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On-line concentration of *s*-triazine herbicides in micellar electrokinetic chromatography using a cationic surfactant

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Abstract

On-line concentration of neutral species of *s*-triazine herbicides in micellar electrokinetic chromatography using tetradecylammonium bromide (TTAB) as a cationic surfactant was investigated. Factors affecting the stacking of analytes were examined. The results indicate that the stacking efficiency is markedly improved with addition of phosphate buffer in the sample matrix. It was found that, depending on the nature of the analytes, the most effective stacking of these analytes occurs when the ratio of the conductivity of buffer electrolyte to that of sample matrix is in the range 1.4–1.2, with sample matrix containing phosphate buffer. Micelle concentration in the separation buffer is also a crucial factor to enhance the stacking efficiency and detection sensitivity of analytes. Moreover, the stacking efficiency of each individual analyte depends on its binding constant to TTAB micelles. The concentration effect is primarily based on sweeping mechanism which is operated in a normal stacking mode with reversed electrode polarity in the presence of reversed electroosmotic flow. As a result of concentration enhancement, the detection limits of these herbicides can reach about 9–15 ng/ml with UV detection.

Keywords: Sample stacking; Sweeping; Triazines; Pesticides; Methylthio-*s*-triazines

1. Introduction

Capillary electrophoresis (CE) has been proven to be a powerful analytical tool for separating charged species of diverse samples, due to many of its advantageous features such as extremely high column efficiency, rapid analysis and small volumes of sample consumption in comparison with HPLC. For separating neutral analytes, micellar electrokinetic chromatography (MEKC) is the method of choice. However, the very limited optical path length, due to small inner diameter of the capillary (100–25 μm) and low sample volume injected (nL), make the detection of low-concentration samples with a UV detector difficult or even impossible without sample preconcentration.

To achieve more sensitive detection, fluorescence detection, particularly laser-induced fluorescence detection [1,2] may be used. Unfortunately, this type of detection is not generally applicable to environmental analysis because only a few compounds have native fluorescence and most analytes need to be derivatized with an appropriate fluorescent tag. In addition, only a limited number of laser sources are available.

Alternatively, on-line sample concentration by either field-amplified sample stacking [3–16] or isotachophoretic sample stacking [17–19] can be employed for enhancing detectability in capillary electrophoresis. For field-amplified sample stacking,

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upon the introduction of a long plug of low-conductivity sample solution into the capillary previously filled with a high-conductivity buffer electrolyte, sample stacking occurs at the concentration boundary between the low conductivity of sample zone and the high conductivity of separation zone when a high voltage is applied across the capillary. For neutral analytes, effective mobility necessary for stacking is provided by charged micelles in MEKC. Stacking with reversed migration micelles by large-volume sample injection may give enrichment factors of more than 500 [16–18]. Recently, head-column field-amplified sample stacking in binary system capillary electrophoresis has been demonstrated to provide over 1000-fold sensitivity enhancement [20,21]. Moreover, Terabe and co-workers [22–29] have reported that the stacking of neutral or ionic analytes in MEKC could be achieved based on sweeping technique. Depending on the nature of sample analytes, this technique can provide up to a 5000-fold concentration enhancement [22] or even approaching a million-fold sensitivity increase by applying cation-selective exhaustive injection and sweeping technique [28]. All these efforts make the detection of environmental analytical samples by UV absorption at trace concentration levels becoming possible.

s-Triazines are important selective pre- and post-emergence herbicides used widely for the control of broadleaf and grassy weeds [30]. These herbicides may contaminate drinking water sources. Six triazine herbicides, including prometryn and terbutryn are on the priority list in European Union drinking water guideline [31]. Simetryn and the two triazine herbicides aforementioned are on the priority list of pesticides in the USA national pesticide survey for a monitoring program on pesticides [31,32]. Because of their extensive use, relatively high persistence and toxicity in environmental matrices [31–33], *s*-triazines are of great environmental concern. Thus, the development of new analytical methods is desirable.

Several papers on the separation of *s*-triazines by MEKC have been reported [34–39]. The analysis of simazine and atrazine in samples of river water [35] and the determination of four chloro- and three methylthio-*s*-triazines in water [36] were conducted using sodium dodecyl sulfate (SDS) as an anionic surfactant. The separation of three chloro- and two methylthio-*s*-triazines was performed by partial filling micellar electrokinetic chromatography using SDS micelles [37]. The separation of prometon, prometryn and propazine was investigated using anionic octylglucoside-borate micelles at alkaline pH [34,38]. Recently, we reported the separation of thirteen *s*-triazines, including five chloro-, three methoxy- and five alkylthio-*s*-triazines, in MEKC using a cationic surfactant [39].

In this study, four methylthio-*s*-triazines are selected as test compounds. We demonstrate the influences of the concentration of both sample matrix and separation buffer on the stacking efficiency and detection sensitivity of these analytes by sweeping technique using a cationic surfactant. The concentration enhancement of neutral species of these *s*-triazines at pH 6.0 is studied and the limits of detection are determined.

2. Experimental

2.1. Chemicals and reagents

Four methylthio-*s*-triazines, including simetryn, ametryn, prometryn and terbutryn, were purchased from Supelco (Bellefonte, PA, USA). Tetradecyltrimethylammonium bromide (TTAB) was acquired from Tokyo Kasei Kogyo (TCI, Tokyo, Japan). All other chemicals were of analytical-reagent grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA)

Standard solutions of *s*-triazines were prepared at a concentration of 10 μg/ml in a solution containing 4% (v/v) acetonitrile. Further dilution of sample solution with deionized water down to 0.05 μg/ml was carried out in the determination of the limits of detection. The pH of the buffer and sample solutions was adjusted by mixing various proportions of a certain concentration of trisodium phosphate with the same concentration of phosphoric acid solutions to attain pH 6.0. All solutions were filtered through a membrane filter (0.22 μm) before use.

2.2. Apparatus

Electrophoretic experiments were performed using a Beckman Coulter (Fullerton, CA, USA) Model P/ACE MDQ capillary electrophoresis system,

equipped with a diode array UV–visible detector and an automatic injection system. The CE system with MDQ softwares was interfaced with a microcomputer and a Hewlett-Packard deskjet 670C printer. The fused-silica capillaries of 50 μm, I.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA). The total length of capillary is 60 cm and the position of UV detector is 10 cm from the anodic end. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of 0.01 pH unit. For conductivity measurements, a conductivity meter (Suntex SC-170, Taipei, Taiwan) calibrated with a 0.01 *M* KCl solution to a value of 1.413 mS/cm (at 25°C) was used.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed 20 min with sodium hydroxide solution (1.0 *M*) at 25°C, followed by sodium hydroxide solution (0.1 *M*) for 20 min and then deionized and purified water for another 20 min at 25°C.

To ensure reproducibility, all experiments were performed at 25°C, and measurements were run at least in triplicate. The capillary was prewashed with running buffer for 5 min before each injection and postwashed for 5 min with deionized water to maintain proper reproducibility for run-to-run injections. Sample injections were made in pressure injection mode at a pressure of 68.9 mbar (1 p.s.i.) or indicated elsewhere. An applied voltage of −20 kV was selected in the electrophoretic separation. Detection was performed at 222 nm.

The stacking was performed by injecting sample solutions for a much longer time compared to the usual hydrodynamic injection. Sample solutions were introduced at the cathodic end of the capillary. Varied plug lengths of sample solution up to 18.1% occupancy of the capillary were assessed by introducing sample solution for a varied injection time up to 105 s.

3. Results and discussion

The addition of a cationic surfactant to the electrophoretic buffer may induce the reversal of the electroosmotic flow (EOF) in the electrophoretic separation. In fact, the EOF was reversed when the concentration of TTAB added in the phosphate buffer (70 m*M*) at pH 6.0 exceeded 0.2 m*M* [40]. In this study, on-line concentration of *s*-triazines was performed under the conditions of reversed EOF, as the concentrations of TTAB employed were much greater than the critical micelle concentration of TTAB which was determined to be 1.6±0.2 m*M* at pH 6.0 [41].

3.1. Effect of sample matrix on stacking efficiency

In a previous report [39], thirteen *s*-triazines, including the four chloro-*s*-triazines and four methylthio-*s*-triazines selected in this work, were completely separated by MEKC in a phosphate buffer (70 m*M*) containing TTAB (15 m*M*) as a cationic surfactant at pH 6.0. For a large volume of sample injection, however, reoptimization of separation parameters, such as phosphate concentration in the sample matrix and micelle concentration in the separation buffer, in particular, is necessary in order to achieve an effective and efficient stacking.

To examine the influences of sample matrix on the stacking efficiency and detection sensitivity of sample analytes, sample analytes were dissolved in an aqueous solution containing varied concentrations of either phosphate buffer in the range 10~70 m*M* and 4% acetonitrile as well. Fig. 1 shows some typical electropherograms of four methylthio-*s*-triazines obtained with sample solutions containing varied concentrations of phosphate buffer (0, 30, 50, 70 m*M*), while a separation buffer is composed of 40 m*M* phosphate buffer and 40 m*M* TTAB at pH 6.0. Sample analytes at a concentration of 10 μg/ml were injected for 30 s. As shown in Fig. 1A, the peaks of the four *s*-triazines obtained without addition of phosphate buffer in the sample matrix are rather broad and are poorly resolved. Apparently, the analytes are not in the efficient stacking conditions. The resolutions of peaks become even worse with a longer injection time. In contrast, as shown in Fig. 1B–D, with addition of phosphate buffer in the sample matrix, the peaks become sharpened and the peak height of these sample analytes increases with increasing phosphate concentration up to 50 m*M*. However, the peak height of these *s*-triazines de-

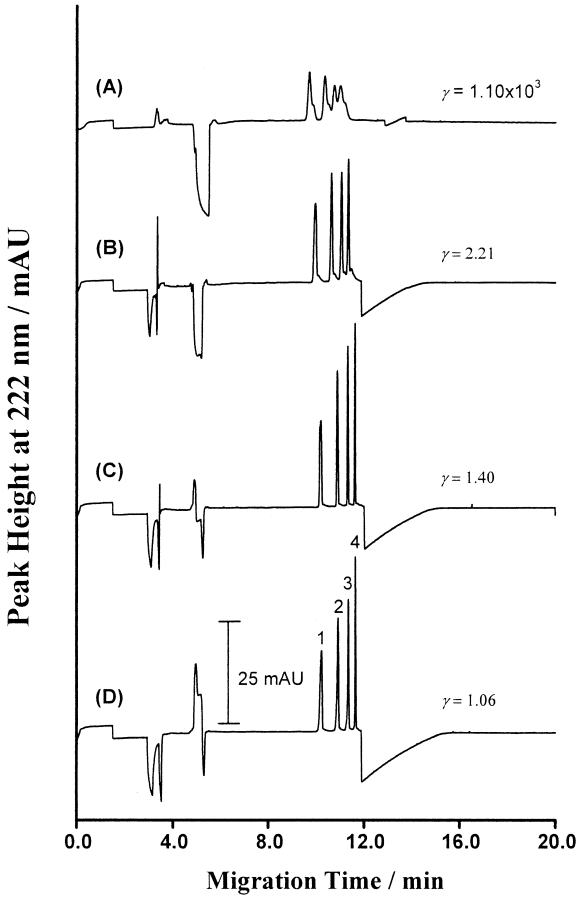

Fig. 1. Effect of sample matrix on the stacking efficiency and detection sensitivity of methylthio-*s*-triazines. Sample matrix: (A) water; (B) 30 m*M* phosphate buffer; (C) 50 m*M* phosphate buffer; (D) 70 m*M* phosphate buffer. Separation buffer, 40 m*M* TTAB in 40 m*M* phosphate buffer at pH 6.0; injection pressure, 1 p.s.i.; injection time, 30 s; capillary, 70 cm H 50 μm I.D; applied voltage, −20 kV; detection wavelength, 222 nm; temperature, 25°C; sample concentration, 10 μg/ml, sample dissolved in a sample matrix containing 4% acetonitrile solution. Peak identification, 1=simetryn, 2=ametryn, 3=prometryn, 4=terbutryn.

creases with further increasing the concentration of phosphate buffer in the sample matrix.

The conductivity values of sample matrices and those of buffer electrolytes at varied concentration were measured. The values of enhancement factor (γ) defined as the ratio of the conductivity of buffer electrolyte to that of sample matrix are indicated in Fig. 1. It should be noted that the γ value of the most effective stacking of analytes is in the range 1.4–1.2, instead of 1.0. Similar phenomena were observed for chloro-*s*-triazines as for methylthio-*s*-triazines and the γ value for most efficient stacking of these analytes was found to be 1.19 [42]. Evidently, the afore-mentioned results reveal that the stacking of analytes is primarily due to sweeping mechanism proposed by Quirino and Terabe [22], although, in this study, it is operated in a normal stacking mode with reversed electrode polarity in the presence of reversed electroosmotic flow. As the γ values of the most effective stacking are not closed to 1.0, the contribution of field-amplified sample stacking to the enhancement of detection sensitivity may not be completely ignored.

3.2. Stacking of analytes by sweeping

Fig. 2 depicts the schematic stacking mechanism of a neutral analyte dissolved in a sample matrix containing phosphate buffer with a separation buffer containing a cationic surfactant. As illustrated in Fig. 2A, the capillary column is initially filled with a micellar background electrolyte (BGE). A sample zone containing nonmicellar sample matrix or simply water is injected hydrodynamically with pressure for a period much longer than usual. By application of voltage at negative polarity (Fig. 2B), the electroosmotic flow is directed toward the anode (as cationic micelles is adsorbed on the capillary wall) and the micelles migrate toward the cathode. During

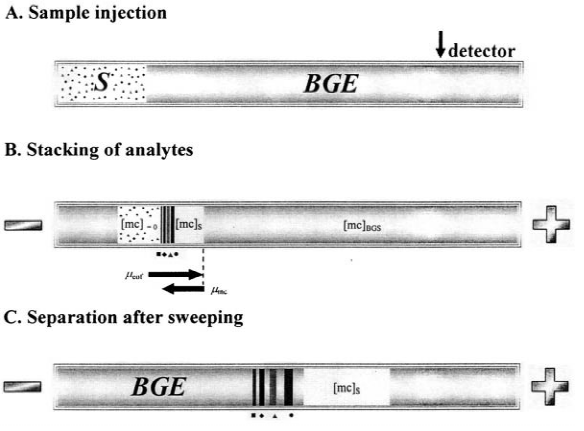

Fig. 2. Schematic diagram of a stacking mechanism by sweeping using a cationic surfactant (with sample matrix containing phosphate buffer).

sweeping, the analytes stacked at the concentration boundary between the region of $[mc]_s$ and that of [mc]=0 in the sample zone, where $[mc]_s$ denotes the concentration of micelles in the sample zone after sweeping and [mc]=0 indicates that no micelles are present in the original sample zone. The separation is then achieved via MEKC (Fig. 2C).

3.3. Effect of micelle concentration

The stacking efficiency and detection sensitivity of these test analytes are greatly affected by TTAB concentration. The peak height of each individual analyte increases with increasing micelle concentration until reaching the maximum, then it decreases with further increasing micelle concentration. Fig. 3 shows the variations of the peak height of methylthio-*s*-triazines as a function of TTAB concentration. As can be seen, the optimal TTAB concentrations determined for terbutryn, prometryn, ametryn and simetryn are about 30, 45, 50 and 70 m*M*, respectively, at the sample concentration of 10 μg/ml for a 30-s injection. In the present study, the optimal TTAB concentration for a simultaneous detection of methylthio-*s*-triazines is 40 m*M* for a 30-s injection and 60 m*M* for a 60-s or longer injection time. It should be noted that, with TTAB micelles at a concentration less than 20 m*M*, a satisfactory stacking of terbutryn for a 30-s injection is difficult.

3.4. Stacking efficiency versus sample plug length

The stacking efficiencies in terms of peak height (SE_{height}) for a neutral analyte is defined as the peak of the analyte for varied lengths of sample plug (H_{stack}) divided by the peak height of the corresponding analyte obtained for 1-s injection (H_{1s}). As a certain minimum injection time is required with Beckman A/PCE MDQ system for a particular injection pressure, H_{1s} is defined as the stacking efficient of a minimal injection time (H_{min}) divided by the minimal injection time. For example, with an injection pressure of 1 p.s.i., the minimum injection time is 3.5 s. Then H_{1s} is equivalent to $H_{3.5s}$ divided by 3.5.

For pressure injections, the length of sample plug in a capillary is directly proportional to the product of the injection pressure and injection time. Thus a 30-s injection of sample solution with a pressure of 1 p.s.i. corresponds to a sample plug length of 4.23 cm, which is 6.04% occupancy of the capillary.

Fig. 4 shows the effect of sample plug length on

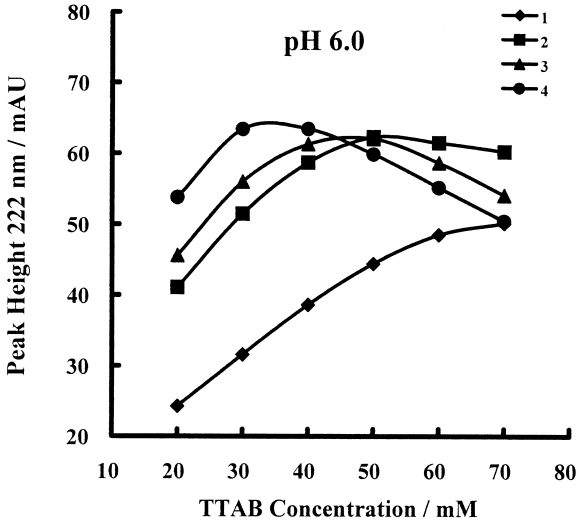

Fig. 3. The variation of peak heights of sample analytes as a function of TTAB concentration. The electrophoretic conditions are the same as for Fig. 1C.

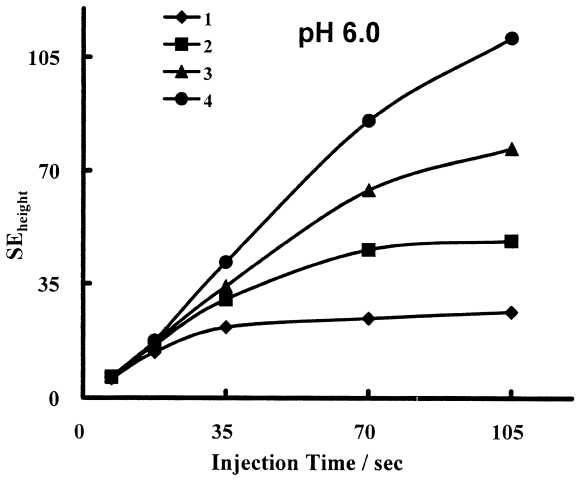

Fig. 4. Plots of SE_{height} versus injection time with a mixture of four methylthio-*s*-triazines at sample concentration of 1.0 μg/ml. Electrophoretic conditions are the same as for Fig. 1C, except sample concentration.

the SE_{height} using a mixture of four methylthio-*s*-triazines at a concentration of 1.0 μg/ml. As illustrated, the stacking efficiency of these test analytes increases linearly with increasing injection time up to about 70, 60, 35, and 20 s for terbutryn, prometryn, ametryn and simetryn, respectively, then increase gradually with further increasing injection time. Consequently, the maximum detection sensitivity of terbutryn, prometryn, ametryn, and simetryn are obtained with a duration of injection time of 70, 60, 35, and 20 s, respectively.

To demonstrate the stacking efficiency and the enhancement of detection sensitivity, Fig. 5 shows a typical electrophoreogram of the four methylthio-*s*-triazines obtained for a 30-s sample injection together with an electrophoreogram obtained for a 2.5-s injection under the optimal condition of usual injection time for comparison. Evidently, by the application of sweeping technique, the detection sensitivity of analytes can be greatly enhanced.

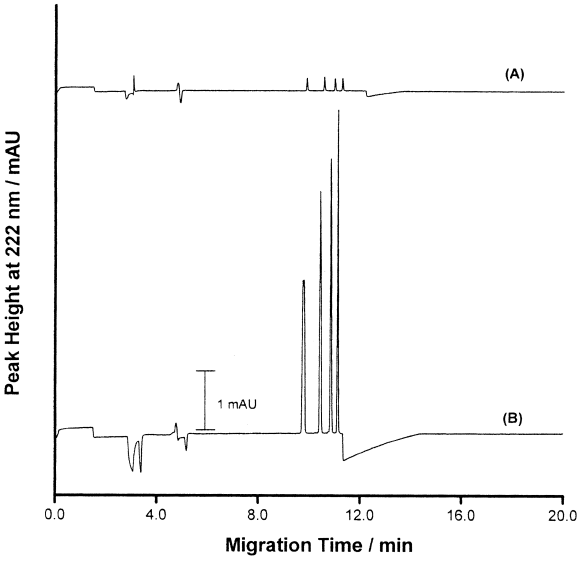

Fig. 5. Detection sensitivity of analytes measured under two different separation conditions: (A) without sample stacking (2.5-s injection with an injection pressure of 0.4 p.s.i.; sample concentration, 1.0 μg/ml); (B) with sweeping-stacking (30-s injection with an injection pressure of 1 p.s.i.; sample concentration, 1.0 μg/ml) Other operating conditions are the same as for Fig. 1C.

3.5. Peak width versus binding constant

It is of interest to note that, under the effective stacking conditions, the peak widths of these *s*-triazines increase in the order: simetryn>ametryn>prometryn>terbutryn. As the magnitudes of binding constants of methylthio-*s*-triazines to TTAB micelles also increase in the same order as for the peak width of the analytes [39], the dependence of the stacking of these analytes on their binding constants is evident. As a matter of fact, it is observed that the stronger the interaction between the analytes and the micelles, the narrower the peak width. As the binding constant of a sample analyte is linearly related to its retention factor, the result is qualitatively consistent with the finding obtained by Quirino and Terabe [22].

3.6. Detection limits and reproducibility

The limits of detection (LODs) at a signal-to-noise ratio (S/N) of 3, as well as the reproducibility of migration times and peak heights, for these *s*-triazines were determined. The migration times of these analytes were quite reproducible, with relative standard deviations (RSDs) varying in the range 0.8–1.0% ($n=8$). Variations of the peak height with RSDs less than 10.5% were obtained. The LODs determined for these methylthio-*s*-triazines with sample concentration in the range of 1000–50 ng/ml for a 30-s injection time are ranging from 9 ng/ml for simetryn to 15 ng/ml for terbutryn. Table 1 gives the data of analysis for these four analytes.

4. Conclusion

On-line concentration of neutral species of *s*-triazine herbicides in MEKC using a cationic surfactant is demonstrated. The stacking efficiency of analytes can be greatly enhanced by sweeping with addition of buffer electrolyte in the sample matrix and with an appropriate micelle concentration in the separation buffer. Reoptimization of separation parameters is necessary for a large-volume sample injection. For analytes with considerably different binding constants to the micelles, the optimal micelle concen-

Table 1
Limits of detection (LOD, $S/N=3$) and reproducibility of methylthio-*s*-triazines for 60-s injection time[a]

	Methylthio-*s*-triazines			
	Simetryn	Ametryn	Prometryn	Terbytryn
Equation of line	$y = 2.2324x - 0.0159$	$y = 2.3910x - 0.0232$	$y = 2.0220x - 0.0294$	$y = 1.9795x + 0.0488$
Coefficient of variation (R^2)	0.9998	0.9999	0.9991	0.9987
LOD (ng/ml)	13	9	14	15
RSD (%)				
Migration time	1.01	1.00	0.80	0.83
Peak height	8.7	9.1	9.4	10.5

[a] Sample matrix: 50 m*M* phosphate buffer containing less than 5% CH_3CN. Buffer electrolyte: 40 m*M* phosphate buffer containing 40 m*M* TTAB at pH 6.0.

tration for an efficient stacking may be different from one analyte to the other.

Acknowledgements

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