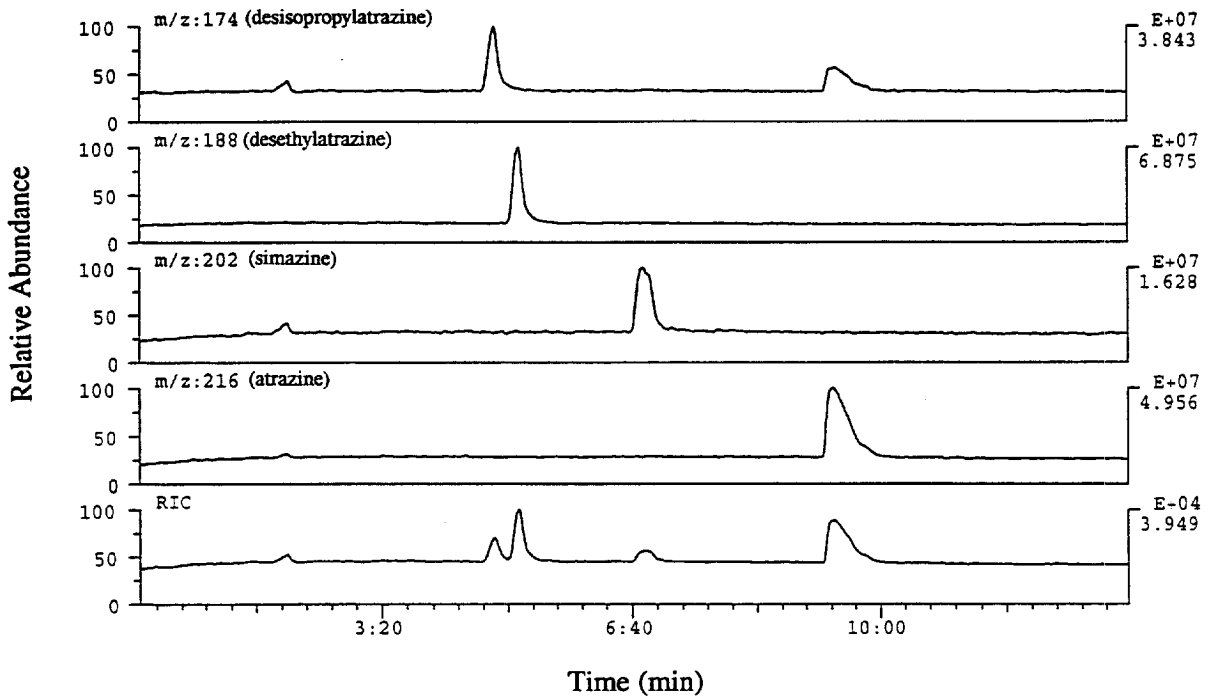

Fig. 8. Selected ion electropherogram of chlorotriazine herbicides in MEKC–ESI-MS using anodically migrating micelles. Electrophoresis buffer, 10 m*M* SDS and 10 m*M* ammonium acetate at pH 5.9; capillary, 25 cm×50 μm I.D. fused-silica; applied voltages, 12 kV at the capillary inlet, 5 kV at the ESI probe; sheath liquid, methanol–water–acetic acid (50:49:1, v/v/v) at pH 2.6, 5 μl/min. (From Ref. [40]. With permission).

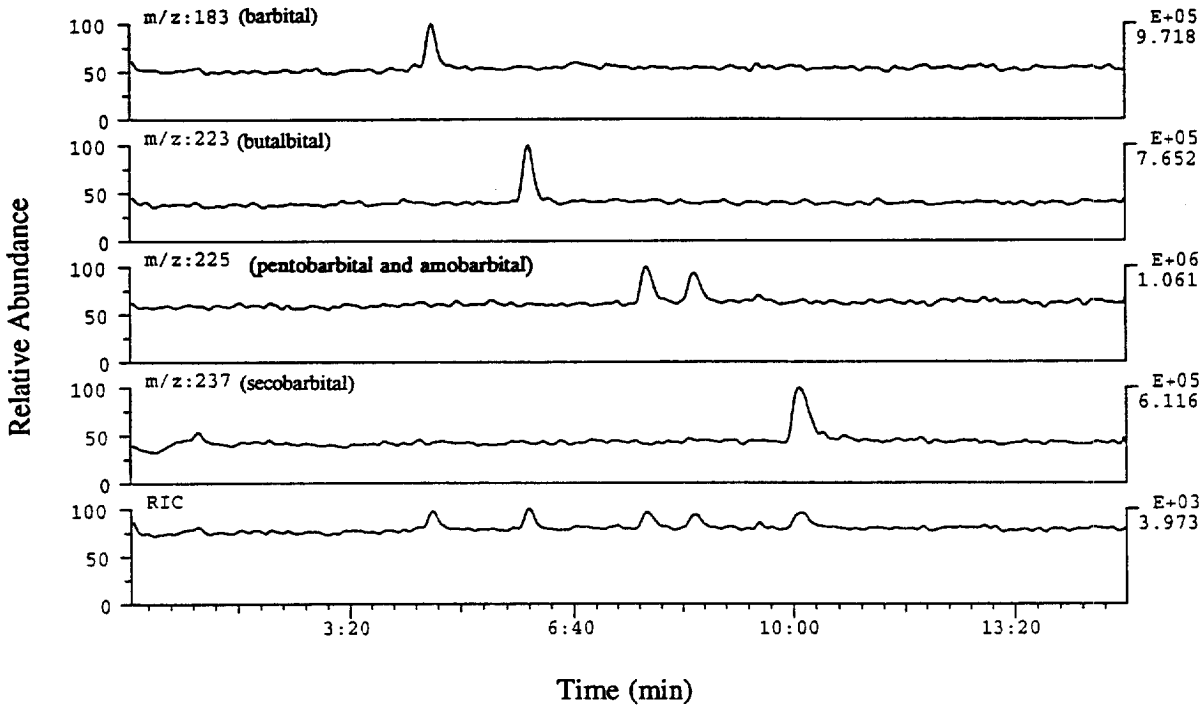

Fig. 9. Selected ion electropherogram of barbiturates in MEKC–ESI-MS using anodically migrating micelles. Electrophoresis buffer, 15 m*M* SDS and 10 m*M* ammonium acetate at pH 5.9; capillary, 20 cm×50 μm I.D. fused-silica; applied voltage, −4.5 kV at the ESI probe and grounding at the inlet reservoir; sheath liquid, methanol–water–ammonium hydroxide (50:49.75:0.25, v/v/v) at pH 10.5, 5 μl/min. (From Ref. [40]. With permission).

Once the analytes migrated across the moving SDS boundary, the neutral analytes were transported by the electroosmotic flow to ESI–MS at the end of the MEKC capillary. The extent of decrease in migration time of triazine herbicides contributed by the moving SDS boundary was greater for late eluting compounds than for early migrating analytes.

The mixing between the capillary eluent and the sheath liquid at the capillary tip might result in the decrease of separation efficiency and resolution of herbicides in MEKC–ESI-MS. The presence of an additional band broadening phenomenon at the interface between the moving SDS phase and the electrophoresis buffer further contributed to the generally lower separation efficiencies of triazine herbicides. Briefly, the analyte band in the SDS phase migrated slower due to its partitioning with the micelle and the presence of a lower electric field. Thus, all analyte molecules that migrated past the micellar phase encountered a higher electric field in the electrophoresis buffer, and therefore, migration speed driven by electroosmosis increased. Further detail on band broadening of analyte solutes across the interface can be found in the mechanistic studies of partial-filling MEKC [38].

To reduce or eliminate the moving SDS boundary in MEKC–ESI-MS, it is possible to raise the electrophoresis pH and the electroosmotic flow against the negative electrophoresis of SDS micelles. In the work of Yang et al. [40], the electroosmotic mobility was adjusted to be slightly smaller than the absolute value of micellar electrophoretic mobility. The resulted migration of SDS micelles toward the anodic end not only ensured the absence of SDS surfactant in ESI-MS, but also illustrated the negative effects of the moving SDS boundary on the separation per-

formance of MEKC–ESI-MS using anodically migrating micelles.

4. Conclusions

The combination of MEKC with MS with the aims of selectivity enhancement and structure confirmation can be hazardous due to the negative influence of MEKC micelles on MS performance, including the loss of analyte sensitivity and ion source contamination. The possibility of off-line coupling between MEKC and MALDI–MS for the analysis of compounds which are difficult to be resolved in CZE remains to be investigated. It is well known that MALDI–MS is a powerful analytical technique capable of excellent sensitivity and tolerant of diverse analysis conditions.

Among various on-line coupling techniques for MEKC–ESI-MS, the use of an ES-CI interface is most promising for routine analysis of MEKC analytes in the presence of nonvolatile salts and surfactants. The use of high-molecular-mass surfactant is able to form a micellar phase at very low surfactant concentrations and avoids the generation of a high level of background ions at low m/z region. On the other hand, the applications of partial-filling MEKC and anodically migrating micelles eliminate the introduction of MEKC micelles into ESI-MS and provide alternative approaches for the direct coupling of MEKC with ESI-MS. It is possible to directly transfer the conventional MEKC separations to partial-filling MEKC–ESI-MS and MEKC–ESI-MS using anodically migrating micelles without any instrument modifications. Future applications of MEKC–ESI-MS involve the structure analysis of compounds relevant to biomedical and pharmaceutical applications in a MS–MS mode.

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