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Polyallylamine-supported pseudo-stationary phases for electrokinetic chromatography
Effect of alkyl chain length of the pseudo-stationary phase and methanol content of aqueous buffer on the separation of hydrophobic compounds

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Abstract

Polymeric pseudo-stationary phases having alkyl (C_8–C_{16}) and carboxylate groups were prepared from commercially available polyallylamine (PAA). PAA was first alkylated with an alkyl bromide, then reacted with methyl acrylate, followed by the hydrolysis of the ester functionality. The PAA-supported pseudo-stationary phases, especially those with long alkyl chains, provided high efficiencies for alkyl phenyl ketones and polynuclear aromatic hydrocarbons in a wide range of buffer–methanol mixtures. Migration times of the hydrophobic compounds, limited by the migration time of the carrier (t_C) at low methanol content, showed a large increase at 40–60% methanol, and decreased due to the decrease in k' values at higher methanol content where large t_C values were observed. The expansion of the migration time window occurred at a higher methanol content with the pseudo-stationary phase with the longer alkyl groups. Difference in the effect of methanol addition on the behavior of PAA derivatives with different alkyl groups can be attributed to the structural change of the polymeric pseudo-stationary phase resulting in the greater electrophoretic mobility relative to electroosmotic flow, and in turn large t_C values. Simple preparation of the PAA-supported pseudo-stationary phase and the high efficiency will make electrokinetic chromatography using polymeric pseudo-stationary phases a promising tool for the separation of a wide range of compounds including very hydrophobic polynuclear aromatic hydrocarbons in simple water–methanol mixtures.

Keywords: Pseudo-stationary phases; Polyallylamine supports; Electrokinetic chromatography; Polynuclear aromatic hydrocarbons; Ketones

1. Introduction

High-performance chromatographic separation methods utilizing capillary electrophoresis techniques, namely capillary electrochromatography (CEC) and micellar electrokinetic chromatography (MEKC), have been under intensive research. These high-performance separation methods can easily generate a number of theoretical plates greater than 100 000 in a relatively short time. CEC is very attractive, because one can fully utilize the knowledge and experience in reversed-phase liquid chro-

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matography (RPLC) by using aqueous buffer–organic solvent mixtures in combination with fused-silica capillaries, either packed with ODS silica particles [1–4] or filled with polymer rods prepared in situ [5,6]. The column preparation and the optimization of separation conditions in CEC, however, will still need extensive studies before its practical application.

MEKC has been shown to provide high speed and high efficiency separations. The practical utility of this method has been proven for the separation of compounds with significant solubility in aqueous systems [7,8]. The separation of hydrophobic compounds, however, gives some problems, because of their poor solubility in water. The addition of organic solvents or cyclodextrins to MEKC systems provided some success by increasing the partition of hydrophobic compounds into the aqueous phase [9–18]. The addition of cyclodextrins to MEKC systems, however, added complexity, while the addition of organic solvents has also limitations with respect to the stability of micelles. The use of polymeric pseudo-stationary phases has been studied [19–36] in order to increase the applicability of electrokinetic chromatography (EKC) especially for the separation of hydrophobic compounds in simple aqueous buffer–organic solvent mixtures as in RPLC or CEC.

Polymeric pseudo-stationary phases for EKC which utilize hydrophobic partition of solutes for separation can be prepared either by polymerizing a micelle structure or by the derivatization of a polymer support. By taking the former approach, Palmer and coworkers [19,20,29,30], and Wang and Warner [26] showed the successful use of micelle polymers prepared from monomers containing undecenyl or undecenoyl functionality. Although these products gave high-performance separation of a wide range of compounds including polynuclear aromatic hydrocarbons (PAHs) as well as chiral compounds, the preparation and purification methods are relatively involved. Monomer structures are limited, because of the limited availability of suitable unsaturated surfactants and the necessity of micelle formation prior to polymerization. Other polymeric pseudo-stationary phases include polyvinylpyrrolidone [24] and commercially available micelle polymers consisting of methacrylic acid and alkyl methacrylate copolymers [22,23,27,28,31].

The second approach for the preparation of polymeric pseudo-stationary phases includes the attachment of interacting groups, namely alkyl groups, as well as ionic groups to a polymer support such as dendrimers [32–37]. One has more freedom in the structure of the pseudo-stationary phase, because the alkyl groups can be either long or short, and densely or lightly populated on the support. The disadvantage of this approach is the slow and often incomplete reaction of polymers. We previously reported the use of a starburst dendrimer (SBD) as a support of alkyl groups which worked as pseudo-stationary phases in a full range of water–methanol (0–90%) mixtures [32–34]. The SBD-supported pseudo-stationary phase with dodecyl (C_{12}) groups showed similar selectivity as the SDS micellar system in aqueous solutions, and greater hydrophobic selectivity to give high-performance separation for PAHs in water–methanol mixtures. Practical application, however, will not be realistic, because the preparation of the SBD skeleton and its derivatization were very time-consuming, in spite of the considerable attention paid to this type of polymers in EKC [32–37].

Here we report the use of polyallylamine (PAA) as a support to attach alkyl groups of C_8 to C_{16} as well as carboxylate groups. PAA is commercially available and the attachment of alkyl groups and carboxylate groups can be easily carried out. In this report the performance of PAA-supported pseudo-stationary phases of various chain lengths will be shown for the separation of compounds having a wide range of hydrophobicity including the 16 polynuclear aromatic hydrocarbons (PAHs), EPA priority pollutants, in buffer–methanol mixtures. The high-performance pseudo-stationary phases based on a commercially available polymer support are expected to expand the range of application of EKC.

2. Experimental

2.1. Preparation of alkylated PAA with carboxylate groups (PAA-C_nH_{2n+1})

The preparation scheme of the pseudo-stationary phases from PAA is shown in Fig. 1, and described below for the case of PAA-C_{16}. PAA hydrochloride (4.6 g) of molecular mass of ca. 10 000, which is

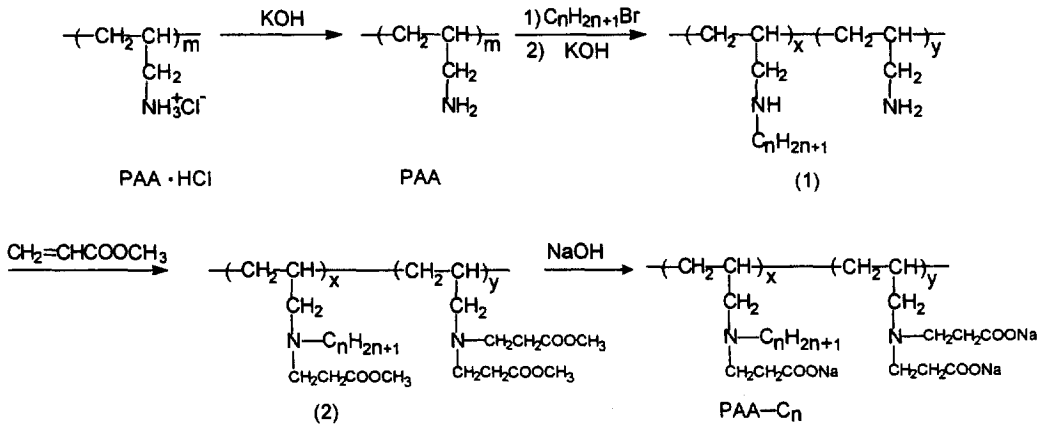

Fig. 1. Preparation of polymeric pseudo-stationary phases from PAA.

commercially available (Nacalai Tesque, Kyoto, Japan), was neutralized with potassium hydroxide (3 g) in methanol (200 ml). After stirring at room temperature for 24 h, the mixture was concentrated by evaporation to ca. 30 ml. Ethanol (100 ml) was added to the solution, then evaporated again to ca. 30 ml. The salt was filtered by using a PTFE filter (2 μm). To the filtrate was added hexadecyl bromide (1.5 g), and the mixture stirred for 48 h at 50°C. The mixture was cooled and neutralized with KOH (0.3 g) in methanol (10 ml). Ethanol (70 ml) was added to the solution, followed by evaporation to ca. 40 ml. After filtration with a PTFE filter, the filtrate was added dropwise to ethyl acetate (500 ml) under stirring. The precipitate (intermediate 1, Fig. 1) was filtered with a PTFE filter and dried under vacuum at 50°C. The intermediate (1: 2.0 g) in methanol (50 ml) was added to a solution of methyl acrylate (23 ml) in methanol (100 ml), and stirred for 10 days. After the evaporation of methanol and methyl acrylate, benzene (25 ml) was added, and the mixture stirred. The supernatant was decanted from the small amount of precipitate, and freeze-dried to produce the intermediate 2 (Fig. 1) which is a very viscous oil at room temperature. A methanol solution of sodium hydroxide (1.0 *M*, 40 ml) was added to the solution of the intermediate (2: 5.1 g) in methanol (100 ml). The mixture was stirred for 24 h, and evaporated. PAA-C_{16} thus obtained was dried under vacuum at 50°C. ^{1}H NMR measurement of the intermediate 2 was carried out by using an XL-200 NMR instrument (Varian, Sunnyvale, CA, USA) to estimate the alkyl group/carboxyl group ratio from the signal intensities.

2.2. Samples

PAHs were purchased from AccuStandard (New Haven, CT, USA), and other chemicals from Nacalai Tesque.

2.3. Equipment and measurement

The same equipment was used as in the previous study [34]. Detection was carried out at 254 nm. The capillary (50 μm I.D., 375 μm O.D.) length was 48 cm with an effective length of 33 cm. PAA-supported pseudo-stationary phase was used at a concentration of 20 mg/ml in 20 m*M* borate buffer (pH 9.3) or buffer–methanol mixtures, unless noted otherwise. The solutions were filtered with a membrane filter (0.2 μm) before use. The t_0 value was obtained by the injection of methanol, and t_C values by the iteration method [38,39] using a series of alkyl phenyl ketones.

3. Results and discussion

3.1. Preparation of pseudo-stationary phases

The pseudo-stationary phases are listed in Table 1

Table 1
Pseudo-stationary phases prepared from PAA

Carrier	R %[a]	R/$COOCH_3$ ratio[b]
PAA-C_8	20	0.13
PAA-C_{10}	20	0.16
PAA-C_{12}-I	10	0.06
PAA-C_{12}-II	20	0.18
PAA-C_{16}	10	0.14

Prepared from PAA hydrochloride (molecular mass = 10 000).
[a] Degree of alkylation; ratio of an alkyl halide to the amino group in PAA in feed.
[b] From NMR measurement.

with the feed ratios of the reagents for the preparation of PAA–C_8–PAA–C_{16}. Also shown in Table 1 are the ratios of the number of alkyl (R) groups to that of $COOCH_3$ groups in the intermediate (2) (Fig. 1) calculated from the signal intensities of the terminal methyl group in the alkyl moiety and the methyl group in the ester moiety determined by NMR, which indicate the population density of the alkyl groups. The ratios of the alkyl groups to that of the carboxylate groups in PAA–C_n can be estimated from these values, assuming the complete hydrolysis and complete recovery of the product. The ratios of R/$COOCH_3$ are greater than expected from the feed ratios, but still much smaller than unity, which is the case with ordinary micelles consisting of ionic surfactants. A high degree of alkylation leads to products insoluble in methanol–water mixtures, especially with PAA derivatives having long alkyl groups.

PAA-C_8, PAA-C_{10}, and PAA-C_{12}, except PAA-C_{16} for solubility reasons, gave separations of alkyl phenyl ketones in borate buffer, as shown in Fig. 2. PAA-C_{10} and PAA-C_{12}-I showed high efficiency and a similar separation profile for the alkyl phenyl ketones with narrow peak spacings at the earlier and later part of the separation window. The migration of hydrophobic compounds close to t_C is due to the predominant partition of the hydrophobic solutes into the pseudo-stationary phase. PAA-C_8 showed poor efficiency with increasing band spacings with the more hydrophobic solutes. The migration time window is wider with the short alkyl derivatives, which gave a separation profile closer to that in RPLC.

The chromatograms in Figs. 3 and 4 show the characteristics of EKC with PAA-supported polymeric pseudo-stationary phases. The migration times of alkyl phenyl ketones are limited by t_C at low methanol concentration. At 20% methanol both PAA-C_{10} and PAA-C_{16} carriers showed features common to a SDS micellar system in aqueous buffer, showing the predominant partition of hydrophobic compounds into the carrier phase. The migration range of these compounds, however, suddenly expanded at 40% methanol with PAA-C_{10}, followed by the compression of the range with further increase in methanol content. The latter observation is similar to

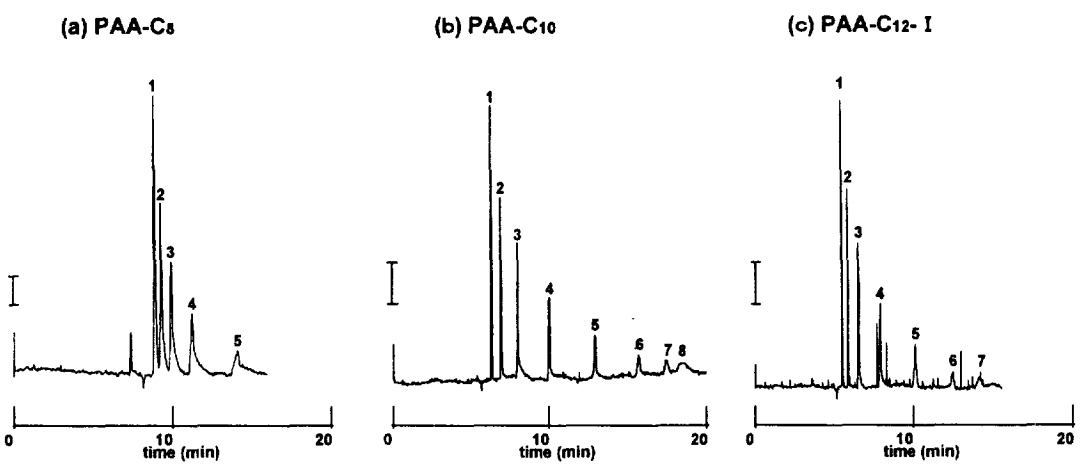

Fig. 2. Separation of alkyl phenyl ketones (C_6H_5-CO-C_nH_{2n+1}, $n = 1–8$) with (a) PAA-C_8 40 mg/ml, (b) PAA-C_{10}, 20 mg/ml, and (c) PAA-C_{12}-I, 20 mg/ml. Field strength = 200 V/cm. Buffer solution: 20 m*M* borate, pH 9.3. Peak numbers indicate the number of carbon atoms in the alkyl group. Scale bars correspond to 0.002 AU.

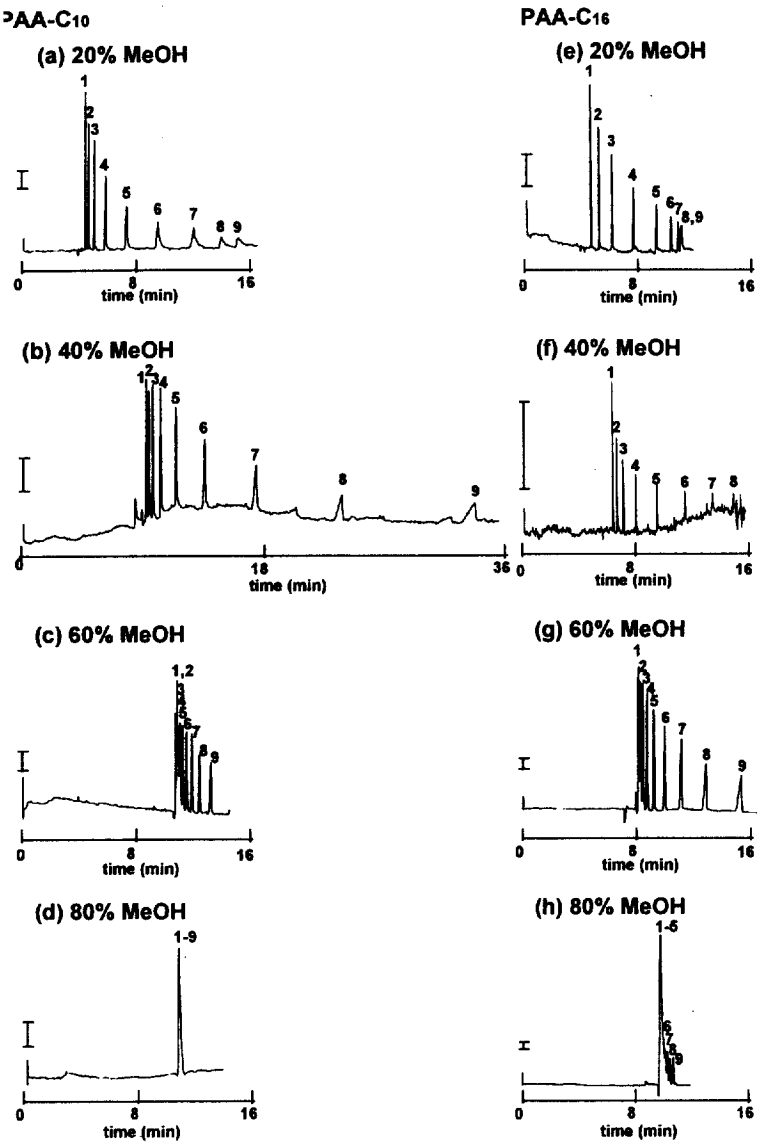

Fig. 3. Effect of methanol addition on the separation of alkyl phenyl ketones (C_6H_5-CO-C_nH_{2n+1}, $n=1$–9) in 20–80% methanol with PAA-C_{10} (a–d) and PAA-C_{16} (e–h). Field strength=400 V/cm. Carrier: 20 mg/ml. Separation solution: 20 m*M* borate buffer–methanol mixture. Peak numbers indicate the number of carbon atoms in the alkyl group. Scale bars correspond to 0.002 AU.

that in RPLC. Above a certain methanol content, 40% for PAA-C_{10} and 60% for PAA-C_{16}, the migration times of the solutes are no longer limited by t_C.

Similar results were obtained for aromatic hydrocarbons. Long migration times were observed for the aromatic hydrocarbons at 40% methanol with PAA-C_{10}, followed by the compression of the migration range with the increase in methanol content. PAA-C_{16} showed a narrow migration range in 40% methanol, and a much wider migration range and better separation than PAA-C_{10} in 60% methanol, indicating its greater hydrophobic property. The results indicate that a migration range of solutes is limited by t_C below a certain methanol content as in

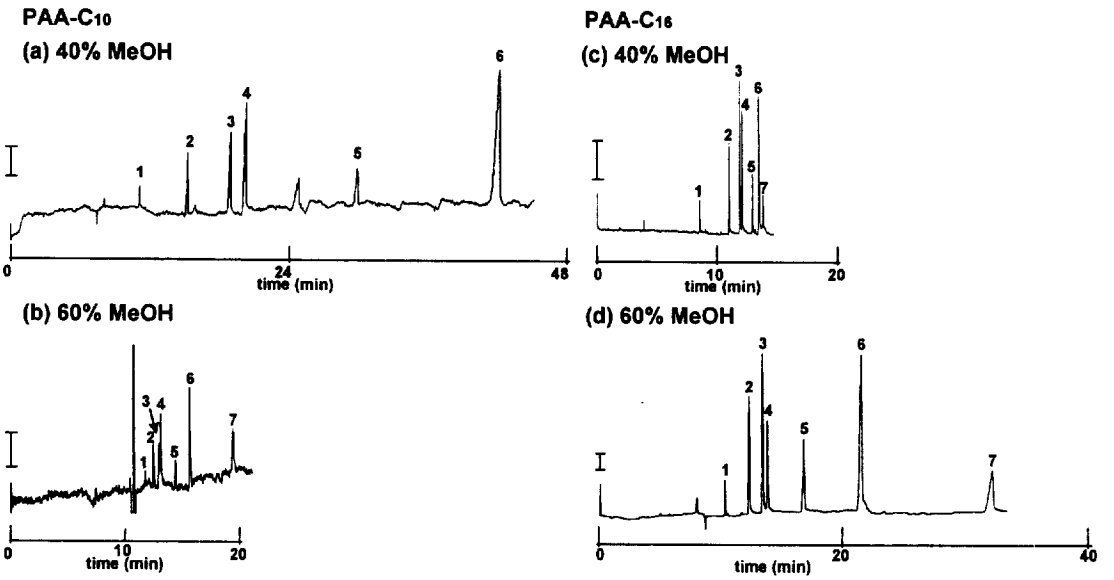

Fig. 4. Effect of methanol addition on the separation of aromatic hydrocarbons with PAA-C_{10} (a, b) and PAA-C_{16} (c, d) in 20 m*M* borate buffer–methanol mixtures. Field strength=400 V/cm. Carrier: 20 mg/ml. Solutes; naphthalene (1), fluorene (2) phenanthrene (3), anthracene (4), pyrene (5), triphenylene (6), benzo(*a*)pyrene (7). Scale bars correspond to 0.002 AU.

a typical MEKC system, and by k' values at a high methanol content as in a RPLC system. The difference in the effect of methanol addition on t_C appears as the difference in a migration profile with PAA-supported pseudo-stationary phase with different alkyl chain length. With the increase in methanol content, the t_0 showed a gradual increase, indicating the gradual decrease in electroosmotic flow, for all pseudo-stationary phases, as shown in Fig. 5. Although the migration time of the alkylated PAAs (t_C) also showed a gradual increase at low methanol content, it showed a sudden increase at certain, and different, methanol concentrations for each carrier with different alkyl chain length. The difference between the two curves for t_0 and t_C corresponds to a separation time window for the solutes, which becomes much wider at a certain methanol content. The expansion of the separation time window

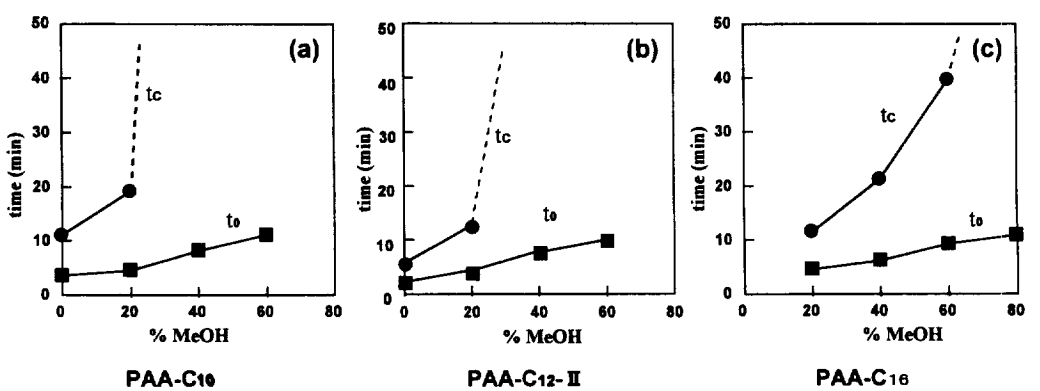

Fig. 5. Variation of migration times of PAA-C_n (t_C) and of an unretained solute (t_0) with methanol content of the separation solutions. 20 m*M* borate buffer–methanol mixtures. Field strength=400 V/cm. Carrier: 20 mg/ml. (a) PAA-C_{10}, (b) PAA-C_{12}-II, and (c) PAA-C_{16}. The t_C values were estimated by the iteration method.

occurred at a higher methanol content and more gradually with the PAA derivatives having longer alkyl groups. The results suggest a structural change of the polymeric pseudo-stationary phases at a certain methanol concentration, as suggested by Palmer and McNair with micelle polymers [20].

The expansion of the migration time windows indicates that the mobility of the PAA-C_n is very small above a certain methanol content. This indicates that the term in the brackets becomes negligible in Eq. (3) [7], where μ_{eo} stands for the electroosmotic mobility and μ_{ep} for the electrophoretic mobility of the carrier, ε for a dielectric constant and η for the viscosity of the medium, ζ_1 for a zeta potential of the fused-silica surface, ζ_2 for a zeta potential and $f(\kappa a)$ for a parameter related to the size and shape of a solute ion, or the carrier in the present consideration.

$$\mu_{eo} = -(\varepsilon/\eta)\zeta_1 \quad (1)$$

$$\mu_{ep} = (\varepsilon/\eta)(2\zeta_2 f(\kappa a)/3) \quad (2)$$

$$\mu_{eo} + \mu_{ep} = -(\varepsilon/\eta)[\zeta_1 - 2\zeta_2 f(\kappa a)/3] \quad (3)$$

The mobility of a carrier, $(\mu_{eo}+\mu_{ep})$, is reduced either by a relative decrease in ζ_1 or by a relative increase in the second term in the brackets. The change in the electroosmotic mobility, determined by ζ_1, ε, and η cannot explain the results, because a similar change of electroosmotic flow was observed for all the PAA derivatives with the change in methanol content, while the variation of the total mobility of the carrier was affected by the alkyl chain length. Therefore the reduction in the mobility of PAA-C_n is presumably caused by the change of ζ_2 and/or the shape parameters of the carrier. This is most likely achieved by swelling of the random coil structure of alkylated PAAs due to the increased solvation resulting in the exposure of more ionic groups as well as alkyl groups to the medium. EKC with micelle polymers [20] and SDS micellar system [40] also showed similar behavior with the addition of methanol. This is understandable, because the micelle-like structure of the micelle polymers or micelles will not be maintained in the presence of high concentrations of organic solvents. The control of separation can be carried out in a much wider range of organic solvents with polymeric pseudo-stationary phases than with micelles [40].

With the expansion of the migration time windows, the migration times of the hydrophobic compounds first increase and then decrease with the increase in methanol content. Thus maxima in migration times and in peak spacings were observed for the hydrophobic solutes at certain methanol content, followed by the compression of the migration range at higher methanol concentration, as shown in Fig. 3 and Fig. 4, and Fig. 5 and Fig. 6. Maxima in migration ranges may not be seen at medium methanol content, unless the sample contains solutes which are hydrophobic enough to show very large k' values at the transition from the region where migration times are limited by t_C to the region controlled by k' values.

When log k' values are plotted against methanol content, a decrease in log k' was always observed with the increase in methanol content, but slight differences were seen from similar plots in RPLC. Upper deviation from a linear relation, or a smaller decrease in k' values, was seen for the more hydrophobic alkyl phenyl ketones at 40% methanol with PAA-C_{12}-II and at 60% with PAA-C_{16}, as shown in Fig. 7. The effect was more clearly seen with the more hydrophobic PAHs, which showed humps, or smaller decreases in k' values, at 40% with PAA-C_{10} and PAA-C_{12}-II, and at 60% with PAA-C_{16}, respectively, where the expansion of a solute migration range was observed. Such non-linear plots were also reported in EKC in acetonitrile–water mixtures [20], but not in RPLC.

The results suggest that a structural change caused by the selective solvation of a polymeric pseudo-stationary phase with methanol starts from less hydrophobic regions and leaves the more hydrophobic sites less solvated where solutes with high hydrophobicities are partitioned. This process, accompanied by the increasing exposure of ionic groups to the medium, is compatible with the decrease in total mobility of PAA-C_n at a higher methanol content, and with the greater methanol concentrations required for PAA-C_{16} to show a change similar as that for the PAAs with the shorter alkyl groups.

Comparison between the pseudo-stationary phases having a difference in the degree of alkylation can be

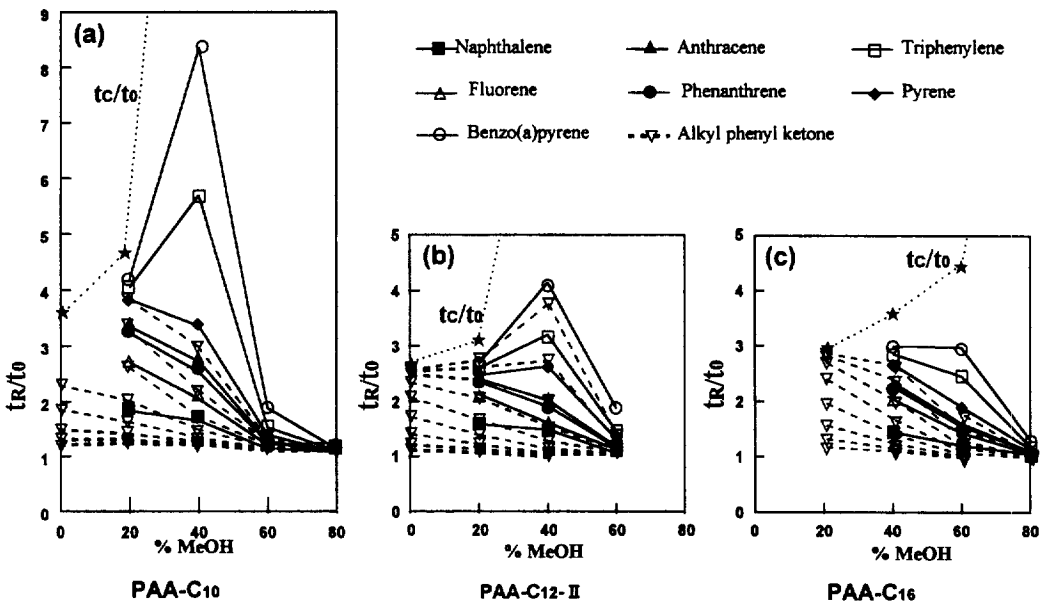

Fig. 6. Variation of migration times of solutes (t_R/t_0) and the PAA-C_n (t_C/t_0) with methanol content. For experimental conditions, see Figs. 3–5. Solutes: alkyl phenyl ketones (dashed lines), and PAHs.

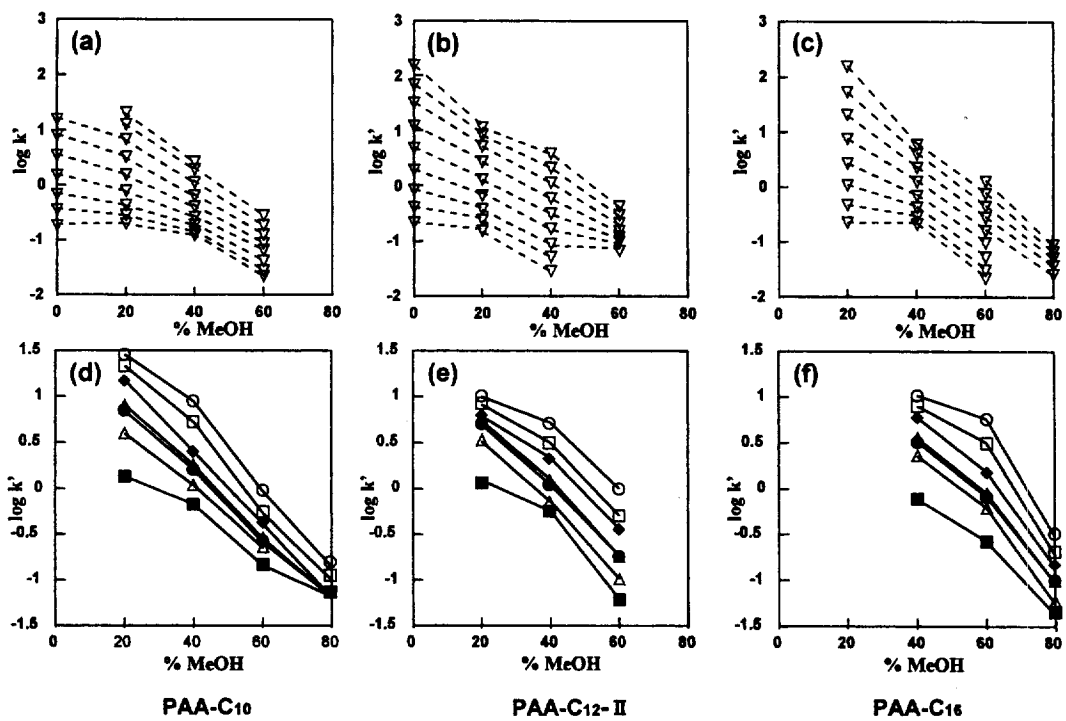

Fig. 7. Plots of log k' values against methanol content. (a–c) Alkyl phenyl ketones (C_6H_5-CO-C_nH_{2n+1}, $n=1$–9), (d–f) aromatic hydrocarbons. See Figs. 3–5 for experimental conditions.

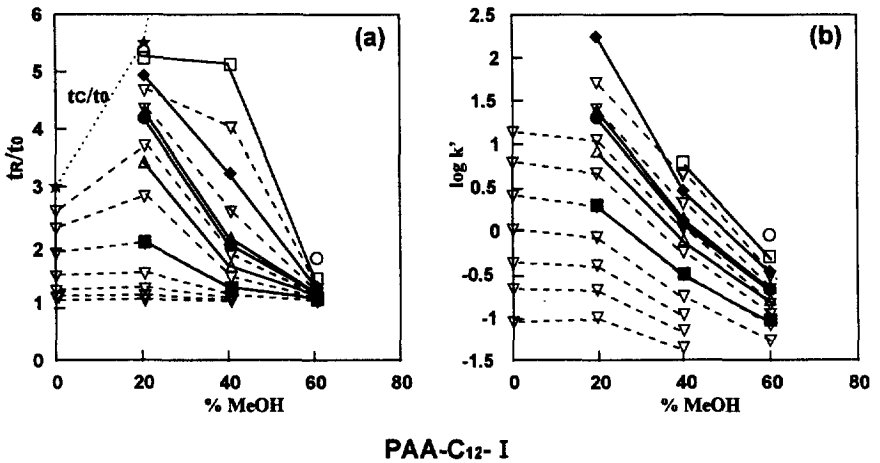

Fig. 8. Plots of (a) (t_R/t_0) and (b) log k' values on PAA-C_{12}-I against methanol content. Solutes: alkyl phenyl ketones (dashed lines) and aromatic hydrocarbons. Field strength = 400 V/cm. Carrier: 20 mg/ml. Separation solution: 20 m*M* borate buffer–methanol mixture.

seen in Figs. 6–8 for PAA-C_{12}-I and PAA-C_{12}-II. PAA-C_{12}-I showed a much wider migration range of solutes due to the greater t_C at low methanol concentrations based on the smaller ratio of the number of alkyl groups to that of carboxylate groups than for PAA-C_{12}-II. The k' values on PAA-C_{12}-I are similar or smaller compared to those on PAA-C_{12}-II except for 20% methanol, where PAA-C_{12}-I resulted in maxima in the t_R/t_C values and large k' values presumably due to the increased solvation. In 60% methanol, PAA-C_{12}-II showed migration profiles and k' values very similar to those for PAA-C_{12}-I. A high degree of alkylation is not necessarily advantageous for the performance in terms of peak capacity and efficiency. The partition behavior of solutes, or the k' values, are influenced by the alkyl chain length and the solvation, while the migration time window is dominated by the contribution of the ionic groups. As shown in Fig. 6, maximum peak spacings were observed at a higher methanol content for the more hydrophobic compounds and on the more hydrophobic pseudo-stationary phase. Optimum separations were obtained in a methanol concentration range which provides wide peak spacings at the descending portions of the plots of t_R/t_0 against methanol content rather than at the ascending portions or near the maxima in Fig. 6 where broad peaks were observed. Efficiencies greater than 100 000 theoretical plates were typically generated with PAA-C_{16}. For relatively hydrophilic solutes, high-performance separations were obtained in an aqueous buffer, and for the more hydrophobic solutes at the higher methanol concentrations, just like in RPLC.

Fig. 9 shows the separation of the 16 PAHs, EPA priority pollutants, with PAA-C_{10}. This pseudo-stationary phase gave a high efficiency separation for the first eight compounds in 50% methanol, but showed relatively poor performance for the more hydrophobic ones in spite of the adequate migration time window. The poor performance is probably due to the low sample loading capacity of this pseudo-stationary phase.

PAA-C_{16} also showed poor resolution for the larger PAHs in 50% methanol, as shown in Fig. 10a. This is due to the small t_C which caused a narrow migration time window for the hydrophobic solutes with large k' values. With a wider migration time window in 60% methanol, the separation was greatly improved, and 15 peaks were clearly observed for the 16 compounds, as shown in Fig. 10b. The first half of the chromatogram was a little compressed compared to the results in 50% methanol, but the latter half was considerably expanded. At 65% methanol the migration range was compressed, and the resolution was lost especially at the beginning of the chromatogram.

Although the two compounds, No. 14 and No. 15, were not resolved in spite of the peak broadening

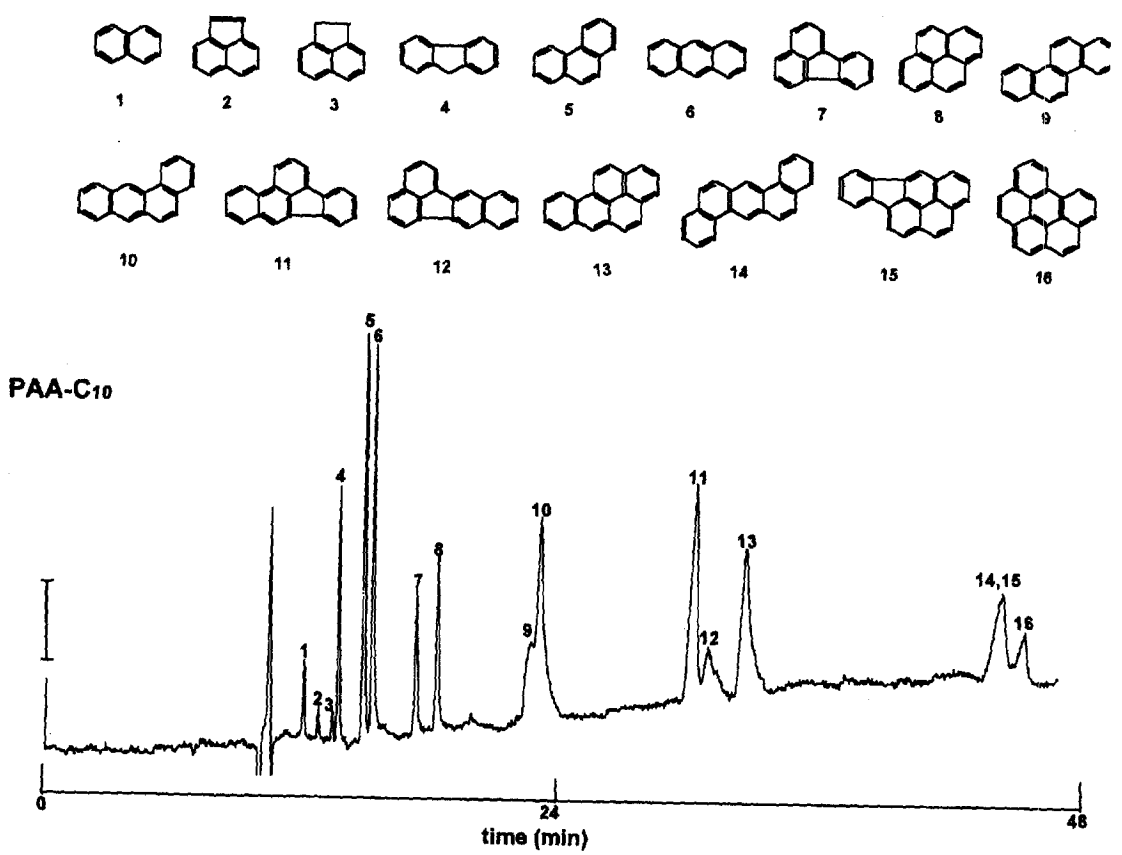

Fig. 9. Separation of 16 PAHs, priority pollutants designated by EPA, with PAA-C_{10}. Borate buffer (20 m*M*)–methanol (50:50). Field strength=400 V/cm. Carrier: 20 mg/ml. Separation solution: 20 m*M* borate buffer–methanol mixture. The scale bar corresponds to 0.002 AU.

indicating a partial separation, the results in 60% methanol compare favorably with the reported separations of similar mixtures by CEC or RPLC using small particles [4,41,42]. Although these methods provided high-performance, the early part of the chromatogram with small k' values did not have much peak capacity, and the later part has wide peak spacings indicating the necessity of gradient elution in RPLC [42,43]. As shown in Fig. 10b, overall separation was excellent with EKC by using PAA-C_{16}. Large peak capacities at low k' values and relatively narrow peak spacings at high k' values are the inherent advantages of EKC [7] for the separation of a series of compounds with widely different hydrophobic properties. EKC with the PAA-supported polymeric pseudo-stationary phases seems to be an attractive alternative to CEC for the separation of a wide range of compounds.

4. Conclusions

(1) A pseudo-stationary phase having alkyl (C_8–C_{16}) and carboxylate groups can be prepared from a commercially available hydrophilic polymer, polyallylamine (PAA). Chain length and population density of alkyl groups are controllable.

(2) PAA-C_n with a relatively low degree of alkylation, particularly PAA-C_{16}, provided high-performance for a wide range of compounds including very hydrophobic PAHs in water–methanol mixtures.

(3) Structural change of pseudo-stationary phase

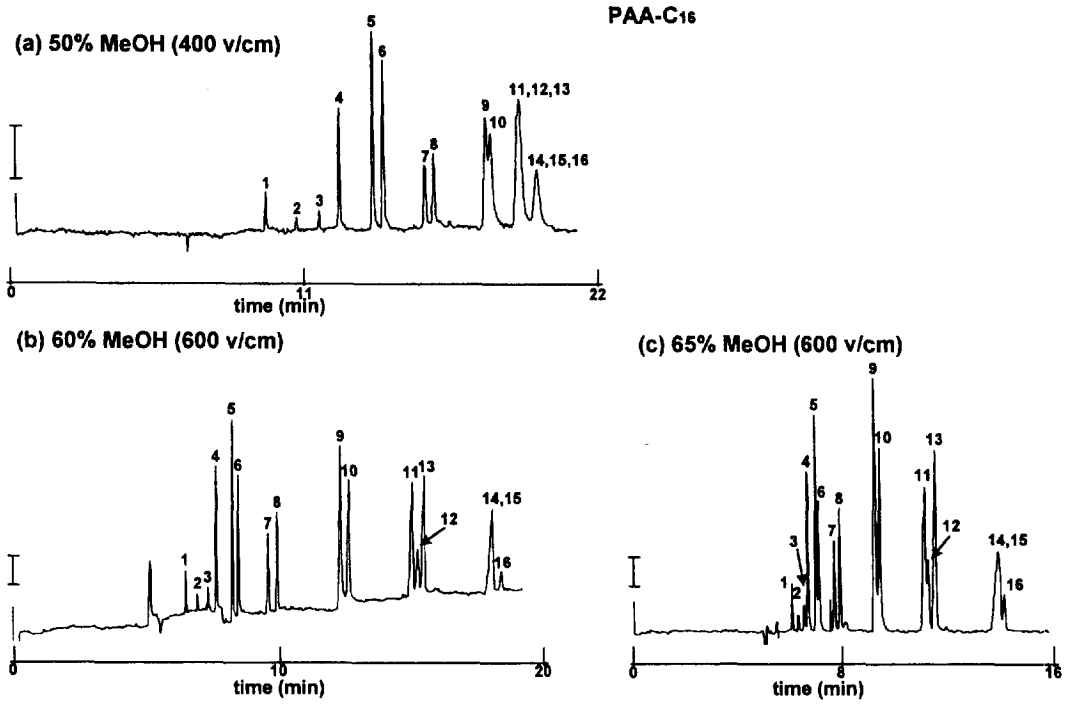

Fig. 10. Separation of 16 PAHs, priority pollutants designated by EPA, with PAA-C_{16}. (a) Borate buffer (20 m*M*)–methanol (50:50). Field strength = 400 V/cm. (b) 40/60, field strength = 600 V/cm. (c) 35/65, field strength = 600 V/cm.. Carrier: 20 mg/ml. Separation solution: 20 m*M* borate buffer–methanol mixture. Scale bars correspond to 0.002 AU.

was suggested with the addition of methanol to cause the expansion of separation time windows, (t_C/t_0). Migration times of hydrophobic solutes are limited by t_C at a low methanol concentration, and controlled by k' values at a high methanol content, showing a transition at 40–60% methanol with t_R/t_0 maxima for hydrophobic compounds. Optimum performance was obtained at a methanol content slightly higher than the composition corresponding to t_R/t_0 maximum.

(4) By selecting an organic solvent content and the chain length of the pseudo-stationary phase, one can realize the inherent advantage of EKC that both an initial part and a later part of a separation can have suitable peak capacity within a reasonable separation time.

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References

[1] H. Soni, T. Tsuda, M.V. Novotny, J. Chromatogr. 559 (1991) 547–558.
[2] H. Yamamoto, J. Baumann, F. Erni, J. Chromatogr. 593 (1992) 313–319.
[3] S. Li, D.K. Lloyd, J. Chromatogr. A 666 (1994) 321–335.
[4] C. Yan, R. Dadoo, H. Zhao, R.N. Zare, D.J. Rakestraw, Anal. Chem. 67 (1995) 2026–2029.
[5] S. Hjerten, K. Nakazato, J. Mohammad, D. Eaker, Chromatographia 37 (1993) 287–294.
[6] C. Fujimoto, J. Kino, H. Sawada, J. Chromatogr. A 716 (1995) 107–113.
[7] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834–841.
[8] S. Terabe, N. Chen, K. Otsuka, Adv. Electrophoresis 7 (1994) 87–153.
[9] K. Otsuka, S. Terabe, T. Ando, Nippon Kagaku Kaishi, 1986, pp. 950–955.

[10] A.T. Balchunas, M.J. Sepaniak, Anal. Chem. 59 (1987) 1466–1470.
[11] J. Vindevogel, P. Sandra, Anal. Chem. 63 (1991) 1530–1536.
[12] P. Lukkari, H. Vuorela, M.-L. Riekkola, J. Chromatogr. A 655 (1993) 317–324.
[13] C. Stathakis, P.L. Warburton, R.M. Cassidy, J. Chromatogr. A 659 (1994) 443–447.
[14] K. Otsuka, M. Higashimori, R. Koike, K. Karuhaka, Y. Okada, S. Terabe, Electrophoresis 15 (1994) 1280–1283.
[15] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr. 516 (1990) 23–31.
[16] Y.F. Yik, C.P. Ong, S.B. Khoo, H.K. Lee, S.F.Y. Li, J. Chromatogr. 589 (1992) 333–338.
[17] S. Terabe, Y. Miyashita, Y. Ishihama, O. Shibata, J. Chromatogr. 636 (1993) 47–55.
[18] C.L. Copper, M.J. Sepaniak, Anal. Chem. 66 (1994) 147–154.
[19] C.P. Palmer, H.M. McNair, J. Microcol. Sep. 4 (1992) 509–514.
[20] C.P. Palmer, M.Y. Khaled, H.M. McNair, J. High Resol. Chromatogr. 15 (1992) 756–762.
[21] S. Terabe, T. Isemura, Anal. Chem. 62 (1990) 650–652.
[22] S. Terabe, H. Ozaki, Y. Tanaka, J. Chin. Chem. Soc. 41 (1994) 251–257.
[23] H. Ozaki, S. Terabe, A. Ichihara, J. Chromatogr. A 680 (1994) 117–123.
[24] W. Schutzner, S. Fanali, A. Rizzi, E. Kenndler, J. Chromatogr. 639 (1993) 375–378.
[25] S. Hara, A. Dobashi, Jpn. Pat. Hei-149206, 1992.
[26] J. Wang, I.M. Warner, Anal. Chem. 66 (1994) 3773–3776.
[27] H. Ozaki, A. Ichihara, S. Terabe, J. Chromatogr. A. 709 (1995) 3–10.
[28] H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi, H. Koizumi, J. Chromatogr. A. 716 (1995) 69–79.
[29] C.P. Palmer, S. Terabe, J. Microcol. Sep. 8 (1996) 115–121.
[30] C.P. Palmer, S. Terabe, Anal. Chem. 69 (1997) 1852–1860.
[31] S. Yang, J.G. Bumgarner, M.G. Khaledi, J. Chromatogr. A. 738 (1996) 265–274.
[32] N. Tanaka, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki, S. Terabe, Chem. Lett. (1992) 959–962.
[33] N. Tanaka, T. Fukutome, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki, K.K. Unger, J. Chromatogr. A 699 (1995) 331–341.
[34] N. Tanaka, T. Fukutome, K. Hosoya, K. Kimata, T. Araki, J. Chromatogr. A 716 (1995) 57–67.
[35] P.G.H.M. Muijselaar, H. Claessens, C.A. Cramers, J.F.G.A. Jansen, E.W. Meijer, E.M.M. de Brabander-van den Berg, S. van der Wal, J. High Resol. Chromatogr. 18 (1995) 121–123.
[36] M. Castagnola, L. Cassiano, A. Lupi, I. Messana, M. Patamia, R. Rabino, D.V. Rossetti, B. Giardina, J. Chromatogr. A 694 (1995) 463–469.
[37] S.A. Kuzdzal, J.J. Hagen, C.A. Monnig, J. High Resol. Chromatogr. 18 (1995) 439–442.
[38] M.M. Bushy, J.W. Jorgenson, J. Microcol. Sep. 1 (1989) 125–130.
[39] M.M. Bushy, J.W. Jorgenson, Anal. Chem. 61 (1989) 491–493.
[40] P.G.H.M. Muijselaar, H.A. Claessens, C.A. Cramers, J. Chromatogr. A. 696 (1995) 273–284.
[41] J.R. Chen, M.T. Dulay, Y.C. Dulay, R.N. Zare, D.J. Rakestraw, D.S. Anex, Ninth International Symposium on High-performance Capillary Electrophoresis, P113, Anaheim, January, 1997.
[42] T.J. Barder, D.L. Gooding, P.J. Wohlman, P.D. DuBois, M.N. Schmunk, C. Thrall, K.D. Bischoff, 20th International Symposium on High-performance Liquid Phase Separations, San Francisco, June, 1996.
[43] C. Yan, R. Dadoo, R.N. Zare, D.J. Rakestraw, D.S. Anex, Anal. Chem. 68 (1996) 2726–2730.