ELSEVIER

Journal of Chromatography A 817 (1998) 15–23

JOURNAL OF CHROMATOGRAPHY A

Characterization of poly(dimethylacrylamide) and the combination of poly(vinyl alcohol) and cetyltrimethylammonium bromide as dynamic electroosmotic flow suppression agents in capillary electrophoresis

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Abstract

Methods that allow reduction of the electroosmotic flow (EOF) to a negligible value in a simple and reproducible manner are of great interest for many potential applications in capillary electrophoresis. In the absence of an EOF the apparent mobility is equal to the effective mobility, making possible identification on the basis of literature values of mobility. In the present work we characterize two dynamic coatings based on the addition of water soluble additives to the running buffer (i) poly(dimethylacrylamide) and (ii) a combination of poly(vinyl alcohol) and cetyltrimethylammonium bromide. The performances of the two systems are evaluated under different experimental conditions.

Keywords: Electroosmotic flow; Background electrolyte composition; Poly(dimethylacrylamide); Cetyltrimethylammonium bromide; Poly(vinyl alcohol); Organic acids

1. Introduction

Capillary zone electrophoresis (CZE) is a powerful technique for the separation of hydrophilic charged compounds. The potential of the technique to provide rapid, efficient and automated separations has attracted significant attention in the last decades. Most of the features of a CE system derive from the minute size of the capillary which allows an efficient dissipation of Joule heat and the use of high field strengths. In uncoated capillaries migration order and run-to-run reproducibility are significantly influenced by the electroosmotic flow (EOF). The presence of EOF with its flat velocity profile has some positive features on the separation allowing simultaneous detection of positively and negatively charged species. However, in many cases the presence of acidic silanols on the capillary surface dramatically affects efficiency and transit time reproducibility. A marked reduction in efficiency, for instance, is observed in the analysis of proteins when cationic macromolecules interact electrostatically with the surface [1,2]. The control of EOF and the suppression of wall adsorption is also a key aspect in isoelectric focusing [3] and in the analysis of DNA or sodium dodecyl sulfate (SDS)–protein complexes [4]. The analysis of fast moving anionic analytes cannot be accomplished in a fused-silica capillary

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0021-9673/98/$19.00
PII: S0021-9673(98)00246-5

since these species migrate against the EOF with a velocity comparable to, or even greater than, that of the EOF, therefore their apparent mobility is close to zero or even negative and the analytes require an infinite amount of time to emerge from the column. The problem is particularly relevant for organic acids whose selectivity is maximum at low pH where the EOF transport is reduced. A great deal of work has been done to suppress EOF including buffer manipulation through addition of salts [5,6], organic solvents [7], additives [8–12] or chemical derivatization of the silica wall [3,13–17].

Before widespread application of CZE, suppression of electroosmosis was an important operational strategy in isotachophoresis (ITP), because in the ITP equipment of the early 1970s use consisted of hydrodynamically closed systems [18,19]. In such systems, electroosmosis generally led to disturbance of the sharp, self-corrected zone boundaries. Failure to suppress EOF to a sufficient extend was one of several reasons why zone electrophoresis in closed equipment was not successful at first.

Dynamic suppression agents used were mostly water-soluble polymers, such as poly(vinyl alcohol) (PVA), methylhydroxyethyl cellulose (MHEC), hydroxyethylcellulose (HEC), hydroxypropylmethyl cellulose (HPMC), polyvinylpirrolidone (PVP) and Triton X-100. In earlier ITP studies, it was shown that these additives acted similarly in both PTFE and fused-silica capillaries [20]. Generally adsorption of polymers to the capillary wall results in viscosity increase of the liquid near the electric double layer. This is a favourable event since, without such wall adsorption, bulk viscosity would increase proportionally leading to lower mobilities and longer analysis times. Some of these polymers have found wide application in CE in the suppression of EOF due to their ability to bind to the capillary wall and change the structure of the silica–water interface [21–23]. The vast majority of the polymers used so far as ‘dynamic’ coatings are hydrophilic linear polymers which are adsorbed to the wall from the buffer solutions via hydrogen bonding.

The effectiveness of these and other additives is often measured using streaming potential measurements. It is the ratio of the zeta potential and the viscosity of the liquid in the vicinity of the electric double-layer which is measured.

$$\mu_{EOF} = -\epsilon\zeta/\eta \quad (1)$$

in which μ_{EOF} is the electroosmotic mobility, ϵ is the dielectric constant near the electric double layer, ζ is the zeta-potential of the capillary wall and η is the viscosity of the liquid near the electric double layer. It is most likely that the additives mentioned mainly increase the latter, although decrease of zeta potential cannot be excluded.

The ability to control and eventually suppress EOF is one of the most important factors in the optimization of CE separations. Chemical derivatization of surface silanols represents an effective way to modify the capillary properties. Our group has been deeply involved in the production of coatings covalently linked to the capillary wall. In an effort to overcome one of the major defects of chemically bonded phases, that is their relative instability towards alkaline hydrolysis, we have recently proposed polymeric coatings based on new acrylic monomers, N-acryloylaminoethoxyethanol (AAEE) [24] and N-(acryloylaminoethoxy)ethyl-β-D-glucopyranoside (AEG) [25] which offer, compared to acrylamide, a higher chemical resistance and hydrophilicity. Although satisfactory results have been obtained with these and with other types of chemically bonded phases, there are many reasons to prefer ‘dynamic’ coatings over permanent ones. Most of the coating procedures are time consuming and require specific skills whereas commercially available columns often show a lack of consistency in inter-column performance and are still fairly expensive. Therefore, methods that allow reduction of EOF to a negligible value in a simple and reproducible manner are of great interest for many potential applications.

The hydrophilicity of the capillary surface is an essential requirement in the analysis of proteins therefore, many research groups have proposed modification of the wall through hydroxylic polymers. However, the analytical application of hydroxylic polymers suffers from a strong dependence of its efficacy on the surface properties, which are in turn significantly affected by several factors, such as pH, ionic strength and capillary history. There are many cases in which a marked hydrophilic character of the wall surface is not essential. Examples are separations of DNA in sieving polymers and separations of small organic ions. The aim of our work is

to extend the choice of potential EOF modifiers and we propose two new systems, based on the addition of poly(dimethylacrylamide) (DMA) or PVA–cetyltrimethylammonium bromide (CTAB) to the running buffer.

To our knowledge there is only one report on the use of poly(DMA) to suppress EOF in the separation of nucleic acids [26]. In particular, poly(DMA) was used as an EOF modifier added to several other polymers currently used as DNA sieving matrices. The use of PVA is well documented in the literature [27–29]; in the present work, we investigate the performances of a system comprised of a PVA–CTAB combination [20,30] and evaluate the sensitivity of the system to operational parameters such as PVA–CTAB concentration and pH. The ability of CTAB to decrease and even reverse the sign of the zeta potential potentiates the effects of PVA which acts on the viscosity of the liquid near the double layer.

The features of the two systems are investigated in the separation of organic acids in different background electrolytes (BGE) containing variable amounts of EOF modifiers. The influence of experimental parameters on the separation are evaluated by comparing reproducibility of the apparent mobilities of the model compounds.

2. Experimental

2.1. Apparatus

CZE experiments were carried out in Milan and Eindhoven. In both cases they were performed in a Beckman P/ACE 5500 automated CE system (Fullerton, CA, USA). Data collection was performed on a PC computer utilizing P/ACE versions 3.0 software. In all the experiments fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA), 460 mm (390 mm to the window)×75 μm I.D. were used. New capillaries were rinsed with 0.1 *M* NaOH for 3 min, then rinsed with deionized water for 5 min and finally washed with BGE. Before each run the capillary was washed for 5 min. The samples were injected by hydrodynamic pressure for 2 s. All separations were carried out at 25°C and the UV adsorption was monitored at 200 nm. All separations were performed at 20 kV with a negative polarity at the inlet in case of dynamic EOF suppression.

2.2. Materials

N,N-dimethylacrylamide, *tert*.-butanol, acetic, citraconic, benzoic, 2-nitrobenzoic, 3-nitrobenzoic, *o*-toluic, *p*-toluic, sulphanilic, hippuric, chromic acid, were from Aldrich (Stanheim, Germany). Ammonium persulfate (AP) and N,N,N′N′-tetramethylethylendiamine (TEMED) were purchased from Bio-Rad (Hercules, CA, USA); tris(hydroxymethyl)aminomethane (Tris), N-methyl-D-glucamine, γ-aminobutyric acid were purchased from Sigma (St. Louis, MO, USA). The water used was deionized using a Milli-Q water purification system (Millipore, USA). Other solvents were of analytical grade. Two test mixtures of anions were made, their properties are listed in Table 1. Values of p*K* and mobilities were taken from the literature [31], effec-

Table 1
Physicochemical parameters of test sample components

Name	pK_{a1}	pK_{a2}	μ_1	μ_1	μ_{eff} at pH 4.0	μ_{eff} at pH 10.2
Citraconic acid	2.46	6.15	−28	−56	−25.6	
o-Nitrobenzoic acid	2.17		−33.6		−30.9	
m-Nitrobenzoic acid	3.49		−32.3		−23.0	
o-Toluic acid	3.91		−29.1		−15.0	
Benzoic acid	4.17		−33.74		−12.7	
p-Toluic acid	4.37		−29.10		−8.1	
Chromic acid	0.75	6.49	−59.3	−81.10		−72.7
Sulphanilic acid	3.23		−33.70			−31.2
p-Nitrobenzoic acid	3.52		−32.30			−29.9
Hippuric acid	3.70		−26.1			−24.1

The data are taken from [34], μ_{eff} values calculated with HPCESIM [35], mobility is given in units of 10^{-9} m^2/V s.

tive mobilities under the respective operational conditions were calculated with the HPCESIM simulation program [32]

2.3. Synthesis and characterization of linear poly(dimethylacrylamide)

Linear poly(DMA) of different relative molecular mass, M_r 230 000, and M_r 600 000, was used in the present investigation. Poly(DMA) of reduced chain length was synthesized according to Grossman [33] by using 2-propanol as a chain transfer agent to control the molecular mass of the product. A solution of freshly distilled N,N-dimethylacrylamide (1 g) was dissolved in 9.7 ml of water and degassed under vacuum for 30 min. Isopropanol (0.3 ml) was then added to the reaction vessel. Next, 100 μl of 10% (v/v) TEMED in water and 10 μl of a 40% (w/v) AP in water were added. The mixture was allowed to react for 1 h at 50°C. To remove any unreacted monomer and contaminants, the reaction mixture was dialyzed against water using a 12 000 molecular mass cut-off dialysis membrane from Sigma. The solution was lyophilized to give 0.8 g of a white solid. A similar procedure was adopted to produce long-chain poly(DMA) in the absence of isopropanol. The average molecular masses of the different polymers were determined by gel permeation chromatography (GPC). Samples and standards were run in a Ultrahydrogel linear column (Waters, Milford, MA, USA) connected to a refractometer and UV Bruker detectors injecting 0.2% (w/v) sample solutions. The mobile phase was 0.2 *M* Na_2SO_4. Five polyacrylamide standards were used to calibrate the GPC (Polysciences, Warrington, PA, USA).

2.4. Procedures

Three different BGEs were used. A pH 4.0 system was made using 50 m*M* acetic acid, with Tris. For the determination of residual EOF in a 50-μm capillary, the following system was used: 100 m*M* N - tris(hydroxymethyl)methyl - 3 - aminopropanesulfonic acid (TAPS) with Tris to pH 8.5 and addition of 2.5 m*M* Na_2EDTA. In a third BGE, another test mixture of chromate, sulphanilate, *p*-nitrobenzoate and hippurate was analyzed. This last BGE consisted of 20 m*M* N-methyl-D-glucamine and 20 m*M* γ-aminobutyric acid at pH 10.2.

In one set of experiments the EOF was suppressed by adding 0.05% (w/v) of poly(DMA) to the running buffer whereas in another set of experiments EOF was suppressed by using BGE solutions containing PVA and CTAB in different concentrations. PVA and CTAB were added to the running buffer from concentrated stock solutions of 5% (w/v) and 5 m*M*, respectively. As adding the latter would lead to 2–3% dilution of the BGE, the initial acetic acid concentration was made up in such a way as to ensure a final concentration of 50 m*M*.

3. Results and discussion

In a pH 8.5 BGE poly(DMA) synthesized by conventional techniques, is used as surface interaction component. There are several ways to measure the silica-adsorbing quality of a polymer. In the present investigation we measured the degree of adsorption by monitoring the reduction of EOF in different buffer systems. The EOF mobility was calculated from the transit time of benzyl alcohol in a pH 8.5 buffer in a 50 μm I.D. capillary. As shown in Table 2, even at alkaline pH, poly(DMA) was effective in suppressing EOF in a concentration range from 0.005 to 0.1%. A concentration of 0.05% gave the best compromise between EOF suppression and buffer viscosity and it was therefore used in subsequent experiments.

The viscosities of pH 4.0 BGEs with different EOF suppressing agents were determined in a P/ACE instrument at 25°C by low pressure displacement in 50 m*M* acetic acid with Tris to pH 4.00 using a 398/464 mm capillary of 75 μm I.D.. Viscosity

Table 2
Variation of μ_{eof} as a function of M_r 230 000 poly(DMA) concentration

Poly(DMA) (%, w/v)	μ_{eof} (10^{-9} $m^2/V\,s$)
0.005	2.02
0.01	1.7
0.03	1.33
0.05	1.07
0.1	<1.06

Table 3
Viscosity of pH 4.0 BGEs containing different EOF suppressing agents, see Section 2

Additive	Viscosity at 25°C ($kg\,m^{-1}\,s^{-1}$)
None	0.0089
0.05% PVA, $5\cdot10^{-5}$ *M* CTAB	0.0090
0.05% M_r 230 000 poly(DMA)	0.0094
0.05% M_r 600 000 poly(DMA)	0.0113

values are summarized in Table 3. Bulk viscosity of the PVA–CTAB system is not significantly increased. The poly(DMA) systems show a slightly increased bulk viscosity.

The test mixture was next analyzed in an uncoated capillary at pH 4.0. The analytes with higher electrophoretic mobility, citraconic and *o*-nitrobenzoic acid required a long time to emerge from the column. R.S.D. values, shown in Table 4, are quite acceptable. Comparison with values from literature, see Table 1, are given only for qualitative purposes.

These results were compared with those obtained in dynamically coated capillaries, using the same pH 4.0 BGE, to which different EOF suppressing agents had been added.

In the second system, a mixture of PVA and CTAB is added to the running buffer. In this case, the effect of PVA, becomes stronger due to the presence of the cationic detergent which acts by changing the zeta potential. Cationic detergents, such as CTAB and Priminox, can decrease and even reverse the sign of the zeta potential, depending on their concentration [34]. Likewise, EOF in micellar electrokinetic chromatography (MECC) is determined by SDS adsorption on the capillary wall. This was known before the beginning of CE [35]. For CTAB, at $5\cdot10^{-5}$ *M*, the silica surface is near-neutral [20,30]. The effect of viscosity increasing and of zeta potential decrease is apparently additive, as was concluded from a study on CZE in open and closed systems [30]. In these experiments, PVA was added to 0.05% and CTAB to $5\cdot10^{-5}$ *M*. In addition, different combinations of PVA and CTAB around these values were used. Fig. 1 shows an example of the separation of the test mixture carried out at pH 4.0 in a BGE containing 0.05% PVA and $5\cdot10^{-5}$ *M* CTAB. Although this combination gave slightly lower R.S.D. of apparent mobility, PVA and CTAB concentrations do not appear to be critical under these conditions as clearly demonstrated by the reproducibility obtained with different concentrations of the two ingredients. Results are summarized as average apparent mobilities and their R.S.D. values in Table 5. The R.S.D. values are as good as or better than those obtained in an uncoated capillary in the cationic mode but the analysis time was three times shorter.

The same sample was separated using the same buffer, but now containing 0.05% of poly(DMA). Two types of poly(DMA) polymers with relative molecular masses of 230 000 and 600 000 were evaluated, the best performance was given by the one with longer chains. In capillaries dynamically coated with short chain poly(DMA) a residual EOF of 10^{-9} $m^2/V\,s$ was measured whereas zero or undetectable EOF was seen in those coated with long chain poly(DMA). Fig. 2 shows a representative electropherogram of organic acids separated at pH 4.0 in poly(DMA) dynamic coating. The suppression of EOF here also led to a faster analysis time and improved the reproducibility of transit times and mobility. Reproducibility data obtained with short and long chain poly(DMA) are summarized together with the PVA–CTAB results in Table 5. The small difference of mobilities in the two cases reflects the presence of a residual EOF in the capillaries coated with poly(DMA) M_r 230 000, the long chain polymer, interacting more strongly with the wall, gave a

Table 4
Average and relative standard deviation (R.S.D., $n=6$) of effective mobilities in units of 10^{-9} $m^2/V\,s$ of test components in an uncoated capillary (pH 4.0) BGE

	DMSO	1	2	3	4	5	6
Mean	38.828	−33.50	−31.31	−26.40	−19.41	−15.92	−11.76
R.S.D. (%)	0.57	1.16	0.95	0.88	0.74	0.75	0.75

Six analytes are listed as in Fig. 1. DMSO = dimethylsulfoxide.

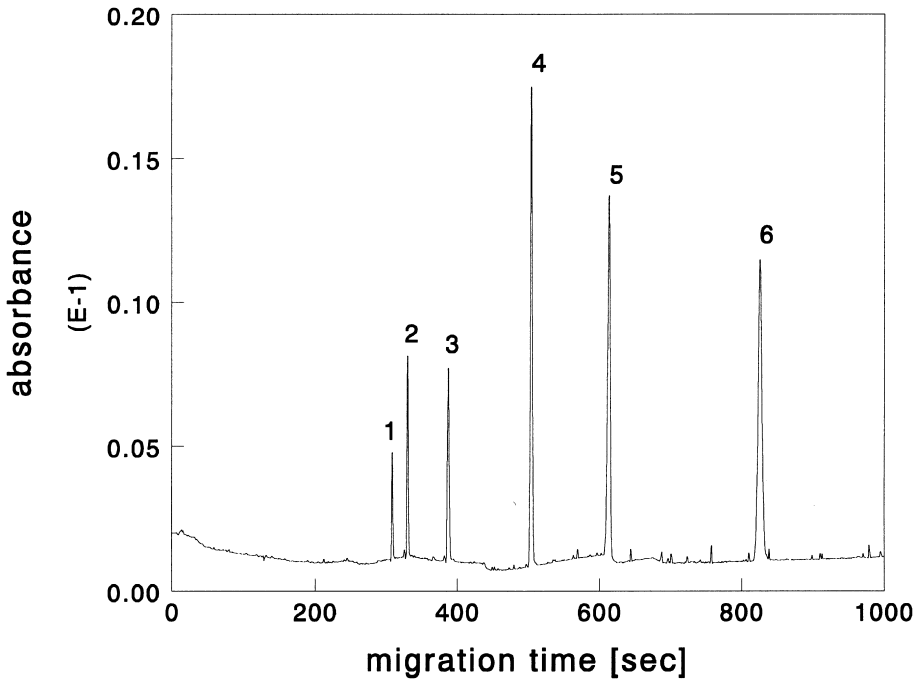

Fig. 1. Analysis of a standard mixture; 1=citraconic acid, 2=*o*-nitrobenzoic acid, 3=*m*-nitrobenzoic acid, 4=*o*-toluic acid, 5=benzoic acid and 6=*p*-toluic acid in a P/ACE 5500 using a pH 4.0 BGE with 0.05% PVA and $5\cdot10^{-5}$ *M* CTAB.

better reproducibility as seen by the lower R.S.D. values of apparent mobility observed for all the samples.

Since EOF influence is more pronounced at higher pH values, it is probable that it would be more difficult to dynamically suppress EOF under such

Table 5
Average and relative standard deviation (R.S.D., $n=6$) of apparent mobilities in units of 10^{-9} $m^2/V\,s$ of test components in capillaries 'dynamically' coated with different additives at pH 4.0

	1	2	3	4	5	6
0.03% (w/v) PVA 5.10^{-5} *M* CTAB	−29.29	−27.53	−22.66	−17.66	−14.36	−10.28
R.S.D. (%)	0.27	0.27	0.55	0.26	0.27	0.67
0.05% (w/v) PVA $5\cdot10^{-5}$ *M* CTAB	−29.58	−27.65	−23.57	−18.11	−14.88	−11.01
R.S.D. (%)	0.27	0.30	0.31	0.33	0.41	−0.60
0.1% (w/v) PVA $5\cdot10^{-5}$ *M* CTAB	−29.53	−27.52	−22.64	−17.67	−14.40	10.32
R.S.D. (%)	0.26	0.27	0.20	0.21	0.21	0.40
0.05% (w/v) PVA $2.5\cdot10^{-5}$ *M* CTAB	−29.42	−27.47	−23.09	−17.56	−14.25	−10.31
R.S.D. (%)	0.40	0.46	0.43	0.53	0.58	0.91
0.05% (w/v) PVA $1\cdot10^{-4}$ *M* CTAB	−29.42	−27.34	−21.80	−17.58	−14.29	−10.12
R.S.D. (%)	0.39	0.51	1.08	0.61	0.73	1.5
0.05% M_r 230 000 Poly(DMA)	−28.41	−26.46	−22.35	−16.60	−13.29	−9.44
R.S.D. (%)	0.35	0.34	0.48	0.68	0.82	0.98
0.05% M_r 600 000 Poly(DMA)	−29.43	−27.51	−23.45	−17.77	−14.49	−10.67
R.S.D. (%)	0.14	0.13	0.26	0.43	0.61	0.84

Six analytes listed as in Fig. 1.

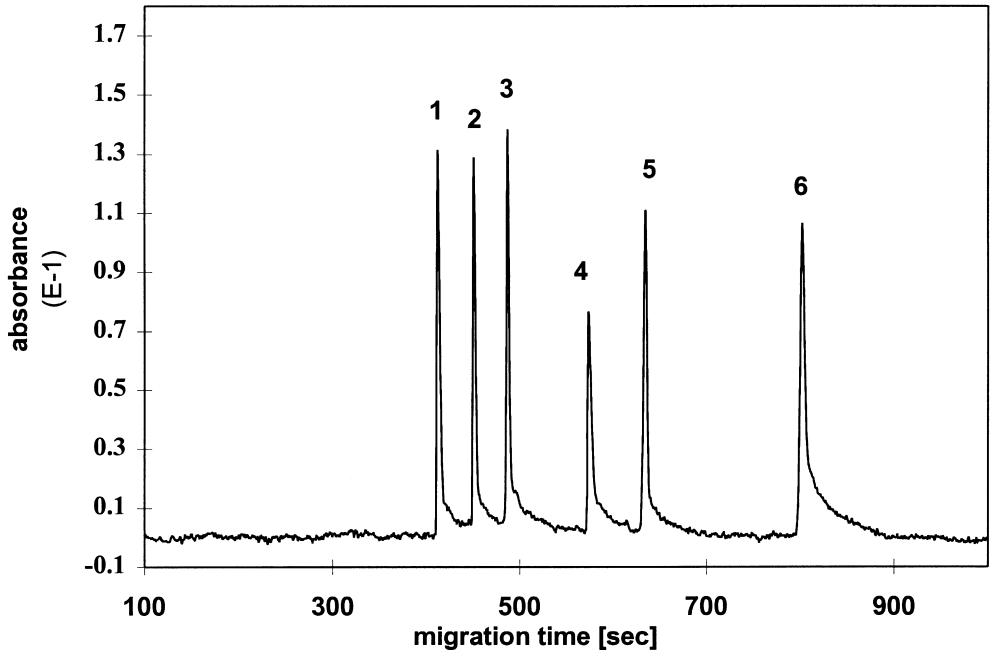

Fig. 2. Analysis of a standard mixture; 1=citraconic acid, 2=*o*-nitrobenzoic acid, 3=*m*-nitrobenzoic acid, 4=*o*-toluic acid, 5=benzoic acid and 6=*p*-toluic acid in a P/ACE 5500 using a pH 4.0 BGE with 0.05% poly(DMA) with a relative molecular mass of 600 000.

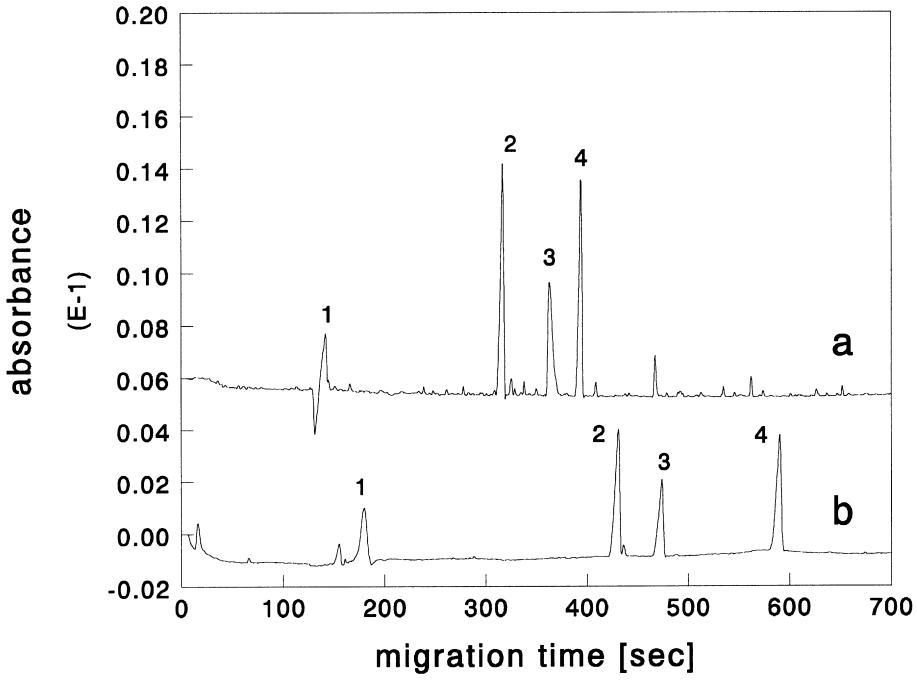

Fig. 3. Analysis of a test mixture; 1=chromate, 2=sulphanilate, 3=*p*-nitrobenzoate and 4=hippurate in a P/ACE 5500 using a pH 10.2 BGE with (a) 0.05% PVA and $5 \cdot 10^{-5}$ *M* CTAB or (b) 0.05% poly(DMA) M_r 230 000.

conditions. This was verified as follows. The ability of both additives to suppress EOF at high pH was investigated by carrying out separations of chromate, sulphanilate, *p*-nitrobenzoate and hippurate in a buffer at pH 10.2. Fig. 3 shows the separations obtained in both cases. The PVA–CTAB combination appears to suppress EOF more effectively than poly(DMA). At such a alkaline pH a simple increase of local viscosity does not sufficiently suppress EOF, so that effectively reducing the zeta potential using CTAB is also required.

4. Conclusions

A simple way to control EOF is described based on the addition to the running buffer of water soluble components that rapidly adsorb to the silica wall and modify its surface properties. This article discusses the main parameters influencing the performances of poly(DMA) and PVA–CTAB used as dynamic coatings for CE. A small residual flow is still present as indicated by the fact that apparent mobilities of test samples measured in dynamically coated capillaries do not correspond to their electrophoretic mobilities, however both systems suppress EOF effectively. The presence of a residual EOF does not seriously affect reproducibility of mobility. The PVA–CTAB system, whose components act simultaneously on the viscosity across the wall and the zeta potential, appears to be more efficient in suppressing EOF than poly(DMA), however both systems are effective. At pH$>$10 the PVA–CTAB combination suppresses EOF more efficiently than poly(DMA). This is because at such alkaline pH, decreasing the zeta potential is an essential additional requirement to suppress EOF.

Acknowledgements

We thank the graduate students (Eindhoven University of Technology) B.M. van Meerendonk and L. van Ravenstein for performing the initial experiments.

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