

Nonaqueous capillary electrophoresis using a zirconia-coated capillary

Li Xu, Yu-Qi Feng*, Zhi-Guo Shi, Shi-Lu Da, Jia-Ming Gu

Department of Chemistry, Wuhan University, Wuhan 430072, PR China

Received 3 February 2004; received in revised form 30 March 2004; accepted 30 March 2004

Abstract

A zirconia-coated capillary was prepared by sol–gel technique. Its electroosmotic flow (EOF) properties were investigated in a variety of nonaqueous media (methanol and mixtures of methanol and acetonitrile). The zirconia-coated capillary exhibited the switchable EOF whose direction and magnitude were strongly dependent on the properties of nonaqueous solvent composition, their apparent pHs (pH^*), and the electrolytes (tris(hydroxymethyl) aminomethane and ammonium acetate). It was found that the isoelectric point (pI) of the zirconia-coated capillary moved to higher pH^* with increasing acetonitrile content from 0 to 75% (v/v) when tris(hydroxymethyl) aminomethane was used as the electrolyte. The nonaqueous capillary electrophoresis (NACE) with the positively charged zirconia-coated capillary was investigated for separations of several alkaloids. The effect of changing the binary solvent composition on the separation was also studied. Finally, the separation between coated and uncoated capillaries in nonaqueous media was compared.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Zirconia; Nonaqueous capillary electrophoresis; Alkaloid

1. Introduction

Nonaqueous capillary electrophoresis (NACE), capillary electrophoresis with only organic solvents as the background electrolyte (BGE) solvents, has become a widely used separation technique since early 1990s. The use of nonaqueous media in the capillary electrophoresis (CE) can offer significant advantages over aqueous capillary electrophoresis such as reducing Joule heating, decreasing wall adsorption and improving selectivity [1]. In addition, it is especially beneficial for the separation of hydrophobic compounds that cannot be dissolved in aqueous background electrolytes.

Although the probability of adsorption of analytes on the capillary wall could be reduced in nonaqueous media, it is still necessary to coat the capillary to suppress analyte–wall interaction in some cases. For example, hexadecyltrimethyl ammoniumbromide [2] has been dynamically adsorbed and polyvinyl alcohol coating [3,4] have been chemically bonded to the capillary wall.

Another important purpose for coating the capillary is to control the EOF because the magnitude and direction

of the EOF are of crucial importance for optimizing the separation in NACE. Various polymer coatings [5–12] have been employed in NACE to reduce or reverse the EOF for such purposes including fluorocarbon [5], polyethylene glycol [6,7], polyglycidylmethacrylate-co-*N*-vinylpyrrolidine [8], hexadimethrine bromide [9,10], polyacrylamide and amino-propyl [11] coatings, etc.

Various coated capillaries have been applied in NACE as discussed above, until nowadays, few applications in NACE have been found for the inorganic oxide-coated capillaries. In aqueous media, Xie et al. [13,14], for the first time, reported the preparation of zirconia-coated and magnesia–zirconia-coated capillaries used to control the EOF and to achieve good separations. The amphoteric character of zirconia provided the modified capillaries with a switchable EOF by changing the pH of running electrolytes. Subsequently, Bellaistre et al. [15] investigated the EOF of the so-called ‘zirconia-like’ capillary under various aqueous conditions and used a triple layer model to explain the observed EOF behavior. Additionally, Fujimoto [16] reported the capillary electrophoresis using a titania-coated capillary in aqueous system, which exhibited great EOF in both anodic and cathodic directions. Recently, we have reported the nonaqueous capillary electrophoretic behavior of titania-coated capillaries [17].

* Corresponding author. Tel.: +86-27-87867564;
fax: +86-27-87647617.

E-mail address: yqfeng@public.wh.hb.cn (Y.-Q. Feng).

In this paper, we report further studies on nonaqueous capillary electrophoresis using a zirconia-coated capillary, its EOF behavior in a variety of organic solvents and with a series of electrolytes. We demonstrate that anodic EOF in NACE can improve some separations of positively charged compounds, such as alkaloids. Results also reveal that the zirconia-coated capillary electrophoresis provides a convenient and efficient method to control the EOF and improve the separation resolution in NACE.

2. Experimental

2.1. Reagents and materials

Fused-silica capillaries with 50 μm i.d. and 365 μm o.d. were obtained from Hebei Yongnian Optical Fiber Factory, Hebei, China. Zirconium oxychloride, tris(hydroxymethyl) aminomethane (Tris), ammonium acetate (NH_4OAc), methanesulphonic acid ($\text{CH}_3\text{SO}_3\text{H}$), methanol (MeOH), ethanol, acetonitrile (ACN) and thiourea were purchased from Shanghai General Chemical Reagent Factory, Shanghai, China. Ephedrine, pilocarpine, papaverine, berberine, atropine and matrine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (NICBP, Beijing, China). Distilled water was from a quartz apparatus.

2.2. Instrumentation

CE experiments were carried out at room temperature (20°C) on a NT 1229 HPCE (Beijing Institute of New Technology Application, China), equipped with a $\pm 30\text{ kV}$ high voltage power supply and a UV detector. Data collection and manipulation were carried out on a TL 9900 chromatographic workstation (Teleh Electronics Tech Co. Ltd., Beijing, China). Apparent pH (pH^*) values of the BGE solutions were measured with a Delta 320-S pH Meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China). After standardization in water, measurement of pH in the (hydro) organic solvent was carried out. The highest pH^* in different solvent systems was obtained by dissolving NH_4OAc (or Tris) in the solvent directly and the concentrations were fixed at 25 mM. To lower the pH^* values, methanesulphonic acid was added to the above NH_4OAc (or Tris) solution. All electrolyte solutions were filtered and degassed before immediate use. Vial (inlet and outlet) replenishment was made every three runs. When the buffer electrolyte was changed, the capillaries were flushed with MeOH for 30 min, then new electrolyte for 30 min and finally conditioned under voltage for 20 min in the new electrolyte solution. Between two measurements, the capillaries were flushed with the electrolyte solution for 2 min. Electrokinetic injection (1–5 kV for 2 s) was used for sampling the analytes. All experiments were repeated at least twice until the R.S.D. was less than 2%.

The effective length of the coated capillary was 35.8 cm and the total length 51.1 cm. The effective length of the uncoated capillary was 40.2 cm and the total length 55.6 cm. Thiourea was used as the EOF marker.

2.3. Preparation of the zirconia-coated capillary

A fused-silica capillary was activated at ambient temperature by rinsing it sequentially with 1 M sodium hydroxide for 2 h, water for 30 min, 1 M hydrochloric acid for 2 h and water for 30 min. After a GC oven connected with nitrogen regulator, the capillary was placed in the GC oven at 433 K and purged with nitrogen for 4 h.

Zirconia sol was used to accomplish the coating process, and it was prepared according to previous work [18]. Briefly, 1.0 g of zirconium oxychloride was dissolved in 20 ml of a 5:3 (v/v) mixture of ethanol and water and kept at 343 K for 2 h. The zirconia sol was introduced into the pre-activated capillary by a syringe. After the solution stayed in the capillary for 1 h, it was forced out by pressure, leaving a thin film on the inner wall of the capillary. Afterwards, the solvent was removed by passing nitrogen through the capillary under 1 bar for 10 min and then under 0.2 bar for 60 min. The above coating sequence was repeated three times. Finally, the zirconia-coated capillary was heated at 573 K for 8 h in a muffle furnace, completing the zirconia coating. A narrow window (2 mm) was created for detection by using a frit burner.

3. Results and discussion

3.1. The EOF

3.1.1. The EOF in methanolic BGE

To investigate the EOF characteristics of the zirconia-coated capillary in organic media, MeOH, the neutral amphotropic solvent with equal capability of proton acceptance and donation, was chosen as a BGE solvent.

Fig. 1 shows the EOF behavior of the zirconia-coated capillary in methanolic (b) and aqueous BGEs (a) with Tris

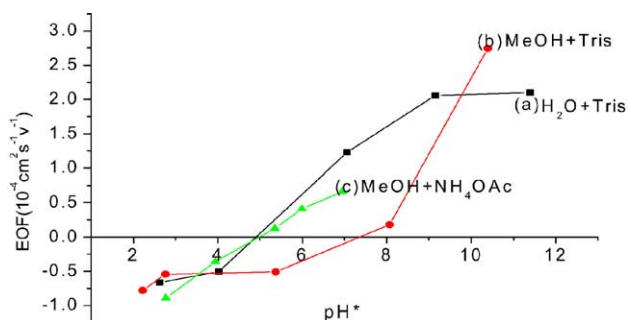


Fig. 1. Comparison of EOF profiles obtained on the zirconia-coated capillary in aqueous and methanolic BGEs with different electrolytes; applied voltage: 25 kV.

electrolyte. Obviously, both the magnitude and direction of the EOF changed with varying pH^* of the running electrolyte (either in MeOH or in aqueous solution). It indicated that the surface hydroxyl groups of zirconia could also deprotonate or protonate in MeOH media, as shown in the following equations:



Fig. 1 also shows that the cathodic flow as well as anodic flow in methanolic BGE is lower than those in aqueous at pH^* below 9.8. It has been pointed out that the EOF in fused-silica capillaries always reduces by increasing percentage of organic modifier in the aqueous buffer [19]. According to the Smoluchowski equation, this effect is not only related to the decreasing ratio of dielectric constant to the viscosity, but also related to a decrease of the ζ potential. As suggested in previous papers [1,15], nonionic structures are more stabilized than ionic structures in organic media. It is therefore expectable that equilibrium (I) in nonaqueous media shifts to the left and equilibrium (II) shifts to the right; both of which would result in the reduced charge on the surface, leading to the decrease in ζ potential. And then EOF decreased in MeOH.

However, at pH^* above 9.8, the cathodic flow in methanolic BGE increased to surpass that in aqueous BGE, as shown in Fig. 1. It can be attributed to the electrostatic interaction between Tris and the zirconia surface. As illustrated previously [13], Tris, in the case, can be adsorbed to the negative charged zirconia surface in a protonated form to change the surface charge. Compared to the aqueous system, the protonation degree of Tris can be restrained in methanolic BGE [1,15]. As a result, the adsorption of protonated Tris on the zirconia surface can reduce, leading to the increase of cathodic EOF in nonaqueous media.

It can also be found that, the pI value of zirconia-coated capillary in methanolic media shifted to the higher pH^* of 7.4 in comparison with 4.9 in aqueous. It has been reported that pK_a values in organic solvents are greater than that in aqueous media because of the poor solvation ability of most organic solvents [1,20,21]. It is thus reasonable that a lifted pI value was observed with methanol replacing aqueous buffer because of the enhanced basic character of ZrOH group in nonaqueous media.

3.1.2. Effect of electrolyte on the EOF

The EOF property of the zirconia-coated capillary was also investigated in MeOH– NH_4OAc system, as shown in Fig. 1(c).

It can be seen from Fig. 1(b) and (c) that EOF– pH^* curves exhibit similar behavior in both systems with different electrolytes. Both are switchable. However, the magnitude of their EOFs is different, and the pI value for MeOH– NH_4OAc system is at lower pH^* than that for MeOH–Tris system. The result was in good agreement with those in aqueous system

Table 1

Physicochemical data of the solvents applied

Solvent	pK_{auto}	ε_{rel}	η (cP)	Polarity	$\varepsilon_{\text{rel}}/\eta$
MeOH	17.2	32.7	0.54	5.1	60.56
ACN	— ^a	37.5	0.34	5.8	110.29
H ₂ O	14.0	80	0.89	10.2	89.89

η : viscosity; ε_{rel} : relative permittivity; pK_{auto} : autoprotolysis exponent.

^a No detectable autoprotolysis.

[13–15]. It can be mainly ascribed to the adsorption of acetate onto the surface of zirconia by strong Lewis acid–base interaction [22]. At pH^* above pI , the hydroxyl groups of zirconia surface deprotonated and the surface should carry negative charge. Therefore, acetate adsorption increased negative charge on the surface, leading to the increase of the cathodic flow. At pH^* below pI , zirconia was protonated, forming positive surface charges as Eq. (2). However, the adsorption of acetate on the surface would decrease the positive charge, leading to the decreased anodic flow. Therefore, pI value, a pH^* related to a net zero charge, shifted to lower pH^* .

3.1.3. Effect of organic solvent composition on the EOF

ACN is traditionally classified as a dipolar aprotic solvent, and very different from both water and MeOH. The properties of these solvents are listed in Table 1 [12,23]. Because of the very low solubility of electrolytes in pure ACN, a binary mixture of ACN and MeOH in different proportion was employed to investigate the effect of the solvent composition on the EOF.

As shown in Fig. 2, the zirconia-coated capillary exhibited switchable EOF in binary systems except in 25% MeOH–75% ACN (within the selected pH^* range, only anodic flow was observed). Compared to the pure methanolic system in Fig. 1(b), the cathodic flow decreased with the increase in ACN, while anodic flow firstly increased and then decreased above pH^* 4.6 and reduced below pH^* 4.6. At the same time, the corresponding pI increased from 8.7 to 9.8 when the content of ACN increased from 25 to 50%. From

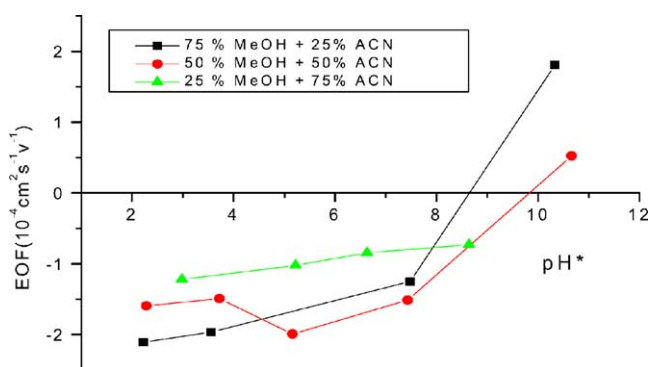


Fig. 2. Comparison of EOF profiles obtained on the zirconia-coated capillary in binary BGEs. Electrolyte: 25 mM Tris + $\text{CH}_3\text{SO}_3\text{H}$; applied voltage: 25 kV.

Table 2

Comparison of several parameters for the MeOH–ACN binary solvent systems

Content of ACN (%)	pH*	η (cP)	μ ($\times 10^{-4}$ cm ² s ⁻¹ V ⁻¹)	ζ (mV)
0	10.4	0.54	2.7	50.8
25	10.3	0.49	1.8	30.4
50	10.7	0.44	0.52	7.9
75 ^a	–	0.39	–	–

^a In this system, desired amount of Tris cannot dissolve completely.

the trend of the curve in Fig. 2, it is reasonable to predict that pI value would be greater than 9.8 in 75% content ACN system.

All the observed distinctive phenomena are related to the complication of nonaqueous systems, in which many factors affect the EOF, respectively, or mutually. In order to investigate the effect of nonaqueous solvent composition on the EOF, Tris BGE was used at constant concentration (25 mM) without the addition of methanesulphonic acid. Related parameters such as pH*, viscosity (η), the electroosmotic mobility (μ_{eo}) and ζ potentials calculated from Smoluchowski equation are listed in Table 2.

As shown in Table 2, all the solutions have the similar pH*. However, viscosity decreased as the content of ACN increased. The dielectric constant of the binary systems almost remains constant [24] because of the very close dielectric constant values of MeOH and ACN, so that the ratio of the dielectric constant to viscosity increased as the ACN content increased. Therefore, the change of the viscosity should result in the increase in the EOF (positive effect). However, the observed EOF decreased unexpectedly. The result suggested that the addition of ACN would cause the change of zirconia surface charge. The deprotonation degree of hydroxyl groups on the zirconia surface was further suppressed with the increasing proportion of ACN [6], leading to a reduction of surface negative charges. It might be a major factor (negative effect) on the decrease of the EOF under the condition. However, the anodic flow showed different trend with the increasing content of ACN at low pH* range. It implies that there are two competing effects, positive one and negative one, as mentioned above, under different conditions. When positive effect played a predominant role over the negative, the faster EOF was produced and vice versa. It is not easy to give a detailed clarification to the complex situation yet.

The same explanation can be employed for the increasing pI values of zirconia-coated capillary with the increasing content of ACN as that in Section 3.1.1. It was due to the enhanced basic character of ZrOH with the increasing ACN content.

It is noteworthy to point out that in organic media the anodic flows occur at higher pH* than in aqueous media. This gives a bright prospect for the separation of analytes in NACE that would not be feasible at high pH in aqueous media.

3.2. Separation

Alkaloids are an important class of the secondary metabolites, many of which are pharmacologically active compounds in herbal medicines. It is of crucial importance to develop efficient methods for the purification and structure elucidation of these compounds. In our study, six alkaloids were used to investigate the effectiveness of the positively charged zirconia-coated capillary with the anodic flow in nonaqueous media. In this mode only positively charged analytes are able to enter the capillary and migrate to the detector, as long as the electrophoretic mobilities of analytes are larger than the reversed electroosmotic mobilities.

In order to investigate the impact of the solvent composition on the selectivity and resolution, the separation was carried out under the condition that the anodic flow remained almost the same in different proportion MeOH–ACN binary mixtures. Fig. 3 shows electropherograms of the six alkaloids obtained on the zirconia-coated column in different solvent compositions. It has been reported that the separation selectivity, the migration order and the resolution of the tested analytes could be altered considerably by varying the MeOH–ACN proportion [21,24]. Fig. 3(a)–(c) illustrates that the electrophoretic mobilities of the majority of the analytes investigated decreased with the increasing ACN content from 25 to 50%, then increased while ACN increasing from 50 to 75%. The mobility of pilocarpine was the only exception, which always reduced. Furthermore, the migration order also changed. In 25% MeOH–75% ACN system, berberine, ephedrine, pilocarpine, papaverine, atropine, matrine migrated out in order; while in 50% MeOH–50% ACN system, pilocarpine migrated out before ephedrine, the migration order of the others remained unchanged. In 75% MeOH–25% ACN system, berberine still remained the first, but the others moved out in different order and papaverine did not come out at all. Many factors can affect the migration rate, including the varying pH* and the ACN content. On the one hand, the protonation degree of the test analytes was dependent on the pH* value. The lower pH* was beneficial to protonate the analytes and made them carry more positive charges, leading to the increased electrophoretic mobilities of the analytes. On the other hand, ACN content variation changed the properties of the system, as discussed above. From the calculation in Table 2, viscosity sharply reduced as the ACN content increased. However, the ionization will be restrained with the increasing ACN. Both the pH variation and the ACN content change contribute to the different separation behavior.

In addition, variation of the BGE's solvent composition has the other multiple effects on the separation process, including pK_a values of molecules [1,25,26], the degree of solvation [20,24,25], the change of hydrodynamic radii of ions [3,24,27], ion-pair formation [3] and so on. All these effects will have considerable impacts on the electrophoretic mobility of individual analytes. But great majority of the

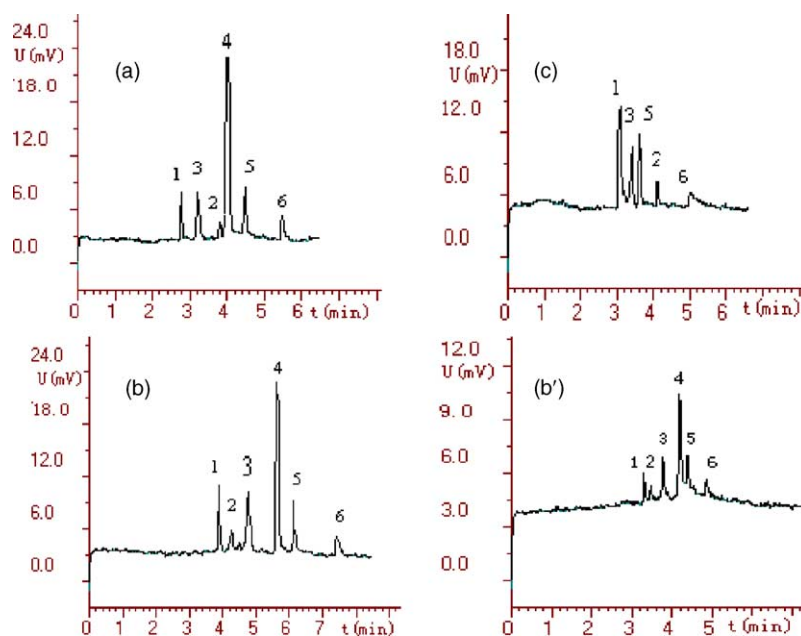


Fig. 3. Separation of alkaloids under different conditions on zirconia-coated (a–c) and uncoated (b') capillaries. Conditions, electrolyte: 25 mM Tris + $\text{CH}_3\text{SO}_3\text{H}$; solvents: (a) 25% MeOH–75% ACN, pH* 1.23; (b, b') 50% MeOH–50% ACN, pH* 3.78; (c) 75% MeOH–25% ACN, pH* 5.98. Samples: (1) berberine; (2) pilocarpine; (3) ephedrine; (4) papaverine; (5) atropine; (6) matrine.

effects remains unclear. The further extensive study is needed.

Excellent resolution was accomplished in 50% MeOH–50% ACN system (as shown in Fig. 3(b)). All analytes were baseline separated, and tailing of analytes was almost eliminated. Compared to the separation (Fig. 3(b')) on the uncoated capillary under the same condition, the zirconia-coated capillary with reversed EOF has the potential to broaden the peak capacity for positively charged analytes. The existence of a positively charged wall eliminates tailing of positively charged analytes. However, the decreased mobilities on the coated capillaries give rise to long diffusion time, which contributes partly to band broadening.

4. Conclusion

The zirconia coating exhibiting switchable EOF could provide more manipulating parameters to improve the separation that would not be feasible in an unmodified capillary. Such a switchable EOF can facilitate the separation of analytes of widely different mobilities using a single capillary. Additionally, inorganic oxide coatings in many aspects are superior over polymer coatings: for example, the presence of organic solvents in the running buffer is detrimental to the efficiency and stability of the polymeric coatings because of the swelling of the polymer layer. The use of nonaqueous systems clearly enhances the scope of inorganic oxide-coated capillary electrophoresis methods, but there remains much to be understood before routine use.

Acknowledgements

This research was supported in part by the National Natural Science Foundation of China (Grant No. 20275029) and Excellent Teacher Program of MOE, PRC.

References

- [1] M.-L. Riekkola, *Electrophoresis* 23 (2002) 3865.
- [2] L.D. Gallaher, E. Johnson Jr., E.M. Johnson, *Anal. Chem.* 72 (2000) 2080.
- [3] C. Czerwenka, M. Lämmerhofer, W. Lindner, *Electrophoresis* 23 (2002) 1887.
- [4] E. Zarbl, M. Lämmerhofer, M. Petracs, W. Lindner, *Electrophoresis* 22 (2001) 3297.
- [5] S. Li, G.S. Weber, *J. Am. Chem. Soc.* 122 (2000) 3787.
- [6] D. Belder, J. Warnke, *Langmuir* 17 (2001) 4962.
- [7] D. Belder, H. Husmann, J. Warnke, *Electrophoresis* 22 (2001) 666.
- [8] P.S. Porras, K.S. Wiedmer, S. Strandman, H. Tenhu, M.-L. Riekkola, *Electrophoresis* 22 (2001) 3805.
- [9] S.H. Hansen, M.E. Jensen, I. Bjørnsdóttir, *J. Pharm. Biomed. Anal.* 17 (1998) 1155.
- [10] A.G. Jensen, S.H. Hansen, *J. Pharm. Biomed. Anal.* 27 (2002) 167.
- [11] Y. Carlsson, M. Hedeland, V. Bondesson, C. Pettersson, *J. Chromatogr. A* 922 (2001) 303.
- [12] F. Steiner, M. Hassel, *Electrophoresis* 24 (2003) 399.
- [13] M.J. Xie, Y.Q. Feng, S.L. Da, *J. Sep. Sci.* 24 (2001) 62.
- [14] M.J. Xie, Y.Q. Feng, S.L. Da, D.Y. Meng, L.W. Ren, *Anal. Chim. Acta* 428 (2001) 255.
- [15] M.C. Bellaistre, O. Mathieu, J. Randon, J.L. Rocca, *J. Chromatogr. A* 971 (2002) 199.
- [16] C. Fujimoto, *Electrophoresis* 23 (2002) 2929.
- [17] L. Xu, Y.Q. Feng, Z.G. Shi, S.L. Da, Y.Y. Ren, *J. Chromatogr. A* 1028 (2004) 164.

- [18] J.C. Bao, D.P. Xu, Q.F. Zhou, Z. Xu, Y.Y. Feng, Y.M. Zhou, *Chem. Mater.* 14 (2002) 4709.
- [19] P.B. Wright, A.S. Listeo, J.G. Dorsey, *Anal. Chem.* 69 (1997) 3251.
- [20] D. Barrón, A. Irlles, J. Barbosa, *J. Chromatogr. A* 871 (2000) 367.
- [21] L.P. Wright, J.P. Aucamp, Z. Apostolides, *J. Chromatogr. A* 919 (2001) 205.
- [22] J.A. Blackwell, P.W. Carr, *Anal. Chem.* 64 (1992) 863.
- [23] M.-L. Riekkola, K.S. Wiedmer, E. ValkóIstván, H. Siren, *J. Chromatogr. A* 792 (1997) 13.
- [24] S.P. Porras, I.E. Valkó, P. Jyske, M.-L. Riekkola, *J. Biochem. Biophys. Meth.* 38 (1999) 89.
- [25] D.R. Ellis, M.E. Palmer, L.W. Tetler, C. Eckers, *J. Chromatogr. A* 808 (1998) 269.
- [26] S.X. Gong, X.D. Su, T. Bo, X. Zhang, H.W. Liu, K.A. Li, *J. Sep. Sci.* 26 (2003) 549.
- [27] S.H. Hansen, I. Bjørnsdottir, J. Tjørnelund, *J. Chromatogr. A* 792 (1997) 49.