ELSEVIER

Journal of Chromatography A, 912 (2001) 335–342

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Chromatographic behavior of poly(styrene–divinybenzene) encapsulated packing material for capillary electrochromatography

Guodong Zhang, Bingjiu Xu*

Department of Analytic Chemistry, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China

Received 3 October 2000; received in revised form 9 January 2001; accepted 12 January 2001

Abstract

Poly(styrene–divinylbenzene) encapsulated silica (PS-DES) was synthesized and its characteristics as capillary electrochromatography stationary phase were studied. On capillary columns packed with this stationary phase, the effects of acetonitrile concentration as well as pH values on electroosmotic flow, and the effect of the latter on column efficiency were evaluated. Aromatic hydrocarbons, polar and basic medicinal compounds were successfully separated by using reversed-phase elution. It was evident that selectivity based on π–π electronic interaction between solute and stationary phase molecules contributed to the separation. Peaks in the chromatograms obtained were generally symmetrical. At a pH of the mobile phase as high as 11.2, the stationary phase was still stable after more than 160 h of exposure.

Keywords: Stationary phases, CEC; Electroosmotic flow; Poly(styrene–divinylbenzene); Hydrocarbons, aromatic

1. Introduction

Capillary electrochromatography (CEC), whose potential as a powerful separation technology was demonstrated by Pretorius et al. as early as 1974 [1], is attracting the attention of more and more researchers nowadays. Reports on theoretical study and application of this technique are frequently seen in the literature. In their studies most people used high-performance liquid chromatography (HPLC) stationary phases, especially ODS [1–15]. Smith and Evans [16] reported the highest column efficiency (up to $8{\cdot}10^6$ plates m^{-1}) in CEC for the separation of highly polar tricyclic compounds by using strong cation-exchange packing. Other columns packed with silica-based stationary phases with chiral molecules such as cyclodextrins, proteins, macrocyclic antibiotics, quinine and cellulose derivatives bonded on their surfaces, as well as Pirkle type stationary phases, were used for CEC separation of enantiomers [17–23].

Bonded silica stationary phases are very extensively and successfully used in HPLC separations, yet their drawbacks, first of all their narrow applicable pH range, makes them less than satisfactory for many applications [24]. Besides, the residual acidic silanol groups on their surfaces might cause adsorption of strongly polar compounds, especially basic ones, leading to tailing peaks or even loss of components [25]. Another kind of stationary phase, polymer microspheres, have high chemical stability, however, they have drawbacks of slow transference rates, hence low column efficiencies, as well as low

*Corresponding author. Tel.: +86-10-6209-1590.
E-mail address: bingjiuxu@mail.bjmu.edu.cn (B. Xu).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00571-4

rigidities [26]. A third kind, polymer encapsulated stationary phases, are composed of inorganic carriers covered with thin films of polymer. They have the advantages of both high efficiency and rigidity of bonded phases, as well as the chemical stability of the polymer phases, thus it is not surprising that the studies on these type of phases have been rather active recently [27–30].

In this paper, the result of a study on the chromatographic behavior of poly(styrene–divinylbenzene) encapsulated stationary phase (PS-DES) used as a CEC stationary phase is reported. It was found it has all the above-mentioned advantages of polymer encapsulated stationary phases, besides, it showed characteristic selectivity due to π–π electronic interactions between its molecules and the solutes, as Zuo et al. [29] also reported.

2. Experimental

2.1. Instruments and reagents

The CEC experiments were carried out in a laboratory-made apparatus, equipped with a 9323 type high-voltage source (0–30 kV, Beijing Institute of New Technologies, Beijing, China) and a CV^4 UV–Vis detector (ISCO, USA), a TL9900 chromatographic workstation (Tele Electronic Technology, Beijing, China) was used for data acquisition and procession. The pump used for packing was from Spectra-Physics (San Jose, CA, USA). The fused-silica capillaries (250 μm, I.D.) used for preparing columns were obtained from Yongnian Optic Fiber Plant (Hebei, China). Spheric silica (5 μm) and YWG-ODS packing material (5 μm) were supplied by Tianjin Second Chemical Reagents Factory (Tianjin, China). SE-30 was obtained from Chrompack (The Netherlands). Acetonitrile (chromatographic grade) was obtained from Fisher Scientific (USA). All other reagents were from Beijing Chemical Reagent Plant (Beijing, China). Vinyltriethoxylsilane, styrene and divinylbenzene were all treated by distillation before use. Azoisobutyronitrile was purified by recrystallization in ethanol before use. Ribavirin, caffeine, 4-aminoantipyrine and codeine phosphate were presented by Professor Sun Zengpei, (The Chinese National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China). Water as a component of mobile phase was deionized.

2.2. Synthesis of packing material

Synthesis of packing material used in this study was similar with that reported by Zuo et al. [29] with the exception of the monomers used and the quantities of the reagents. In our first step, a mixture of 5 g of silica gel, 3 ml of vinyltriethoxysilane and 50 ml of dry toluene was heated under reflux for 18 h. The product of vinylsilylated silica gel was filtered out and washed with toluene and acetone. Subsequently, 1 g of vinylsilylated silica, 0.3 ml of divinylbenzene, 0.3 ml of styrene, 2.8 mg of azoisobutyronitrile and 2 ml of hexanol were added to 10 ml of acetonitrile, and heated to 80°C for 18 h. The product was separated and then extracted with acetonitrile and acetone, respectively, and finally dried in vacuo over P_2O_5 at 80°C.

2.3. Preparation of CEC columns

Upon packing of the columns we used the technique developed in this group [31], i.e., first to make inlet frit by sintering immobilized SE-30 coated ODS particles (SE-30-ODS); and then to pack the column by sucking the slurry of the stationary phase into the column rather than conventional pressing-in method, followed by infusing a segment of SE-30-ODS by pressurizing a slurry of the material into the column, and subsequently to pressurize the column bed with water from a Spectra-Physics pump, at approximately 350 bar; finally, to make the outlet frit by sintering the SE-30-ODS.

2.4. Separation conditions

The mobile phases were prepared by mixing the buffer of desired pH with an appropriate amount of acetonitrile. The mobile phase was filtered through a 0.25-μm filter and degassed with ultrasonication for about 5 min before use.

The packed capillary column was rinsed with mobile phase using a manual syringe pump (Unimicro Technologies, USA). Prior to separation, the

capillary was conditioned at a relatively low voltage (3 kV) until a constant current was achieved. Electrokinetic injections were run at 3 kV for 5 s. All experiments were carried out at room temperature, both the inlet and the outlet of the column were kept at atmospheric pressure.

3. Results and discussion

3.1. Electroosmotic flow in columns packed with polymer encapsulated packing

It has been suggested that in packed CEC columns, electroosmotic flow (EOF) characteristics were similar to those in open tubular capillary columns [4,5]. EOF relationship is generally derived as:

$$u_{eo} = \frac{\epsilon_r \epsilon_0 \zeta V}{\eta L} = \mu_{eo} E \quad (1)$$

where ϵ_r and ϵ_0 are the relative and vacuum permittivities, respectively, and u_{eo} is the electroosmotic flow velocity, μ_{eo} is the electroosmotic flow mobility, V is the applied voltage, E is the electric field strength, L is total column length, ζ is the zeta potential and η is the viscosity of the solvent.

From Eq. (1) it can be derived that when μ_{eo} is constant, u_{eo} is proportional to the electric field strength. If significant heating takes place during separation, parameters ϵ_r, ϵ_0, ζ and η, especially the latter, will change with temperature. In this case the proportionality between E and u_{eo} will not be observed. By measuring E vs. current (I) through a PS-DES column, a linear relationship was obtained ($R^2 = 0.9993$, see Fig. 1), this would suggest that the conductivity of the solution did not change significantly with E, which in turn suggested that the temperature of the mobile phase in the column did not change significantly. This notion can also be derived from the linear relationship between E and u_{eo}, that we also observed. In summary, under the conditions we adopted in our experiments, heating effect is negligible, as reported in the case of reversed-phase CEC columns packed with ODS [5,6,9].

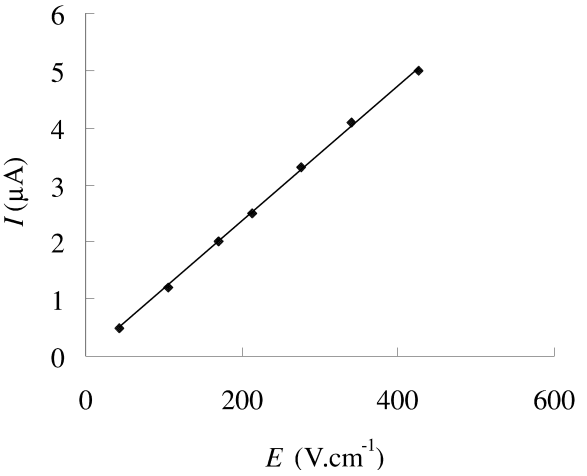

Fig. 1. Plot of current (I) vs. field strength (E). Stationary phase: poly(styrene–divinylbenzene) encapsulated silica gel (5 μm). Column: 47 cm (effective length 22 cm)×250 μm. Mobile phase: CH_3CN–5 mM Tris–HCl, pH 7.5 (70:30, v/v).

3.2. Effect of acetonitrile concentration on EOF

In capillary zone electrophoresis (CZE), it was reported [32] that EOF decreased with higher acetonitrile content in the mobile phase, this was attributed to a concomitant decrease in the zeta potential. With a PS-DES packed column, the present authors found quite the reverse, i.e., EOF increased with acetonitrile content (30 to 90%, v/v, added to 5 mM Tris–HCl buffer, pH 7.5). Choudhary and Horváth [10] observed the same trend, this observation was also supported by Rebscher and Pyell [6] and Dittmann and Rozing [9]. However, Yamamoto et al. [5] observed a decrease of EOF with increasing acetonitrile content. Judging from these contradictory experimental phenomena in the literature, the effect of acetonitrile content on EOF still needs to be elucidated, it is probable that there are factors whose function has not been clarified in reversed-phase packed CEC, for example, the zeta potential.

3.3. Effect of pH on EOF

A comparison of the effect of pH on EOF in columns packed with PS-DES, vinylsilylated silica gel and bare silica gel is shown in Fig. 2. It can be seen that firstly, in all the packed columns compared, the EOF increased with pH, which is similar with the

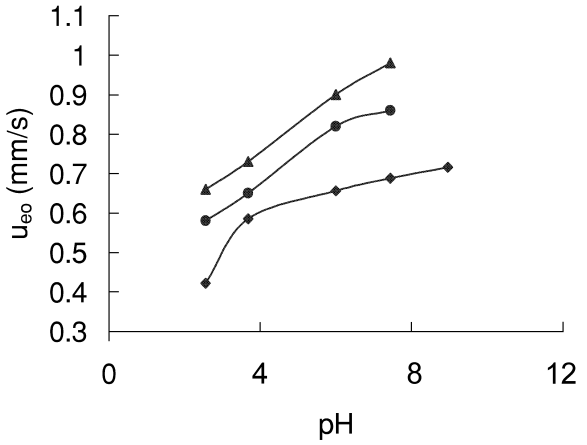

Fig. 2. Effect of pH on electroosmotic flow. Columns were packed with following stationary phase: (♦) 5 μm PS-DES [47 cm (effective length 22 cm)×250 μm], (●) 5 μm vinylsilylated silica gel [47 cm (effective length 22 cm)×250 μm], (▲) 5 μm silica gel [47 cm (effective length 22 cm)×250 μm]; mobile phases: CH_3CN–5 m*M* buffer (70:30, v/v); phosphate, pH 3.2; phosphate, pH 4.6; Tris–HCl, pH 7.5; Tris, pH 9.3; phosphate–NaOH, pH 11.2 (not used for silica and vinylsilylated silica packed columns). Detection: 254 nm. Injection: 3 kV for 5 s. Applied voltage: 15 kV. Thiourea as the EOF marker, except in the case of silica gel packed column, where toluene was used.

observation on ODS packed columns [8,9]; secondly, under the same pH condition the EOF on columns packed with bare silica, vinylsilylated silica and PE-DES decreased in order. In CEC, EOF was mainly contributed to the packing surface, presumably due in part to the very large surface area of the packing material, and in part to incomplete double layer overlap on packing surface; whereas on the capillary surface, it is probable that double layer overlap occurs because of its contact with the packing particles [34]. From the above it could be derived that EOF changed with surface silanol group population on the packing surface indeed: on the vinylsilylated silica surface, the number of the surface silanol groups was reduced, whereas on the PS-DES surface, the remaining silanol groups was further masked by the polymer. If it was true that in the columns packed with vinylsilylated silica and PS-DES, EOF was caused according to the conventional double layer theory, the increase in EOF rate with pH could be explained by higher degree of ionization of silanol groups at higher pH, hence more surplus hydrogen ions in the bulk of the mobile phase.

3.4. Effect of electroosmotic flow on column efficiency

Just as in all types of chromatography, column efficiency in CEC is determined by several factors, i.e., eddy diffusion, axial diffusion, resistance to mass transfer and extra-column effects (connective tubing, frit, detection window, etc.) [33]. Fig. 3 shows the relationship between the reduced plate height of thiourea (commonly used as non-retaining compound in reversed-phase CEC) and EOF. It can be seen in Fig. 3 that, had it been possible to obtain an EOF of higher than 0.9 mm s^{-1}, the curve would rise on its right wing (apparently a result of slow exchange of the solute across the stationary phase–mobile phase boundary), just as an ordinary Van Deemter curve of a retained solute. Judging from this, thiourea seemed to be an unsatisfactory EOF marker.

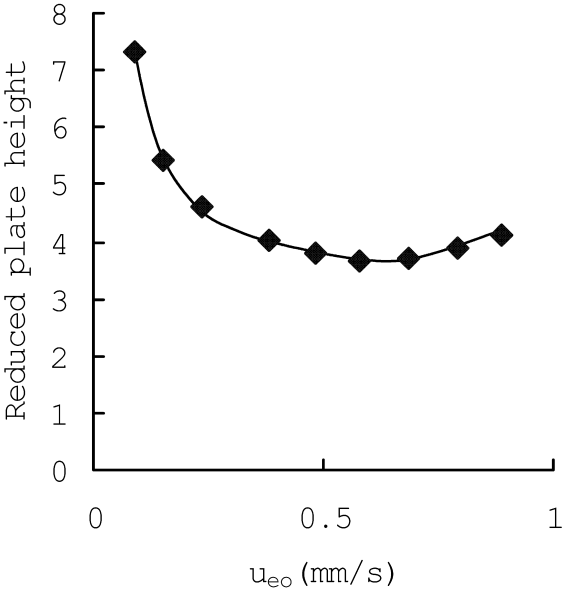

Fig. 3. Reduced plate height versus electroosmotic velocity on a column packed with 5 μm PS-DES stationary phase [47 cm (effective length 22 cm)×250 μm]. Mobile phase: CH_3CN–5 m*M* Tris–HCl, pH 7.5 (70:30, v/v). Detection: 254 nm. Injection: 3 kV for 5 s. Thiourea as the EOF marker.

3.5. Chromatographic characteristics of PS-DES

Due to its capability of bringing about π–π electronic interactions with solute molecules, PS-DES showed characteristic selectivity against aromatic compounds. In Fig. 4A, ethylbenzene and naphthalene were better than baseline separated on the PS-DES packed column, whereas in Fig. 4B, they are not separated on the ODS packed column under otherwise the same conditions. It should be noticed that the capacity factors of all the retained compounds were unanimously higher on the PS-DES than on ODS, see Table 1. This might be due to the π–π interaction of the polystyrene molecules on the former stationary phase with the solutes, probably also to a higher hydrophobicity of the former. The elution orders of the four aromatic compounds suggested the reversed-phase retention mechanism on the polymer encapsulated silica gel. A calculation of the EOF mobilities (μ_{eo}) on the two phases showed figures of $0.83 \cdot 10^{-8}$ and $1.37 \cdot 10^{-8}$ $m^2\ V^{-1}\ s^{-1}$ under the conditions described in Fig. 4, respectively. The former was smaller than the latter, probably suggesting less silanol groups on the surface of the former phase, hence its hydrophobicity was stronger.

On these types of encapsulated stationary phases, most of silanol groups on silica surface were covered by a layer of inert polymer, thereby greatly alleviating the adsorption of basic compounds on stationary phase surface. Figs. 5–7 are chromatograms of several polar, basic and basic medicinal compounds, respectively. Excellent separations were realized.

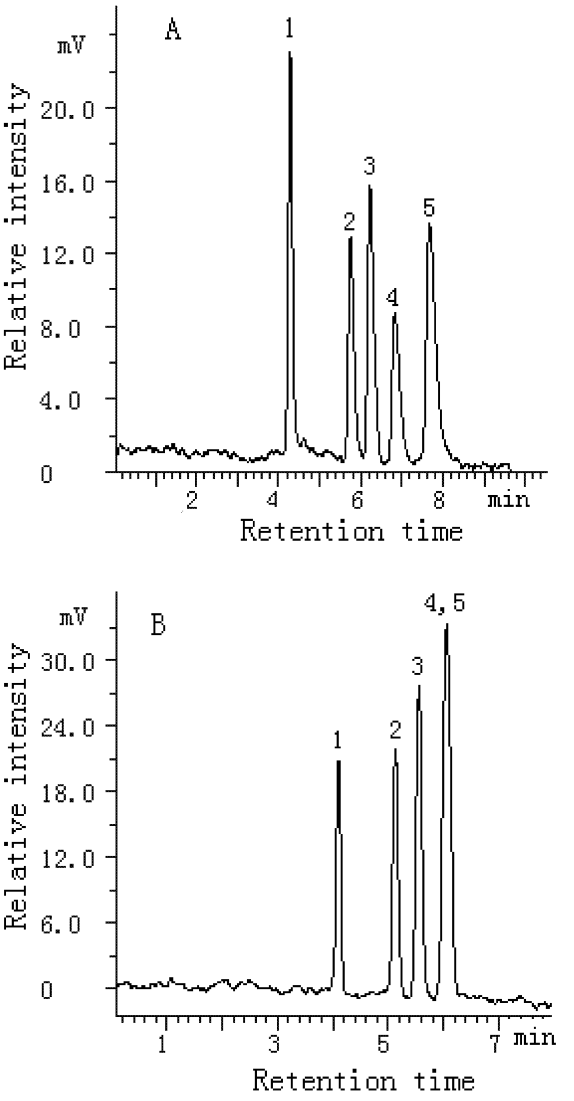

Fig. 4. Electrochromatograms of a mixture of aromatic compounds. Columns were packed with following stationary phases: (A) 5 μm PS-DES [47 cm (effective length 22 cm)×250 μm]; (B) 5 μm ODS [51 cm (effective length 26 cm)×250 μm]. Mobile phase: CH_3CN–5 m*M* Tris–HCl, pH 7.5 (70:30, v/v). Applied voltage: 20 kV. Detection: 254 nm. Injection: 3 kV for 5 s. Peaks: 1=thiourea, 2=benzene, 3=toluene, 4=ethylbenzene, 5=naphthalene.

3.6. Stability of PS-DES stationary phase under alkaline conditions

In a test of alkaline resistance of the encapsulated silica phase, the mobile phase [CH_3CN–5 m*M* Na_2HPO_4–NaOH, pH 11.2 (70:30, v/v)] was driven through the column by EOF for a total time of 160 h, after which time the stationary phase still showed good chromatographic performance, and the capacity factors of solutes were essentially the same, as can be seen in Fig. 8, which is a plot of the capacity factors of the four solutes vs. exposure time of the column to high-pH mobile phase. It was evident that the polymer cover prevented alkaline mobile phase from being in direct contact with silica, resulting in excellent alkaline-resistance, even at a pH as high as 11.2. In a test of the stability of the stationary phases (ODS as well as a polymer encapsulated phase), the

Table 1
Comparison of retention factors on ODS and PS-DES stationary phases[a]

Compound	Retention factor (k')	
	ODS	PS-DES
Benzene	0.252	0.344
Toluene	0.356	0.453
Ethyl benzene	0.478[b]	0.595
Naphthalene	0.478[b]	0.792

[a] Experimental conditions as in Fig. 4.
[b] The two compounds were co-eluted.

HPLC columns were continuously flushed with a pH 7.5 buffer, and the capacity factors of solutes etc. were measured to show if there were differences after flushing [27]. In our case, we more practically measured the changes of the solute retention times on the column with real mobile phase flowing through it. It might be interesting to see whether the stationary phase could stand a pH 11.2 (or even higher alkalinity) buffer flowing through it for a certain period of time; that will be carried out in our further work.

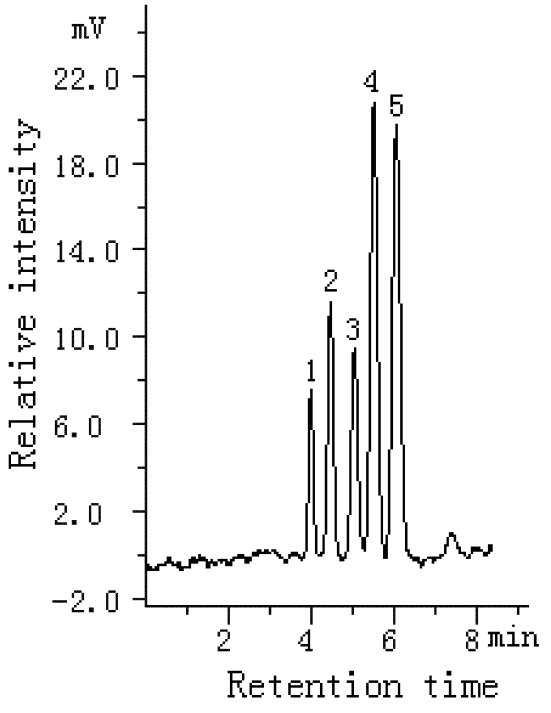

Fig. 5. Electrochromatogram of a mixture of polar compounds. Stationary phase: PS-DES (5 μm), column: 47 cm (effective length 22 cm)×250 μm. Mobile phase: CH_3CN–5 m*M* Tris–HCl, pH 7.5 (70:30, v/v). Applied voltage: 20 kV. Detection: 254 nm. Injection: 3 kV for 5 s. Peaks: 1=thiourea, 2=acetanilide, 3=β-naphthol, 4=methyl benzoate, 5=ethyl benzoate.

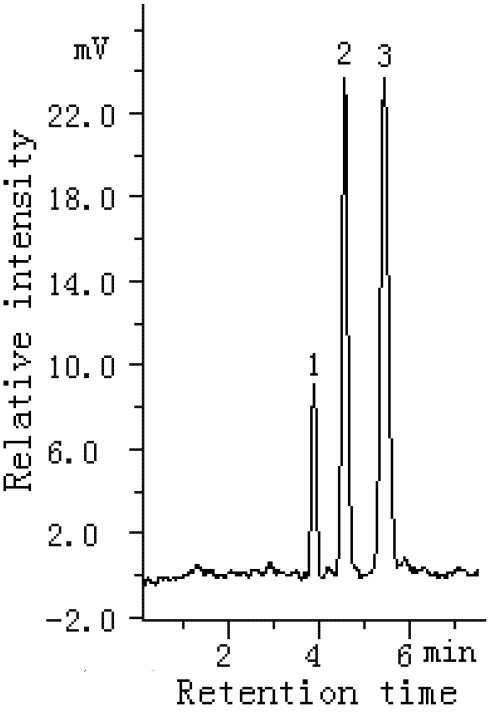

Fig. 6. Electrochromatogram of an amine mixture. Stationary phase: PS-DES (5 μm), Column: 47 cm (effective length 22 cm)×250 μm. Other conditions as in Fig. 5. Peaks: 1=thiourea, 2=aniline, 3=1-naphthylamine.

3.7. Study on the low adsorptivity of basic compounds on PS-DES

For further study on the low adsorptivity of basic compounds on the polymer encapsulated stationary phase, caffeine was chosen as basic compound and ribavirin as internal standard. A series of standard solutions containing 1000, 800, 600, 400, 200, 100 μg ml^{-1} of caffeine and 800 μg ml^{-1} internal standard (ribavirin), respectively, were prepared. Each solution was injected five times. Regression equation was obtained by plotting the logarithm of ratios of the peak areas of caffeine A_s to those of the internal standard A_i [$y=\log_{10}(A_s/A_i)$] against the logarithm of caffeine concentration C_s [$x=$

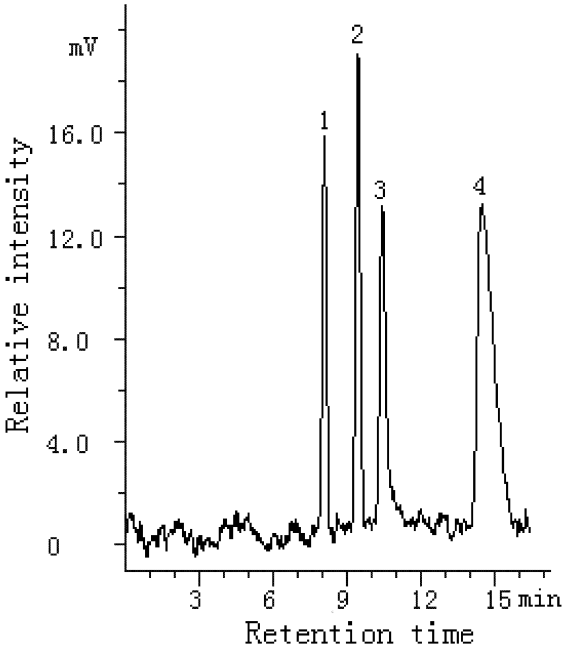

Fig. 7. Electrochromatogram of basic medicinal compounds. Stationary phase: PS-DES (5 μm), column: 50 cm (effective length 25 cm)×250 μm. Mobile phase: CH_3CN–5 m*M* NaH_2PO_4, pH 4.6 (70:30, v/v). Applied voltage: 15 kV. Detection: 214 nm. Injection: 3 kV for 5 s. Peaks: 1=ribavirin, 2=caffeine, 3=4-aminoantipyrine, 4=codeine phosphate.

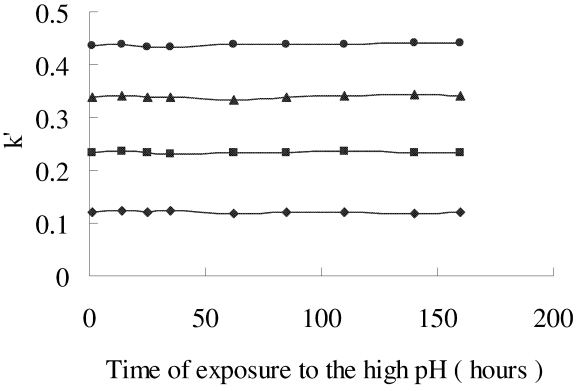

Fig. 8. Stability of PS-DES stationary phase in strongly alkaline (pH 11.2) mobile phase in CEC. Stationary phase: PS-DES (5 μm), column: 50 cm (effective length 25 cm)×250 μm. Mobile phase: CH_3CN–5 m*M* Na_2HPO_4–NaOH, pH 11.2 (70:30, v/v). Applied voltage: 15 kV. Detection: 254 nm. Injection: 3 kV for 5 s. Sample: (♦) acetanilide, (■) β-naphthol, (▲) methyl benzoate, (●) ethyl benzoate.

$\log_{10}(C_s)$]. The relationship was linear in the range 100~1000 μg ml^{-1} with the regression equation obtained as follows: $y=-2.7308+1.0364x$ ($r=0.9967$), the number of data points is 6, the standard error for the regression is 0.03601, the standard deviations of the slope and intercept are 0.04206 and 0.1102, respectively. It can be justified to say that the results showed a good linearity, taking into consideration the fact that the peak height–noise ratio was not large in a CEC measurement (see Figs. 4–7), noise alone already caused significant errors in peak area determination.

With this log–log regression, the effect of deviations in low-side data on the linearity is amplified: if there was significant adsorption of stationary phase surface, low concentration samples would lose larger portion of solutes, causing worse linearity of the log–log regression. The result obtained above suggested the low adsorptivity of basic compounds on PS-DES.

4. Conclusions

Experimental results showed that the polymer encapsulated stationary phase did have some interesting characteristics: due to its π–π electronic interaction with solutes, it shows characteristic selectivity especially on aromatic compounds, and its selectivity could be further tailored by derivatizing its phenyl groups. This type of stationary phase shows reversed-phase retention mechanism in CEC, the adsorptivity of basic solutes on their surface is low, generally leading to symmetrical peaks. The stationary phase is stable even at a pH of the mobile phase as high as 11.2. This stationary phase can be a good alternative used in HPLC and CEC. Further study is under way by the present authors to modify the procedure to synthesize the stationary phase, to improve the resulting efficient as well as the peak shape of the columns packed with the phase.

Acknowledgements

The authors would like to thank Professor Zengpei Sun (The Chinese National Institute for the Control of Pharmaceutical and Biological Products, Beijing,

China) for his encouragement of our work, and kindly donating medicine samples.

References

[1] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[2] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[3] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[4] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[5] H. Yamamoto, J. Baumann, F. Erni, J. Chromatogr. 593 (1992) 313.
[6] H. Rebscher, U. Pyell, Chromatographia 38 (1994) 737.
[7] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[8] R.J. Boughtflower, T. Underwood, C.J. Paterson, Chromatographia 40 (1995) 329.
[9] M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 744 (1996) 63.
[10] G. Choudhary, Cs. Horváth, J. Chromatogr. A 781 (1997) 161.
[11] R.M. Seifar, W.Th. Kok, J.C. Kraak, H. Poppe, Chromatographia 46 (1997) 131.
[12] A.S. Lister, C.A. Rimmer, J.G. Dorsey, J. Chromatogr. A 828 (1998) 105.
[13] B. Xin, M.L. Lee, Electrophoresis 20 (1999) 67.
[14] C. Desiderio, L. Ossicini, S. Fanali, J. Chromatogr. A 887 (2000) 489.
[15] M.M. Dittmann, K. Masuch, G.P. Rozing, J. Chromatogr. A 887 (2000) 209.
[16] N.W. Smith, M.B. Evans, Chromatographia 41 (1995) 197.
[17] S. Li, D.K. Lloyd, J. Chromatogr. A 666 (1994) 321.
[18] D.K. Lloyd, S. Li, P. Ryan, J. Chromatogr. A 694 (1995) 285.
[19] C. Wolf, P.L. Spence, W.H. Pirkle, E.M. Derrico, D.M. Cavender, G.P. Rozing, J. Chromatogr. A 782 (1997) 175.
[20] A. Dermaux, F. Lynen, P. Sandra, J. High Resolut. Chromatogr. 21 (1998) 575.
[21] A.S. Carter-Finch, N.W. Smith, J. Chromatogr. A 848 (1999) 375.
[22] M. Lämmerhofer, W. Lindner, J. Chromatogr. A 839 (1999) 167.
[23] K. Krause, M. Girod, B. Chankvetadze, G. Blaschke, J. Chromatogr. A 837 (1999) 51.
[24] R.V. Arenas, J.P. Foley, Analyst 119 (1994) 303.
[25] H. Engelhardt, M. Czok, R. Shultz, E. Schweinheim, J. Chromatogr. 458 (1988) 29.
[26] Y.B. Yang, M.J. Verzele, J. Chromatogr. 387 (1987) 197.
[27] H. Engelhardt, H. Low, W. Eberhardt, M. Mauβ, Chromatographia 27 (1989) 535.
[28] A. Kurganow, O. Kuzmenko, V.A. Dauankou, J. Chromatogr. 506 (1990) 391.
[29] Y.M. Zuo, B.R. Zhu, Y. Liao, M.D. Gui, Z.L. Pang, J.X. Qi, Chromatographia 38 (1994) 756.
[30] Y.M. Zuo, K. Chang, W.S. Lu, X.W. Xu, Z.L. Pang, J.X. Qi, Chem. J. Chin. Univ. 18 (1997) 1479.
[31] B.M. Ning, B.J. Xu, J. Chin. Pharm. Sci., submitted for publication.
[32] C. Schwer, E. Kenndler, Anal. Chem. 63 (1991) 1801.
[33] J.H. Knox, I.H. Grant, Chromatographia 26 (1988) 329.
[34] N. Smith, M.B. Evans, J. Chromatogr. A 832 (1999) 41.