Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1033 (2004) 161–166
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Preparation of a sulfonated fused-silica capillary and its application in capillary electrophoresis and electrochromatography

Li Xu, Yu-Qi Feng*, Zhi-Guo Shi, Shi-Lu Da, Fang Wei

Department of Chemistry, Wuhan University, Wuhan 430072, China

Received 23 September 2003; received in revised form 9 December 2003; accepted 20 January 2004

Abstract

In the present paper, two new methods, sol–gel and chemical bonding methods, were proposed for preparation of sulfonated fused-silica capillaries. In the sol–gel method, a fused-silica capillary was coated with the sol solution obtained by hydrolysis of 3-mercaptopropyltrimethoxysilane (MPTS) and tetramethoxysilane, and followed by age; while in the chemical bonding method, a capillary was chemically bonded directly with MPTS. Then, both the resulting capillaries were oxidized with an aqueous solution of hydrogen peroxide solution (H_2O_2) (30%, m/m) to obtain the sulfonated capillaries. The electroosmotic flow (EOF) for the sulfonated capillaries was found to remain almost constant within the studied pH range, and greater than that of the uncoated capillary. However, the coating efficiency of the capillary prepared by chemical bonding method was higher than that by sol–gel method, by comparing their magnitude of the EOF, the degree of disguise of the silanol and reproducibility of preparation procedure. The effects of the electrolyte's concentration and the content of methanol (MeOH) on the EOF were also studied. Especially, the study of the apparent pH (pH*) on the EOF in a water–MeOH system was reported. Finally, capillary electrophoretic separation of seven organic acids was achieved within 6.5 min under optimal condition using the chemically bonded sulfonated capillary. Moreover, separation of four alkaloids on the sulfonated capillary was compared with that on uncoated capillary in different conditions. Ion-exchange mechanism was found to play a key role for separation of these four basic analytes on the sulfonated capillary.

Keywords: Capillary columns; Coated columns; Electroosmotic flow; Electrochromatography, ion-exchange; Sol–gel; Berberine; Jateorrhizine; Topotecan; Caffeine

1. Introduction

Capillary electrophoresis (CE) has been extensively used in the separation of charged compounds [1–5]. Usually, successful CE separations require one to carefully control and reproduce the electroosmostic flow (EOF) in the capillary, such as using external electric field [6–8]. In addition, the use of capillary coatings in CE to manipulate the EOF has generated much interest. Currently, ionic and nonionic coatings have been applied to obtain the requisite EOF. For example, nonionic coatings are generally used to suppress the EOF and analyte–wall adsorption, while positive coatings are used to reverse the EOF and negative coatings to increase the cathodic EOF [9–17].

CE separations of basic compounds have been widely reported. But it is difficult to analyze strong acids on a bare fused-silica capillary because the direction of the EOF and the electrophoretic velocity are generally opposite and, in most cases, the EOF is slower than the analytes' electrophoretic migration. Consequently, some of the anions will never reach the point of detection [18]. This problem can be overcome by following methods. First, the EOF is reversed by the addition of a special surfactant to the electrolyte buffer [19]; or the capillary containing positive coating is employed [20]. In this way, the analysis of organic acids can be obtained in a coelectroosmotic mode. Second, at certain pH some acids have sufficient electrophoretic mobility to overcome the EOF, thus separations can be achieved in the counter-EOF mode [18]. Additionally, if the EOF is larger enough than electrophoretic mobilities, analysis could be succeeded in a counter-electroosmotic mode. Sulfonated capillary seems to satisfy the requirement [21,22]. Sulfonic acid is a strong acid with pK_a ($pK_a = 0.699$) less than 2. When buffer pH is greater than 2, the sulfonic acid is totally ionized, leading to a stable charge density and thus, nearly constant EOF velocity which is independent of the buffer

* Corresponding author. Tel.: +86-27-87867564; fax: +86-27-87647617.
E-mail address: yqfeng@public.wh.hb.cn (Y.-Q. Feng).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.01.020

pH. Especially at lower pH, the EOF is much larger than that of the bare capillary while the ionization of organic acids is restrained to some extent. In the case, adjustment of buffer pH can be used to control the dissociation of organic acids but has no effect on the EOF. So the EOF can be larger than the mobilities of organic acids when pH is lowered to some extent. Thus, a sulfonated capillary is a preferable capillary to separate organic acids. Furthermore, due to the anion nature of sulfonic acid, it can also be used for the separation of basic compounds in the open-tubular mode of ion-exchange capillary electrochromatography (IE-OT-CEC).

Currently, several studies on sulfonated capillaries have been reported and the capillaries are also commercially available [21,22]. Moreover, the application of sulfonic acid moiety in capillary electrochromatography to reproduce enough EOF was reported [23,24]. But all these are related to polymer coatings. Though polymer coatings may increase the double layer viscosity at the silica support, which reduces the variance of the EOF caused by SiO^- groups and minimizes zone broadening, however, the presence of some organic solvents in the running buffer may be detrimental to the efficiency and stability of the coating due to the swelling of the polymer layer. To overcome the disadvantages of the polymer coating, in this paper we put forward a new way to obtain sulfonated inorganic coating. Taking 3-mercaptopropyltrimethoxysilane (MPTS) as the sulfonic acid group source, sol–gel or chemical bonding methods can be applied [25,26]. EOF characteristics were evaluated as a function of pH, the amount of organic modifier and electrolyte's concentration. At last, successful separations of aromatic acids in the capillary zone electrophoresis (CZE) mode and alkaloids in IE-OT-CEC mode were achieved under optimized experimental conditions.

2. Experimental

2.1. Reagents and materials

Fused-silica capillaries of 50 μm i.d. × 365 μm o.d. were obtained from Hebei Yongnian Optical Fiber Factory (Hebei, China). Tetramethoxysilane (TMOS), MPTS, sodium hydroxide, hydrochloric acid (HCl), toluene, acetic acid, disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide solution (H_2O_2, 30%, m/m), methanol (MeOH), ethanol, *p*-aminobenzoic acid (*p*-ABA), benzoic acid (BA), *p*-nitrobenzoic acid (*p*-NO_2-BA), *m*-nitrobenzoic acid (*m*-NO_2-BA), *o*-chlorobenzoic acid (*o*-Cl-BA), *o*-bromobenzoic acid (*o*-Br-BA), 3,5-nitrobenzoic acid (3,5-NO_2-BA) and thiourea were purchased from Shanghai General Chemical Reagent Factory (Shanghai, China). Berberine, jateorrhizine, topotecan and caffeine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (NICPBP, Beijing, China). Distilled water was from a quartz apparatus. Thiourea was used as EOF marker.

2.2. Instrumentation

CE experiments were carried out at room temperature with NT 1229 HPCE from Beijing Institute of New Technology Application (Beijing, China), which consisted of a ±30 kV high voltage power supply and a UV detector. Data collection and manipulation were carried out on an N-2000 chromatographic workstation from Zhejiang University (Zhejiang, China). The pH (or pH*, the apparent pH) values of the background electrolyte (BGE) solutions were measured with a Delta 320-S pH Meter from Mettler Toledo Instruments (Shanghai, China).

2.3. Preparation of sulfonated capillaries

Fused-silica capillaries were activated at ambient temperature by rinsing sequentially with 1 mol l^{-1} sodium hydroxide for 2 h, water for 30 min, 1 mol l^{-1} HCl for 2 h and water for 30 min. After connecting with nitrogen regulator, capillaries were placed in a GC oven at 433 K and purged with nitrogen for 4 h.

2.3.1. Chemical bonding method

One milliliter of MPTS and 3 ml of anhydrous toluene were mixed and introduced into the pre-treated capillary by a syringe. After being rinsed for 2 h, the capillary was sealed and placed in an oven at 378 K. Ten hours later, the capillary was taken out and flushed with toluene and then MeOH. Finally, it was flushed with H_2O_2 (30%, m/m) for 24 h and then washed with H_2O. The sulfonated capillary thus obtained was referred to as capillary A.

2.3.2. Sol–gel method

One milliliter of TMOS was dissolved in 8 ml mixture of ethanol–aqueous acetic acid water (pH 4) (3:1, v/v). After added 0.25 g MPTS, the solution was stirred at ice bath for 30 min, leading to the formation of silica sol. The sol was introduced into the pre-treated capillary by a syringe and was kept in situ for 1 h. Subsequently, the capillary was connected to a nitrogen regulator, and the extra sol solution was removed by passing nitrogen through the capillary under 1 bar for 10 min and then under 0.2 bar for 60 min. Afterwards, the capillary was heated at 393 K for 8 h in a muffle furnace, leading to the formation of mercapto coating. Finally, the capillary was flushed with H_2O_2 (30%, m/m) for 24 h and then washed with water. The sulfonated capillary obtained in this way was referred to as capillary B.

3. Results and discussion

3.1. Characterization of the coating efficiency

Because of the extremely tiny structure of capillaries, the traditional physical and chemical methods are not suitable for their characterization. An effective way to measure

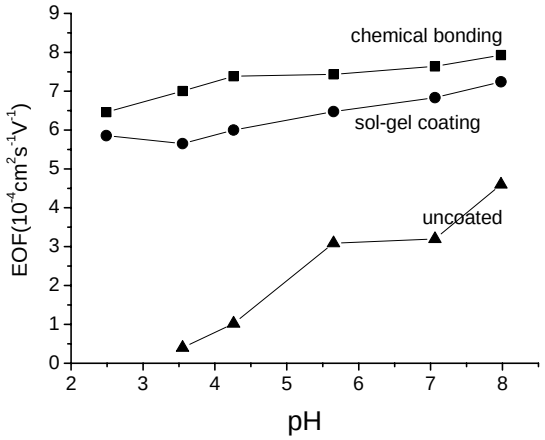

Fig. 1. Comparison of EOF mobilities obtained on sulfonated capillaries prepared from chemical bonding and sol–gel methods and uncoated capillary in aqueous BGEs. Electrolyte: 5 mM Na_2HPO_4 + HCl; applied voltage: 20 kV.

changes on the inner wall surface of the capillary induced by chemical reactions is the determination of the magnitude and the direction of the EOF, which is generally viewed as the major characteristic property of the coating [27]. Based on the method, changes of the charge density and charge type on the surface can be determined. Because of the possible breaking of Si–O–Si at high pH and Si–C at low pH, phosphate buffers in the pH range from 2.5 to 8 were used exclusively in the present study.

In Fig. 1, the EOF–pH curves of the uncoated fused-silica capillary and the sulfonated capillaries, prepared from different methods, are compared. Both capillaries with sulfonated coatings show larger EOF than the uncoated capillary at studied pH range. It indicates that sulfonic acid group has been successfully grafted onto the capillary wall, whether by chemical bonding or by sol–gel method. It can also be found that the two sulfonated capillaries show relatively constant EOF over a wide pH range, and the EOF of capillary A is more stable than that of capillary B. Furthermore, in comparison to capillary B, the magnitude of the EOF for capillary A is larger at the same pH. These results suggest that the coating procedure by the chemical bonding method is more efficient. It can also be seen from Fig. 1 that the EOF in the both sulfonated capillaries increases with increasing pH especially in higher pH range. This result can be ascribed to an incomplete coverage of the capillary surface and an exposure of some unreacted silanols. Because the residual silanols can deprotonate and the degree of deprotonation increases with pH increasing especially in high pH range, which can increase the negative charge on the inner wall surface, leading to the increase of the EOF.

The reproducibility for the capillaries obtained by the two proposed methods was investigated by measurement of EOF. Capillaries from column-to-column and batch-to-batch were underwent EOF measurement using 5 mM phosphate of pH 3.55 as buffer solution. As for the chemical bonding method, the variation of the EOF mobilities altered between 7.0×10^{-4} and 7.3×10^{-4} cm^2 V^{-1} s^{-1} with a 2.0% R.S.D. ($n = 3$), while for sol–gel method, the variation of the EOF mobilities changed from 5.6×10^{-4} and 5.9×10^{-4} cm^2 V^{-1} s^{-1} with a 3.1% R.S.D. ($n = 3$).

Stability of the sulfonated capillaries was also examined. Capillary A was chosen for the experiment due to its relatively large EOF at studied pH range. Reproducibility tests were performed to determine both intra-day and inter-day variation in migration times. The R.S.D. ($n = 4$) of EOF velocities for intra-day and inter-day was found to be 1.9 and 5.2%, respectively. Considering the small dimension size of the capillary columns, the variation is reasonable and can be accepted. Therefore, capillary A was chosen for the following study.

3.2. The EOF characteristic

The effect of MeOH content and the electrolyte's concentration (Na_2HPO_4) on the EOF were also studied. In agreement with previous reports [21,22], the EOF decreases as the MeOH content or the electrolyte's concentration increases.

Organic modifiers are generally different from water in their viscosity, dielectric constant, electrical and thermal conductivity, self-dissociation constant, polarity and boiling point etc. Naturally, these differences will influence the EOF and the separation efficiency directly or indirectly [28]. So here, particular emphasis is laid on the EOF characteristic of the sulfonated capillary in hydro-organic mixture solvent. MeOH, a commonly used organic modifier, was used in our investigation.

At the same pH (pH*), the EOF in the binary system is lower than that of aqueous system, which may be ascribed to the suppression of ionization of the sulfonic acid in the presence of organic solvent [29]. It can also be found, whether in aqueous system or in binary system, the EOF keeps almost constant. This result indicates that ionization of sulfonic acid is also independent of the buffer pH in hydro-organic system. Therefore, hydro-organic system can be utilized in sulfonated capillary to improve solubility of analytes and their selectivity, while it keeps the constant EOF at 2–8 pH range.

3.3. Separation of organic acids in CZE mode

Fig. 2 shows an electropherogram for the separation of a seven-component mixture of benzoic acid derivatives on capillary A. It can be seen from Fig. 2 that the benzoic acids except BA and *p*-NO_2-BA are baseline separated.

The organic solvent content, pH and electrolyte's concentration in the buffers are effective parameters influencing the migration time, the resolution and the analysis time of analytes [20,21]. Experiments demonstrate that the organic solvent restrains the ionization of sulfonic acid more than benzoic acids. When the methanol content increases up to 70%, the electrophoretic velocities of several analytes are greater than the EOF, but with opposite direction. And then

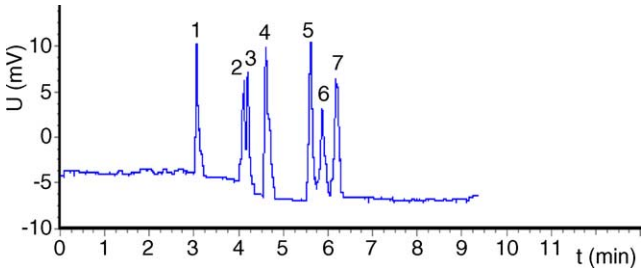

Fig. 2. Separation of benzoic acids on the sulfonated capillary. Conditions: electrolyte, 15 mM Na_2HPO_4 + HCl, pH 2.5. Capillary: 48.3 cm; effective length: 36.8 cm; applied voltage: 20 kV; injection: 5 kV for 2 s; sample concentration at 1 mM; UV detection 214 nm. Samples: (1) *p*-ABA; (2) BA; (3) *p*-NO_2-BA; (4) *m*-NO_2-BA; (5) *o*-Cl-BA; (6) *o*-Br-BA; and (7) 3,5-NO_2-BA.

they cannot migrate out. Furthermore, separation efficiency decreases with increasing methanol content.

The buffer pH was also investigated for the separation of aromatic acids. At pH 4.13, 3,5-NO_2-BA cannot come out; while at pH 3.55, several analytes cannot be baseline separated. Electrolyte's concentration control is generally used to improve resolution in CZE separation of analytes that have similar electrophoretic mobilities. Therefore, disodium hydrogen phosphate concentrations at 5, 10, 15 and 20 mM were investigated for the separation of analytes. Fifteen mM was found to be the most efficient for the separation.

3.4. Separation of alkaloids in IE-OT-CEC mode

Alkaloids are an important class of pharmacologically active compounds in herbal medicines. It is of crucial importance to develop efficient methods for the analysis of these compounds. As basic compounds, alkaloids possess positive charges under acidic conditions, which could provide favorable interaction with negative charged sulfonic groups. Therefore, we attempted to separate alkaloids on the sulfonated capillary in IE-OT-CEC mode. The structures of studied alkaloids are shown in Fig. 3.

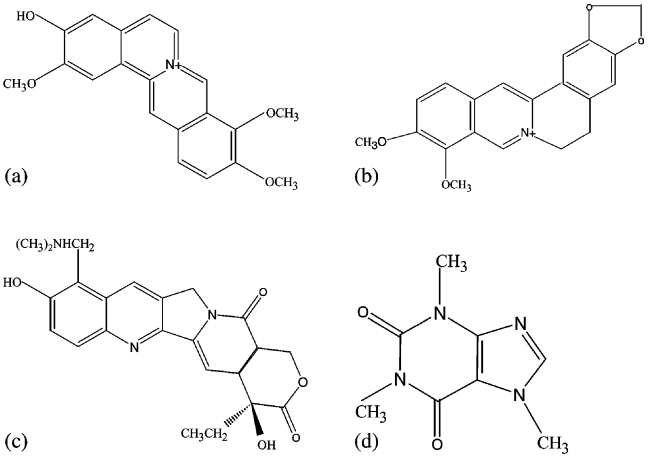

Fig. 3. Structures of alkaloids. (a) jateorrhizine; (b) berberine; (c) topotecan; and (d) caffeine.

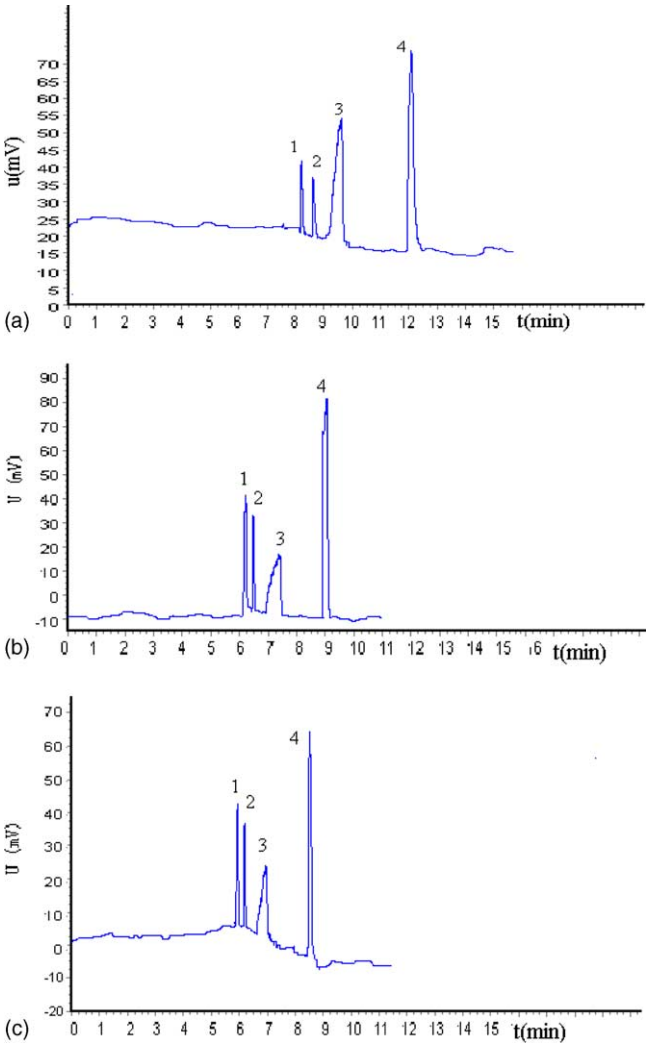

Fig. 4. Separation of alkaloids under different conditions on the sulfonated capillary. Conditions: electrolyte, 5 mM Na_2HPO_4 + HCl; solvent, MeOH–water (50:50): (a) pH* 2.46; (b) pH* 3.49; and (c) pH* 4.13; capillary: 62 cm; effective length: 50 cm; applied voltage: 20 kV; injection: 5 kV for 6 s; sample concentration at 2 mM; UV detection 214 nm. Samples: (1) berberine (2) jateorrhizine (3) topotecan (4) caffeine.

Fig. 4 depicts the separation of four alkaloids, berberine, jateorrhizine, topotecan and caffeine at pH* 2.52, 3.49 and 4.13 in the hydro–MeOH system as discussed in Section 3.2 for the sulfonated capillary. Obviously, The four components are easily separated under above three conditions, the peak symmetries are good and no obvious peak tailing is observed except topotecan. In order to make sure if ion-exchange behavior occurs, we performed the separation of the analytes on a conventional uncoated capillary with the same buffers. Because of serious peak tailing, the separation cannot be accomplished in the bare fused silica capillary. Instead, we list electrophoretic mobilities of four analytes under above three conditions on the uncoated and sulfonated capillaries in Table 1. Though the migration time of the four analytes on the uncoated capillary is larger than that on the sulfonated

Table 1
Comparison of electrophoretic mobilities (10^{-4} cm^2 V^{-1} s^{-1}) on sulfonated and uncoated capillaries under different conditions

pH*	Capillary	Berberine	Jateorrhizine	Topotecan	Caffeine
2.52	Uncoated	1.9	1.2	1.5	–
	Sulfonated	0.96	0.92	−0.025	−0.057
3.49	Uncoated	1.9	1.8	1.6	–
	Sulfonated	0.98	0.79	0.38	−0.34
4.13	Uncoated	2.0	1.6	1.6	–
	Sulfonated	1.4	1.0	0.95	−0.049

(–) shows the ananlyte cannot be detected.

capillary, caffeine could not come out under these conditions on the uncoated capillary. The electrophoretic mobilities, deducted the electroosmotic mobilities from the apparent mobilities, on the uncoated capillary are larger than those on the sulfonated capillaries without exception, indicating ion-exchange behavior occurs. Especially at pH 2.52, there is a striking difference in selectivity; jateorrhizine eluted after topotecan on the uncoated capillary, while with the sulfonated capillary, jateorrhizine eluted before topotecan, which further demonstrates an ion exchange mechanism contributing to the separation. It is surprising that the efficiency obtained in IE-OT-CEC is so much greater than that for CZE. Li et al. [30] attributed this phenomenon to a chromatofocusing, in which the analytes are highly retained in a fine band at the head of the column, and which is responsible for boosting the efficiency to such a high value.

To eliminate the peak tailing of topotecan, the binary system with higher content of MeOH was used. Fig. 5 depicts the electropherogram of separation of four alkaloids at pH 2.52 with 70% MeOH. Clearly, the four analytes are baseline separated, peak tailing of topotecan is completely eliminated and an excellent resolution is achieved.

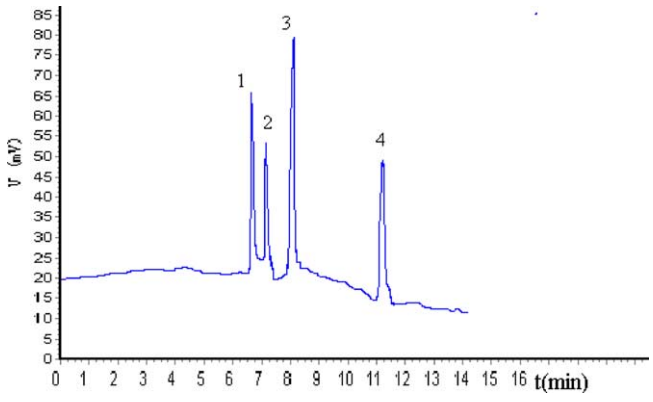

Fig. 5. Separation of alkaloids on the sulfonated capillary under optimal conditions. Conditions: electrolyte, 5 mM Na_2HPO_4 + HCl; solvent, MeOH–water (70:30), pH* 2.46. Other conditions as in Fig. 4. Samples: (1) berberine; (2) jateorrhizine; (3) topotecan; and (4) caffeine.

4. Conclusion

In this paper, capillaries with a surface sulfonated layer were successfully prepared by sol–gel and chemical bonding methods. The comparison between these two methods demonstrated that the capillaries prepared by chemical bonding method showed higher efficiency than those prepared by sol–gel method. The EOF of the sulfonated capillary in a hydro–MeOH binary system was independent of the buffer pH, as that in aqueous system. At last, applications of the sulfonated capillary in CZE mode and CEC mode were demonstrated. The separation of aromatic acids in CZE mode under optimal condition was given. Comparing the electrophoretic mobilities of the studied alkaloids on the sulfonated capillary with those on the uncoated capillary, cation-exchange interactions between the cationic analytes and the anionic sulfonated stationary were found to be the predominant mechanism.

Acknowledgements

The authors gratefully acknowledge the support of the National Nature Science Foundation of China (Grant: 20275029) and the Excellent Young Teachers Program of MOE, China.

References

[1] S. Terabe, M.J. Markuszewski, N. Inoue, K. Otsuka, T. Nishioka, Pure Appl. Chem. 73 (2001) 1563.
[2] S. Hu, N.J. Dovichi, Anal. Chem. 74 (2002) 2833.
[3] M.G. Khaledi (Ed.), High-Performance Capillary Electrophoresis: Theory, Techniques, and Applications, Wiley, New York, 1998.
[4] P.G. Righetti (Ed.), Capillary Electrophoresis in Analytical Biotechnology (CRC Series in Analytical Biotechnology), CRC Press, Boca Raton, FL, 1996.
[5] V. Kaika, Electrophoresis 22 (2001) 4139.
[6] Y. Chen, Y. Zhu, Electrophoresis 20 (1999) 1817.
[7] V. Kasicka, Z. Prusik, P. Sazelova, M. Chiari, I. Miksik, Z. Deyl, J. Chromatogr. B 741 (2000) 43.
[8] N.K. Hartley, M.A. Hayes, Anal. Chem. 74 (2002) 1249.
[9] J. Horvath, V. Dolnik, Electrophoresis 22 (2001) 644.
[10] P.G. Righetti, C. Gelfi, B. Verzola, L. Castelletti, Electrophoresis 22 (2001) 603.
[11] C. Finkler, H. Charrel, H. Engelhardt, J. Chromatogr. A 822 (1998) 101.
[12] B.A. Musial, M.N. Martin, N.D. Danielson, J. Sep. Sci. 25 (2002) 311.
[13] M. Chiari, M. Cretich, F. Damin, L. Ceriotti, R. Consonni, Electrophoresis 21 (2000) 909.
[14] Y.Y. Hsieh, Y.H. Lin, J.S. Yang, G.T. Wei, P. Tien, L.K. Chau, J. Chromatogr. A 952 (2002) 255.
[15] W. Jiang, J.N. Awasum, K. Irgum, Anal. Chem. 75 (2003) 2768.
[16] J. Charvatova, V. Kasicka, Z. Deyl, V. Kral, J. Chromatogr. A 990 (2003) 111.
[17] M.W. Kamande, C.P. Kapnissi, X.F. Zhu, C. Akbay, I.M. Warner, Electrophoresis 24 (2003) 945.
[18] J. Senior, D. Rolland, D. Tolson, C. Sotiri, V.D. Biasi, J. Pharm. Biol. Med. Anal. 22 (2000) 413.

[19] T. Soga, A.G. Ross, J. Chromatogr. A 837 (1999) 231.
[20] M. Chiari, L. Ceriotti, G. Crini, M. Morcellet, J. Chromatogr. A 836 (1999) 81.
[21] E. Minnoor, Y. Liu, D.J. Pietrzyk, J. Chromatogr. A 884 (2000) 297.
[22] Y. Liu, D.J. Pietrzyk, J. Chromatogr. A 804 (1998) 337.
[23] A.V. Pirogov, W. Buchberger, J. Chromatogr. A 916 (2001) 51.
[24] Q.L. Tang, M.L. Lee, J. Chromatogr. A 887 (2000) 265.
[25] D. Margolese, J.A. Melero, S.C. Christiansen, B.F. Chmelka, G.D. Stucky, Chem. Mater. 12 (2000) 2448.
[26] W.M. Van Rhijn, D.E. De Vos, B.F. Sels, W.D. Bossaert, P.A. Jacobs, Chem. Commun. (1998) 317.
[27] C.-Y. Liu, Electrophoresis 22 (2001) 612.
[28] S. Morales, R. Cela, J. Chromatogr. A 896 (2000) 95.
[29] P.B. Wright, A.S. Listeo, J.G. Dorsey, Anal. Chem. 69 (1997) 3251.
[30] D. Li, H.H. Knobel, T.V. Remcho, J. Chromatogr. B 695 (1997) 169.