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Migration behavior and separation of sulfonamides in capillary zone electrophoresis
III. Citrate buffer as a background electrolyte

Ching-Erh Lin*, Chia-Chich Chang, Wei-Chen Lin

Department of Chemistry, National Taiwan University, P.O. Box 23–34, Roosevelt Road Sect. 4, No. 1, Taipei 10764, Taiwan

Abstract

To separate sulfonamides using capillary zone electrophoresis, citrate buffer is superior to phosphate buffer as a background electrolyte. Separation parameters affecting the selectivity and resolution of sulfonamides were optimized. Complete separation of thirteen sulfonamides was rapidly and efficiently achieved within 2.1 min with citrate buffer at pH 6.9. The resolution of peaks between sulfamethoxypyridazine and sulfathiazole, is enhanced either on heating the capillary to 35°C or on adding methanol to the buffer electrolyte in an appropriate proportion. In combination with mobility data obtained at low pH, two pK_a values of each individual sulfonamide are satisfactorily determined. Electrophoretic mobilities of sulfonamides measured at optimum pH of the buffer correlate well with those calculated from Offord's equation. Thus the order of migration of these sulfonamides, reflected in the magnitudes of their electrophoretic mobilities, depends on their ratios of charge to mass, and is primarily determined by their pK_a values.

Keywords: Background electrolyte composition; Sulfonamides; Citrate

1. Introduction

Capillary electrophoresis (CE) is a powerful separation technique and attracts much attention because it provides high resolution, great efficiency, rapid analysis and small consumption of both sample and solvent in comparison with HPLC [1–5]. For this reason, the development of capillary electrophoretic methods to separate diverse analytical samples continues unabated.

Sulfonamides are antibacterial compounds commonly used to prevent and to treat diseases in medical and veterinary practice. The separation and monitoring of these analytes have drawn much attention [6–8]. Among various analytical methods, including GC [9,10], GC–MS [11], HPLC [12–22], HPLC–MS [23], CE [24–32] and CE–MS [30], CE is a sensitive method to separate and to identify sulfonamides.

We previously examined the influence of buffer pH and electrolyte modifier on the migration behavior and separation of thirteen sulfonamides as negatively charged species by capillary zone electrophoresis (CZE) using a phosphate–borate buffer solution [31]. Closely migrating sulfonamides were effectively separated with a phosphate–borate buffer containing an organic modifier (methanol or acetonitrile) or a low concentration of β-cyclodextrin at pH 6.85 and an applied voltage of 20 kV. Peaks for compounds between sulfathiazole and sulfamethoxypyridazine were particularly well resolved.

The tested sulfonamides possess two dissociation

*Corresponding author.

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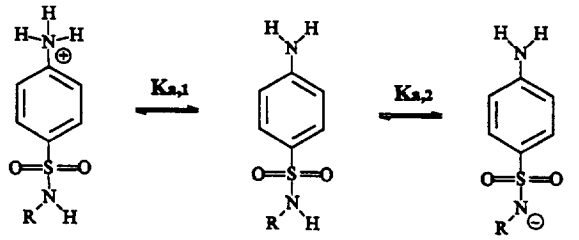

Fig. 1. Dissociation equilibria of sulfonamide involving $pK_{a,1}$ and $pK_{a,2}$.

equilibria. As shown in Fig. 1, $K_{a,1}$ is the dissociation constant for an equilibrium between the positively charged, protonated amino group of sulfonamide and its electrically neutral conjugate base, whereas $K_{a,2}$ refers to an equilibrium involving loss of the sulfonamide proton to yield its negatively charged conjugate. Therefore, depending on the pH of the buffer employed, sulfonamides can be separated by CZE either as negatively charged, deprotonated species [31] or as positively charged, protonated species [33].

We reported that citrate buffer is an excellent background electrolyte for separation of sulfonamides [33] or β-blockers [34] as positively charged species at low pH. It is desirable to investigate separation parameters affecting the migration behavior and separation of sulfonamides as negatively charged species with citrate buffer in the pH range 4.0–8.0 so that comparisons of analysis time and resolution can be made with phosphate–borate buffer. In combination with mobility data measured at low pH [33], the two pK_a values of each individual sulfonamide can be simultaneously and properly determined with the same buffer system. A correlation between the electrophoretic mobility of sulfonamides measured at optimum pH and that calculated from Offord's equation is examined and the factors responsible for the migration order of sulfonamides are studied.

2. Experimental

2.1. Chemicals and reagents

Thirteen sulfonamides are tested in this work (Fig. 2). These compounds originally purchased from Sigma (USA), were supplied as a gift from the Taiwan Meat Development Foundation. Citric acid (Shimakyu, Japan) and trisodium citrate dihydrate (Showa, Japan) were obtained from the indicated suppliers. Methanol of HPLC grade (Mallinckrodt, USA) 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 of about 0.25 m*M* in methanolic solution. The pH of the buffer at a certain concentration in the range 10–60 m*M* was adjusted to a desired pH on mixing appropriate proportions of citric acid and trisodium citrate solutions or a 1 *M* HCl solution. All solutions were filtered through a membrane filter (0.22 μm) before use.

2.2. Apparatus

Separations were made with a capillary electrophoresis system described previously [31]. The capillary dimensions were 43 cm×50 μm I.D.. The UV detection position is 7.0 cm from the cathodic end. Sample injection was done in a hydrodynamic mode during 2 s. 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 precision ±0.01 pH unit.

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed using a standard sequence described previously [34] for 50 min with sodium hydroxide solution (1.0 *M*) at 60°C, followed with sodium hydroxide solution (0.1 *M*) at 60°C for 10 min and with deionized and purified water at 25°C for 10 min.

To ensure reproducibility, all experiments were performed at 25°C, except otherwise indicated, 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 repro-

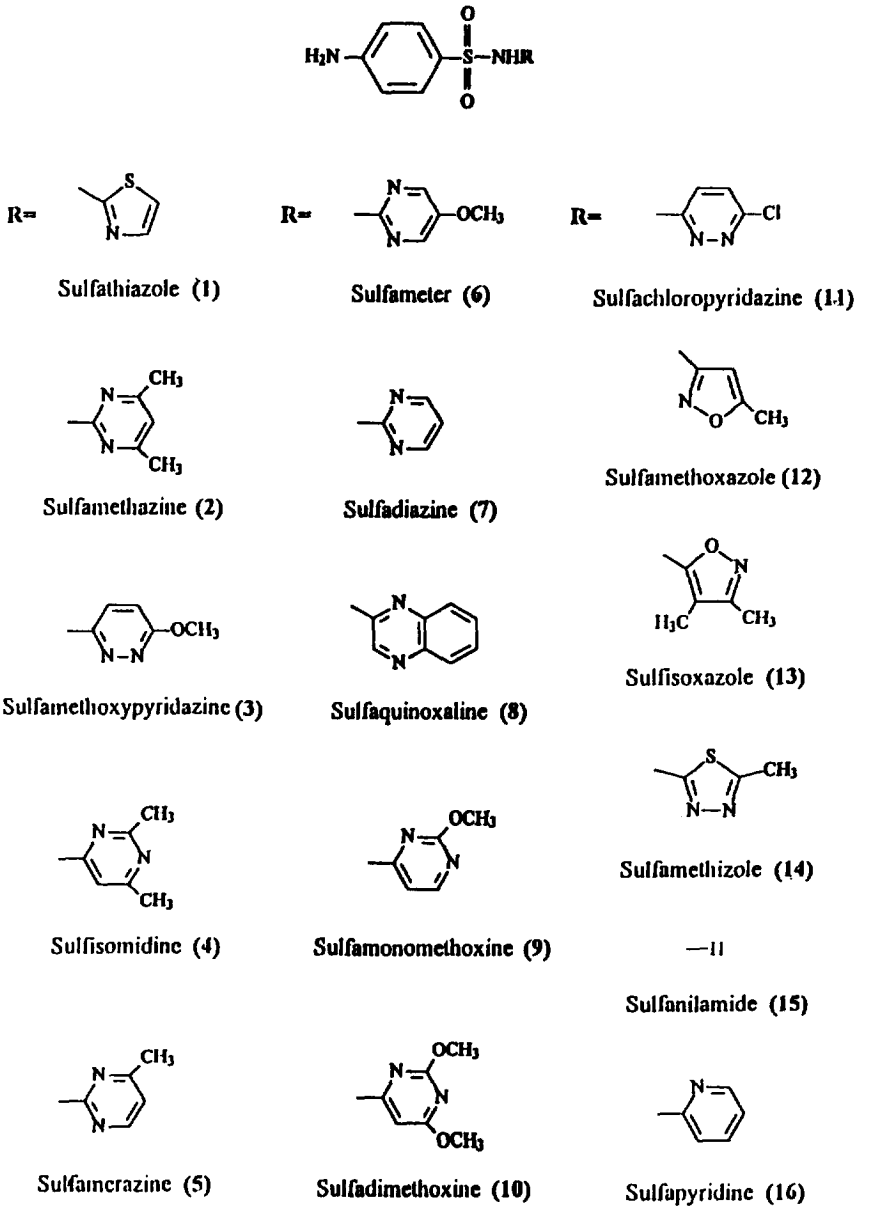

Fig. 2. Structures of sulfonamides.

ducibility for run-to-run injections. The detection wavelength was set at 254 nm.

2.4. Calculations

The electrophoretic mobility of 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.

The net charge of a negatively charged sulfonamide was calculated from the $pK_{a,2}$ value determined in this work with the equation [35]:

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

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

3. Results and discussion

3.1. Optimization of separation parameters

3.1.1. Buffer pH

In CZE, manipulation of buffer pH becomes a key strategy to optimize a separation [34,36]. As illustrated previously [31], buffer pH is a sensitive parameter in the separation of sulfonamides. Thus precise optimization of buffer pH is crucial to improve the separation of closely migrating sulfonamides.

Fig. 3 shows the electrophoretic mobility of sulfonamides obtained at pH varied in the range 5.5–7.3, with citrate buffer (30 m*M*) at 20 kV. The trends observed in the variation of the electrophoretic mobility of sulfonamides as a function of buffer pH were basically similar to those reported previously [31]. The negative electrophoretic mobility (migrating toward the anode) of sulfonamides, with the exception of sulfisoxazole (13), increases with increased pH of the buffer. As illustrated, all thirteen sulfonamides can be separated completely at pH 6.9 without further modification of background electrolyte.

3.1.2. Buffer concentration and applied voltage

Increasing the buffer concentration has a favorable effect on the resolution [33,34,36,37]. Improved separation of sulfonamides was achieved with increased phosphate buffer concentrations from 30 to

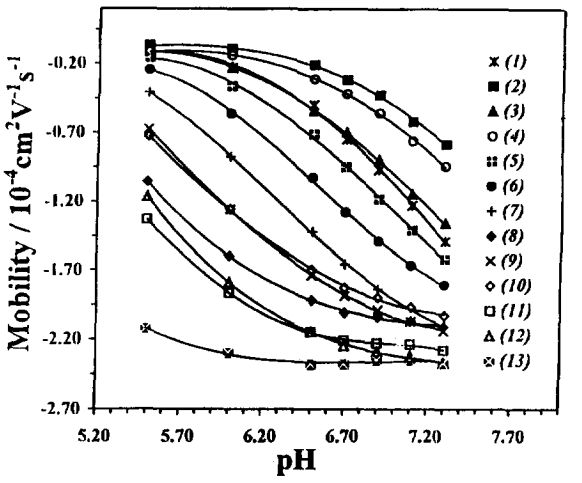

Fig. 3. Electrophoretic mobility of sulfonamides obtained at pH varied in the range 5.5–7.3. Buffer: citrate (30 m*M*). Capillary: 43 cm×50 μm I.D. fused-silica. Operating conditions: 20 kV, 25°C. Peaks: 1 = sulfathiazole; 2 = sulfamethazine; 3 = sulfamethoxypyridazine; 4 = sulfisomidine; 5 = sulfamerazine; 6 = sulfameter; 7 = sulfadiazine; 8 = sulfaquinoxaline; 9 = sulfamonomethoxine; 10 = sulfadimethoxine; 11 = sulfachloropyridazine; 12 = sulfamethoxazole; 13 = sulfisoxazole.

100 m*M* [29]. The electrophoretic mobility of sulfonamides migrating toward the anode increases with increasing concentration of citrate buffer. The pair of peaks between sulfamethoxypyridazine (3) and sulfathiazole (1) that are most affected by increased buffer concentration are completely resolved when the concentration of citrate buffer is increased to 30 m*M* at pH 6.9 and applied voltage 20 kV. This result is not achievable with phosphate–borate buffer [31].

To avoid excessive Joule heating, the buffer concentration is restricted to less than 60 m*M* at 15 kV, or to a smaller concentration at a higher applied voltage. Fig. 4 presents electropherograms of sulfonamides obtained under three optimum conditions. As shown in Fig. 4A, complete separation of thirteen sulfonamides was successfully achieved within 2.1 min. Hence, citrate buffer is superior to phosphate–borate buffer to separate sulfonamides.

3.1.3. Temperature

The separation efficiency and resolution can in some cases be improved by altering the capillary temperature in the separation of a mixture of analytes

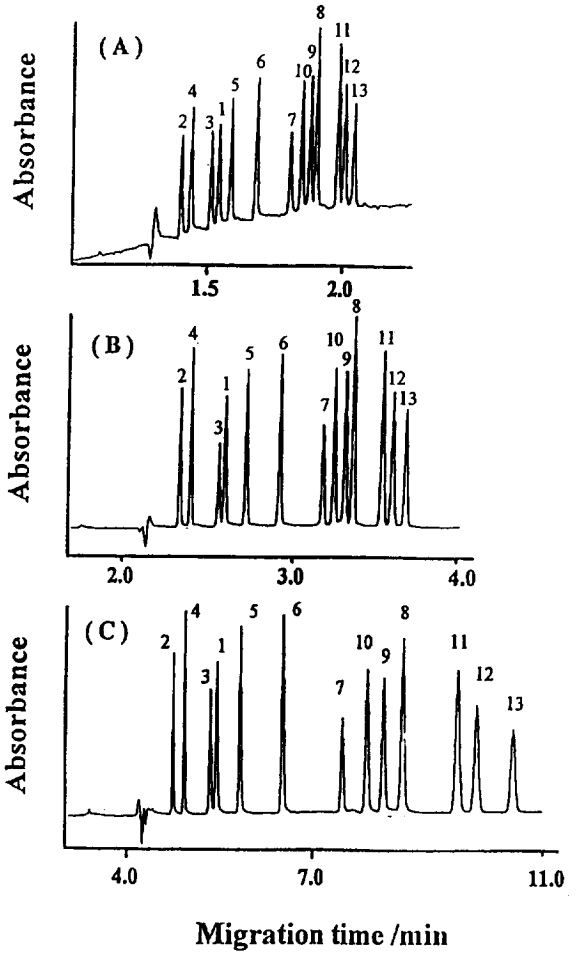

Fig. 4. Electropherograms of sulfonamides obtained at pH 6.9 at varied buffer concentration and applied voltage: (A) 20 m*M*, 30 kV; (B) 30 m*M*, 20 kV and (C) 60 m*M*, 15 kV. Other operating conditions and peak numbering as for Fig. 2.

[36,38]. Fig. 5 shows the effect of temperature on the electrophoretic mobility of sulfonamides at temperature varied in the range 15–45°C. Because viscosity varies with temperature, both electroosmotic flow and electrophoretic mobility of sulfonamides increase with increasing temperature. As the increase of electrophoretic mobility of sulfathiazole (1) is greater than that of sulfamethoxypyridazine (3), the resolution of peaks between sulfamethoxypyridazine (3) and sulfathiazole (1) improves as temperature is increased. However, the resolution of peaks between sulfamonomethoxine (9) and sulfaquinoxaline (8)

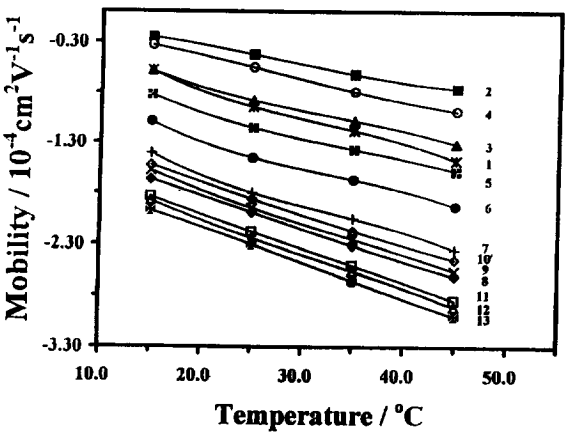

Fig. 5. Electrophoretic mobility of sulfonamides at varied temperature with citrate buffer (30 m*M*) at pH 6.9. Other operating conditions and peak numbering as for Fig. 2.

deteriorates when the capillary temperature exceeds 35°C. Thus, the best resolution of sulfonamides is expected to occur in the temperature range 25–35°C.

3.1.4. Organic modifier

The addition of organic modifiers to the buffer electrolyte in CE serves to enhance the separation and resolution [39–44]. We demonstrated previously [31] that, upon the addition of methanol to phosphate–borate buffer, the resolution of the pair of components (3) and (1) was enhanced considerably.

With the addition of methanol in an appropriate proportion to citrate buffer, a similar effect of organic modifier was observed. Fig. 6 illustrates the separation of sulfonamides with and without addition of methanol (15%, v/v) to the citrate buffer (40 m*M*) at pH 6.9 and applied voltage 15 kV. The resolution of sulfonamides, except two components (9) and (10), is considerably enhanced.

3.2. Determination of pK_a values

Capillary electrophoresis is applied as a convenient method for precise pK_a determination [34,36,45,46]. For an analyte with dissociation equilibria involving dissociation constants, $K_{a,1}$ and $K_{a,2}$,

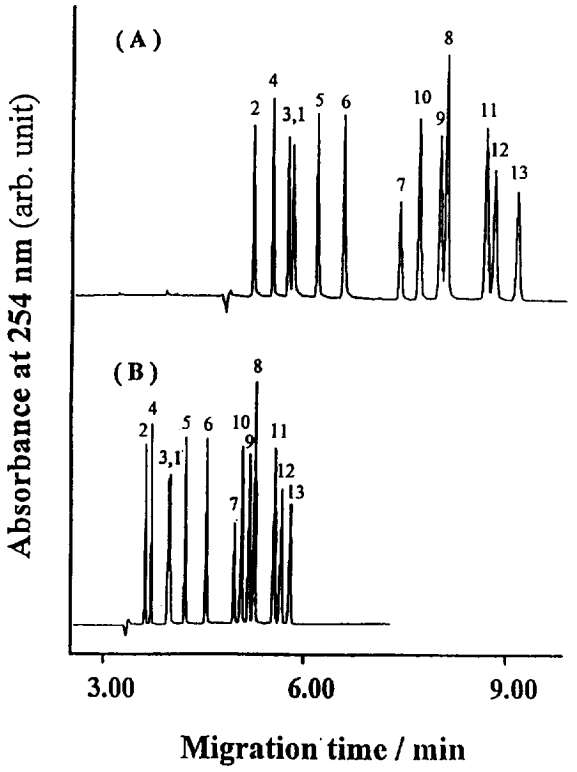

Fig. 6. Electropherograms of sulfonamides obtained with citrate buffer (40 m*M*) as a background electrolyte at pH 6.9 with (A) and without (B) addition of methanol (15%, v/v) to the buffer solution. Other operating conditions and peak numbering as for Fig. 2.

$$H_2A^+ + H_2O \overset{K_{a,1}}{\rightleftharpoons} HA + H_3O^+$$

$$HA + H_2O \overset{K_{a,2}}{\rightleftharpoons} A^- + H_3O^+$$

the electrophoretic mobility of this analyte is given by

$$\mu_{ep} = \left(\frac{[H_3O^+]/K_{a,1}}{C_A}\right)\mu_{H_2A^+} + \left(\frac{K_{a,2}/[H_3O^+]}{C_A}\right)\mu_{A^-} \quad (3)$$

where $C_A = [H_2A^+] + [HA] + [A^-]$ is the analytical concentration of HA and $\mu_{H_2A^+}$ and μ_{A^-} are the limiting electrophoretic mobilities of H_2A^+ and A^- species, respectively. At $pH \leq pK_{a,1} - 2$ at which H_2A^+ is almost the only ionized species, the electrophoretic mobility of the analyte is approximately described with the equation:

$$\mu_{ep} = \frac{[H_3O^+]/K_{a,1}}{1 + [H_3O^+]/K_{a,1}} \cdot \mu_{H_2A^+} \quad (4)$$

At $pH \geq pK_{a,2} + 2$ at which A^- is almost the only ionized species, the electrophoretic mobility of the analyte is approximately given by

$$\mu_{ep} = \frac{K_{a,2}/[H_3O^+]}{1 + K_{a,2}/[H_3O^+]} \cdot \mu_{A^-} \quad (5)$$

In order to determine the two pK_a values of sulfonamides properly, we measured electrophoretic mobilities of sulfonamides over a wide range of pH. With the aid of plots of electrophoretic mobility versus buffer pH, we determined the estimated values of four parameters (i.e., $K_{a,1}$, $K_{a,2}$, $\mu_{H_2A^+}$ and

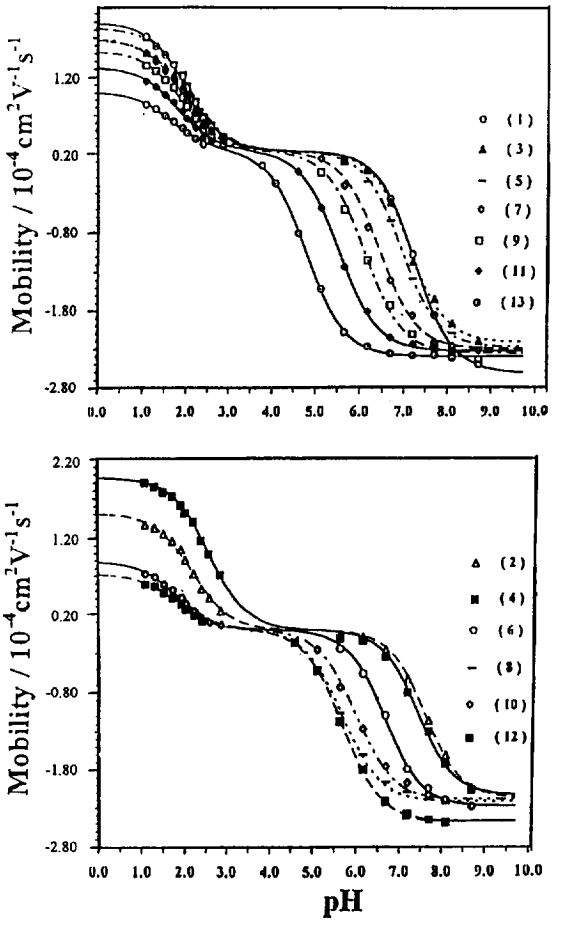

Fig. 7. Plots of electrophoretic mobility of sulfonamides as a function of buffer pH.

μ_{A^-}) involved in Eq. (3). The two pK_a values and the two limiting mobilities of each individual sulfonamide are then determined on fine adjustment of trial values of these four parameters and by curve-fitting the experimental mobility data as a function of buffer pH through the utilization of Excel software until the best fit is obtained. Fig. 7 shows the best fit of mobility curves for these thirteen sulfonamides. The pK_a values and limiting mobility data of these sulfonamides appear in Table 1.

3.3. Migration order

The net charge (q) of sulfonamides at pH 6.9 was calculated with Eq. (2), and Offord's parameter ($q/M^{2/3}$), where M is the molar mass of sulfonamide, was evaluated [47,48]. As shown in Fig. 8, an excellent correlation with coefficient $r^2 = 0.994$ was obtained from plots of electrophoretic mobility against $q/M^{2/3}$ value for these thirteen sulfonamides. As reflected by the magnitudes of their electrophoretic mobilities, these results demonstrate that the migration order of these sulfonamides depends on their ratios of charge to mass. As the net charge of sulfonamide depends on the degree of ionization given by its pK_a and the pH of the buffer [35], these results also indicate that the migration order of sulfonamides is primarily determined by their pK_a values at optimum buffer pH.

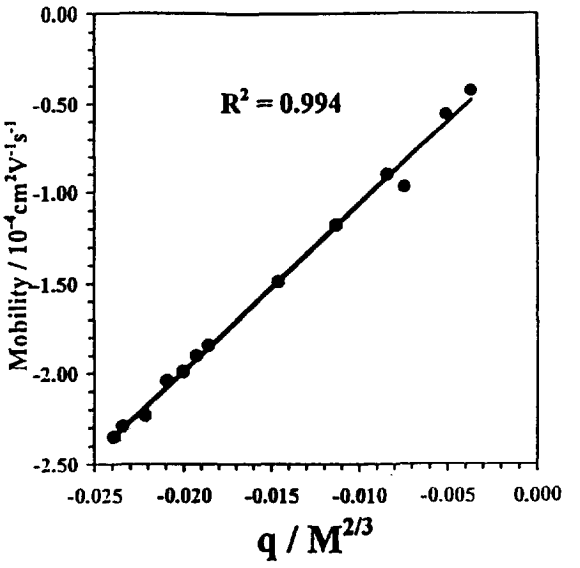

Fig. 8. Correlation of electrophoretic mobility of sulfonamides with Offord's parameter $q/M^{2/3}$.

4. Conclusion

Citrate buffer is superior to phosphate–borate buffer as a background electrolyte to separate sulfonamides with CZE. Thirteen sulfonamides were effectively and rapidly separated under optimum conditions without further modification of the background electrolyte. The effects of temperature and organic modifiers on the resolution of some sul-

Table 1
pK_a values and limiting electrophoresis mobilities of sulfonamides

Sulfonamide	Literature values[a]		p$K_{a,1}$	p$K_{a,2}$	$\mu_{H_2A^+}$[b]	μ_{A^-}[b]
	p$K_{a,1}$	p$K_{a,2}$				
(1) Sulfathiazole	–	7.2	2.08	7.07	1.53	−2.63
(2) Sulfamethazine	2.4	7.4	2.28	7.42	1.50	−2.17
(3) Sulfamethoxypyridazin	–	6.7	2.09	6.95	1.32	−2.25
(4) Sulfisomidine	–	–	2.68	7.26	1.94	−2.13
(5) Sulfamerazine	2.3	7	2.17	6.77	1.46	−2.31
(6) Sulfamete	–	6.8	1.87	6.50	0.96	−2.28
(7) Sulfadiazine	2	6.5	2.10	6.28	1.33	−2.33
(8) Sulfaquinoxaline	–	5.5	1.86	5.56	0.90	−2.21
(9) Sulfamonomethoxine	–	–	1.98	5.96	1.20	−2.39
(10)Sulfadimethoxine	–	6.2	1.87	5.86	0.90	−2.19
(11)Sulfachloropyridazine	–	5.5	1.90	5.40	1.00	−2.36
(12) Sulfamethoxazole	–	5.6	1.83	5.57	0.74	−2.47
(13) Sulfisoxazole	1.5	5.1	1.66	4.71	0.71	−2.43

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

fonamides are noticeable. The pK_a values of sulfonamides are satisfactorily determined. The electrophoretic mobilities of sulfonamides are described with Offord's equation. The results indicate that the migration order of sulfonamides is primarily determined by their pK_a values and depends on their ratios of charge to mass.

Acknowledgments

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References

[1] F. Foret, L. Krivankova and P. Bocek, Capillary Zone Electrophoresis, VCH, Weinheim, 1993.
[2] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Applications, Elsevier, Amsterdam, 1993.
[3] N.A. Guzman (Editor), Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993.
[4] P. Camilleri (Editor), Capillary Electrophoresis: Theory and Practice, CRC Press, Boca Raton, FL, 1993.
[5] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer-Verlag, New York, 1993.
[6] N. Littlefield, Technical Report, Chronic Toxicity and Carcinoginecity studies of Sulfamethazine in B6CF$_1$ Mice, National Center for Toxicological Research, Jefferson, AR, 1988.
[7] V.K. Agenwal, J. Chromatogr., 624 (1992) 411.
[8] D. Guggisberg, A.E. Mooser and H. Koch, J. Chromatogr., 624 (1992) 425.
[9] S. Kmostak and M. Dvorak, J. Chromatogr., 503 (1990) 260.
[10] J.D. Henion, B.A. Thomson and P.H. Dawson, Anal. Chem., 54 (1982) 451.
[11] 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.
[12] M.M.L. Aerts, W.M.J. Beek and U.A.T. Brinkman, J. Chromatogr., 435 (1988) 97.
[13] S.C. Su, A.V. Hartkopf and B.F. Larger, J. Chromatogr., 119 (1976) 523.
[14] R.F. Cross, J. Chromatogr., 478 (1989) 422.
[15] J. Unrch, E. Piotrowski, D.P. Schartz and R. Barford, J. Chromatogr., 519 (1990) 179.
[16] A.R. Long, C.R. Shart and S.A. Barker, J. Chromatogr., 502 (1990) 87.
[17] R.F. Cross, J.L. Ezzell and B.E. Richter, J. Chromatogr. Sci., 31 (1993) 162.
[18] N. Furusawa and T. Mukai, J. Chromatogr. A, 677 (1994) 81.
[19] N. Takeda and Y. Akiyama, J. Chromatogr., 607 (1992) 31.
[20] J.R. Perkins, C.E. Parker and K.B. Tomer, J. Am. Soc. Mass Spectrom., 3 (1992) 139.
[21] S. Yang and M.G. Khaledi, J. Chromatogr. A, 692 (1995) 311.
[22] J. Abian, M.I. Churchwell and W.A. Korfmacher, J. Chromatogr. A, 629 (1993) 267.
[23] S. Porter, Analyst, 119 (1994) 2753.
[24] M.T. Ackermans, J.L. Beckers, F.M. Everaerts, H. Hoogland and M.J.H. Tomassen, J. Chromatogr., 596 (1992) 101.
[25] C.L. Ng, H.K. Lee and S.F. Li, J. Chromatogr., 598 (1992) 133.
[26] C.L. Ng, C.P. Ong, H.K. Lee and S.F.Y. Li, J. Microcol. Sep., 5 (1993) 191.
[27] C.L. Ng, H.K. Lee and S.F. Li, J. Chromatogr., 632 (1993) 165.
[28] Q.X. Dang, Z.P. Sun and D.K. Ling, J. Chromatogr., 603 (1992) 259.
[29] M.C. Ricci and R.F. Cross, J. Microcol. Sep., 5 (1993) 207.
[30] R.F. Cross and M.C. Ricci, LC·GC Int., 8 (1995) 399.
[31] C.E. Lin, W.C. Lin, W.C. Chiou, E.C. Lin and C.C. Chang, J. Chromatogr. A, 755 (1996) 261.
[32] I.M. Johansson, R. Pavellka and J.D. Henion, J.Chromatogr., 559 (1991) 515.
[33] C.E. Lin, C.C. Chang and W.C. Lin, J. Chromatogr. A, (1997) in press.
[34] C.E. Lin, C.C. Chang, W.C. Lin and E.C. Lin, J. Chromatogr. A, 753 (1996) 133.
[35] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer-Verlag, New York, 1993, p. 77.
[36] S.C. Smith and M.G. Khaledi, Anal. Chem., 65 (1993) 193.
[37] H.T. Rasmussen and H.M. McNair, J. Chromatogr., 516 (1990) 223.
[38] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann and H.M. Widmer, Anal. Chem., 63 (1991) 1541.
[39] E.D. Lee, W. Muck, J.D. Henion and T.R. Covey, Biomed. Environ. Mass Spectrom., 18 (1989) 844.
[40] I.M. Johansson, E.C. Hung, J.D. Henion and J. Zweigenbaum, J. Chromatogr., 554 (1991) 311.
[41] G.M. McLaughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Mossis, J.A. Morrison and T.J. Bronzert, J. Liq. Chromatogr., 15 (1992) 961.
[42] C. Schwer and E. Kenndler, Anal. Chem., 63 (1991) 1801.
[43] X. Hung, J.A. Luckey, M.J. Gordon and R.N. Zare, Anal. Chem., 61 (1987) 766.
[44] C.E. Lin, W.C. Chiou and W.C. Lin, J. Chromatogr. A, 722 (1996) 345.
[45] J. Cai, J.T. Smith and Z. El Rassi, J. High Resolut. Chromatogr., 15 (1992) 30.
[46] J.A. Cleveland, Jr., M.H. Benko, S.J. Gluck and Y.M. Walbroehl, J. Chromatogr. A, 652 (1993) 301.
[47] R.E. Offord, Nature, 5049 (1966) 591.
[48] E.C. Rickard, M.M. Strohl and R.G. Nielsen, Anal. Biochem., 197 (1991) 197.