ELSEVIER

Journal of Chromatography A, 749 (1996) 219–226

JOURNAL OF CHROMATOGRAPHY A

On-line partial filling micellar electrokinetic chromatography–electrospray ionization mass spectrometry

Wendy M. Nelson, Qing Tang, A. Kamel Harrata, Cheng S. Lee*

Department of Chemistry and Ames Laboratory USDOE, Iowa State University, Ames, IA 50011, USA

Received 20 February 1996; revised 29 April 1996; accepted 29 April 1996

Abstract

On-line combination of partial filling micellar electrokinetic chromatography (PF-MEKC) and electrospray ionization mass spectrometry (ESI-MS) is demonstrated for the analysis of triazine herbicides including atrazine, propazine, ametryne and prometryne. In comparison with conventional micellar electrokinetic chromatography (MEKC), PF-MEKC involves filling a small portion of the capillary with a sodium dodecyl sulfate (SDS) micellar solution for achieving the separation. In PF-MEKC, the triazine analytes first migrate into the micellar plug where the separation occurs and then into the electrophoresis buffer which is free of surfactant. Consequently, the electroosmotic transfer of neutral triazine herbicides to ESI-MS at the end of PF-MEKC capillary is comparable to conventional capillary zone electrophoresis ESI-MS. Therefore, PF-MEKC–ESI-MS provides a mechanism for the separation and mass detection of neutral molecules without the interference of surfactant.

Keywords: Mass spectrometry; Partial filling micellar electrokinetic chromatography; Herbicides; Triazines; Atrazine; Propazine; Ametryne; Prometryne

1. Introduction

The potential interference of surfactants used in MEKC with on-line electrospray ionization (ESI) mass spectrometry (MS) has been investigated by several research groups [1–4]. Direct coupling of MEKC to MS is hazardous due to the elution of non-volatile surfactant from the MEKC capillary into the MS. The presence of a relatively high concentration of non-volatile surfactant results in a significant loss of electrospray efficiency and sensitivity of analytes relative to those observed in the absence of surfactant. The choice of surfactant added to the running buffer in MEKC is also highly significant, because surfactants are non-volatile and in many cases may cause background ions in the mass spectrum. Both positive and negative ESI mass spectra of sodium dodecyl sulfate (SDS), which is the most frequently used surfactant in MEKC, have been observed by Smith et al. [5]. To date, the use of a low molecular mass surfactant, such as SDS, has not yet been successfully demonstrated for the direct coupling of MEKC with ESI-MS.

The combination of MEKC with ESI-MS is very attractive for the possibility of selectivity enhancement, for the direct identification of analytes and for the structure analysis of analyte molecules in a MS–MS mode. The need for coupling MEKC with ESI-MS has led to several new approaches including the use of high molecular mass surfactants [2], a semi-

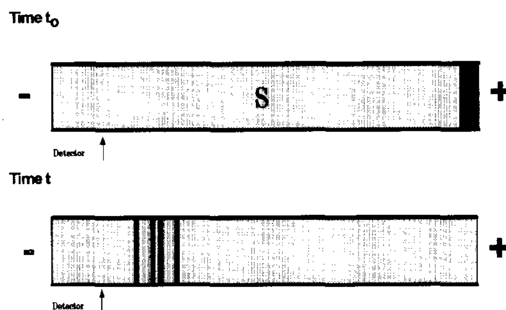
*Corresponding author.

0021-9673/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
PII S0021-9673(96)00455-4

permeable membrane interface [3] and a voltage switching and buffer renewal system [4]. Ozaki et al. [2] demonstrated the use of high molecular mass surfactants such as butyl acrylate–butyl methacrylate–methacrylic acid copolymer sodium salt and as a result avoided the production of high levels of low mass background ions in the mass spectrum. The interface introduced by Foley and Masucci [3] utilized a semi-permeable membrane that selectively permeates small analyte molecules to the mass spectrometer, while retaining large buffer additives such as surfactants. The voltage switching and buffer renewal system introduced by Lamoree et al. [4] allowed on-line heart-cutting of the analyte zones in the MEKC capillary with subsequent transfer via a second capillary zone electrophoresis (CZE) capillary for introduction to the mass spectrometer.

Successful demonstration of on-line coupling of MEKC to ESI-MS involves the elimination of surfactant introduction into the mass spectrometer. PF-MEKC was first introduced by Terabe et al. [6] as a potential solution for interfacing MEKC separations with ESI-MS. In comparison with conventional MEKC (see Fig. 1), PF-MEKC involves filling a small portion of the capillary with a micellar solution to achieve a separation. The capillary in PF-MEKC is filled with electrophoresis buffer, followed by the introduction of micellar solution and finally a sample injection. In PF-MEKC, the analytes first migrate into the micellar plug where the separation occurs and then into the electrophoresis buffer, which is free of surfactant. The analytes in the electrophoresis buffer sequentially elute out of the MEKC capillary and are subsequently introduced into ESI-MS, while the surfactant plug remains behind in the capillary. Once the analytes are detected by the mass spectrometer, electrophoresis is terminated to avoid the surfactant plug eluting into the detector.

Mechanistic studies of PF-MEKC and its comparison with conventional MEKC were discussed by Nelson and Lee [7]. The effects of micellar concentration, plug length and overall length on separation efficiency and resolution of PF-MEKC were investigated both experimentally and theoretically. A thorough understanding of the separation mechanism in PF-MEKC offered the opportunity for on-line coupling of PF-MEKC to ESI-MS. In this paper, PF-MEKC–ESI-MS is presented using triazine herbicides as a model system.



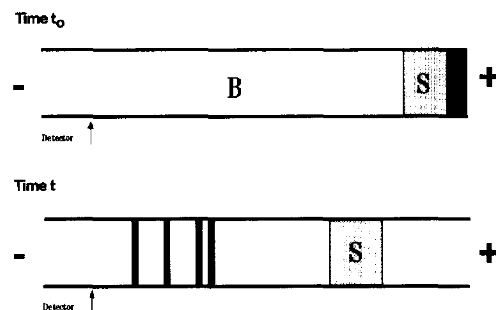


Fig. 1. Schematic diagrams comparing conventional MEKC and PF-MEKC. (A) sample analytes; (B) background electrophoresis buffer; (S) surfactant in background electrophoresis buffer.

2. Experimental

2.1. Micellar electrokinetic separations: UV measurements

Electrokinetic separations were performed using fused-silica tubing from Polymicro Technologies (Phoenix, AZ, USA). Capillary dimensions were 50 μm I.D. and 360 μm O.D., with a total length of 45 cm, 30 cm to the detector. Detection was carried out using a Linear UVIS 200 detector from Linear Instruments (Reno, NV, USA) that had been modified for on-column detection. The detection wavelength was set at 226 nm. A Spellman CZE 1000R High

Voltage Power Supply (Plainview, NY, USA) delivered −9.0 kV to the detector end of the capillary for electrokinetic injection and for electrophoretic separation. All experiments were performed at room temperature, 24°C. Data collection was performed using a Hewlett Packard HP 35900D analog-to-digital interface board with the HP G1250C General Purpose Chemstation Software (Hewlett Packard, Fullerton, CA, USA).

2.2. Mass spectrometer and electrospray interface

The mass spectrometer used in this study was a Finnigan MAT TSQ700 (San Jose, CA, USA) triple quadrupole, equipped with an electrospray ionization source. The Finnigan MAT electrospray adapter kit, containing both gas and liquid sheath tubes, was used to directly couple PF-MEKC with ESI-MS without modifications. The electrospray needle was maintained at 5 kV for all ESI-MS measurements. The first quadrupole was used in selected ion monitoring mode at m/z ratios of 216 (atrazine), 228 (ametryne), 230 (propazine) and 242 (prometryne), at a scan rate of 0.125 s/scan. The electron multiplier was set at 1.4 kV, with conversion dynode at −15 kV. Tuning and calibration of the mass spectrometer were established by using an acetic acid solution (methanol–water–acetic acid, 50:49:1, v/v) containing myoglobin and a small peptide of methionine–arginine–phenylalanine–alanine (MRFA).

Interfacing PF-MEKC to ESI-MS was accomplished using a 45 cm long, 50 μm I.D. and 192 μm O.D. fused-silica capillary mounted within the electrospray probe. The capillary tip was fixed at 0.5 mm outside the electrospray needle. The inlet reservoir contained a 20 m*M* ammonium acetate buffer, pH 6.8, and was maintained at the same height as the electrospray probe. The mass spectrometer operated in the positive ion mode, therefore requiring an acidic sheath liquid to assist positive ion formation. The sheath liquid was a water–methanol–acetic acid solution (50:49:1, v/v) delivered by a Harvard Apparatus 22 Syringe Pump (South Natick, MA, USA) at 5 μl/min. No sheath gas was employed during the PF-MEKC–ESI-MS measurements. Two Spellman CZE 1000R High Voltage Power Supplies (Plainview, NY, USA) delivered electric potentials of 14 and 5 kV at the inlet electrode and electrospray needle, respectively. Therefore, a constant electric field of 200 V/cm was maintained during the PF-MEKC separation. A resistor that was wired in parallel with the high voltage electrode connecting the electrospray needle was incorporated as a current sink to protect the high voltage power supply.

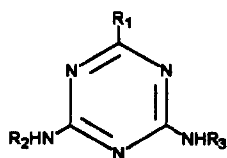
2.3. Chemicals

SDS of protein research grade was purchased from Boehringer Mannheim (Mannheim, Germany) and was used as received. Background electrophoresis buffer was prepared by titrating a 20 m*M* ammonium acetate solution with a 50% (v/v) sodium hydroxide solution to pH 6.8. Ammonium acetate is needed to better maintain the efficiency and stability of the electrospray process, even though the buffer is within 0.2 pH units of it's relative minimum buffering capacity at pH 7.0. HPLC-grade methanol and quinine hydrochloride (Aldrich, Milwaukee, WI, USA) were used as the electroosmotic flow marker and the micellar marker, respectively. Ammonium acetate, sodium hydroxide and methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA).

Atrazine, propazine, ametryne and prometryne were purchased from ChemService (West Chester, PA, USA). The chemical structures of four triazine herbicides are included in Fig. 2. A standard triazine herbicide solution with an individual concentration of 10^{-4} *M* was prepared in 20 m*M* ammonium acetate buffer containing 10% (v/v) methanol. The standard herbicide solution did not contain any SDS surfactant. All solutions were prepared using water purified by a NANOpure II system (Dubuque, IA, USA).

3. Results and discussion

In order to obtain a conventional MEKC separation of the triazine herbicides, a fused-silica capillary was filled with electrophoresis buffer containing 25 m*M* SDS and 20 m*M* ammonium acetate buffer at pH 6.8. The standard herbicide solution with the addition of 2.8×10^{-5} *M* quinine hydrochloride was electrokinetically injected at the anodic end of the



Triazines	R1	R2	R3
Atrazine	Cl	Isopropyl	Ethyl
Propazine	Cl	Isopropyl	Isopropyl
Ametryne	S-methyl	Ethyl	Isopropyl
Prometryne	S-methyl	Isopropyl	Isopropyl

Fig. 2. Structures of the four triazine herbicides examined in this study.

capillary by applying −9.0 kV for 1 s to the cathodic end (the UV detector end). The same −9.0 kV was applied for the separation of triazine herbicides shown in Fig. 3. The elution order and capacity factor of triazine herbicides are summarized in Table 1, with the more hydrophobic herbicides eluting later in the micelle phase.

For comparison with conventional MEKC, PF-MEKC was accomplished by employing a fused-silica capillary filled with 20 m*M* ammonium acetate buffer at pH 6.8 containing no SDS. A 25 m*M* SDS solution (in 20 m*M* ammonium acetate buffer, pH 6.8) and a standard herbicide solution were sequentially injected at the anodic end of the capillary by applying −9.0 kV for 25 s and 1 s, respectively. The separation of triazine herbicides in PF-MEKC is shown in Fig. 4. To identify the elution of the

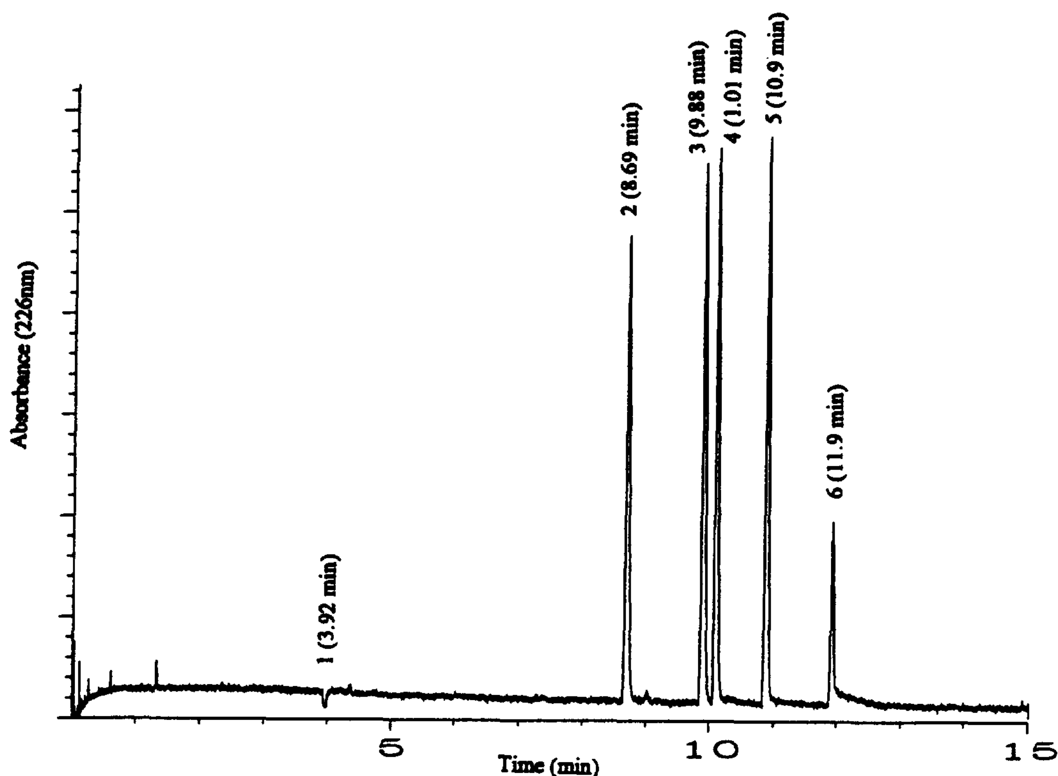


Fig. 3. Conventional MEKC separation of 10^{-4} *M* each of atrazine (2), propazine (3), ametryne (4) and prometryne (5). Methanol (1) and quinine hydrochloride (6) were used as the electroosmotic flow marker and the micellar marker, respectively. Electrophoresis buffer: 20 m*M* ammonium acetate and 25 m*M* SDS, pH 6.8. Capillary: 50 μm I.D. and 360 μm O.D., with a total length of 45 cm and with a length of 30 cm to the detector. Applied voltage: −9.0 kV and 1 s for injection; −9.0 kV for electrophoresis. UV detection at 226 nm.

Table 1
Elution order and capacity factor for triazine herbicides

Analyte peak	Elution order	Conventional MEKC retention time (min)	Capacity factor
Methanol	1	3.92	
Atrazine	2	8.69	4.48
Propazine	3	9.88	8.84
Ametryne	4	10.1	10.1
Prometryne	5	10.9	19.8
Quinine hydrochloride	6	11.9	

micellar plug, quinine hydrochloride was added to a 25 m*M* SDS solution with a final concentration of 4.0×10^{-4} *M*. By assuming that each SDS micelle contained approximately 62 monomers [8], the addition of 4.0×10^{-4} *M* quinine hydrochloride gave a ratio of one quinine hydrochloride molecule to one SDS micelle. To mimic the injection conditions given in Fig. 4, a SDS solution containing quinine hydrochloride and an ammonium acetate buffer that was free of SDS and triazine herbicides was injected sequentially by applying −9.0 kV for 25 s and 1 s, respectively. As shown in Fig. 5, a SDS plug with a peak width of approximately 26 s eluted at 8.5 min.

Several dramatic differences between conventional- and PF-MEKC were observed when comparing the electropherograms shown in Figs. 3–5. A decrease in the migration times and separation efficiencies of triazine herbicides was observed in PF-MEKC when compared to conventional MEKC. In addition, the migration time of the SDS micelle, t_{MC},

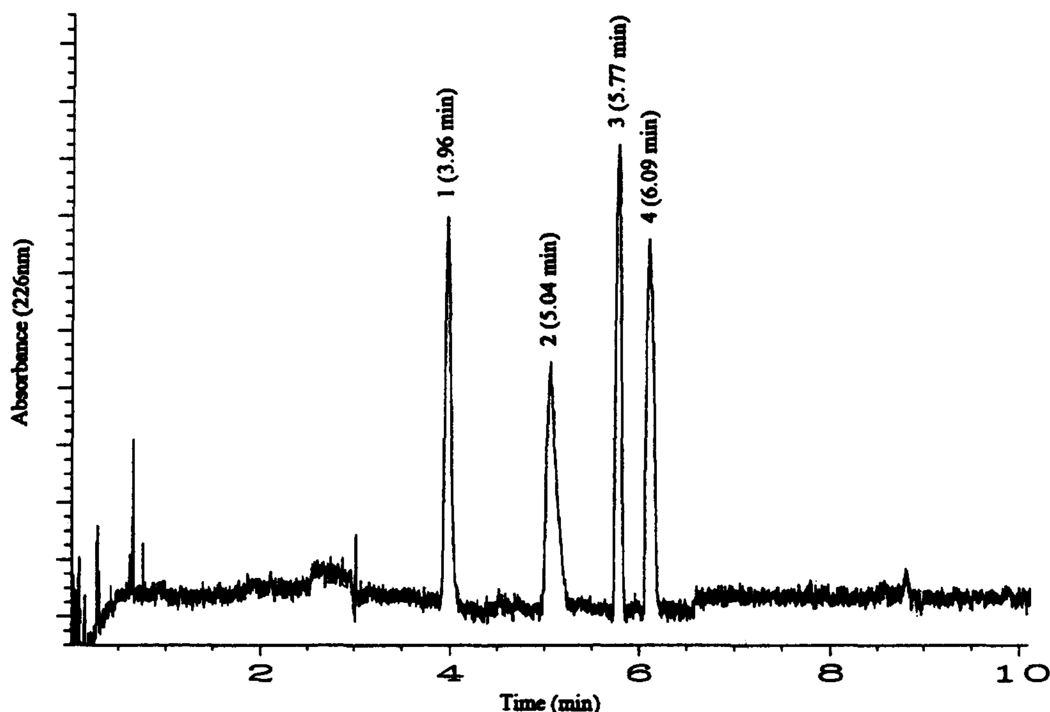


Fig. 4. PF-MEKC separation of 10^{-4} *M* each of atrazine (1), propazine (2), ametryne (3) and prometryne (4). Electrophoresis buffer: 20 m*M* ammonium acetate, pH 6.8. Applied voltage: −9.0 kV for 25 s SDS injection (25 m*M* SDS in 20 m*M* ammonium acetate buffer, pH 6.8); −9.0 kV for 1 s triazine injection and −9.0 kV for electrophoresis. Other conditions are the same as in Fig. 3.

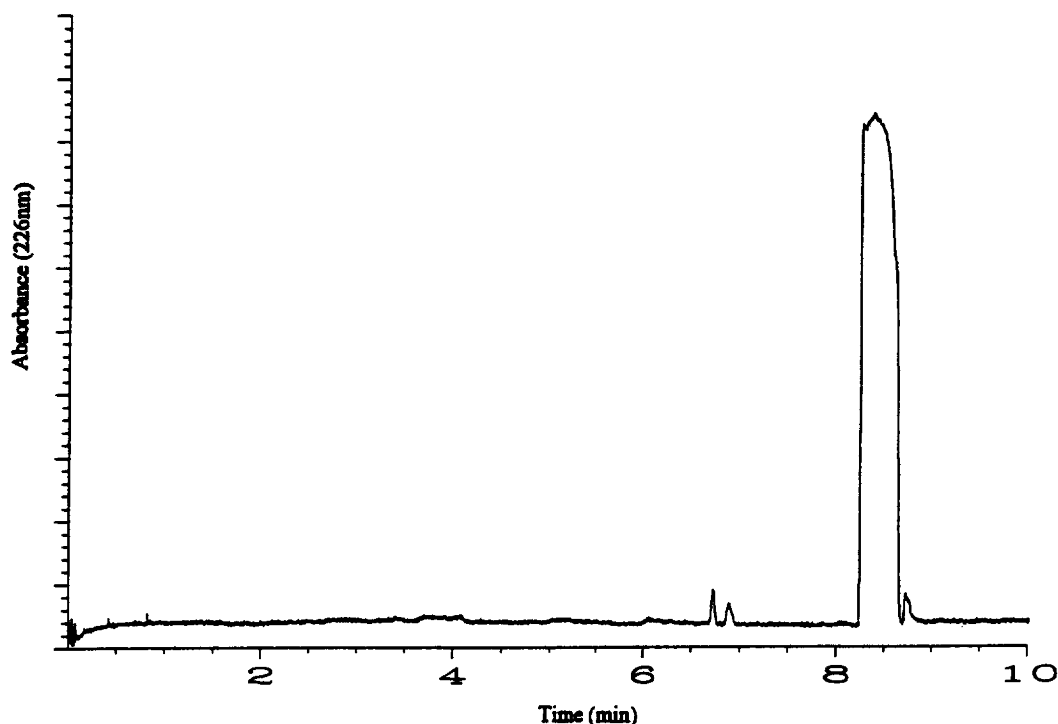


Fig. 5. PF-MEKC separation of 4.0×10^{-4} *M* quinine hydrochloride in 25 m*M* SDS, 20 m*M* ammonium acetate buffer, pH 6.8. Electrophoresis buffer: 20 m*M* ammonium acetate buffer, pH 6.8. Applied voltage: −9.0 kV for 25 s quinine hydrochloride–SDS injection; −9.0 kV for 1 s electrophoresis buffer injection and −9.0 kV for electrophoresis. Other conditions are the same as in Fig. 3.

was significantly reduced from 11.9 min in conventional MEKC to 8.5 min in PF-MEKC. Mechanistic studies of PF-MEKC and a theoretical model for the prediction of separation behavior in PF-MEKC were presented by Nelson and Lee [7]. Optimization techniques, possible applications and advantages of PF-MEKC were similarly addressed.

Interfacing PF-MEKC with ESI-MS was similar to CZE–ESI-MS with respect to the electroosmotic transfer of the neutral triazine herbicides to ESI-MS at the end of the MEKC capillary. The analyte zones in PF-MEKC were first separated in the SDS micellar plug and subsequently migrated into the electrophoresis buffer, which was free of SDS surfactant. In the electrophoresis buffer, the neutral triazine herbicides were driven towards the electrospray tip as a result of electroosmotic flow. In the PF-MEKC–ESI-MS experiments, the separation conditions, including capillary dimensions and electrokinetic voltages, were identical to those employed in PF-MEKC–UV studies.

The choice of the electrophoresis buffer in PF-MEKC has proven to be important with regard to the maintenance and efficiency of the electrospray process. On the basis of volatility, the ammonium acetate buffer was chosen over phosphate buffer for PF-MEKC–ESI-MS experiments. As shown in Fig. 6, the triazine herbicides including atrazine, propazine, ametryne and prometryne were sequentially eluted and monitored by ESI-MS at m/z values of 216, 230, 228 and 242, respectively. In comparison with PF-MEKC–UV experiments (see Fig. 4), the longer migration distance towards the end of the capillary in PF-MEKC–ESI-MS accounted for the increase in the migration time. There was no further enhancement in the separation resolution of triazine herbicides after their migration into the electrophoresis buffer. Prometryne appeared as a shoulder and

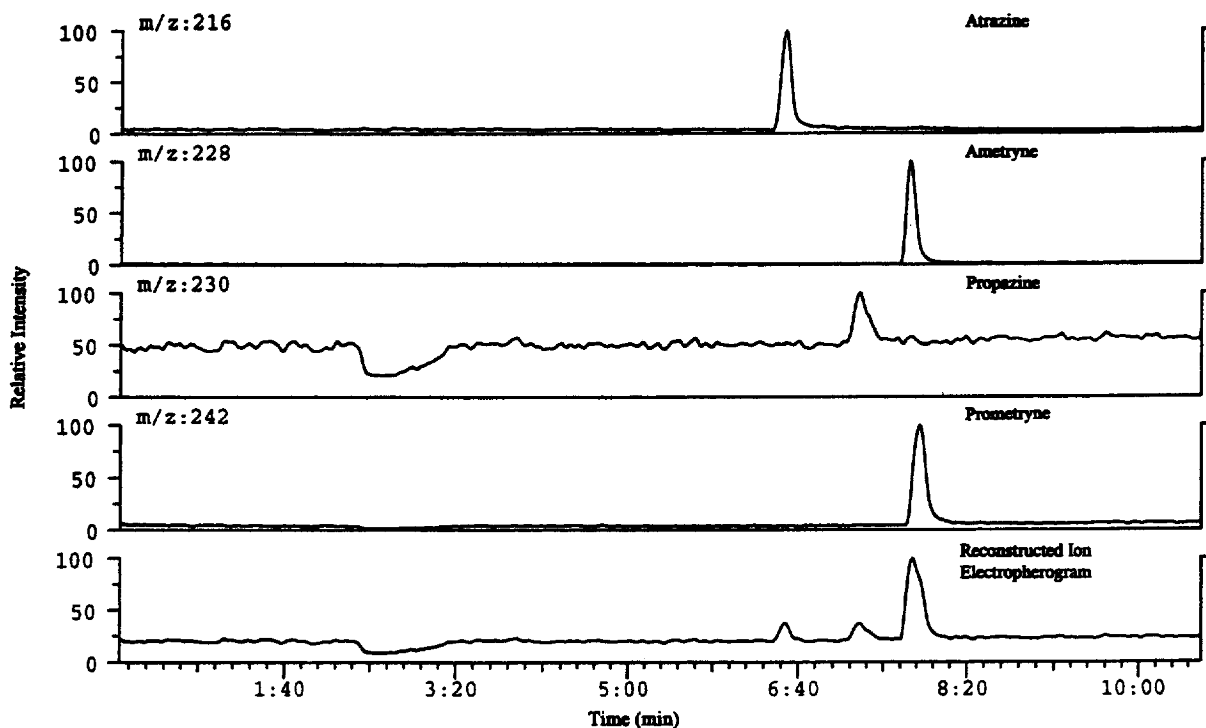


Fig. 6. Positive electrospray ionization mass electropherogram of triazine herbicides in PF-MEKC–ESI-MS. Applied voltage: 5 kV for electrospray and 14 kV for electrophoresis. Sheath liquid: methanol–water–acetic acid (50:49:1, v/v) at pH 2.6, 5 μl/min flow-rate. Separation conditions of PF-MEKC are the same as in Fig. 4.

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 resolution of triazine herbicides in PF-MEKC–ESI-MS. However, the selected ion monitoring of ESI-MS provided the direct identification of triazine herbicides.

As demonstrated in this study, the application of PF-MEKC provides an alternative approach for the direct coupling of MEKC separation with ESI-MS. Based on the mechanistic studies of PF-MEKC [7], it is possible to directly transfer the conventional MEKC separations to PF-MEKC–ESI-MS measurements without any instrument modifications. Due to the absence of surfactant in the electrospray process, long-term stable operation of PF-MEKC–ESI-MS is ensured. In conclusion, PF-MEKC–ESI-MS enables the separation and mass detection of neutral triazine herbicides that are difficult to analyze by CZE–ESI-MS to be carried out. Further applications of PF-MEKC–ESI-MS involve obtaining structure information of analyte molecules in a MS–MS operation mode. The utilization of MS–MS would be particularly important for the analysis of unknown analytes.

Acknowledgments

This work was supported by an EPA Grant (R 823292-01) and the Microanalytical Instrumentation Center of the Institute for Physical Research and Technology at Iowa State University is gratefully acknowledged. W.M.N. is a recipient of the Graduate Assistantship in Areas of National Need (GAANN) fellowship from the US Department of Education.

C.S.L. is a National Science Foundation Young Investigator (BCS-9258652).

References

[1] J. Varghese and R.B. Cole, J. Chromatogr. A, 652 (1993) 369.
[2] H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Shkairi and K. Hideaki, J. Chromatogr. A, 716 (1995) 69.
[3] J.P. Foley and J.A. Masucci, Proceedings of the Seventeenth International Symposium on Capillary Chromatography and Electrophoresis, 1995, pp. 278.
[4] M.H. Lamoree, U.R. Tjaden and J. van der Greef, J. Chromatogr. A, 712 (1995) 219.
[5] R.D. Smith, C.J. Barinaga and H.R. Udseth, Anal. Chem., 60 (1988) 1948.
[6] S. Terabe, H. Ozaki, Y. Takada, M. Sakairi and H. Koizun, Proceedings of the Seventh International Symposium on High Performance Capillary Electrophoresis, 1995, pp. 53.
[7] W.M. Nelson and C.S. Lee, Anal. Chem., in press.
[8] J.H. Fendler, Membrane Mimetic Chemistry: Characterizations and Applications of Micelles, Microemulsions, Monolayers, Bilayers, Vesicles, Host–Guest Systems, and Polyions, John Wiley and Sons, New York, 1982, Ch. 2.