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Migration behavior and selectivity of sulfonamides in capillary electrophoresis

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Abstract

The migration behavior and selectivity of thirteen sulfonamides in capillary electrophoresis (CE), with emphasis on micellar electrokinetic chromatography (MEKC) were systematically investigated using a phosphate–borate buffer electrolyte, with sodium dodecyl sulfate (SDS) as an anionic surfactant in MEKC. The optimization strategies for the separation of sulfonamides in capillary zone electrophoresis (CZE) and in MEKC are described. The migration behavior and selectivity of sulfonamides in CZE are mainly manipulated by the pH of the buffer. The migration order of sulfonamides depends on the ratios of charge to mass ($q/M^{2/3}$) and is primarily determined by their pK_a values. Thus precise optimization of buffer pH is crucial to further improve the separation of some closely migrating sulfonamides. On the other hand, buffer pH and micelle concentration greatly affect the migration and selectivity of sulfonamides in MEKC. The migration order of sulfonamides is mainly determined by their pK_a values and the magnitude of the binding constants of solutes-to-micelles. The influences of buffer pH and micelle concentration correlate with each other. The magnitude of the binding constants correlates with the differences between the electrophoretic mobility of sulfonamides measured at a pH below pK_a-2 in CZE and that in MEKC. In this work, acid dissociation constants of these sulfonamides and binding constants of sulfonamides to SDS micelles in a phosphate–borate buffer are reported. © 1997 Elsevier Science B.V.

Keywords: Migration behavior; Selectivity; Electrophoretic mobility; Sulfonamides

1. Introduction

Capillary electrophoresis (CE) is a popular and powerful separation technique nowadays. Mainly because of many advantageous features of this technique, including high resolution, great efficiency, rapid analysis and small consumption of both sample and solvent in comparison with high-performance liquid chromatography (HPLC), the development of CE methods to separate diverse analytical samples has been growing very rapidly over the past decade [1–7].

Sulfonamides are anti-bacterial and anti-infective drugs commonly used to treat diseases in medicine and veterinary practice. As recent evidence has implicated sulfamerazine as a possible thyroid carcinogenic agent [8] and sulfonamide residues in food and animal tissues may be present in minute concentrations and may pose a health treat to consumers, the separation and monitoring of these analytes have drawn much attention. Various chromatographic and capillary electrophoretic methods, including GC [9,10], GC–MS [11], HPLC [12–22], HPLC–MS [23], CE [24–34] and CE–MS [35], have been applied. As CE is a sensitive analytical method to identify and to separate these compounds, the development of CE methods continues unabated.

The separation of sulfonamides by CE has been performed in the modes of capillary zone electrophoresis (CZE) [24–33] and micellar electrokinetic

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chromatography (MEKC) [25,34]. These compounds were usually separated as negatively charged species by CZE at an optimum pH in the range 6.0–7.5 using various types of buffer, with or without the addition of electrolyte modifier [25–31,33]. Eighteen sulfonamides were separated within 22 min using 50 m*M* phosphate buffer (pH 7.5), but the peaks between sulfathiazole and sulfamethoxypyridazine and those between sulfadiazine and sulfadimethoxime were not resolved [29]. In our previous work, the migration behavior and selectivity of sulfonamides using CZE were systematically examined [31–33]. Precise optimization of buffer pH is crucial to further improve the separation of sulfonamides. With the addition of either an appropriate amount of an organic modifier or a low concentration of β-cyclodextrin to a phosphate–borate buffer at pH 6.85, effective separations of thirteen sulfonamides were successfully achieved within 5.5 min [31]. Citrate buffer is considered to be superior to phosphate or phosphate–borate buffer because it can be used as a background electrolyte at high concentrations in a wide pH range. Therefore, complete separation of thirteen sulfonamides as negatively charged species is more efficiently achieved in a shorter analysis time at pH 6.8–6.9 [33]. Sulfonamides as positively charged, protonated species can be also separated with citrate buffer by CZE at low pH. In fact, with citrate buffer at high concentrations (320–500) m*M* and at a pH in the range 2.1–2.6, complete separation of sixteen sulfonamides was achieved [32].

In contrast to CZE, very few papers on the separation of sulfonamides in MEKC were reported. Two sulfonamides (sulfadimidine and sulfadiazine), together with some other drugs, were separated by MEKC using tris(hydroxymethyl)aminomethane (20 m*M* Tris) as a buffer electrolyte with sodium dodecyl sulfate (100 m*M* SDS) at pH 8.5 [25]. Seven sulfonamides were separated on addition of SDS at high concentrations to a phosphate–borate buffer solution with or without the addition of tetrabutylammonium bromide as electrolyte modifier [34]. Dang et al. [34] found that the migration time of sulfonamides increased with increasing SDS concentration, but varied only slightly with buffer pH in the range 5.0–9.0. This finding seems to be questionable because the influences of buffer pH and micelle concentration on the migration behavior correlate with each other [36,37] and the variation of the electrophoretic mobility as a function of pH is sigmoidal in the pH range from pK_a-2 to pK_a+2 [36]. Therefore, a systematic investigation of the influences of micelle concentration and buffer pH on the electrophoretic mobility of sulfonamides in MEKC is desirable.

In this paper, factors that optimize the selectivity and subsequent separation of sulfonamides in CZE and in MEKC are discussed. The results of the influences of buffer pH and micelle concentration on the migration behavior and selectivity of sulfonamides in MEKC are presented. Moreover, acid dissociation constants of individual sulfonamides and binding constants of thirteen sulfonamides to SDS micelles are determined.

2. Experimental

2.1. Chemicals and reagents

Thirteen sulfonamides originally purchased from Sigma (USA) were supplied as a gift from the Taiwan Meat Development Foundation. Sodium dodecyl sulfate (SDS), β-cyclodextrin (β-CD), and anhydrous disodium tetraborate were obtained from Merck (Germany). Sodium dihydrogenphosphate dihydrate was purchased from Showa Chemicals (Japan). Sudan III was obtained from Janssen (Belgium). Methanol (MeOH) was of HPLC grade (Mallinckrodt, USA), and were used without further purification. All other chemicals were of analytical-reagent grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of sulfonamides were prepared at a concentration of about 0.15 m*M* in methanolic solution. Phosphate–borate buffer solution was prepared by mixing 50 m*M* disodium tetraborate with 50 m*M* sodium dihydrogenphosphate solution. An appropriate amount of SDS surfactant was added to the buffer solution in the case of MEKC separation. The pH of the buffer solution was adjusted with 0.1 *M* sodium hydroxide or 0.1 *M* hydrochloric acid to a desired value. All solutions were filtered through a membrane filter (0.22-μm) before use.

2.2. Apparatus

Separations were made with a capillary electrophoretic 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. Before installation in a capillary cartridge for on-column detection, the capillary dimensions were 44 cm×50 μm I.D. A 0.3-cm segment of polyimide coating was burned off the tubing and the detection distance was 7.0 cm from the cathodic end. Sample injection was done in a hydrodynamic mode for 1.0 s. The voltage applied was 20 kV. The CE system was interfaced with a microcomputer and printer with software CE 1000 1.05A. For pH measurements, a pH meter (Suntex SP-701, Taipei, Taiwan) was employed with a precision of 0.01 pH unit.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed using a standard sequence described previously [37]: 10 min with deionized water at 60°C, 60 min with 1.0 *M* NaOH at 60°C and then 10 min with deionized water at 25°C. When changing the buffer solution, the capillary was washed for 10 min with 1.0 *M* NaOH at 60°C, followed by deionized water for 5 min at 25°C.

To ensure reproducibility, all experiments were carried out at least in triplicate. In order to maintain proper reproducibility of run-to-run injections, the capillary was prefilled for 3 min with running buffer before each injection and post-washed for 2 min with deionized water, 3 min with 1.0 *M* sodium hydroxide solution and 2 min with deionized water. The detection wavelength was monitored at 214 nm.

2.4. Calculation

The electrophoretic mobility of test solutes was calculated with the equation:

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

in which t_{eo} is the migration time of a neutral marker (methanol), L_t is the total length of capillary, L_d is the length of the capillary between injection and detection and V is the applied voltage.

The net charge of negatively charged sulfonamides was calculated from their pK_a values determined in this work with Eq. (2) [38]:

$$q = \frac{10^{(pK_a - pH)}}{10^{(pK_a - pH)} + 1} - 1 \qquad (2)$$

where q is the net charge of a negatively charged species.

3. Results and discussion

3.1. Basic consideration on mobility

In the electrophoretic separation of ionizable solutes, pH plays an important role as it determines the extent of ionization of individual solutes. In CZE, the effective mobility (μ_{eff}) as a function of buffer pH for an acidic solute (HA) can be described by the following relationship [39]:

$$\mu_{eff} = \frac{K_a/[H^+]}{1 + K_a/[H^+]}\mu_{A^-} \qquad (3)$$

where μ_{A^-} is the electrophoretic mobility of the fully dissociated form of the acid (A^-) and K_a is the acid dissociation constant. Accordingly, sigmoidal behavior for the variation of the mobility as a function of buffer pH is predicted. The mobility curve for each individual solute can then be simulated, provided that the values of K_a and μ_{A^-} are known.

On the other hand, the migration behavior of an acidic solute in MEKC can be predicted according to the following relationships [36,40]:

$$\mu_{eff} = \frac{\mu_{HA} + (K_{a,app}/[H^+])\mu'_{A^-}}{1 + (K_{a,app}/[H^+])} \qquad (4)$$

where

$$K_{a,app} = \frac{K^m_{A^-}[M] + 1}{K^m_{HA}[M] + 1} \times K_a \qquad (5)$$

$$\mu_{HA} = \frac{K^{m}_{HA}[M]\mu_{mc}}{1 + K^{m}_{HA}[M]} \quad (6)$$

and

$$\mu'_{A^-} = \frac{\mu_{A^-} + K^{m}_{A^-}[M]\mu_{mc}}{1 + K^{m}_{A^-}[M]} \quad (7)$$

in which μ'_{A^-} is the electrophoretic mobility of the anionic form of the solute in the presence of micelles in the aqueous solution, μ_{mc} is the mobility of micelles, $K_{a,app}$ is the apparent dissociation constant of the solute in micellar solution, K^{m}_{HA} and $K^{m}_{A^-}$ are the binding constants of the neutral form (HA) and the anionic form (A^-) of sulfonamides to the micelles, respectively and [M] is the micelle concentration which is the total surfactant concentration minus the critical micelle concentration (CMC). As illustrated in Eqs. (4)–(7), buffer pH and micelle concentration are the two most important experimental parameters that can greatly affect the electrophoretic mobility of acidic solutes in MEKC. At a given micelle concentration, the electrophoretic mobility of a selected solute as a function of buffer pH depends strongly on the magnitude of the binding constant of solutes-to-micelles (K^{m}_{HA} and $K^{m}_{A^-}$). By knowing the values of binding constants, acid dissociation constants and mobility data (μ_{A^-}), the mobility of the solutes can be predicted for any pH and micelle concentration according to Eq. (4).

3.2. Selectivity and separation of sulfonamides in CZE

Fig. 1 shows the structures of thirteen sulfonamides studied. Fig. 2 shows the effect of buffer pH on the electrophoretic mobility of thirteen sulfonamides obtained using 50 m*M* phosphate–50 m*M* borate buffer solution in the pH range 3.0–10.0 at 20 kV. As expected, sigmoidal behavior for each individual sulfonamide was observed. Through the utilization of EXCEL software, acid dissociation constants (K_a) of individual sulfonamides were determined by varying the value of pK_a until the predicted mobility curves calculated from Eq. (3) were best fitted with the observed mobility curves obtained by plotting the electrophoretic mobilities as a function of buffer pH. As demonstrated in Fig. 2, the solid lines are the predicted mobility curves of individual sulfonamides calculated from Eq. (3). The correlations between experimental and predicted mobility data for individual sulfonamides are excellent with the correlation coefficient (r^2) greater than 0.995. Table 1 lists the pK_a values and electrophoretic mobilities (μ_{A^-}) determined for these sulfonamides. The literature pK_a values of sulfonamides are also included. This information is necessary for a better understanding of the influence of buffer pH on the electrophoretic mobility of sulfonamides in CZE and in MEKC.

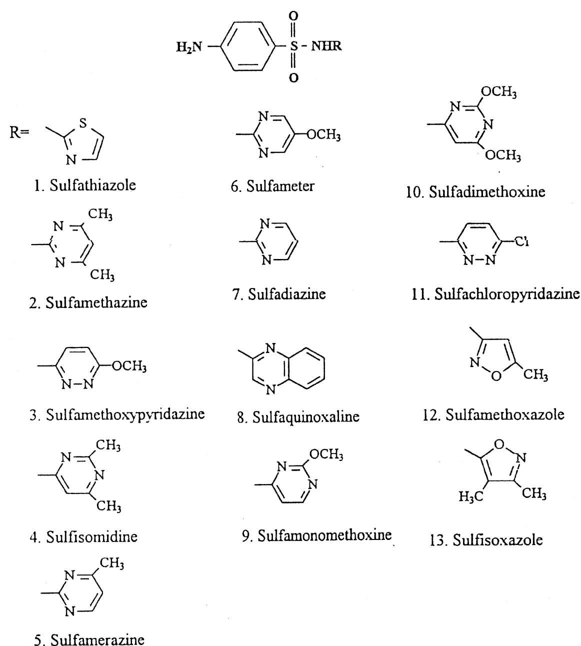

Fig. 1. Structures of sulfonamides.

As demonstrated previously [31], peaks between sulfamethoxy pyridazine (3) and sulfathiazole (1) become poorly resolved at pH 7.0 and even unresolvable at pH values below 6.9; sulfadiazine (7) and sulfadimethoxine (10) merge at pH 7.1; sulfaquinoxaline (8) and sulfadimethoxine (9) become poorly resolved or unresolvable in the pH range 7.0–7.3; sulfachloropyridazine (11) and sulfamethoxazole (12) are not well-resolved at pH about 6.8; sulfamethoxazole (12) and sulfisoxaole (13) merge at pH 7.2. Thus, the selectivity and resolution of closely migrating sulfonamides are quite sensitive to the pH of the buffer in the pH range 6.8–7.3 and precise

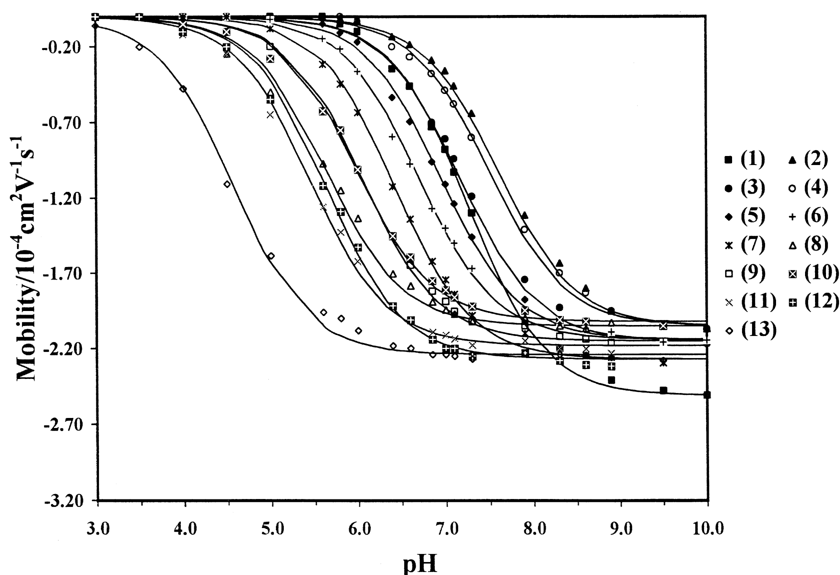

Fig. 2. Electrophoretic mobility of sulfonanides obtained as a function of buffer pH in the range 3.0–10.0 in CZE. Buffer: 50 m*M* phosphate –50 m*M* borate. Operating conditions: 20 kV, 25°C. Capillary: 44 cm× 50 μm, I.D. Curve identification: 1 = sulfathiazole; 2 = sulfamethazine; 3 = sulfamethoxypyridazine; 4 = sulfisomidine; 5 = sulfamerazine; 6 = sulfameter; 7 = sulfadiazine; 8 = sulfaquinoxaline; 9 = sulfamonomethoxine; 10 = sulfadimethoxine; 11 = sulfachloropyridazine; 12 = sulfamethoxazole; 13 = sulfisoxazole.

optimization of buffer pH is crucial for further improving the separation of sulfonamides.

As described previously [33], the electrophoretic mobilities of sulfonamides measured at optimum pH of the buffer correlate very well with those calculated from Offord's equation [41–43]. Thus, the migration order of sulfonamides in CZE depends on their ratios of charge to mass (i.e. $q/M^{2/3}$). Fig. 3 confirms this result using a phosphate–borate buffer system. For sulfonamides with small differences in molecular mass, the migration order is primarily determined by their corresponding pK_a values. How-

Table 1
Values of acid dissociation constant (pK_a) and electrophoretic mobility (μ_{A^-}) of sulfonamides determined in 50 m*M* phosphate–50 m*M* borate buffer solution[a]

Sulfonamides	pK_a		μ_{A^-}
	Literature[b]	This work	
(1) Sulfathiazole	7.2	7.24	−2.45
(2) Sulfamethazine	7.4	7.65	−2.05
(3) Sulfamethoxypyridazine	6.7	7.19	−2.13
(4) Sulfisomidine	–	7.50	−2.03
(5) Sulfamerazine	7	6.94	−2.12
(6) Sulfameter	6.8	6.68	−2.11
(7) Sulfadiazine	6.5	6.43	−2.24
(8) Sulfaquinoxaline	5.5	5.65	−2.03
(9) Sulfamonomethoxine	–	6.03	−2.12
(10) Sulfadimethoxine	6.2	5.99	−2.01
(11) Sulfachloropyridazine	5.5	5.49	−2.18
(12) Sulfamethoxazole	5.6	5.65	−2.28
(13) Sulfisoxazole	5.1	4.57	−2.22

[a] Mobility data in units of 10^{-4} cm^2 V^{-1} s^{-1}
[b] Literature values obtained from [29].

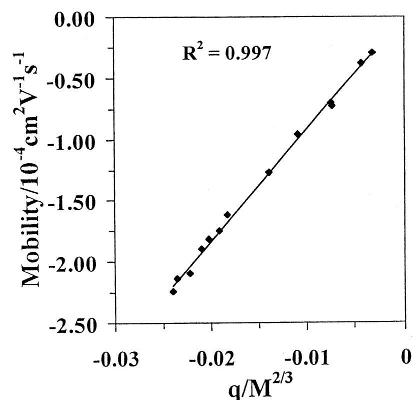

Fig. 3. Correlation of the electrophoretic mobility of sulfonamides with Offord's parameter ($q/M^{2/3}$) at pH 6.85.

ever, for sulfonamides with close values of $q/M^{2/3}$, further improvement of the separation should be achieved by manipulating additional separation parameters, such as concentration and type of organic modifiers, concentration and type of electrolyte modifiers and temperature. In fact, on addition of an appropriate amount of methanol or acetonitrile as organic modifier [31,33], a low concentration of β-CD as electrolyte modifier to a phosphate–borate buffer at pH 6.85 [31], or even raising the temperature of the capillary to 35–40°C at pH 6.85 [33], the selectivity of sulfonamides alters and the resolution of peaks between sulfathiazole (1) and sulfamethoxypyridazine (3), in particular, is markedly enhanced, thus leading to a complete separation of thirteen sulfonamides. Fig. 4 shows some electropherograms

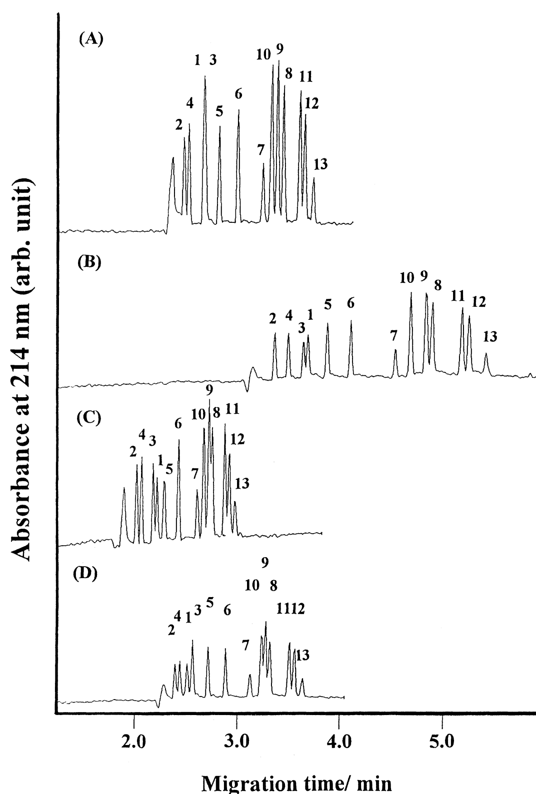

Fig. 4. Electropherogram of sulfonamides obtained at pH 6.85 in CZE, (A) without further modification of electrolyte, (B) with the addition of methanol (16.6%, v/v), (C) by raising capillary temperature to 40 °C, (D) with the addition of 0.05 m*M* β-CD. Buffer: 50 m*M* phosphate–50 m*M* borate. Operating conditions as for Fig. 2. Peak identification as for curve identification in Fig. 2.

of sulfonamides obtained at the optimum buffer pH by manipulating one of the aforementioned separation parameters.

The extent of the variation in the electrophoretic mobility of solutes is directly proportional to the magnitude of the formation constant when an inclusion complexing agent is added to an electrolyte buffer. By knowing the formation constants of sulfonamides with β-CD, the enhancement in the resolution of peaks between sulfathiazole (1) and sulfamethoxypyridazine (3) on addition of β-CD is understandable, because the formation constants of these two sulfonamides with β-CD are much greater than the other sulfonamides [31]. In fact, the formation constants of sulfathiazole (1) and sulfamethoxypyridazine (3) with β-CD evaluated are 1100 and 560 M^{-1}, respectively, whereas those of the rest of sulfonamides with β-CD are in the range 50–310 M^{-1}. Therefore, knowing the formation constants of inclusion complexes is important for a better understanding of the effect of β-CD on the migration behavior and selectivity of sulfonamides.

3.3. Selectivity and separation of sulfonamide in MEKC

The influences of buffer pH (in the range 4.0–9.0) and of micelle concentration (in the range 20–40 m*M*) on the electrophoretic mobility of thirteen sulfonamides were systematically investigated. The binding constants of these sulfonamides to the SDS micelles were determined so that the mobility and selectivity of sulfonamides in MEKC can be predicted for any pH and micelle concentration.

3.3.1. Effect of buffer pH

The influence of buffer pH on the electrophoretic mobility and selectivity of thirteen sulfonamides with SDS concentration at 30 m*M* in a phosphate–borate buffer in the pH range 5.6–7.3 is shown in Fig. 5. The electrophoretic mobility of sulfonamides (moving towards anode), with the exception of sulfadimethoxine (10) and sulfaquinoxaline (8), increases with increasing the pH of the buffer. As demonstrated, the selectivity of these sulfonamide is quite sensitive to the pH of the buffer selected and the migration window becomes wider at lower pH

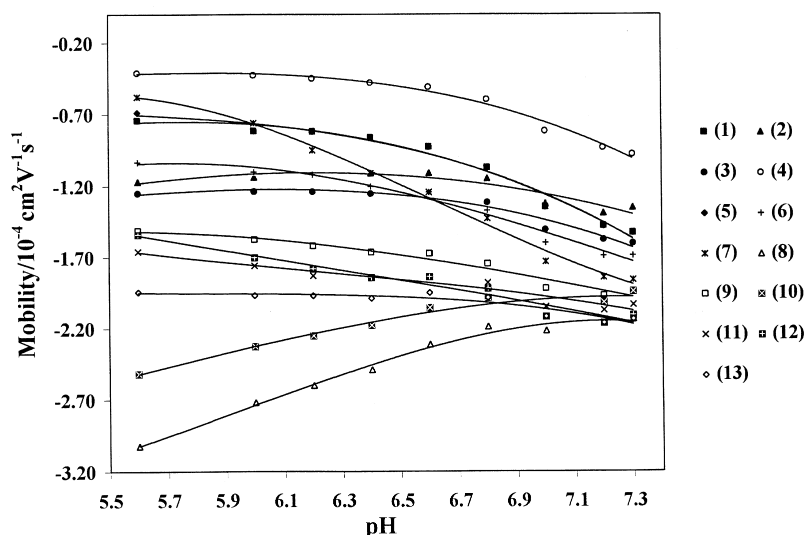

Fig. 5. Electrophoretic mobility of sulfonamides obtained as a function of buffer pH in the range 5.6–7.3 in MEKC. Buffer: 50 m*M* phosphate–50 m*M* borate containing 30 m*M* SDS. Operating conditions and curve identification as for Fig. 2.

values. Thus the optimum buffer pH for the separation of sulfonamides in MEKC seems to lie in the range 6.0–5.6. Fig. 6 shows the electropherograms of sulfonamides obtained at three different pH values of the buffer.

It should be noted that, as shown in Fig. 5, the electrophoretic mobility of sulfathiazole (1), sulfisomidine (4), sulfadiazine (7) and sulfadimethoxine (10) varies considerably in the pH range 5.6–7.3. The migration behavior of these four sulfonamides differs considerably from those obtained by Dang et al. [34]. We question the correctness of their results.

In order to have a better understanding on the migration behavior of each individual sulfonamide, the binding constants (K^{m}_{HA} and $K^{m}_{A^-}$) of sulfonamides to SDS micelles are determined. These values are determined by curve-fitting the predicted mobility data as a function of buffer pH with the experimental mobility data through the utilization of EXCEL software. The binding constants ($K^{m}_{A^-}$) of the anionic form of sulfonamides are determined to be zero or nearly equal to zero because the electrophoretic mobility of anionic sulfonamides in MEKC at pH≥9.0 are almost equal to the corresponding values obtained in CZE. Fig. 7 shows the results of the best fit for sulfathiazole (1) and sulfaquinoxaline (8) as two typical examples. Table 2 presents the binding constants (K^{m}_{HA}) and mobility data (μ_{HA} and μ_{A^-}) of sulfonamides evaluated.

Once the binding constants of individual sulfonamides are determined, the mobility curve describing the variation of the electrophoretic mobility as a function of buffer pH for each individual sulfonamide can then be simulated and the variation of the selectivity with buffer pH is understandable. For instance, sulfaquinoxaline (8) and sulfadimethoxine (10) interact strongly with the SDS micelles and the binding constants of these two sulfonamides determined are 113 and 53 M^{-1}, respectively. Since these two values exceed 33 and 30 M^{-1}, which are the borderline for the inversion of the sigmoidal curves for sulfaquinoxaline (8) and sulfadimethoxine (10), respectively. Consequently, the phenomenon of the electrophoretic mobility of these two sulfonamides increasing as the pH of the buffer decreases from pK_a-2 to pK_a+2 can be reasonably explained. Fig. 8 illustrates how the shape of the mobility curve for sulfaquinoxaline (8) was affected by the magnitude of the binding constant.

It is interesting to note that the differences between the electrophoretic mobility of sulfonamides in MEKC measured at a pH below $pK_{a,app}-2$ and that of the corresponding sulfonamides in CZE can be used to estimate the magnitude of binding constants.

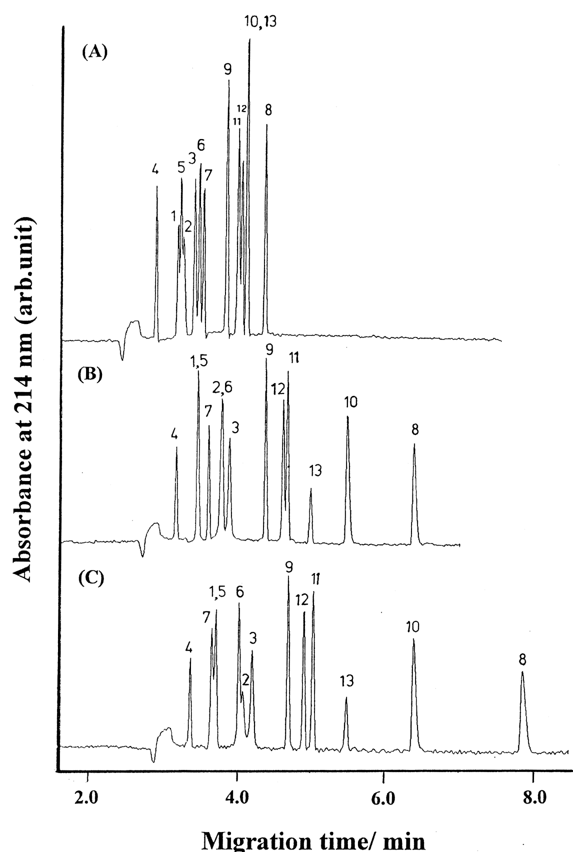

Fig. 6. Electropherograms of sulfonamides obtained at varied pH in MEKC, (A) 6.8, (B) 6.2 and (C) 6.0. Buffer as for Fig. 5. Operating conditions and peak identification as for Fig. 4.

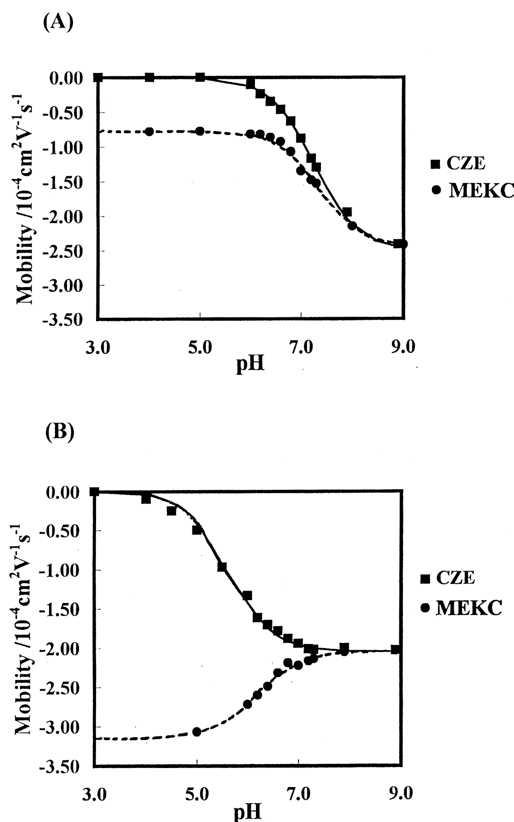

Fig. 7. The fitting of predicted mobility curves (———, CZE; - - -, MEKC) with experimental mobility data ((■), CZE; (●), MEKC) for (A) sulfathiazole (1) and (B) sulfaquinoxaline (8).

Fig. 9 shows the plot of the mobility differences between μ_{HA} (CZE) and μ_{HA} (MEKC) versus the binding constants determined from the curve-fitting. As can be seen, the correlation is excellent and a linear relationship can be obtained for sulfonamides with binding constants less than 20 M^{-1}.

3.3.2. Influence of micelle concentration

As expected, the electrophoretic mobility of sulfonamides increases with increasing micelle concentration and the extent of the variation of the electrophoretic mobility of each individual sulfonamide depends on the magnitude of the binding constant. It is also noted that the larger the binding constant, the greater the extent of the variation in the electrophoretic mobility. Moreover, the selectivity of sulfonamides varies considerably as micelle con-

Table 2
Values of binding constants (K_{HA}) of sulfonamide to SDS micelles and mobility data (μ_{HA} and μ'_{A^-}) of sulfonamides

Sulfonamides	K^{m}_{HA} [a]	μ_{HA} [b]	μ'_{A^-}
(1) Sulfathiazole	8	−0.78	−2.45
(2) Sulfamethazine	13	−1.13	−2.05
(3) Sulfamethoxypyridazine	15	−1.25	−2.13
(4) Sulfisomidine	4	−0.43	−2.03
(5) Sulfamerazine	7	−0.70	−2.12
(6) Sulfameter	10	−0.93	−2.11
(7) Sulfadiazine	2	−0.23	−2.24
(8) Sulfaquinoxaline	113	−3.15	−2.03
(9) Sulfamonomethoxine	13	−1.13	−2.12
(10) Sulfadimethoxine	53	−2.49	−2.01
(11) Sulfachloropyridazine	6	−0.61	−2.18
(12) Sulfamethoxazole	6	−0.61	−2.28
(13) Sulfisoxazole	3	−0.33	−2.22

[a] Binding constants in units of M^{-1}.
[b] Mobility data in units of 10^{-4} $cm^2 V^{-1} s^{-1}$; $\mu_{mc} = -4.1 \cdot 10^{-4}$ $cm^2 V^{-1} s^{-1}$.

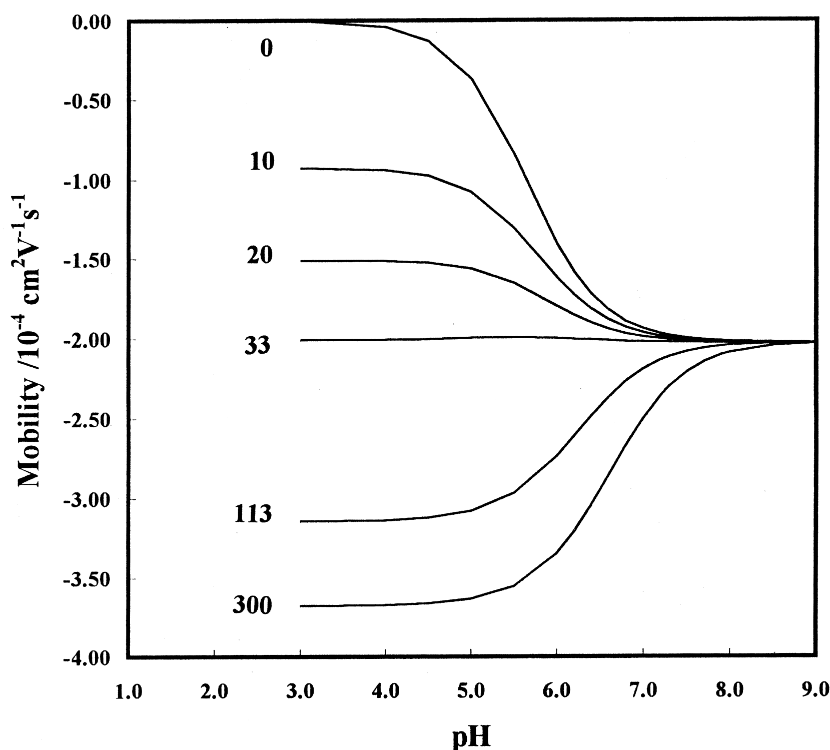

Fig. 8. Mobility curves simulated for sulfaquinoxaline (8) at varied binding constants (K^{m}_{HA}).

centration increases from 0 to 30 m*M*, whereas the mobility increases steadily at pH 6.8 but to a smaller extent at pH 6.2 when micelle concentration increases from 30 to 40 m*M*. The migration order of thirteen sulfonamides with SDS concentration at 40 m*M* and at pH<6.2 remains almost the same as that with SDS concentration at 30 m*M*. Fig. 10 illustrates the effect of micelle concentration by showing the electropherograms of sulfonamides obtained at pH 6.2 with SDS concentrations at 0, 20, 30, and 40 m*M*.

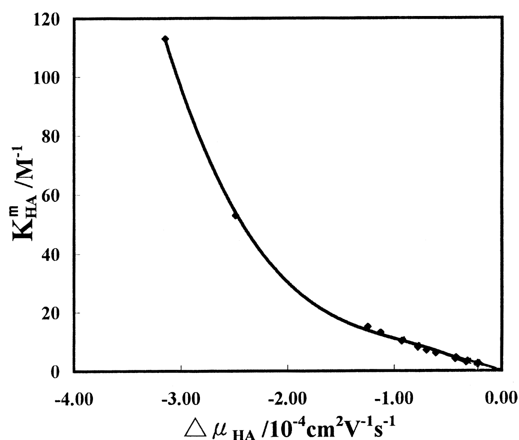

Fig. 9. The plot of binding constants of sulfonamides versus the mobility differences between μ_{HA} (CZE) and μ_{HA} (MEKC).

To separate the thirteen sulfonamides studied at the optimum condition in MEKC, the pH of the buffer should be selected at about 5.8 and the SDS concentration at 40 m*M*. Fig. 11 presents such an electropherogram obtained at this optimum condition. This separation was achieved within 10 min.

4. Conclusion

The optimization strategies for the separation of sulfonamides in CZE and in MEKC are described. Buffer pH is the most important separation parameter

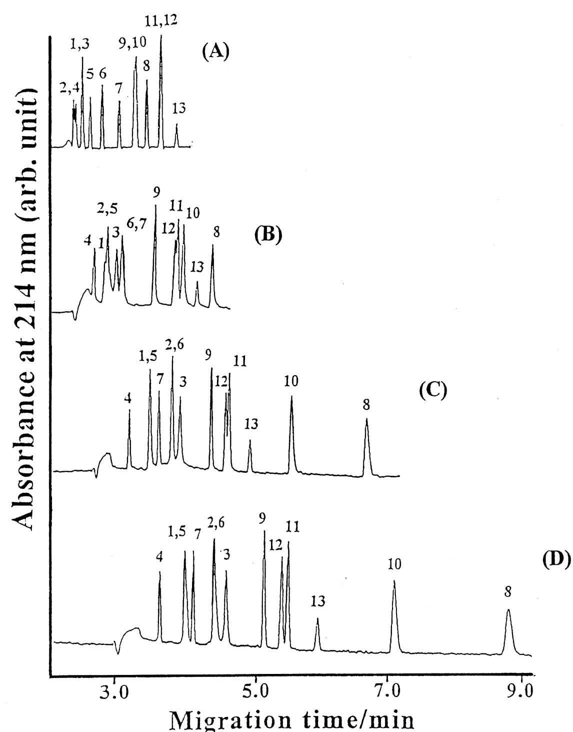

Fig. 10. Electropherograms of sulfonamides obtained at varied SDS concentration at pH 6.2 in MEKC, (A) 0 m*M*, (B) 20 m*M* (C) 30 m*M* and (D) 40 m*M*. Operating conditions and peak identification as for Fig. 4.

that controls the migration behavior and selectivity of sulfonamides in CZE. By knowing or determining the values of acid dissociation constants, the migration behavior of sulfonamides in CZE can be predicted. Buffer pH optimized precisely at about 6.85 is crucial for further improving the separation of sulfathiazole and sulfamethoxypyridazine by manipulating other separation parameters. On the other hand, the migration behavior of sulfonamides in MEKC is more complicated than in CZE. Buffer pH and micelle concentration are the two most important separation variables that affect greatly the migration behavior of sulfonamides in MEKC. For a better understanding of the migration behavior of sulfonamides in MEKC, the evaluation of binding constants of sulfonamides-to-micelles, in addition to acid dissociation constants and mobility data of the fully dissociated species (μ_{A^-}), is essential. The optimized separation of thirteen sulfonamides in MEKC was achieved with SDS concentration at 40 m*M* and with buffer pH at 5.8. In addition to CZE, MEKC provides an alternative method to separate sulfonamides.

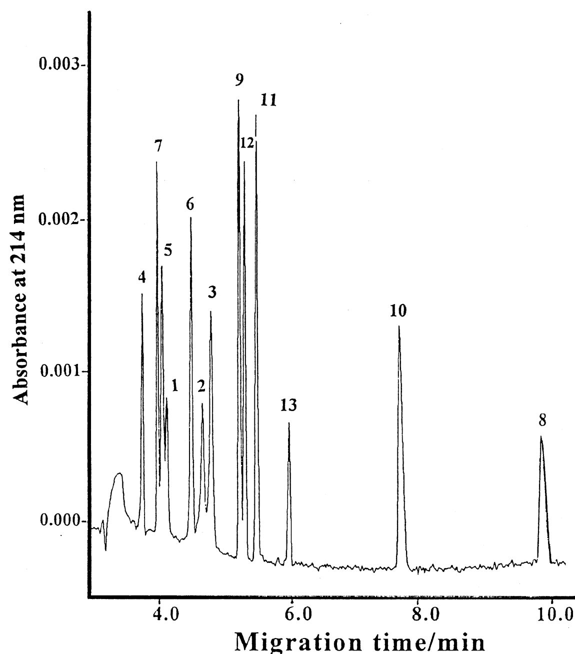

Fig. 11. Electropherograms of thirteen sulfonamides obtained at optimum conditions. Buffer: 50 m*M* phosphate–50 m*M* borate containing 40 m*M* SDS at pH 5.8. Operating conditions and peak identification as for Fig. 4.

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