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Polymeric ionic liquid as additive for the high speed and efficient separation of aromatic acids by co-electroosmotic capillary electrophoresis

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ABSTRACT

A simple and reliable co-electroosmotic capillary electrophoresis system for the fast determination of aromatic acids has been developed by employing poly (1-vinyl-3-butylimidazolium bromide) as the background electrolyte modifier. The polymeric ionic liquid was synthesized by the conventional radical polymerization. The reversed electroosmotic flow was obtained by adding a small amount of the polymeric ionic liquid (0.0006%, w/v) to the electrolyte. To further improve the resolution of aromatic acids, conditions including the concentration of polymeric ionic liquid and pH of background electrolytes were optimized. All eight aromatic acids were baseline resolved in one measurement in a short time (less than 3.5 min) under optimized conditions, 100 mM NaH_2PO_4 buffer containing 0.006% (w/v) polymeric ionic liquid, pH 6.0. Separation efficiencies were in the range from 355,000 to 943,000 (plates/m). Satisfactory reproducibility on the basis of the migration time of analytes was achieved. RSDs ($n = 3$) were less than 0.33% except the p-aminobenzoic acid (0.9%). The applicability of the present method has been demonstrated for the determination of water-soluble aromatic acids in a common drug for external use.

1. Introduction

Capillary electrophoresis (CE) is a rapidly growing, powerful and effective tool in the separation of charged or neutral compounds in a working buffer. It is a novel separation method that combines advantageous features of online detection, high separation efficiency, low sample consumption, rapid analysis, automation and reduced cost of capillaries compared to HPLC columns or GC capillary columns [1]. However, in normal CE, the separation of anions is a quite time-consuming work because electrophoretic mobilities of anions are comparable to or even greater than the electroosmotic flow (EOF) [2]. Usually, analysis times in CE methods can be reduced by applying short capillaries, high potentials or optimizing the buffer composition to establish a high EOF. However, these improvements are often counteracted by the loss of the separation efficiency due to Joule heating [3]. A better mean of reducing the analysis time of anionic compounds with the high efficiency and good reproducibility is the use of co-electroosmotic CE method, in which the direction of reversed EOF is the same as that of the electrophoretic mobility of the anions in negative polarity. This method has been used successfully for the analysis of anions such as inorganic anions, phenolic compounds or carboxylic acids [2].

The direction of EOF can be reversed by the chemical modification of the fused-silica capillary internal wall using background electrolyte (BGE) modifiers [4]. Typical modifiers used in this method are positively charged reagents including quaternary amine surfactants, for example, cetyltrimethylammonium bromide (CTAB), and diquaternary amine compounds such as hexadimethrine bromide (HDB) [5,6]. The cationic modifier is physically adsorbed on the capillary surface and a reversed EOF is generated.

Room temperature ionic liquids (ILs) are compounds formed of organic cations and inorganic or organic anions that are either liquid at room temperature or whose melting points are slightly higher than the ambient temperature [7]. ILs are environmentally benign and have many advantages over common organic solvents such as, high ionic conductivity, wide temperature range as a liquid phase, negligible vapor pressure, good thermal stability, tunable viscosity and miscibility with water and organic solvents, as well as good extractability for various organic compounds and metal ions [8]. To this day, due to their special physicochemical properties, ILs have generated enormous interest in the separation analysis, especially in chromatographic techniques. They are used as suitable GC stationary phase coated on fused-silica capillary column [9], and additive/stationary phase in HPLC [10,11]. In CE, ILs are used either as supporting electrolytes or as additives for BGE in CE [12–17] and nonaqueous capillary electrophoresis [18,19] and are also used as supported coatings of the capillary wall [20,21]. It can be affirmed that, to date, ILs have been basically added to BGE in CE separations

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to modify the EOF, reverse the EOF, and/or avoid the interaction of analytes with the capillary wall [7]. CE based on ILs can lead to fast, highly efficient separations of a wide variety of analytes, e.g., phenols and aromatic acids, metal ions, medicines, enantiomers, biological materials [22].

Recently, interesting in the polymeric form of IL (PIL) is increasing because PIL constitutes a new class of polymer materials with exceptional properties such as thermal stability and mechanical and electrochemical properties [23]. Much attention has been paid to these PILs for their potential applications such as catalytic membranes, polymer electrolytes, ionic conductive materials, stationary phase coatings in solid-phase microextraction and stationary phase of GC [24–28].

Aromatic acids are useful chemical compounds and synthetic precursors widely used in various industries [29]. Nowadays, in CE, quite a number of separations of the charged aromatic acids are conducted by capillary zone electrophoresis (CZE) with reversed EOF. Different ILs have been used as additives for CE separation of aromatic acids [13]. In this study, a kind of PIL, poly (1-vinyl-3-butylimidazolium bromide) ($PVBIm^+$ Br^-) was prepared by the conventional radical polymerization and used as a cationic additive in CE to reverse the EOF. Aromatic acids were chosen as model analytes.

The main purpose in the present research was to develop a new co-electroosmotic CE method using $PVBIm^+$ Br^- as the dynamical BGE modifier, and apply this CE method to the separation of eight negatively charged aromatic acids. The abilities of this PIL in reversing the EOF and improving the separation were evaluated. Conditions of the separation were optimized. The run-to-run reproducibility of the coated capillary in terms of the migration time of the neutral marker was investigated. Furthermore, the determination of the concentration of aromatic acids in a common drug using the proposed CE method was reported.

2. Experimental

2.1. Materials

1-vinyl-3-butylimidazolium bromide (99%) was purchased from the Shanghai Cheng Jie Chemical Co. (China). 2,2′-Azobisisbutyronitrile (AIBN) obtained from the Shanghai Shanpu Chemical Co. (China) was recrystallized in absolute alcohol. Compound salicylic acid and benzoic acid liniment (National medical licensing number: H21023502) were purchased from the Shenyang Dongling Pharmaceutical Co. (China). All other chemicals used in electrophoresis were of analytical grade. Distilled water was used throughout. Phosphate buffer was prepared from sodium dihydrogen phosphate, and pH was adjusted to different values with NaOH. The stork solution of PIL was prepared in the distilled water at a concentration of 0.2% (w/v). BGEs were prepared by diluting the stock PIL solution with phosphate buffer. The real sample solution of compound salicylic acid and benzoic acid liniment was diluting the sample to 100-fold with the distilled water. The lower aqueous phase was injected directly after filtered with a 0.45 μm syringe filter.

2.2. Apparatus and electrophoresis

Capillary electrophoresis was performed on an Agilent CE system (Agilent Technologies) with a diode array UV–vis detector and a ChemStation (Rev.A 09.03) for system control and data processing. An untreated fused-silica capillary (Hebei Ruifeng Instrumental Co., China) of 50 μm i.d., 375 μm o.d, with a total length of 48.5 cm (40 cm to detector) was used. The detection window was obtained by burning off a 0.4 cm section of the polyimide cladding of the capillary and rinsed with acetone. pH values of the buffer were measured using a Sartourius PB-10 pH meter (Beijing, China).

2.3. Preparation of $PVBIm^+$ Br^-

Polymerization of the 1-vinyl-3-butylimidazolium bromide was carried out by the free radical polymerization using procedures described elsewhere [25,27]. Briefly, 3.0 g of the IL monomer ($VBIm^+$ Br^-) was dissolved completely in 30 mL of chloroform in a 100 mL round-bottom flask. Then, 0.06 g of the free radical initiator, AIBN, was added. The solution was refluxed for 3 h at 70 °C under nitrogen atmosphere for the polymerization. After the removal of chloroform and the product was washed three times with ethyl acetate and then dried under vacuum at 80 °C for 12 h.

2.4. Electrophoresis procedure

Fresh capillaries were used for coating to avoid the hysteresis effect. New capillaries were initially conditioned by rinsing with 1 M NaOH for 10 min and then with the distilled water for 10 min. Between runs, if the BGE was changed, the capillary was rinsed with 1 M NaOH for 10 min, distilled water for 10 min, and finally with the BGE for 10 min. Otherwise, the capillary was just rinsed with the BGE for 2 min. All the solutions and samples were degassed and filtered through a 0.45 μm syringe filter. The sample was introduced into the capillary by the hydrodynamic injection for 3 s at 50 mbar. Direct UV detection was employed at a wavelength of 214 nm. The separation voltage was −20 kV. The temperature was thermostatically controlled at 25 °C. Efficiencies were calculated using the half-height method through the Agilent ChemStation software.

2.5. EOF and effective electrophoretic mobility measurement

Mesityl oxide (DMSO) was used as a neutral marker. A constant voltage of ±20 kV was applied (depending on the direction of the EOF). The magnitude of the EOF (μ_{EOF}) and effective electrophoretic mobility (μ_{eff}) were calculated based on the migration time of DMSO and analytes under constant voltage according to:

$$\mu_{EOF} = \frac{lL}{t_{nm}V} \quad (1)$$

$$\mu_{eff} = \mu_{ap} - \mu_{EOF} = \frac{lL}{V}\left(\frac{1}{t_m} - \frac{1}{t_{nm}}\right) \quad (2)$$

where L is the total capillary length, l is the effective capillary length (from the injection end to the detector), t_{nm} is the migration time of the neutral marker, μ_{app} is the apparent electrophoretic mobility, t_m is the migration time of the analyte, and V is the applied voltage.

3. Results and discussion

3.1. Effect of PIL concentration on EOF

In CE, cationic polymers can be strongly adsorbed on the capillary wall so that the EOF can be reversed to the anodic direction. Cations of the PIL used in this work are expected to be electrostatically attached to the negatively charged surface of the fused-silica capillary. Measurement of the EOF can be used as an indirect way to demonstrate the change of the capillary surface. We investigated the effect of the PIL concentration on the EOF. The result is shown in Fig. 1. It can be seen that the EOF changes from cathodic to anodic when the concentration is above 0.0006% (w/v). A strong reversed (anodic) EOF was generated attributed to modification of the interfacial double layer by cationic PIL additives. The reversed EOF first increases to a maximum and then decreases slowly with the increase of the PIL concentration, As the concentration of the

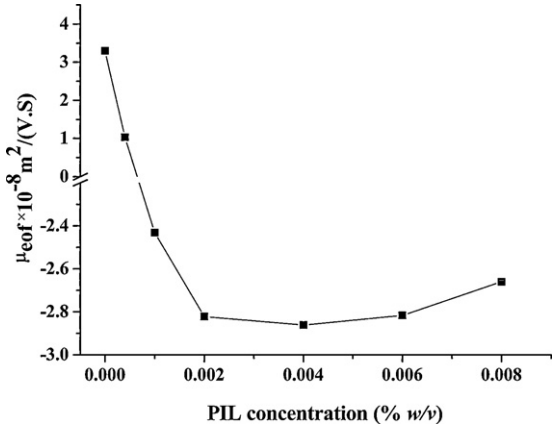

Fig. 1. Effect of PIL concentration on EOF. Conditions: capillary length: 48.5 cm (40 cm to detector); BGE, 100 mM NaH_2PO_4 (pH = 5.0) containing different concentrations of $PVBIm^+$ Br^-; applied voltage, ±20 kV; temperature, 25 °C.

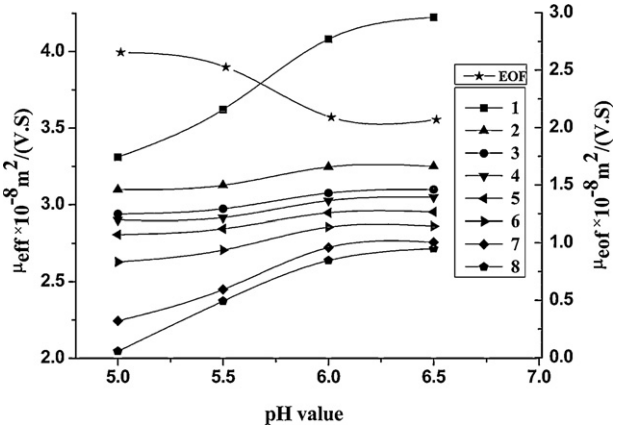

Fig. 3. Effect of BGE pH on EOF and effective electrophoretic mobility. BGE: 100 mM NaH_2PO_4 containing 0.006% (w/v) $PVBIm^+$ Br^- with pH from 5.0 to 6.5; other conditions are the same as in Fig. 1.

PIL increases further up to 0.004% (w/v), more PIL is adsorbed on the capillary inner surface, which results in the increase in ξ potential and the positive charge of the electric double layer, and faster EOF is observed accordingly [30]. When the concentration is 0.004% (w/v), the maximum of EOF can be observed due to the saturation of PIL on the capillary inner surface. The EOF decreases when more PIL are contained in the BGE on account of the higher viscosity and ionic strength.

3.2. Effect of PIL concentration on effective electrophoretic mobility

To establish the optimum separation conditions for the eight aromatic acids studied, BGEs with different concentrations of PIL were investigated. Fig. 2 illustrates the relationship between the μ_{eff}s of aromatic acids and the PIL concentration. The varying trends of μ_{eff} curves are mainly affected by the EOF according to Eq. (2). The μ_{eff} increases when the EOF decreases, and *vice versa*. However, when the concentrations are in the range of 0.002–0.004% (w/v) where EOFs are leveled off, μ_{eff}s of compound 1, 7 and 8 change greatly. This could be related to the association of aromatic acids and the PIL in the BGE [31] which also affects the selectivity of aromatic acids. As shown in Fig. 2 the best selectivity can be attained when the PIL concentration is 0.006% (w/v). Therefore 0.006% (w/v) of PIL was chosen for subsequent experiments.

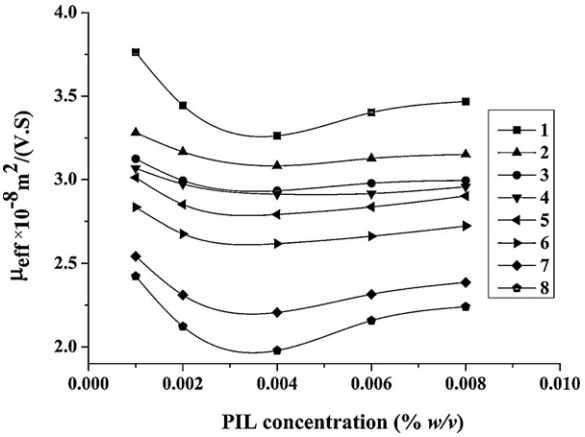

Fig. 2. Effect of PIL concentration on μ_{eff} of analytes. Conditions are the same as in Fig. 1.

3.3. Effect of pH on separation

In the CE separation the buffer pH is one of the most important conditions because its control determines the extent of ionization and mobility of each solute [32]. Fig. 3 shows the effect of pH on the separation of analytes. A pH ranging from 5.0 to 6.5 of the electrolyte system was chosen to achieve electrophoretic mobilities of aromatic acids toward the anode. The μ_{eff}s of aromatic acids increase with pH from 5.0 to 6.5. This is because the EOFs decrease with the increase of pH as shown in Fig. 3. The selectivities are almost unaffected by pH, only slight changes are observed. The reason is that charges of aromatic acids are constant due to the fact that they are fully dissociated in this pH range. The selectivity and efficiency are better at pH 6.0 than at the other pH values investigated.

3.4. Separation of aromatic acids under optimized conditions

Eight aromatic acids were used as model analytes to evaluate the co-electroosmotic CE method using $PVBIm^+$ Br^- as the additive. Fig. 4 illustrates the comparison of the separation of these aromatic acids between the same buffer with and without PIL. As shown in Fig. 4A, only phthalic acid can be detected under the negative polarity. The separation of other seven aromatic acids requires about 30 min under the positive polarity (data not shown). When no of the PIL was added to the BGE, the direction of aromatic acids was opposite to EOF, thus only phthalic acid with the higher electrophoretic mobility than the EOF can be detected under the negative polarity. However, when the PIL was added, eight aromatic acids can be separated in one run with short analysis time. Fig. 4B shows the electropherogram of eight aromatic acids with 0.006% (w/v) PIL as the additive. It can be seen that good separation and peak shapes of aromatic acids are achieved within 3.5 min. The short analysis time is mainly due to the co-electroosmotic mode by the addition of the PIL to the BGE. Analogous to previously reported ILs as BGE additives in CE [12], the separation mechanism relies on the association of aromatic acids with the imidazolium cations of the PIL either adsorbed to the capillary wall or electrophoretically migrated in the bulk solution.

As listed in Table 1, the separation efficiencies (N) of analytes reach as high as 355,000–943,000 (plates/m) when using the PIL as the additive. It can be seen that the efficiency of the EOF is less than that of the analyte, which suggests that high efficiencies could be the result of the association of aromatic acids with the PIL either coating on the capillary inner surface or in the bulk solutions.

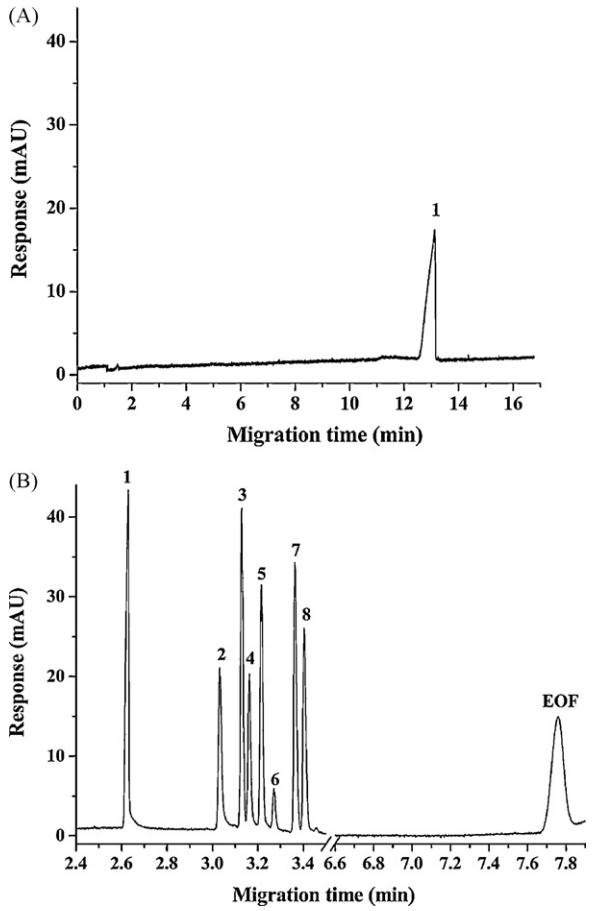

Fig. 4. Separation of eight aromatic acids using the same buffer with and without PIL as additive: (A) without PIL and (B) with PIL. Conditions: BGE, 100 mM NaH_2PO_4 (pH = 6.0) containing 0.006% (w/v) PIL, other conditions are the same as in Fig. 1. Peaks: 1, o-phthalic acid; 2, salicylic acid; 3, 4-aminobenzenesulfonic acid; 4, toluene-p-sulfonic acid; 5, 3-nitrobenzoic acid; 6, 4-chlorobenzoic acid; 7, p-hydroxybenzoic acid; 8, p-aminobenzoic acid.

3.5. Reproducibility study on this method for aromatic acids

The reproducibility study on this method for aromatic acids was investigated in terms of the migration time of analytes. Due to the modification of the EOF and capillary inner wall through the PIL additive in BGE, the reproducibility of analytes can be improved greatly. Table 1 shows the satisfactory run-to-run reproducibility of the migration time. RSDs ($n = 3$) of the migration time of aromatic acids are less than 0.33% except p-aminobenzoic acid (0.9%).

Table 1
Separation efficiency (N) and migration time reproducibility of aromatic acids and EOF[a].

Compound	N (plates/m)	RSD (%)
1	355,000	0.33
2	582,000	0.11
3	943,000	0.15
4	830,000	0.14
5	869,000	0.14
6	756,000	0.11
7	751,000	0.31
8	740,000	0.9
EOF	166,000	0.7

[a] Experimental conditions are the same as in Fig. 4. RSD of the migration time is measured based upon three repeated runs ($n = 3$).

Table 2
Results of regression analysis on calibration curves and detection limits[a].

Compound	Liner range (mg/mL)	Calibration curve	Correlation coefficient	LOD (mg/mL)
Salicylic acid	0.005–0.3	$y = 0.8 + 394.5x$	0.9997	0.0015
Benzoic acid	0.005–0.3	$y = 0.9 + 279.3x$	0.9997	0.002

[a] Experimental conditions are the same as in Fig. 5. The LODs are defined as the concentration where the S/N values are 3. y and x stand for the peak area and the concentration (mg/mL) of the analytes respectively.

3.6. Application

To evaluate the suitability of the proposed CE system for the real sample, it was applied to determine the concentration of salicylic acid and benzoic acid in a common drug for external use (compound salicylic acid and benzoic acid liniment). The optimal conditions in Section 3.5 were used.

3.6.1. Linearity and detection limit

The liner relationship between the concentration of the two analytes and the corresponding peak areas were obtained. The linear range, correlation coefficient and the limit of detection (LOD) are listed in Table 2. It was found that the system has good linearity ($R > 0.999$).

3.6.2. Determination of analytes in real sample

The sample solution of the compound salicylic and benzoic acid liniment was analyzed by the present CE method under the optimized conditions. Fig. 5 illustrates the separation of salicylic and benzoic acids in the standard solution (Fig. 5A) and real sample (Fig. 5B). The peaks were identified by comparing the migration times and spiking the standards to the sample solution. The result shows that the matrix components in the real sample have no interference in the determination of the analytes. From the regression equations in Table 2, concentrations of the salicylic and benzoic acids were 0.36 mg/mL and 0.60 mg/mL respectively. The RSDs ($n = 3$) for peak area of the salicylic acid and benzoic acid are 1.50% and 0.85% respectively. Excellent reproducibility of the migration time (less than 0.3% ($n = 3$)) is also obtained.

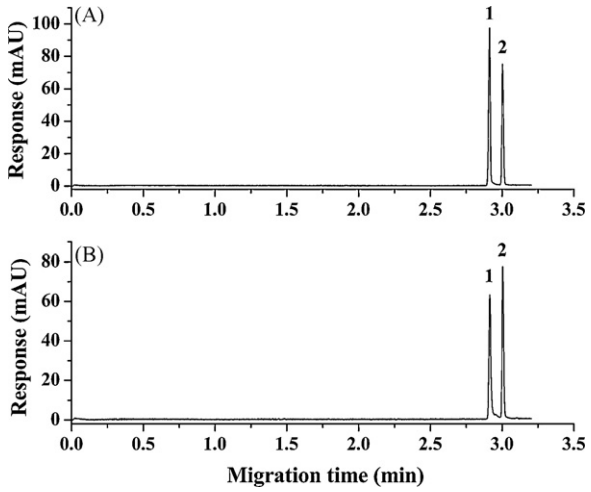

Fig. 5. Typical electropherograms of the standard mixture and real sample: (A) the standard mixture and (B) the sample solution of compound salicylic acid and benzoic acid liniment. Conditions are the same as in Fig. 4. Peaks: 1, salicylic acid; 2, benzoic acid.

4. Conclusions

$PVBIm^+ Br^-$ has been synthesized according to the conventional radical polymerization. Then a co-directional movement of negatively charged analytes and the reversed EOF was accomplished by employing this PIL as a BGE modifier. The co-electroosmotc CE system was further applied in the separation of eight aromatic acids. The electrophoretic conditions including the concentration of PIL and pH of BGE were investigated. The results proved that the co-electroosmotic capillary electrophoresis, which was dynamically modified with polymeric ionic liquid, provided better separation than the normal mode. Successful separation and identification of eight aromatic acids have been achieved under optimized conditions (100 mM NaH_2PO_4, $PVBIm^+ Br^-$ 0.006% (w/v), pH = 6.0). High efficiency (355,000–943,000 (plates/m)) and short analysis time (less than 3.5 min) were obtained. This technique proposed is simple, reliable, and provides good reproducibility in terms of migration time of samples. The RSDs ($n = 3$) were less than 0.33% except p-aminobenzoic acid (0.9%). Analogous to the previously reported ILs used as BGE additives in CE, the separation mechanism relies on the association of aromatic acids with imidazolium cations of the PIL either coated on the capillary wall or electrophoretically migrated in the bulk solution. Furthermore, the present CE method was applied to determine the concentration of salicylic acid and benzoic acid in a common drug for external use. Excellent RSDs for the peak area and migration time of the aromatic acids in the sample solution were obtained.

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References

[1] H.L. Su, M.T. Lan, K.W. Lin, Y.Z. Hsieh, Electrophoresis 29 (2008) 3384–3390.
[2] S. Cortacero-Ramírez, A. Segura-Carretero, M. Hernáinz-Bermúdez de Castro, A. Fernández-Gutiérrez, J. Chromatogr. A 1064 (2005) 115–119.
[3] J. Hernández-Borges, T. Borges-Miquel, G. González-Hernández, M.A. Rodríguez-Delgado, Chromatographia 62 (2005) 271–276.
[4] F. Bianchi, M. Careri, C. Corradini, J. Sep. Sci. 28 (2005) 898–904.
[5] H.B. Mo, L.Y. Zhu, W.J. Xu, J. Sep. Sci. 31 (2008) 2470–2475.
[6] C.A. Lucy, A.M. MacDonald, M.D. Gulcev, J. Chromatogr. A 1184 (2008) 81–105.
[7] M. López-Pastor, B.M. Simonet, B. Lendl, M. Valcárcel, Electrophoresis 29 (2008) 94–107.
[8] B. Buszewski, S. Studzińska, Chromatographia 68 (2008) 1–10, (and the references therein).
[9] J.L. Anderson, D.W. Armstrong, Anal. Chem. 77 (2005) 6453–6462.
[10] L. He, W. Zhang, L. Zhao, X. Liu, S.X. Jiang, J. Chromatogr. A 1007 (2003) 39–45.
[11] H.D. Qiu, S.X. Jiang, X. Liu, J. Chromatogr. A 1103 (2006) 265–270.
[12] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robison, A.M. Stalcup, Anal. Chem. 73 (2001) 3838–3844.
[13] L.J. Yu, W.D. Qin, S.F.Y. Li, Anal. Chim. Acta 547 (2005) 165–171.
[14] S.D. Qi, Y.Q. Li, Y.R. Deng, Y.Q. Cheng, X.G. Chen, Z.D. Hu, J. Chromatogr. A 1109 (2006) 300–306.
[15] M.E. Yue, Y.P. Shi, J. Sep. Sci. 29 (2006) 272–276.
[16] A. Seiman, M. Vaher, M. Kaljurand, J. Chromatogr. A 1189 (2008) 266–273.
[17] X.P. Wu, W.P. Wei, Q.M. Su, L.G. Xu, G.N. Chen, Electrophoresis 29 (2008) 2356–2362.
[18] M. Vaher, M. Koel, M. Kaljurand, Electrophoresis 23 (2002) 426–430.
[19] Y. François, A. Varenne, E. Juillerat, A.C. Servais, P. Chiap, P. Gareil, J. Chromatogr. A 1138 (2007) 268–275.
[20] W.D. Qin, S.F.Y. Li, Analyst 128 (2003) 37–41.
[21] M. Borissova, M. Vaher, M. Koel, M. Kaljurand, J. Chromatogr. A 1160 (2007) 320–325.
[22] Y. Xu, E. Wang, J. Chromatogr. A 1216 (2009) 4817–4823.
[23] S. Ding, H.D. Tang, M. Radosz, Y.Q. Shen, J. Polym. Sci., Part A: Polym. Chem. 42 (2004) 5794–5801.
[24] J.B. Tang, M. Radosz, Y.Q. Shen, Macromolecules 41 (2008) 493–496.
[25] H. Mori, M. Yahagi, T. Endo, Macromolecules 42 (2009) 8082–8092.
[26] F. Zhao, Y.J. Meng, J.L. Anderson, J. Chromatogr. A 1208 (2008) 1–9.
[27] Y.N. Hsieh, R.S. Horng, W.Y. Ho, P.C. Huang, C.Y. Hsu, T.J. Whang, C.H. Kuei, Chromatographia 67 (2008) 413–420.
[28] Y.N. Hsieh, W.Y. Ho, R.S. Horng, P.C. Huang, C.Y. Hsu, H.H. Huang, C.H. Kuei, Chromatographia 66 (2007) 607–611.
[29] H.Y. Huang, Y.R. Lin, S.H. Hsieh, Anal. Chim. Acta 632 (2009) 148–155.
[30] X.J. Huang, Q.Q. Wang, B.L. Huang, Talanta 69 (2006) 463–468.
[31] Y. Tian, R. Feng, L.P. Liao, H.L. Liu, H. Chen, Z.R. Zeng, Electrophoresis 29 (2008) 3153–3159.
[32] M.A. Rodríguez-Delgado, M.L. Pérez, R. Corbella, G. González, F.J. García Montelongo, J. Chromatogr. A 871 (2000) 427–438.