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Migration behavior and separation of *s*-triazines in micellar electrokinetic capillary chromatography using a cationic surfactant

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Abstract

The migration behavior and separation of various *s*-triazines, including five chloro-, three methoxy- and five alkylthio-*s*-triazines, were investigated in micellar electrokinetic chromatography (MEKC) using a cationic surfactant. In this study, tetradecyltrimethyl ammonium bromide (TTAB) was selected as a cationic surfactant. The results indicate that the selectivity of neutral species of *s*-triazines in each class is not significantly influenced by buffer pH and micelle concentration, but the overall selectivity is considerably affected by these two separation parameters when charged solutes are present, particularly, at buffer pH below 5.0. Complete separation of thirteen *s*-triazines was optimally achieved within 6 min on addition of TTAB (15 m*M*) to a phosphate buffer (70 m*M*) at pH 4.75 or 3.8. Based on a model that describes the relationship of the effective electrophoretic mobility of a neutral solute and micelle concentration in MEKC, the migration behavior of chloro-*s*-triazines at pH 6.0 is predicted and the binding constants of *s*-triazines to TTAB micelles are evaluated. The correlation between the binding constants and P_{ow} (the partition coefficient of a solute between 1-octanol and water) reveals that the migration order of *s*-triazines in each class is primarily determined by the hydrophobicity of the solutes.

Keywords: Buffer composition; Triazines; Pesticides; Cationic surfactant

1. Introduction

s-Triazines are important selective pre- and post-emergence herbicides used widely for the control of broadleaf and grassy weeds [1]. Because of their extensive use, relatively high persistence and toxicity in environmental matrices [2–4], *s*-triazines are of great environmental concern.

Herbicides may contaminate drinking water sources [5,6]. Six triazine herbicides, including atrazine, simazine, cyanazine, terbuthylazine, prometryn and terbutryn, are on the priority list in European Union drinking water guidelines [4]. Nine triazine herbicides, including propazine, prometon, simetryne, and the six triazine herbicides aforementioned, are on the priority list of pesticides in the USA national pesticide survey for a monitoring program on pesticides [3,4]. Thus, the development of new analytical methods to separate and characterize these compounds is of importance.

Triazine herbicides and their degradation products have been determined by gas chromatography (GC) [7–10], high-performance liquid chromatography (HPLC) [11–15], and their hyphenated techniques

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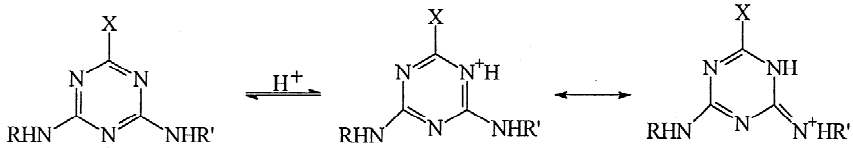
[16–19] over the past decades. In recent years, capillary electrophoresis (CE) has proven to be a powerful separation technique and has been successfully applied to the separation of various herbicides [20–28].

CE in various modes, including capillary zone electrophoresis (CZE) [28–31], isotachophoresis (ITP) [32,33] micellar electrokinetic chromatography (MEKC) [33–36], and its hyphenated technique [37–39] are applied to separate *s*-triazine herbicides and their degradation products. However, reports on the separation of *s*-triazines by MEKC are few. Besides, those reports are confined to the use of anionic surfactants only. The analysis of simazine and atrazine in samples of river water [34] and the determination of four chloro-*s*-triazine and three methylthio-*s*-triazine herbicides in water [35] were conducted using sodium dodecyl sulfate (SDS) as an anionic surfactant. The separation of prometon, prometryn and propazine was investigated using anionic octylglucoside-borate and *N*-D-gluco-*N*-methylalkanamide-borate micelles at alkaline pH [33,36]. Recently, the separation of three chloro- and two methylthio-*s*-triazines was performed by partial-filling micellar electrokinetic chromatography using a buffer electrolyte consisting of 10 m*M* phosphate and 20 m*M* SDS at pH 7.0 [38]. However, the separation of propazine and ametryne was not achieved.

As indicated in Table 1, *s*-triazines are protonated at the nitrogen atom of the *s*-triazine ring in aqueous solution at low pH because the ring nitrogen is more basic than the amino group on the side chain of the *s*-triazine ring [12,40]. Table 1 presents pK_a values and some selected characteristics of *s*-triazines studied, which include five chloro-, three methoxy- and five alkylthio-*s*-triazines [2,3,10–13,31,41]. The pK_a values of chloro-, methoxy-, and alkylthio-*s*-triazines reported in the literature are in the range 1.3–2.0, 4.0–4.4, and 3.1–4.4, respectively [2,10–13]. Since the pK_a values of chloro-*s*-triazines are much lower than those of methoxy-*s*-triazines and

Table 1
Some characteristics of *s*-triazines studied



s-Triazines	Compound No.	Substituents			pK_a[a]	λ_{max} and ε_{max}[b]				Solubility[c]
		X	R	R′		λ_1(nm)	ε_1(M^{-1d} cm^{-1})	λ_2(nm)	$\varepsilon 2$(M^{-1} cm^{-1})	(μg/ml)
Simazine	1	Cl	C_2H_5	C_2H_5	1.65	222	36000	263	3100	5
Cyanazine	3	Cl	C_2H_5	$C(C_2H_5)CN$	1.85					171
Atrazine	4	Cl	C_2H_5	$CH(CH_3)_2$	1.68	222	41000	263	3900	33
Propazine	8	Cl	$CH(CH_3)_2$	$CH(CH_3)_2$	1.85	221	32000	268	3100	5
Terbuthylazine	10	Cl	C_2H_5	$C(CH_3)_3$	1.94	223	19500	263	1500	5
Atraton	2	OCH_3	C_2H_5	$CH(CH_3)_2$	4.2	217				
Prometon	5	OCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$	4.2					620
Secbumeton	6	OCH_3	C_2H_5	$CH(CH_3)(C_2H_5)$						620
Simetryn	7	SCH_3	C_2H_5	C_2H_5	4.0	222	44400			450
Ametryn	9	SCH_3	C_2H_5	$CH(CH_3)_2$	4.0	222	40000			185
Prometryn	11	SCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$	4.1	223	42000			48
Terbutryn	12	SCH_3	C_2H_5	$C(CH_3)_3$	4.4	223	21200			25
Dipropetryn	13	SC_2H_5	$CH(CH_3)_2$	$CH(CH_3)_2$						

[a] Ref. [2,10–13].
[b] Ref. [2].
[c] Ref. [3].
[d] The unit of $(mol/L)^{-1}$.

alkylthio-*s*-triazines, an effective and simultaneous separation by CZE for these three classes of *s*-triazines is difficult.

In this study, the separation of the 13 *s*-triazines was attempted by means of MEKC using a cationic surfactant. We present here an assessment of the effects of buffer pH and micelle concentration on the migration behavior and separation of these thirteen *s*-triazine herbicides. The binding constants of neutral species of these *s*-triazines to micelles at pH 6.0 are evaluated so that the migration behavior of neutral species of these triazines can be better understood.

2. Experimental

2.1. Chemicals and reagents

Thirteen *s*-triazines, specifically including atrazine (Janssen, Belgium), simazine (Lancaster, UK), cyanazine, atraton, dipropetryn, secbumeton and terbuthylazine (Riedel-de Haen, Germany), ametryn, prometryn, propazine, prometryn, simetryn and terbutryn (Supelco, USA), were purchased from the indicated suppliers. Tetradecyltrimethyl-ammonium bromide (TTAB) was acquired from Tokyo Kasei Kogyo (TCI, 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 about 20 μg/ml in a 25% methanolic solution. The pH of the buffer was adjusted by either mixing sodium dihydrogenphosphate buffer solution (70 m*M*) with disodium hydrogenphosphate solution (70 m*M*) or adding hydrochloric acid solution (0.1 *M*) to the phosphate buffer solution (in the pH range 3.7–4.5) to attain the desired value. All solutions were filtered through a membrane filter (0.22 μm) before use.

2.2. Apparatus

Separations were made with a capillary electrophoresis system (Spectra-Physics Model 1000, Fremont CA, USA), equipped with a programmable and high-speed scanning multiple-wavelength UV–Visible detector, a fused-silica capillary cartridge thermostated with a Peltier thermoelectric device, and an automatic injection system. The capillary dimensions were 43 cm×50 μm I.D. With the use of cationic surfactants, the polarity of the electrodes was reversed under the conditions of reversed electroosmotic flow. Thus, the position of UV detection is 7.0 cm from the anodic end. The CE system was interfaced with a microcomputer and printer with software CE 500 1.05A. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of ±0.01 pH unit.

2.3. Electrophoretic procedure

Whenever a new capillary was used, the capillary was washed using a standard sequence described previously [42]: 20 min with sodium hydroxide solution (1.0 *M*) at 60°C, followed by sodium hydroxide solution (0.1 *M*) at 60°C for 10 min and then deionized and purified water at 25°C for another 10 min.

To ensure reproducibility, all experiments were performed at 25°C, and measurements were run at least in triplicate. The capillary was prewashed for 3 min with running buffer before each injection and postwashed for 3 min with deionized water to maintain proper reproducibility for run-to-run injections. An applied voltage of −20 kV was selected and the total current was kept below 65 μA in order to avoid Joule heating. Sample injections were made in the hydrodynamic mode. The sample solution was typically injected for 1 s. The detection wavelength was set at 220 nm.

2.4. Mobility calculations

The electrophoretic mobility of the analytes was calculated from the observed migration time with the equation

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an uncharged solute (methanol

as neutral marker), L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

3. Results and discussion

The addition of a cationic surfactant, such as long-chain alkyltrimethylammonium salts, to the electrophoretic buffer may induce the reversal of the electroosmotic flow (EOF) in a capillary electrophoretic separation [43,44]. The reversal of the EOF is caused by the adsorption of a cationic surfactant onto the charged surface of silica capillary by the electrostatic interaction, forming a primary hydrophobic layer, and then through further adsorption of cationic surfactants by hydrophobic interactions to form a bilayer of cationic surfactants at the capillary walls, thus effectively making the surface charge positively. In the case of TTAB, the EOF is reversed when the concentration of TTAB added in the phosphate buffer (70 m*M*) at pH 6.0 exceeds 0.2 m*M* [46].

In this study, the thirteen *s*-triazine herbicides selected in this work are grouped into three classes with the triazine ring at position 2 substituted with chloro-, methoxy-, and alkylthio-groups, respectively. The electrophoretic mobility of *s*-triazines was measured under the conditions of reversed EOF and the electroosmotic mobility takes place toward the anode.

3.1. Theoretical consideration on electrophoretic mobility

In MEKC, the migration behavior of ionizable analytes is more complicated than that of neutral analytes owing to the possession of their own electrophoretic mobility of ionized solutes and the involvement of additional dissociation and/or association equilibria. In a phenomenological approach, the effective electrophoretic mobility of an ionizable solute in MEKC using a cationic surfactant can be expressed as

$$\mu_{eff} = \alpha_{BH^+}\mu_{BH^+} + \alpha_{CS\cdot BH^+}\mu_{CS\cdot BH^+} + \alpha_{MC\cdot BH^+}\mu_{MC\cdot BH^+} + \alpha_{CS\cdot B}\mu_{CS\cdot B} + \alpha_{MC\cdot B}\mu_{MC\cdot B} \quad (2)$$

where α and μ denote the mole fraction and electrophoretic mobility of the protonated or unprotonated species of a basic solute, respectively, B and BH^+ in the subscript represent the unprotonated and protonated species of a basic solute, respectively, and CS and MC in the subscript represent the cationic surfactant monomers and cationic micelles, respectively. Thus, μ_{BH^+} is the electrophoretic mobility of the protonated species of a solute in the aqueous phase under the conditions of reversed EOF, and $\mu_{CS\cdot BH^+}$ and $\mu_{MC\cdot BH^+}$ represent the electrophoretic mobility of the protonated solute associated with the surfactant monomers and micelles, respectively, whereas $\mu_{CS\cdot B}$ and $\mu_{MC\cdot B}$ represent the electrophoretic mobility of the unprotonated solute associated with the surfactant monomers and micelles, respectively. It should be emphasized that the contribution of mobility in the fourth term in Eq. (2) must be taken into consideration when basic solutes interact strongly with surfactant monomers.

In an electrophoretic separation process involving the following five equilibria:

$$BH^+ + H_2O \overset{K_a}{\rightleftharpoons} B + H_3O^+$$

$$BH^+ + CS \overset{K_{CS\cdot BH^+}}{\rightleftharpoons} CS\cdot BH^+$$

$$BH^+ + MC \overset{K_{MC\cdot BH^+}}{\rightleftharpoons} MC\cdot BH^+$$

$$B + CS \overset{K_{CS\cdot B}}{\rightleftharpoons} CS\cdot B$$

$$B + MC \overset{K_{MC\cdot B}}{\rightleftharpoons} MC\cdot B^+$$

where K_a is the acid dissociation constant of a protonated solute, and K_{CS} and K_{MC} are the binding constants of solutes to surfactant monomers and to micelles, respectively. The analytical concentration of a basic solute is equal to $[B]+[BH^+]+[SC\cdot B]+[SC\cdot BH^+]+[MC\cdot B]+[MC\cdot BH^+]$. The mole fractions of the protonated and unprotonated solutes associated with the surfactant monomers in the aqueous phase and with the micelles in the micellar phase can then be expressed in terms of K_a, $K^+_{CS\cdot BH}$, $K_{CS\cdot B}$, $K^+_{MC\cdot BH}$, $K_{MC\cdot B}$, [CS], and [M]. For example, the mole fraction of the unprotonated solute solubilized in micelles is given by

$$\alpha_{MC\cdot B} = \frac{[MC\cdot B]}{[B]+[BH^+]+[CS\cdot B]+[CS\cdot BH^+]+[MC\cdot B]+[MC\cdot BH^+]}$$

$$= \frac{K_a K_{MC\cdot B}[M]}{[H^+](1+K_{CS\cdot BH^+}[CS]+K_{MC\cdot BH^+}[M])+K_a(1+K_{CS\cdot B}[CS]+K_{MC\cdot B}[M])}$$

In MEKC, [CS]=CMC. Hence, the effective electrophoretic mobility (μ_{eff}) of a basic solute in MEKC using a cationic surfactant can then be specifically expressed as

$$\mu_{eff} = \frac{[H^+](\mu_{BH^+}+K_{CS\cdot BH^+}[CS]\mu_{CS\cdot BH^+}+K_{MC\cdot BH^+}[M]\mu_{MC\cdot BH^+})}{[H^+](1+K_{CS\cdot BH^+}[CS]+K_{MC\cdot BH^+}[M])+K_a(1+K_{CS\cdot B}[CS]+K_{MC\cdot B}[M])}$$

$$+\frac{K_a(K_{CS\cdot B}[CS]\mu_{CS\cdot B}+K_{MC\cdot B}[M]\mu_{MC\cdot B})}{[H^+](1+K_{CS\cdot BH^+}[CS]+K_{MC\cdot BH^+}[M])+K_a(1+K_{CS\cdot B}[CS]+K_{MC\cdot B}[M])} \quad (3)$$

Since almost all of the basic solutes exist as neutral species at pH>pK_a+2, the contributions of mobility from the protonated species of the basic solute are eliminated and Eq. (3) is reduced to ([45])

$$\mu_{eff} = \frac{K_{CS\cdot B}[CS]\mu_{CS\cdot B}+K_{MC\cdot B}[M]\mu_{MC\cdot B}}{1+K_{CS\cdot B}[CS]+K_{MC\cdot B}[M]\mu_{MC\cdot B}} \quad (4)$$

When the concentration is below the CMC, [M] is equal to zero and Eq. (4) is further simplified to ([46])

$$\mu_{eff} = \frac{K_{CS\cdot B}[CS]\mu_{CS\cdot B}}{1+K_{CS\cdot B}[CS]} \quad (5)$$

On the other hand, at pH<pK_a−2, almost all of the solutes are fully protonated and the μ_{eff} can be simplified as

$$\mu_{eff} = \frac{\mu_{BH^+}+K_{CS\cdot BH^+}[CS]\mu_{CS\cdot BH^+}+K_{MC\cdot BH^+}[M]\mu_{MC\cdot BH^+}}{1+K_{CS\cdot BH^+}[CS]+K_{MC\cdot BH^+}[M]} \quad (6)$$

Consequently, according to Eqs. (3)–(6), the migration behavior of a basic solute at varied micelle concentrations or at varied buffer pH can be better understood, provided that the binding constants, the acid dissociation constant, and the necessary mobility data are available.

3.2. Effect of buffer pH

The influence of buffer pH on the electrophoretic mobility for the three classes of *s*-triazines with TTAB at a concentration of 15 m*M* in a phosphate buffer (70 m*M*) in the pH range 3.7–6.0 is shown in Fig. 1. As the pK_a values of methoxy-*s*-triazines are in the range 4.0–4.2 and those of alkylthio-*s*-triazines are in the range 4.0–4.4, these two classes of *s*-triazines are partially protonated in this pH range. The electrophoretic mobilities of these two classes of *s*-triazines, except atraton, were found to increase in the pH range from 3.7 to 4.7.

According to Eq. (3), the effect of buffer pH on the migration behavior of methoxy- and alkylthio-*s*-triazines in this pH range is predictable. By varying the parameters (binding constants and limiting mobilities) through the utilization of Excel software, the influence of each parameter on the variation of electrophoretic mobility at varied pH can be better understood. The curve-fitting analysis of simulated mobility curves reveals that the curve shape depends mainly on the magnitude of the limiting mobility of the protonated species of the solute (μ_{BH^+}). The variations in the electrophoretic mobility resulted from the terms involving $K_{CS\cdot BH^+}$ and $K_{MC\cdot BH^+}$ are small when buffer pH varies from 3.7 to 4.7. This is because $K_{CS\cdot BH^+}$ and $K_{MC\cdot BH^+}$ are very small, due to cationic–cationic repulsive interactions between protonated solutes and cationic surfactant molecules. The contributions of the electrophoretic mobility from the terms involving $K_{CS\cdot B}$ and $K_{MC\cdot B}$ have no influence on the curve shape in this pH range.

On the other hand, as chloro-*s*-triazines (with pK_a values of about 1.6–2.0) exist as neutral species in the pH range studied, the electrophoretic mobilities are expected to remain constant when the pH of the buffer varies. However, the electrophoretic mobilities of *s*-triazines decrease with increasing buffer pH in the range 4.7–6.0 in the phosphate buffer. This is due to the decrease in the electrophoretic mobility of micelles (μ_{MC}) in this pH range. In fact, the μ_{MC} was found to decrease from $3.58\cdot10^{-4}$ cm^2 V^{-1} s^{-1} at pH 4.7 to $2.74\cdot10^{-4}$ cm^2 V^{-1} s^{-1} at pH 6.0. A similar phenomenon was also observed under the conditions of constant ionic strength, but it was not observed when using ammonium acetate solution as a buffer electrolyte [47]. Moreover, a similar phenomenon of decrease in the electrophoretic mobility in the same pH range was also observed for the separation of some neutral aromatic compounds, such as benzene, *m*-xylene and naphthalene by

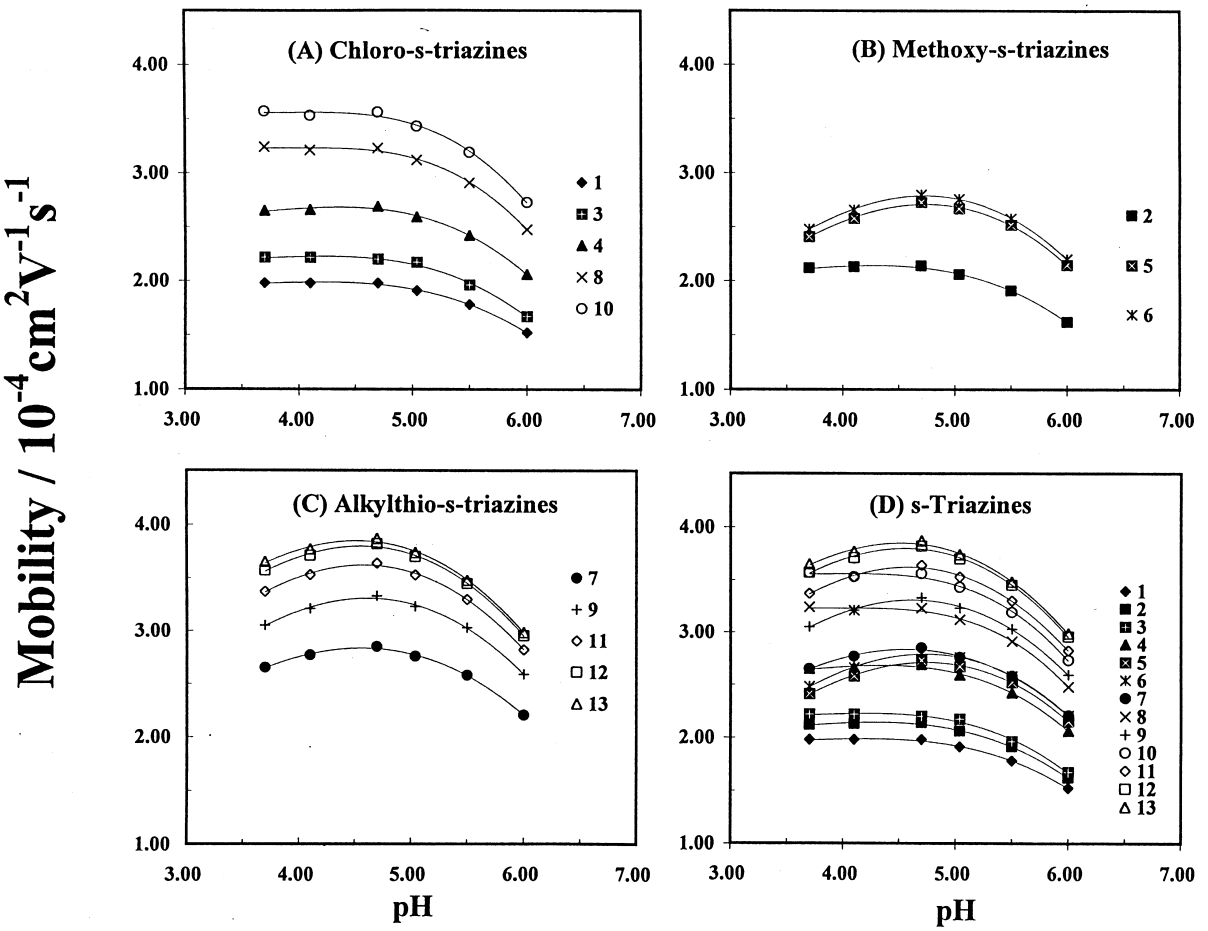


Fig. 1. Effect of buffer pH on the electrophoretic mobility of three different classes of *s*-triazines with 15 m*M* TTAB added in 70 m*M* phosphate buffer in the pH range 3.7–6.0: (A) chloro-*s*-triazines, (B) methoxy-*s*-triazines, (C) alkylthio-*s*-triazines, and (D) overall influence of buffer pH. Other operating conditions: −20 kV, 25°C. Curve identification: the numbers denote the analytes shown in Table 1.

MEKC with TTAB in a phosphate buffer [47]. Apparently, the results reflect that the micellar properties of TTAB micelles are affected by the phosphate buffer, but not the solute itself, in this pH range.

The trends in the variation of electrophoretic mobility as a function of buffer pH for methoxy-*s*-triazines are similar to that of alkylthio-*s*-triazines in the pH range studied, except that methylthio-*s*-triazines migrate toward the cathode more rapidly than the corresponding methoxy-*s*-triazines during electrophoresis. This is probably due to the greater extent of micelle solubilization of alkylthio-*s*-triazines compared to that of methoxy-*s*-triazines, because the P_{ow} values of alkylthio-*s*-triazines are greater than the corresponding values of methoxy-*s*-triazines. Depending on the nature of the substituent at ring position 2, the electrophoretic mobility of *s*-triazines with the same alkylamino groups in these three classes increases in the order methoxy-*s*-triazine< chloro-*s*-triazine<alkylthio-*s*-triazine (methylthio-*s*-triazine<ethylthio-*s*-triazine). For instance, the electrophoretic mobility of *s*-triazines with ethylamino group increases in the order atraton<atrazine< ametryn, whereas that of isopropylamino group increases in the order prometon<propazine< prometryn<dipropetryn. Chloro-*s*-triazines were found to migrate before their corresponding

methylthio-*s*-triazines as observed in the MEKC separation using SDS [24], or *N*-D-gluco-*N*-methylalkanamide-borate complexes [33] as a surfactant, but the migration order was reversed when using octylglucoside–borate complex as a surfactant [36].

The increase in the electrophoretic mobility for chloro-*s*-triazines follows the order simazine< atrazine<propazine<terbuthylazine. Likewise, the migration order of methoxy-*s*-triazines follows atraton<prometon<secumeton, and that of methylthio-*s*-triazines is simetryn<ametryn< propmetryn<terbutryn. Thus, the electrophoretic mobility of *s*-triazine increases with increasing chain length and bulkiness of alkylamino groups at ring positions 4 and 6. Since the electrophoretic mobility of *s*-triazines in each cases was found to increase with increasing P_{ow}, which is an index of the hydrophobicity of the solutes, the migration order of the solutes in each class is determined by the hydrophobicity of the solutes.

Fig. 1D shows the overall influence of buffer pH on the electrophoretic mobility of 13 *s*-triazines with TTAB concentrations at 15 m*M*. As mentioned earlier, the magnitude of the electrophoretic mobility of chloro-*s*-triazines is almost invariant with respect to the pH of the buffer in the pH range 3.7–4.7, whereas those of methoxy-*s*-triazines and alkylthio-*s*-triazines are pH-dependent. Thus, the migration order of some *s*-triazines may be altered by varying buffer pH. For instance, the migration orders of the following three pairs of analytes [i.e., ametryn (9) and propazine (8), prometryn (11) and terbuthylazine (10), and secbumeton (6) and atrazine (4)], are reversed at pH below 4, when compared with those at pH above 4; simetryn (7) and secbumeton (6) are well separated at pH below 4.7, but they migrate together at pH above 5.0. Therefore, for complete separation of these triazines, buffer pH is optimally selected at about 3.8 or 4.75. Fig. 2 shows the electropherograms of 13 *s*-triazines obtained under these two optimum conditions.

When varying buffer pH with TTAB concentration at 25 m*M*, similar trends in the variation of electrophoretic mobility of *s*-triazines as in Fig. 1 were observed, except that simazine (1) migrated after atraton (2) at pH<4.4; atrazine (4) migrated after simetryn (7) at pH<3.8; and atrazine (4) migrated before prometon (5) and secbumeton (6) subsequently at pH greater than 4.7 and 4.4. Thus, depending on the pH of the buffer in the range 3.7–4.75, the migration order of *s*-triazines observed with TTAB concentration at 25 m*M* may be different from that observed at 15 m*M*. In other words, the overall selectivity of charged *s*-triazines is affected not only by buffer pH but also by micelle concentration, particularly at buffer pH below 5.0.

3.3. Effect of micelle concentration

In general, the selectivity of neutral analytes does not depend on micelle concentration in MEKC. However it is not true for ionizable analytes because the effective electrophoretic mobility is the sum of the mobilities of ionized species and neutral analytes solubilized in micelles. Fig. 3 shows the electrophoretic mobility of *s*-triazines as a function of TTAB concentration in the range 5–25 m*M* at pH 4.75. It should be noted that chloro-*s*-triazines are neutral at this buffer pH, whereas methoxy-*s*-triazines and alkylthio-*s*-triazines are partially protonated. As can be seen, the electrophoretic mobility of *s*-triazines increases quite rapidly with increasing TTAB concentration in the range 5–10 m*M* and then increases gradually when TTAB concentration exceeds 10 m*M*. The trends in the variation of electrophoretic mobility of *s*-triazines as a function of TTAB concentration are quite similar in each class. However, the extent of the variation of electrophoretic mobility of chloro-*s*-triazines is somewhat different from those of methoxy-*s*-triazines and alkylthio-*s*-triazines. This is expected because the pK_a values of chloro-*s*-triazines are much lower than those of methoxy- and alkylthio-*s*-triazines.

As shown in Fig. 3, the selectivity of some *s*-triazines changes on varying the concentration of TTAB at pH 4.75. The resolution of the peaks between secbumeton (6) and simetryn (7) improves on varying TTAB concentration from 15 to 25 m*M*. On the contrary, the resolution of peaks between atrazine (4) and prometon (5) becomes unresolvable when TTAB concentration exceeds 15 m*M*. The migration order of *s*-triazines in each class, which depends primarily on the hydrophobicity of the solutes, is not affected by varying micelle concentration, but the overall migration order of some *s*-triazines may alter. For instance, the migration

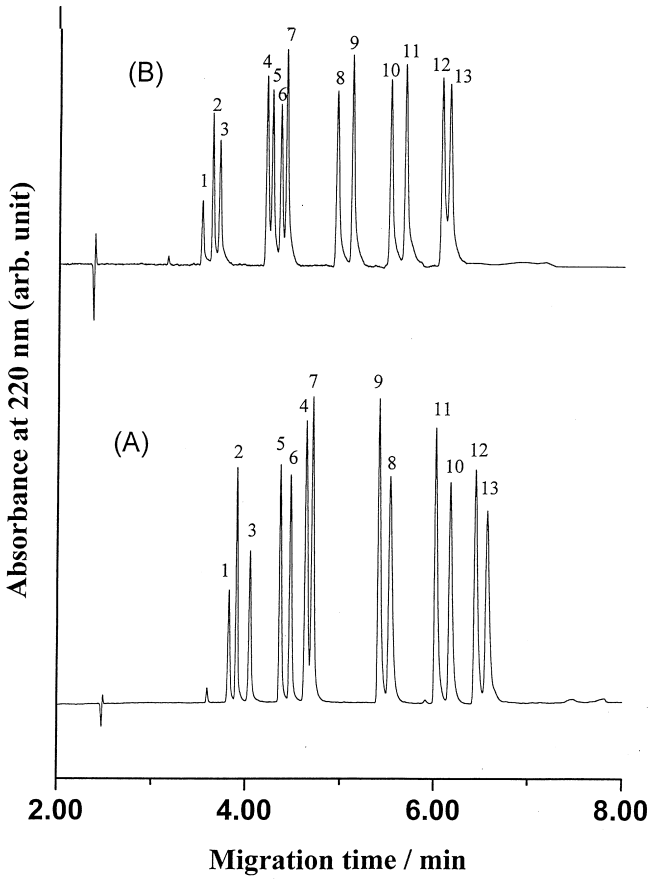


Fig. 2. Electropherograms of thirteen *s*-triazines obtained with TTAB at a concentration of 15 m*M* under optimum pH conditions: (A) pH 3.8; (B) pH 4.75. Other operating conditions are the same as for Fig. 1. Peak identification: the numbers denote the analytes shown in Table 1.

order of atraton (2) and cyanazine (3) is reversed when TTAB concentration varies from below 9 m*M* to above 9 m*M*.

3.4. Prediction of migration behavior of neutral species

As mentioned earlier, the migration behavior of neutral species of *s*-triazines in MEKC at pH 6.0 can be predicted according to Eq. (4), provided that the values of $K_{CS\cdot B}$, $K_{MC\cdot B}$, $\mu_{CS\cdot B}$ and $\mu_{MC\cdot B}$ are known. First of all, the values of $K_{CS,B}$ and $\mu_{CS,B}$ for each individual solute should be evaluated according to Eq. (5) by varying these two parameters through the utilization of Excel software until the predicted mobility curve is best fitted to the observed mobility curve obtained in the concentration range below the CMC [46]. We also need to know the CMC value of the cationic surfactant selected. For TTAB micelles in a phosphate buffer (70 m*M*) at pH 6.0, the CMC value was determined to be 1.6 ± 0.1 m*M* [45]. In MECK, it is generally assumed that the mobility of micelles incorporated with a solute is the same as the mobility of micelles. The value of μ_{MC} can be determined experimentally by measuring the electrophoretic mobility of a compound which is completely solubilizable in the micelles such as sudan III. The binding constant ($K_{MC\cdot B}$) of *s*-triazines can then be evaluated from Eq. (4) by varying the magnitude of this parameter using Excel software until the predicted mobility curve is best fitted to the observed mobility curve obtained in the concentration range

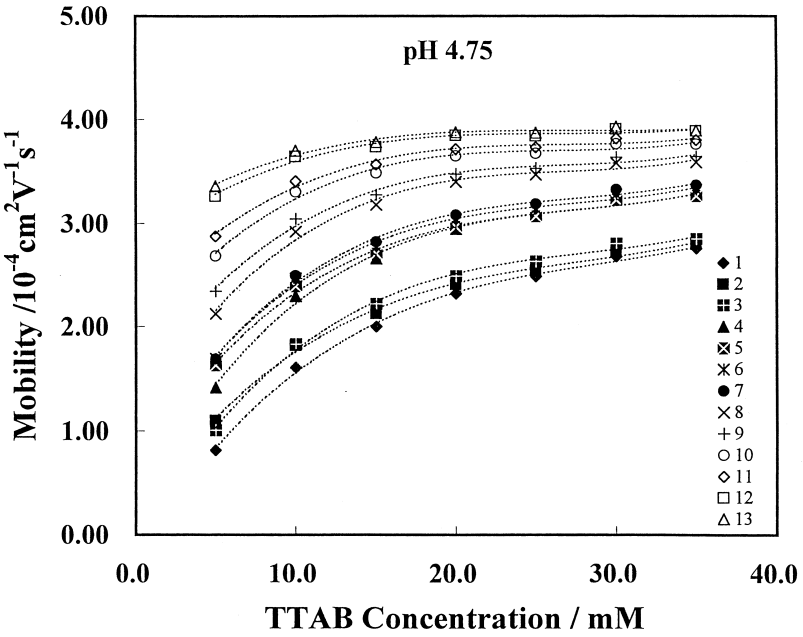


Fig. 3. Effect of TTAB concentration on the electrophoretic mobility of *s*-triazines: at pH 4.75. Other operating conditions are the same as for Fig. 1.

above the CMC. Table 2 presents the binding constants of *s*-triazines to TTAB micelles evaluated, together with the P_{ow} values reported in the literature. For the purpose of demonstration, the agreement between the predicted mobility curves (represented by solid lines) and experimental mobility data (represented by data points) for chloro-*s*-triazines at pH 6.0 is shown in Fig. 4.

Table 2
Binding constants ($K_{MC\cdot B}$) of *s*-triazines to TTAB micelles in a phosphate buffer (70 mM)

s-Triazines	$K_{MC\cdot B}/M^{-1}$	Log P_{ow}^{a}
Simazine (1)	191	2.26
Cyanazine (3)	294	1.8
Atrazine (4)	486	2.61
Propazine (8)	920	2.91
Terbuthylazine (10)	1185	3.06
Atraton (2)	273	2.69
Prometon (5)	535	2.99
Secbumeton (6)	564	–
Simetryn (7)	578	2.80
Ametryn (9)	968	3.07
Prometryn(11)	1260	3.34
Terbutryn (12)	1820	3.74
Dipropetryn (13)		

[a] Ref. [2,41].

By plotting log $K_{MC\cdot B}$ versus log P_{ow}, linear relationships between log $K_{MC\cdot B}$ and log P_{ow} with correlation coefficients (r^2) equal to 0.993 and 0.964 for chloro- and alkylthio-*s*-triazines were obtained, respectively. Cyanazine is precluded in the plot for chloro-*s*-triazines because the nature of the cyano group is different from that of the alkyl group in chloro-*s*-triazine. Thus, we may conclude that, as reflected from their alkyl substituents, the migration order of *s*-triazines in each class is determined by the hydrophobicity of the solutes.

3.5. Effect of buffer concentration

The resolution of peaks between atrazine (4) and prometon (5) and that between terbutryn (12) and dipropetryn (13) were effectively resolved only when using phosphate buffer at 70 m*M*. Peaks between atrazine (4) and prometon (5) and those between terbutryn (12) and dipropetryn (13) are unresolvable when the concentration of phosphate buffer is reduced to 50 and 30 m*M*, respectively.

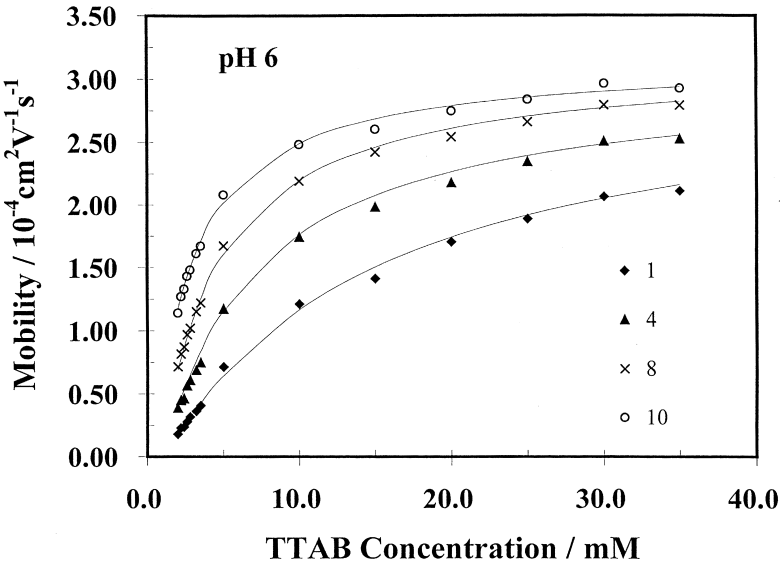


Fig. 4. The agreement between the predicted mobility curves (represented by solid lines) and experimental mobility data (represented by data points) for chloro-*s*-triazines.

Therefore, the use of phosphate buffer concentration at a high ionic strength is more favourable to the separation of atrazine (4) and prometon (5), or to the separation of terbutryn (12) and dipropetryn (13).

3.6. Sample stability, reproducibity, and detection limit

The stock solutions of *s*-triazine standards were prepared at a concentration of 1000 μg/ml in methanol and were stored in a refrigerator at 4°C. The sample solutions were then prepared from stock solutions by mixing with deionized water and diluted into a 25% or 5% methanolic solution before use. The sample solutions containing atrazine and propazine, respectively, were found to be stable over 12 months because no trace of methoxylated products (i.e., atraton and prometon) could be detected in the electropherograms.

The variations in migration time were measured. Migration times for these *s*-triazines were reproducible, with relative standard deviations varying less than 0.5% ($n=6$). The detection limits of simazine and atrazine dissolved in 25% methanolic solution were found to be 0.60 and 0.52 μg/ml, respectively, at a signal to noise ratio of three. These values were in good agreement with the values reported in the literature [40]. The detection limits of both simazine and atrazine could be reduced to 0.1 μg/ml level when samples were dissolved in a methanolic (5%, v/v)–phosphate buffer solution with a duration of injection time of 5 s.

4. Conclusion

Various *s*-triazine herbicides are effectively separated, for the first time, by means of MEKC using TTAB as a cationic surfactant. As chloro-*s*-triazines and some alkylthio-*s*-triazines are difficult to separate by CZE, MEKC provides advantages over CZE. For neutral species of these *s*-triazines, the selectivity is not significantly affected by both buffer pH and micelle concentration, but for charged analytes, the selectivity is influenced not only by buffer pH but also by micelle concentration, particularly at pH below 5.0. The migration order of *s*-triazines in each class in MEKC using cationic surfactant is primarily determined by the magnitude of the hydrophobicity.

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