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Migration behavior and separation of sulfonamides in capillary zone electrophoresis
I. Influence of buffer pH and electrolyte modifier

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Abstract

The influence of buffer pH and electrolyte modifier on the migration behavior and separation of thirteen sulfonamides was investigated by capillary zone electrophoresis. The results indicate that precise optimization of buffer pH is crucial in improving the separation of some closely migrating sulfonamides. On the addition of either an appropriate amount of an organic modifier (methanol or acetonitrile) or a low concentration of β-cyclodextrin (β-CD, 0.5 m*M*) to a phosphate–borate buffer at pH 6.85 and an applied voltage of 20 kV, the resolution of peaks between sulfathiazole and sulfamethoxypyridarine is markedly enhanced and effective separations of thirteen sulfonamides are achieved within a relatively short time. Methanol gives better resolution than acetonitrile as an organic modifier. Weak inclusion complexation occurs between β-CD and sulfonamides, with the exception of sulfathiazole. The formation constants of thirteen sulfonamides with β-CD are reported.

Keywords: Sulfonamides; Buffer composition; pH effects

1. Introduction

Sulfonamides are extensively applied in medicine and veterinary practice as antibacterial drugs. These compounds are commonly used to treat bacterial infections related to the respiratory, intestinal and urinary tracts. They also serve to prevent and to treat certain microbial diseases in animals. However, recent evidence has implicated sulfamerazine as a possible thyroid carcinogenic agent [1]. As sulfonamide residues in food and animal tissues may be present in minute concentrations and may pose a health threat to consumers through allergic or toxic reactions, or through induction of antibiotic resistance in pathogenic organisms [2,3], interest in the development of rapid and sensitive analytical methods to identify and to separate these compounds in trace proportions continues unabated.

Various approaches, including microbiological and immunoassay [4], UV–Vis spectrophotometry [5,6], flow injection analysis [7,8], thin-layer chromatography [9,10], gas chromatography (GC) [11,12], GC–MS [13], high-performance liquid chromatography (HPLC) [14–24], HPLC–MS [25], supercritical fluid chromatography (SFC) [26–29], capillary electrophoresis (CE) [30–35] and CE–MS [36] are applied to determine sulfonamides. Among them, HPLC has attracted much attention because this

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technique tends to be efficient in separating closely related compounds [14–23]. In recent years, CE has become a popular and powerful separation technique which is widely applied to diverse samples because of its high resolution, extremely high efficiency, rapid analysis and small consumption of both sample and solvent in comparison with HPLC [37–42]. Thus, CE is expected to be an excellent alternative method for the identification and separation of sulfonamides.

The separation of sulfonamides by CE has been performed by capillary zone electrophoresis (CZE) [30–34] and micellar electrokinetic capillary chromatography (MECC) [35]. A mixture of some selected sulfonamides was separated by capillary zone electrophoresis at pH 7.0 using either a phosphate (20 m*M*)–borate (20 m*M*) buffer [30] or a phosphate (50 m*M*)–borate (50 m*M*) buffer containing β-cyclodextrin (β-CD, 10 m*M*) [33]. Li and co-workers [31,32], utilizing the overlapping resolution mapping procedure to predict optimum separation conditions, reported that seven selected sulfonamides were best separated with a buffer composed of phosphate (50 m*M*)–borate (50 m*M*) on addition of either β-CD (3 m*M*) at pH 6.0 or β-CD (2 m*M*) at pH 6.4. Ricci and Cross [34] stated that, among twenty-two sulfonamides tested, eighteen of them were resolved using 50 m*M* phosphate buffer at pH 7.5. However, peaks between sulfathiazole and sulfamethoxypyridazine and those between sulfadiazine and sulfadimethoxine were not resolved [34]. In addition, the optimal pH range suggested by Ricci and Cross [34] was higher than the value reported by other investigators [30,33]. As the optimum conditions reported previously are inefficient in separating the thirteen sulfonamides selected in this study and the migration selectivity of sulfonamides is very sensitive to the pH of the buffer, a closer examination in the pH range around 6.8–7.3 is apparently needed, especially for the separation of a wide range of sulfonamides. Moreover, to further improve the separation of sulfonamides selected, we systematically investigated the influence of an organic modifier, such as methanol or acetonitrile, or β-cyclodextrin (β-CD) as an electrolyte modifier on the migration behavior and separation of sulfonamides. Lastly, in order to gain an insight into the interaction between sulfonamides and β-CD, the formation constants of inclusion complexes formed between sulfonamides and β-CD were also evaluated.

2. Experimental

2.1. Chemicals and reagents

Thirteen sulfonamides investigated in the study are shown in Fig. 1. These sulfonamides, originally purchased from Sigma (USA), were supplied as a gift from Taiwan Meat Development Foundation. β-Cyclodextrin and anhydrous disodium tetraborate

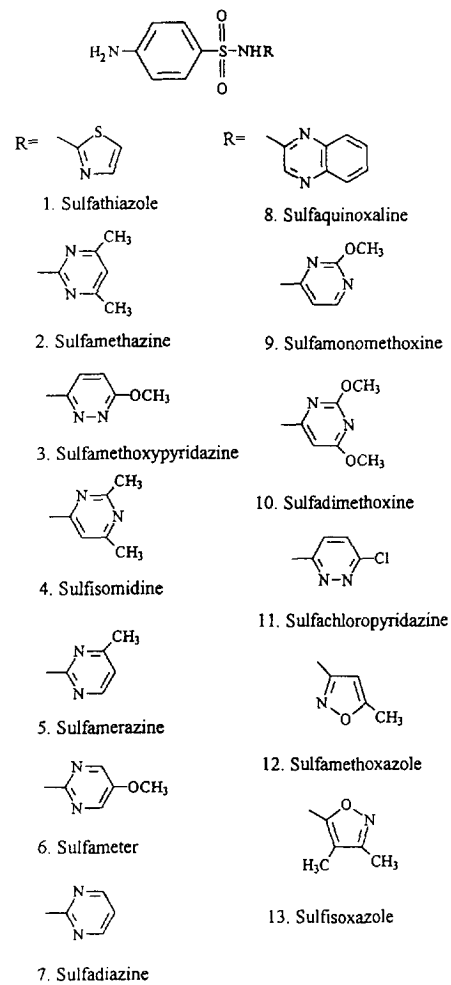

Fig. 1. Structures of sulfonamides.

were obtained from Merck (Germany). Sodium dihydrogenphosphate dihydrate was purchased from Showa Chemicals (Japan). Methanol (MeOH) was of HPLC grade (Mallinckrodt, USA) and was 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 about 0.25 m*M* in methanolic solution. Phosphate–borate buffer solution was prepared by mixing disodium tetraborate with sodium dihydrogenphosphate solution in a appropriate ratio. The pH of the buffer solution was adjusted with sodium hydroxide (0.1 *M*) or hydrochloric acid (0.1 *M*) 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 electrophoresis system (Spectra-Physics Model 1000, Fremont, CA, USA), equipped with a programmable and high-speed scanning multiple-wavelength UV–Vis detector, a fused-silica capillary cartridge thermostated with a Peltier thermoelectric device and an automatic injection system. The capillary dimensions were 44 cm×50 μm, I.D. A 0.3-cm segment of polyimide coating was burned off from the tubing 7.0 cm from the cathodic end before installation in a capillary cartridge for on-column detection. Sample injection was done in a hydrodynamic mode during 1 s. 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 [42]. 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 to maintain proper reproducibility of run-to-run injections.

For peak identification, each individual sulfonamide was injected into the capillary, when necessary. On-column UV spectra (200–300 nm with a 2-nm wavelength increment) of sulfonamides were very helpful and were recorded simultaneously during the electrophoretic separation when necessary. A three dimensional spectral scan of CE separation of a mixture of sulfonamides, as shown in Fig. 2, is presented to show the characteristics of UV absorption spectra of these analytes. The detection wavelength was set at 214 nm so that the peaks between sulfathiazole and sulfamethoxypyridazine could be better resolved. However, the detection sensitivity could be enhanced 1–3 fold by setting the detection wavelength at about 254 nm.

2.4. Mobility calculation

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

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

where μ_S is the electrophoretic mobility of the solute 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.

Formation constants of inclusion complexes formed between sulfonamides and β-CD are evaluated by curve-fitting the experimental mobility data as a function of the β-CD concentration at pH 6.85 through the utilization of SigmaPlot software according to the equation [61]

$$\mu_S = \frac{\mu_S^o + \mu_{S-CD} K_f [CD]}{1 + K_f [CD]} \quad (2)$$

where μ_S^o is the electrophoretic mobility of a solute in the absence of β-CD, $\mu_{S\text{-}CD}$ is the mobility of the inclusion complex formed between a solute and β-CD, K_f is the formation constant of the inclusion complex, and [CD] is the concentration of β-CD.

the buffer electrolyte, the electroosmotic flow decreases as a result of a decrease in zeta potential and a decreased magnitude of the ratio of dielectric constant to viscosity of the buffer [55]. The electroosmotic mobility decreased from $5.9 \cdot 10^{-4}$ to $3.3 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} when methanol (23%, v/v) was added to the borate–phosphate buffer at pH 6.85.

Fig. 5 shows the effect of the addition of methanol to the phosphate–borate buffer on the separation of thirteen sulfonamides. As illustrated, sulfisomidine (**4**) behaves very differently from the rest of sulfonamides because the electrophoretic mobility of this component increases with an increase in the proportion of methanol contained in the buffer solution; the resolution of the pairs of components (**3**,**1**) and (**2**,**4**) enhanced considerably at the expense of that of the pairs of components (**9**,**8**) and (**11**,**12**). Indeed, an effective separation of these sulfonamides was achieved when an appropriate proportion of methanol (16.6%, v/v) was added to the buffer electrolyte. Fig. 6A shows such an electropherogram of sulfonamides obtained.

The effect of the addition of acetonitrile to the buffer electrolyte on the separation of sulfonamides was also examined. The pair of components (**3**,**1**) was almost completely resolved upon the addition of acetonitrile (9%, v/v) to the buffer electrolyte at 20 kV. It should be noted that, although the resolution of the pair of components (**3**,**1**) improves continuously upon further increasing the proportion of acetonitrile, the resolution of the pairs of components (**9**,**8**) and (**11**,**12**) deteriorates. Fig. 6B shows such an electropherogram of sulfonamides obtained when acetonitrile (9%, v/v) was added to the buffer solution.

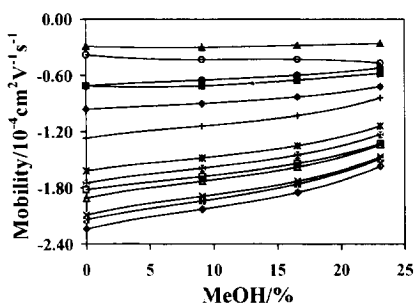

Fig. 5. Electrophoretic mobility of sulfonamides obtained on addition of varied proportion of methanol to the buffer solution. Buffer: phosphate (50 m*M*)–borate (50 m*M*) at pH 6.85. Operating conditions as for Fig. 2 and curve identification as for Fig. 3.

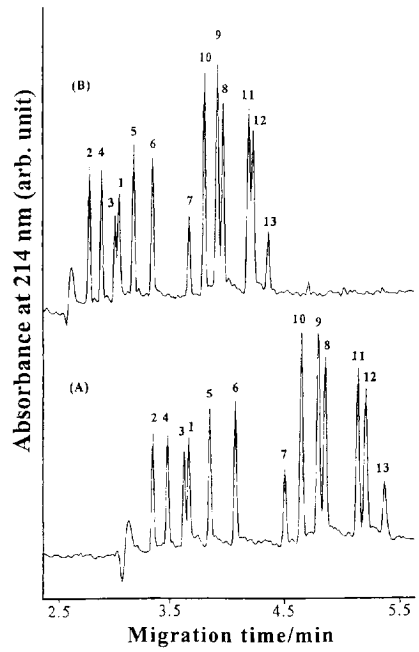

Fig. 6. Electropherograms of sulfonamides obtained with the addition of (A) methanol (16.6%, v/v) and (B) acetonitrile (9%, v/v) to the buffer solution. Buffer: phosphate (50 m*M*)–borate (50 m*M*) at pH 6.85. Operating conditions and peak identification as for Fig. 2.

3.3. Influence of β-cyclodextrin

Cyclodextrins (CDs) have been applied successfully in the separation of many positional, structural and enantiomer isomers in MECC and CZE [56–60]. The addition of β-cyclodextrin to the buffer electrolyte may greatly alter the migration selectivity of analytes when inclusion complexation of the analyte with β-CD takes place.

Fig. 7 shows the electrophoretic mobility of sulfonamides at varied concentrations of β-CD in the range 0–10 m*M* at pH 6.85. In general, the electrophoretic mobility of sulfonamides, with the exception of sulfathiazole (**1**), decreases rather gradually with an increase in β-CD concentration. This phenomenon is attributed to the formation of weak

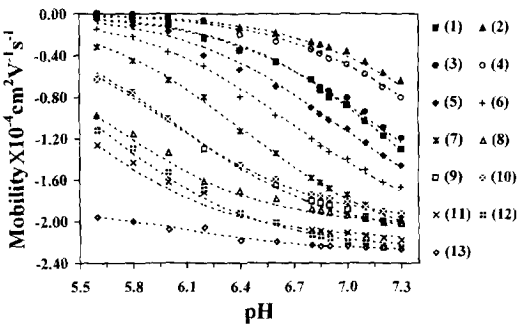

Fig. 3. Electrophoretic mobility of sulfonamides obtained at varied pH in the range 5.6–7.3. Buffer: phosphate (50 m*M*)–borate (50 m*M*). Operating conditions as for Fig. 2.

pH 5.6 to $5.8 \cdot 10^{-4}$cm^2 V^{-1} s^{-1} at pH 6.8, and then levels off in the range 6.8–7.3.

Fig. 3 shows the effect of buffer pH on the electrophoretic mobility of sulfonamides obtained in the pH range 5.6–7.3 with an applied voltage of 20 kV. With the exception of sulfisoxazole (**13**), the electrophoretic mobility of sulfonamides, being in the opposite direction to the electroosmotic flow, increases with an increase in the pH of the buffer. Depending on the pK_a of sulfonamides, the extent of the variation in mobility in the pH range 7.0–7.3 for sulfonamides with pK_a<6.1 becomes relatively small; thus, the peaks between two closely migrating sulfonamides cannot be separated at higher pH. For instance, sulfadimethoxine (**9**) and sulfaquinoxaline (**8**) are well separated at pH below 6.9, but co-migrate at pH above 7.1; sulfamethoxazole (**12**) and sulfisoxazole (**13**) are well resolved at pH below 7.0, but almost merge at pH 7.2. On the other hand, peaks between two consecutively migrating sulfonamides with pK_a>7.0 become unresolvable at pH below 6.8. For instance, peaks between sulfamethoxypyridazine (**3**) and sulfathiazole (**1**) become poorly resolved or even unresolvable at pH below 6.9; peaks between sulfamethazine (**2**) and sulfisomidine (**4**) merge at pH below 6.1. Moreover, electrophoretic mobilities of components **1**, **7** and **13** are affected more than those of consecutively migrating components **3**, **10** and **12**, respectively, by the pH of the buffer. Thus, the resolution of the corresponding pairs of two closely migrating components, such as (**3**,**1**), (**7**,**10**) and (**12**,**13**), is greatly affected by varying buffer pH. As demonstrated, the results indicate that these sulfonamides are best separated at pH about 6.85. Fig. 4 illustrates the electropherograms of sulfonamides obtained at pH 6.8, 6.9, 7.0 and 7.3 which demonstrate the influence of buffer pH on the separation of the thirteen sulfonamides selected.

3.2. Influence of organic modifier

It has been shown that the addition of organic modifiers to the buffer electrolyte in capillary electrophoresis serves to enhance separation and resolution [49–54]. Thus, the addition of an organic modifier, such as methanol and acetonitrile, to the buffer solution was attempted in order to further improve the separation of sulfathiazole (**1**) and sulfamethoxypyridazine (**3**).

As expected, on addition of organic modifier to

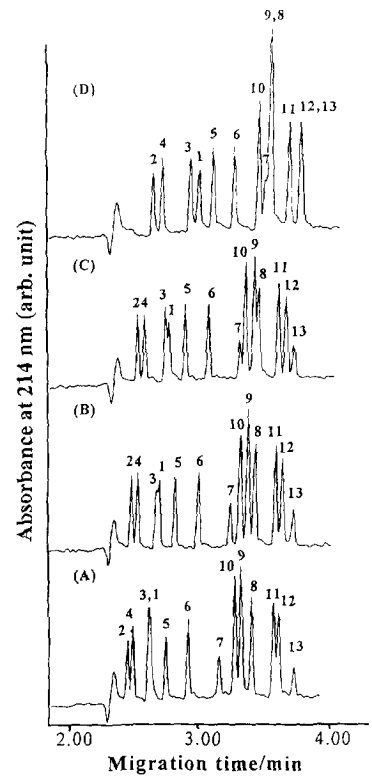

Fig. 4. Electropherograms of sulfonamides obtained at varied pH: (A) 6.8, (B) 6.9, (C) 7.0, and (D) 7.3. Buffer: phosphate (50 m*M*)–borate (50 m*M*). Operating conditions and peak identification as for Fig. 2.

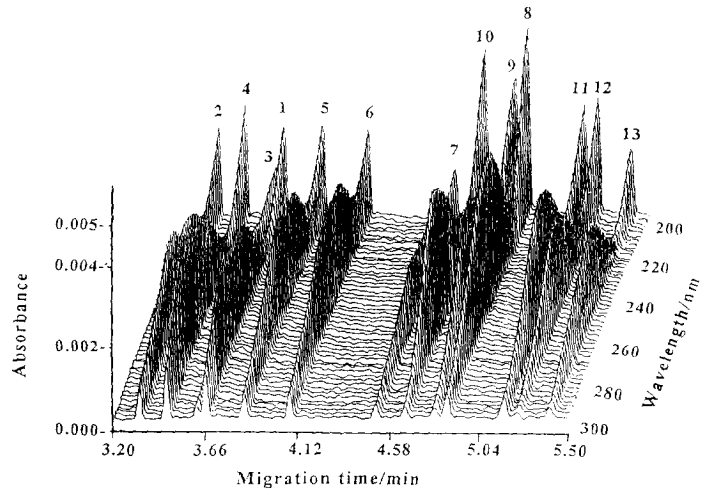

Fig. 2. Three-dimensional spectral scan of CE separation of a mixture of thirteen sulfonamides. Buffer: phosphate (50 m*M*)–borate (50 m*M*) containing methanol (16.6%, v/v) at pH 6.85. Operating conditions: 20 kV, 25°C. Capillary: 44 cm×50 μm I.D. Peak identification: (**1**) sulfathiazole; (**2**) sulfamethazine; (**3**) sulfamethoxypyridazine; (**4**) sulfisomidine; (**5**) sulfamerazine; (**6**) sulfameter; (**7**) sulfadiazine; (**8**) sulfaquinoxaline; (**9**) sulfamonomethoxine; (**10**) sulfadimethoxine; (**11**) sulfachoropyridazine; (**12**) sulfamethoxazole and (**13**) sulfisoxazole.

2.5. Pretreatment of milk samples

Homogenized milk sample was obtained from a local market. In order to eliminate or minimize matrix interference, milk samples were deprotenized first. A 5-ml milk sample fortified with thirteen standard sulfonamides (62.5–77.5 mg/ml) was placed into a small test tube containing 5 ml of 3 *M* HCl solution. The mixture was then centrifuged for 20 min. The upper liquid layer was pipeted to another test tube to which 1 ml diluted HCl solution (3 *M*) was added. The mixed solution was then centrifuged for another 20 min. The resultant clear supernatant was filtered through a 0.45-μm filter and an aliquot (1 ml) was used as sulfonamide fortified milk sample for analysis. Milk samples treated by the same procedures, but without the addition of sulfonamides, were used as blank milk sample.

3. Results and discussion

To achieve satisfactory separations using the CZE technique, the optimization of buffer pH and the modification of background electrolytes are of primary importance. In this work, the combined effects of buffer pH and electrolyte modifier on the migration behavior and selectivity of sulfonamides are taken into consideration for obtaining optimized separations.

3.1. Influence of buffer pH

In CZE, the pH of the buffer plays an important role in the separation of ionizable analytes because it determines the extent of ionization of each individual analyte [42,43]. Therefore, manipulation of buffer pH usually becomes a key strategy to optimize a separation. For the separation of sulfonamides, the optimum pH of the buffer was often chosen at 7.0 [30,33]. As buffer pH is a very sensitive parameter for the separation of sulfonamides, precise optimization of buffer pH is crucial in further improving the separation of sulfonamides by adding electrolyte modifier to a buffer solution. Therefore, measurements of the electrophoretic mobility were performed with an increment of 0.05 or 0.1 pH unit when optimizing buffer pH in the range 6.8–7.3.

The charge of the capillary wall surface and the zeta potential are influenced by buffer pH [44–46]. As excepted, the negative charge is built up at the surface with increasing pH [44–48]. The magnitude of electroosmotic mobility changes from $5.1 \cdot 10^{-4}$ at

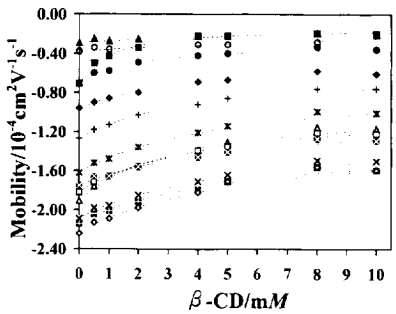

Fig. 7. Electrophoretic mobility of sulfonamides at varied concentration of β-CD in the range 0–10 mM. Buffer: phosphate (50 mM)–borate (50 mM) at pH 6.85. Operating conditions as for Fig. 2 and curve identification as for Fig. 3.

inclusion complexes between sulfonamides and β-CD. With higher concentrations of β-CD, sulfonamides have a higher probability to form inclusion complexes. Table 1 lists the formation constants evaluated for these inclusion complexes. Sulfonamides with relatively high pK_a (p$K_a \geq 7.2$), such as sulfamethazine (**2**) and sulfisomidine (**4**), are only slightly incorporated into β-CD on addition of β-CD because very weak inclusion complexation occurs between these two sulfonamides and β-CD. These two sulfonamides, migrating at a speed very close to the electroosmotic flow, are ionized only slightly at pH 6.85.

On addition of β-CD (<1.5 mM), the variation of the electrophoretic mobility of sulfathiazole (**1**) is much greater than that of sulfamethoxypyridazine (**3**). Thus, the peak resolution between sulfathiazole (**1**) and sulfamethoxypyridazine (**3**) is markedly enhanced when the concentration of β-CD varies from 0 to 1.5 mM. In contrast, peaks among sulfadimethoxine (**10**), sulfamonomethoxine (**9**) and sulfaquinoxaline (**8**), and those between sulfathiazole (**1**) and sulfisomidine (**4**) become poorly resolved or even unresolvable when the concentration of β-CD varies from 0 to 1.5 mM. As a consequence, the thirteen sulfonamides are best separated when 0.5 mM is β-CD added to the buffer electrolyte.

The migration order between sulfathiazole (**1**) and sulfisomidine (**4**) and that between sulfadimethoxine (**10**), sulfamonomethoxine (**9**) and sulfaquinoxaline (**8**) was reversed on addition of higher concentrations of β-CD (1.5–10 mM). No advantages were observed for improving the separation of sulfonamides by increasing β-CD concentration from 4 to 10 mM because peaks between sulfathiazole (**1**) and sulfamethazine (**2**) become unresolvable with higher concentrations of β-CD; peaks between sulfaquinoxaline (**8**) and sulfamonomethoxine (**9**), and those between sulfamethoxazole (**12**) and sulfisoxazole (**13**) are also only partially or barely resolved. As a result, the effectiveness of the separation of thirteen sulfonamides can not be significantly improved by using higher concentrations of β-CD. Fig. 8 illustrates the influence of β-CD on the separation of sulfonamides.

Table 1
Formation constants of inclusion complexes formed between sulfonamides and β-CD at pH 6.85

Peak number	Sulfonamides	K_f	μ_{S-CD}
2	Sulfamethazine	50±3	−0.03
4	Sulfisomidine	60±2	−0.05
1	Sulfathiazole	1100±4	−0.13
3	Sulfamethoxypyridazine	560±4	−0.29
5	Sulfamerazine	270±3	−0.47
6	Sulfameter	260±2	−0.56
7	Sulfadiazine	200±2	−0.70
10	Sulfadimethoxine	180±3	−1.00
9	Sulfamonomethoxine	240±2	−0.96
8	Sulfaquinoxaline	310±3	−0.94
11	Sulfachoropyridazine	190±3	−1.20
12	Sulfamethoxazole	200±3	−1.30
13	Sulfisoxazole	190±3	−1.23

K_f in units of M^{-1}. μ_{S-CD} in units of 10^{-4} cm^2 V^{-1} s^{-1}.

3.4. Analysis of milk samples

Fig. 9 shows the CE analysis of sulfonamide-fortified milk sample, and blank milk sample. As illustrated, a clean background was obtained for blank milk sample, whereas the resolution and migration times of sulfonamide signals were little affected for sulfonamide-fortified milk sample. The results clearly indicate that sulfonamide analysis in milk samples is not much interfered by the matrix of deproteinized milk. Thus the method developed in

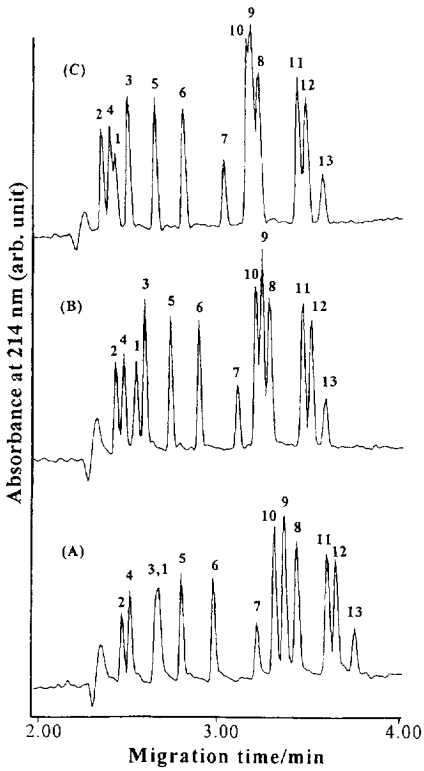

Fig. 8. Electropherograms of sulfonamides obtained with the addition of concentration of β-CD: (A) 0 mM, (B) 0.5 mM and (C) 1.0 mM. Buffer: phosphate (50 mM)–borate (50 mM) at pH 6.85. Operating conditions and peak identification as for Fig. 2.

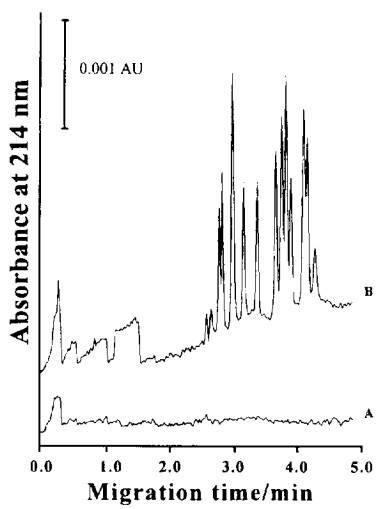

Fig. 9. CE analysis of milk samples; (A) blank milk sample; (B) sulfonamide fortified milk sample. Operation conditions as Fig. 4.

this work is applicable to the analysis of milk samples.

4. Conclusion

The precise optimization of buffer pH is crucial for further improving the separation of thirteen sulfonamides selected. With the addition of an appropriate amount of an organic modifier or a low concentration of β-cyclodextrin (0.5 mM) to the phosphate–borate buffer electrolyte at pH 6.85, effective separations of a mixture of thirteen sulfonamides are successfully achieved within a short time. The present approach is particularly useful in enhancing the separation of sulfamethoxypyridazine and sulfathiazole. Weak inclusion complexations occur between β-CD and sulfonamides, with the exception of sulfathiazole. The separation of sulfonamides was not significantly improved when β-CD with concentration in the range 4–10 mM was added to the buffer solution at pH 6.85.

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References

[1] N. Littlefield, Technical Report, Chronic Toxicity and Carcinogenecity studies of Sulfamethazine in B6CF$_1$ Mice, National Center for Toxicological Research, Jefferson, AR, 1988.
[2] V.K. Agarwal, J. Chromatogr., 624 (1992) 411.
[3] D. Guggisberg, A.E. Mooser and H. Koch, J. Chromatogr., 624 (1992) 425.
[4] E. Martlbauer, E. Usleber, E. Schneider and R. Dietrich, Analyst, 119 (1994) 2543.
[5] F. Salinas and A.E. Mansilla, Anal. Chim. Acta., 223 (1990) 289.
[6] J.J.B. Nevado, J.M.L. Gallego and G.C. Penalvo, Fresenius J. Anal. Chem., 342 (1992) 723.
[7] M.A. Koupparis and P.I. Anagnostopoulou, Anal. Chim. Acta, 204 (1988) 271.

[8] M.T. Tena, M.D. Luque de Castro and M. Valcarcel, Analyst, 119 (1994) 1625.
[9] L.S.G. Van Poucke and G.C.I. Depourcq and C.H. Van Peteghem, J. Chromatogr. Sci., 29 (1991) 423.
[10] G.J. Reimer and A. Suarez, J. Chromatogr., 555 (1991) 315.
[11] S. Kmostak and M. Dvorak, J. Chromatogr., 503 (1990) 260.
[12] J.D. Henion, B.A. Thomson and P.H. Dawson, Anal. Chem., 54 (1982) 451.
[13] W.C. Brumley, Z. Min, J.E. Matusik, J.A.G. Roach, C.J. Barnes, J.A. Sphon and T. Fazio, Anal. Chem., 55 (1983) 1405.
[14] M.M.L. Aerts, W.M.J. Beek and U.A. Th. Brinkman, J. Chromatogr., 435 (1988) 97.
[15] A.R. Long, C.R. Short and S.A. Barker, J. Chromatogr., 502 (1990) 87.
[16] R.F. Cross, J. Chromatogr., 478 (1989) 422.
[17] J. Unruh, E. Piotrowski, D.P. Schwartz and R. Barford, J. Chromatogr., 519 (1990) 179.
[18] J. Wieling, J. Schepers, J. Hempenius, C.K. Mensink and J.H.G. Jonkman, J. Chromatogr., 545 (1991) 101.
[19] R.F. Cross, J.L. Ezzell and B.E. Richter, J. Chromatogr. Sci., 31 (1993) 162.
[20] N. Furusawa and T. Mukai, J. Chromatogr., A, 677 (1994) 81.
[21] N. Takeda and Y. Akiyama, J. Chromatogr., 607 (1992) 31.
[22] S. Pleasance, P. Blay, M.A. Quilliam, and G. O'Hara, J. Chromatogr., 558 (1991) 155.
[23] S. Yang and M.G. Khaledi, J. Chromatogr., A, 692 (1995) 311.
[24] J. Abian, M.I. Churchwell and W.A. Korfmacher, J. Chromatogr., 629 (1993) 267.
[25] S. Porter, Analyst, 119 (1994) 2753.
[26] E.D. Ramsey, J.R. Perkins, D.E. Games, and J.R. Startin, J. Chromatogr., 464 (1989) 353.
[27] S. Schmidt, L.G. Blomberg and E.R. Campbell, Chromatographia, 25 (1988) 775.
[28] J.R. Perkins, D.E. Games, J.R. Startin and J. Gilbert, J. Chromatogr., 540 (1991) 239.
[29] T. Berger, Application Note 228–190, Hewlett-Packard, 1992.
[30] M.T. Ackermans, J.L. Beckers, F.M. Everaerts, H. Hoogland and M.J.H. Tomassen, J. Chromatogr., 596 (1992) 101.
[31] C.L. Ng, H.K. Lee and S.F.Y. Li, J. Chromatogr., 598 (1992) 133.
[32] C.L. Ng, C.P. Ong, H.K. Lee and S.F.Y. Li, J. Microcol. Sep., 5 (1993) 191.
[33] C.L. Ng, H.K. Lee and S.F.Y. Li, J. Chromatogr., 632 (1993) 165.
[34] M.C. Ricci and R.F. Cross, J. Microcol. Sep., 5 (1995) 207.
[35] Q.X. Dang, Z.P. Sun and D.K. Ling, J. Chromatogr., 603 (1992) 259.
[36] I.M. Johansson, R. Pavelka and J.D. Henion, J. Chromatogr., 559 (1991) 515.
[37] F. Foret, L. Křivánková and P. Boček, Capillary Zone Electrophoresis, VCH, Weinheim, 1993.
[38] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Aplications, Elsevier, Amsterdam, 1993.
[39] N. A. Guzman (Editor), Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993.
[40] P. Camilleri (Editor), Capillary Electrophoresis: Theory and Practice, CRC Press, Boca Raton, FL, 1993.
[41] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer-Verlag, New York, 1993.
[42] C.E. Lin, W.C. Lin and W.C. Chiou, J. Chromatogr., A, 705 (1995) 325.
[43] S.C. Smith and M.G. Khaledi, Anal. Chem., 65 (1993) 193.
[44] K.D. Lukacs and J.W. Jorgenson, J. High Resolut. Chromatogr. Commun., 8 (1985) 407.
[45] R.M. McCormick, Anal. Chem., 60 (1988) 2322.
[46] S. Fujiwara, S. Iwase, and S. Honda, J. Chromatogr., 447 (1988) 133.
[47] M.A. Hayes and A.G. Ewing, Anal. Chem., 64 (1992) 512.
[48] G.M. Janini, K.C. Chan, J.A. Barnes, G.M. Muschik and H.J. Issaq, J. Chromatogr. A, 653 (1993) 321.
[49] E.D. Lee, W. Muck, J.D. Henion and T.R. Covey, Biomed. Environ. Mass Spectrom., 18 (1989) 844.
[50] I.M. Johansson, E.C. Huang, J.D. Henion and J. Zweigenbaum, J. Chromatogr., 554 (1991) 311.
[51] G.M. McLaughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison and T.J. Bronzert, J. Liq. Chromatogr., 15 (1992) 961.
[52] C. Schwer and E. Kenndler, Anal. Chem., 63 (1991) 1801.
[53] X. Hung, J.A. Luckey, M.J. Gordon and R.N. Zare, Anal. Chem., 61 (1989) 766.
[54] C.E. Lin, W.C. Chiou and W.C. Lin, J. Chromatogr., A, 722 (1996) 345.
[55] G.M. Jamini, K.C. Chan, J.A. Barnes, G.M. Muschik and H.J. Issaq, Chromatographia, 35 (1993) 497.
[56] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr., 516 (1990) 23.
[57] S. Fanali in N. A. Guzman (Editor), "Capillary Electrophoresis Technology", Marcel Dekker, New York, 1993, p. 731.
[58] S. Terabe, H. Ozaki, K. Otsuka and T. Ando, J. Chromatogr., 332 (1985) 211.
[59] H. Nishi, T. Fukuyama and S. Terabe, J. Chromatogr., 553 (1991) 503.
[60] H. Nishi and S. Terabe, J. Chromatogr. A, 694 (1995) 245.
[61] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 603 (1992) 235.