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Electroosmotic flow suppressing additives for capillary zone electrophoresis in a hydrodynamically closed separation system

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Abstract

Electroosmotic flow in a hydrodynamically closed capillary zone electrophoresis (CZE) separation compartment must be minimized to achieve high efficiency CZE separations. A group of eight potential electroosmotic flow suppressors was investigated in this context for the separations in fluorinated ethylene–propylene capillary tubes. The suppressors included water soluble methylhydroxyethyl derivatives of cellulose, polyvinylalcohol, polyvinylpyrrolidones and polyethyleneglycols of different molecular masses and Triton X-100. Methylhydroxyethylcellulose derivatives and polyvinylalcohol were found to provide the highest separation efficiencies for a group of model anions when the electroosmotic flow suppressors were used as the carrier electrolyte additives. Using a methylhydroxyethylcellulose coated separation compartment very significant improvements in the separation efficiencies were achieved for polyvinylpyrrolidones and polyethyleneglycols applied in the carrier electrolyte solutions. For example, polyvinylpyrrolidone K 90 applied in this way gave for some of the model analytes the plate height values approaching those estimated in the calculations as theoretical limits for our experimental conditions ($H \approx 3.5$ μm). CZE experiments with albumin and γ-globulin showed that the use of methylhydroxyethylcellulose derivative in the carrier electrolyte solution at pH=9.2 was effective in eliminating potential disturbances in the separation efficiencies of the analytes due to adsorption of the proteins.   © 1997 Elsevier Science B.V.

Keywords: Electroosmotic flow; Buffer composition; Hydrodynamically closed separation system; Organic acids

1. Introduction

At present, capillary zone electrophoresis (CZE) separations are almost exclusively carried out in hydrodynamically opened separation compartments provided with capillary tubes of 20–75 μm I.D. [1–4]. This approach introduced by Jorgenson and Lukacs [5] can conveniently combine the electrophoretic separation with the electroosmotic transport of the solution in which the separation is carried out.

Some analytical applications of CZE (e.g., trace determinations; microscale preparative runs) may require that the separations are carried out in the tubes of larger cross-sections. In such instances the use of hydrodynamically opened separation systems must solve problems linked with the hydrodynamic (flow) dispersion (see, e.g., Ref. [6]). For example, a recent work by Yin et al. [7] showed that this can be a main dispersive source in CZE unless relevant measures are taken. These authors reduced its impact on the band broadening by performing the separations in the tubes provided with 50 μm I.D. flow restrictors. Effective for a 180 μm I.D. tube such a solution probably may have limitations for the tubes of larger I.D..

The use of a hydrodynamically closed separation system offers an alternative solution in eliminating the hydrodynamic dispersion. This hydrodynamic

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concept of the separation compartment is favoured in capillary electrophoresis (CE) instruments designed primarily for capillary isotachophoresis (ITP) [8,9]. Typically provided with the capillary tubes of 200–800 μm I.D. these instruments employ semi-permeable membranes to prevent the hydrodynamic dispersion of the separated constituents. This solution, however, is not restricted to the ITP separations and, for example, it was employed in some pioneering works on CZE (see, e.g., Refs. [6,10,11]).

Electroosmotic flow (EOF), in general, does not contribute to the band broadening in the opened CZE separation systems [1–5,12]. On the other hand, it must be suppressed in the closed systems as otherwise a serious electroosmotic dispersion is unavoidable [1,6,10,11]. EOF suppressors (EOS) added to the carrier electrolyte solutions and chemically modified walls of the capillary tubes are general means employed in the suppression of electroosmosis [10,11,13–15].

CZE separations in the tubes of larger I.D. are of our current interest [16–22]. We are interested in this CZE approach as it offers a convenient way in enhancing the sample loadability closely linked with lower concentration limits of detection (LODs). Our CZE separation system is provided with the capillary tube made of fluorinated ethylene–propylene (FEP) copolymer while the hydrodynamic dispersion is prevented via the use of a mechanically supported membrane in the closed separation system [16]. The FEP tube is preferred as it is chemically inert and as such it does not restrict the compositions of the carrier electrolyte solutions. In addition, its physical properties are favourable from the point of view of the on-column detection [23,24]. Its chemical inertness is, however, limiting means applicable to the suppression of electroosmosis (see above). Our previous works [16–22] showed that the presence of EOF suppressors in the carrier electrolyte solutions may be an effective solution for the FEP capillary tubes. In this work we investigated in details EOF suppressing properties of a group of eight additives which were expected to reduce the ζ-potential and/or increase viscosity of the electrolyte solution at the capillary wall under our CZE working conditions. Some of the additives were already proved effective for similar purposes in ITP while others were considered to be less convenient EOF suppressors [9,10,25].

ζ-Potential values in the tubes made of various plastic materials are pH dependent [26]. Therefore, our experiments with a model group of the analytes were performed in the carrier electrolyte solutions of pH values corresponding to different ζ-potential values. The additives were applied in the carrier electrolyte solutions without or with the coating of the capillary tube by a high-molecular-mass methylhydroxyethyl derivative cellulose.

2. Experimental

2.1. Instrumentation

An EA 101 ItaChrom capillary electrophoresis system (Merck, Darmstadt, Germany) was used in a single-column configuration of the separation unit. The samples were injected with the aid of a laboratory developed valve injector with a 350 nl internal sample loop. The column was provided with a 300 μm I.D. FEP capillary tube (O.D.≈700 μm). The length of the capillary tube was 230 mm (190 mm to the detector). The UV-photometric detector was set at a 254 nm detection wavelength.

2.2. Chemicals and the electrolyte solutions

The electrolyte components of the carrier electrolyte solutions were obtained from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA) and Reanal (Budapest, Hungary).

A stock solution of the carrier electrolyte solution having pH of 5.2 was prepared from the components given in Table 1. It contained none of the EOF suppressors. The final carrier electrolyte solution was prepared by appropriately diluting the stock solution and by adding a purified concentrate of the EOF suppressors (see Section 2.3).

The carrier electrolyte solutions of pH=9.2 were prepared from a stock solution of glycine. The final solutions were prepared in the following steps to minimize the contents of CO_2 in the solutions: (i) a required portion of the stock solution of glycine was taken into a 50 ml volumetric flask. (ii) A required volume of the EOF suppressor concentrate was added and the solution in the flask was made up to the mark. (iii) The solution was sonicated and finally a weighed amount of 1,3-bis[tris(hydroxy-

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Table 1
Electrolyte systems

Parameter	Electrolyte system No.	
	1	2
Solvent	Water	Water
Anion	MES	Glycine
Concentration (mmol/l)	100	50
Counter-ion	Histidine	BTP
Concentration (mmol/l)	10	21
pH	5.2	9.2
Additive[a]	MHEC 300, (MHEC 30K, PEG 4K, PEG 5M, PVP 40K, PVP 750K, PVA 80K, TRITON X-100)	MHEC 30K, (PEG 5M, PVP 750K)
Concentration (%, w/v)	0.2	0.2

MES, 2-(N-morpholino)ethanesulfonic acid; BTP, 1,3-bis[tris(hydroxyethyl)methylamino]propane.

[a] Only one of the additives was added to the carrier electrolyte solution, for the assignments of the abbreviations see Section 2.3.

ethyl)methylamino]propane (BTP) (see Table 1) was added to achieve the required pH value (the BTP preparative was kept in a desiccator over NaOH pellets to reduce the amount of adsorbed CO_2). (iv) The flasks containing the carrier electrolyte solutions of pH=9.2 were kept in a large volume desiccator over NaOH pellets.

Chemicals used for the preparation of model mixtures were obtained from the above suppliers with the exception of *p*-aminobenzoic acid (Lachema, Brno, Czech Republic) and 2,4-dihydroxybenzoic acid (Fluka, Buchs, Switzerland). The concentrations of the acids in the solutions of model mixtures were $5 \cdot 10^{-5}$ mol/l with the exception of 2,4-dihydroxybenzoic and 3,5-dihydroxybenzoic acids which were present at 10^{-4} mol/l concentrations.

Albumin A82 and γ-globulin G70 (outdated preparatives) were from Imuna (Šarišské Michal'any, Slovak Republic). Water purified by a Pro-PS water purification system (Labconco, Kansas City, KS, USA) was used throughout.

2.3. Additives used for the suppression of electroosmosis and their purification

The EOF suppressors studied in this work included methylhydroxyethylcellulose 30000 (MHEC 30K), methylhydroxyethylcellulose 300 (MHEC 300), polyethyleneglycol 5000000 (PEG 5M), polyvinylpyrrolidone 90 (PVP 750K) and polyvinylalcohol 25/140 (PVA 80K) from Serva. Polyethyleneglycol 4000 (PEG 4K) was from P-PARK

(Northampton, UK), polyvinylpyrrolidone K 30 (PVP 40K) from Fluka and ethoxylated *p-tert*.-octylphenol with 9–10 ethylene oxide units (Triton X-100) from Koch-Light (Colnbrook, Bucks, UK). Aqueous concentrates of these additives [each at a 0.5% (w/v) concentration] were deionized using an Amberlite MB1 mixed bed ion exchanger (Serva).

2.4. Coating of the inner walls of the capillary tubes

The capillary tube assembled in the separation compartment of the analyzer was filled with a 1% (w/v) aqueous solution of MHEC 30K for approximately 10 min. The solution was then displaced with air and then with the carrier electrolyte solution taken into the investigation.

2.5. Cleaning of the separation compartment

To remove the residues of the EOF suppressors from the separation compartment it was washed with ca. 30 ml of a 2% (v/v) solution of a laboratory detergent (Extran MA 02 neutral, Merck) and then with ca. 150 ml of deionized water.

3. Results and discussion

Considering chemical similarities we can assume that electrokinetic properties of FEP are close to those reported for other fluoroplastics [25–27]. Therefore, our investigations of the EOF suppressors

Methylhydroxyethylcellulose

Polyethyleneglycol   **Polyvinylpyrrolidone**   **Polyvinylalcohol**

$(CH_3)_3CCH_2(CH_3)_2C$ —〈 〉— $(OCH_2CH_2)_xOH$
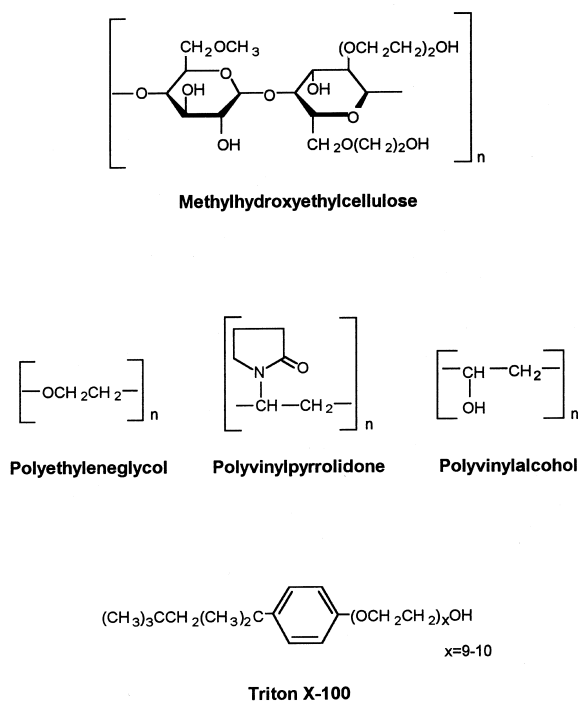
x=9-10

Triton X-100

Fig. 1. Chemical structures of basic units of the studied EOF suppressors.

(Fig. 1) in the FEP capillary tubes were carried out at pH values corresponding to lower and maximum values of the ζ-potential as reported for PTFE tubes [25]. The compositions of the carrier electrolytes providing such acid–base conditions and used throughout are given in Table 1.

In evaluating effects of the studied EOF suppressors in terms of minimization of the electroosmotic dispersion in the closed separation system the separation without the use of EOF suppressors served as a reference to which the suppressing effect of an actual EOF suppressor was related. To guarantee that there was no bias in such an evaluation (e.g., due to a memory effect of the walls of the separation compartment) the following scheme was strictly adhered to in our experiments:

(i) The separation compartment was thoroughly washed in the way described in Section 2.5 to remove the residues of EOF suppressor which could remain on the walls from previous experiments. (ii) CZE runs with a three-component model mixture were carried out in the carrier electrolyte solution

without EOF suppressor to confirm that the separation compartment is in a required electrokinetic state, i.e., providing very low separation efficiencies for the model analytes (Fig. 2). (iii) CZE runs with the electrolyte system containing the studied EOF suppressor were carried out. The separation compartment was filled with the carrier electrolyte solution through a disposable membrane filter (1.2 μm pore size). Each of the carrier electrolyte solutions was filtered through a separate filter to avoid a risk of undesired coating of the separation compartment.

The resulting changes in the plate heights of the analytes were ascribed to the presence of the EOF suppressor in the carrier electrolyte or better to its influence on the ζ-potential and/or viscosity at the wall of the separation compartment (η_w) in accordance with Eq. (1).

3.1. Influences of EOF suppressors on the separation efficiencies at pH=5.2

Following the above scheme the separation efficiency data for the model analytes (Table 2) were obtained using the studied EOF suppressors as the carrier electrolyte solution additives (system No. 1, Table 1). The average values of the plate heights for five repeated CZE runs with each of the EOF suppressors are summarized in Table 3. These as well as illustrative electropherograms in Fig. 3 show that they differed in their EOF suppressing effects. It is apparent that the most significant improvements relative to the reference state (Fig. 2) were achieved
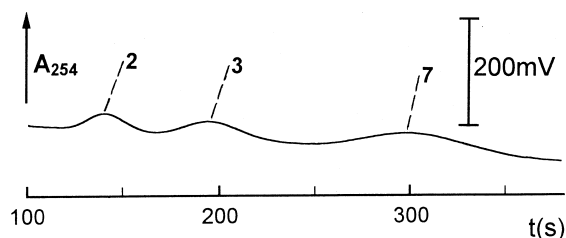

Fig. 2. An electropherogram from the separation of a 3-component model mixture at pH=5.2 (system No. 1, Table 1) in the carrier electrolyte containing no EOF suppressor. The separation compartment before the run was washed as described in Section 2.5. For the peak assignments see Table 2 and the concentrations of the anions in the model mixture are given in Section 2.2. The driving current was stabilised at 140 μA with a 8 kV voltage between the driving electrodes.

Table 2
Migration characteristics of model analytes

No.	Acid	pK_1	pK_2	m_1	m_2
1	2,6-Naphthalenedisulfonic	−1.00	0	−0.01	−54.9
2	2-Naphthylamine-4,8-disulfonic	–	–	–	–
3	Sulfanilic	3.23	–	−39.0	–
4	2,4-Dihydroxybenzoic	3.39	–	−35.0	–
5	3,5-Dihydroxybenzoic	4.30	–	−32.0	–
6	p-Hydroxybenzoic	4.73	9.31	−34.0	−62.0
7	Sorbic	4.77	–	−33.4	–
8	Anthranilic	4.85	–	−31.6	–
9	p-Aminobenzoic	4.94	–	−31.6	–

m_1, m_2, absolute ionic mobilities of single and double negatively charged ionic forms of the acid.

for both cellulose derivatives (MHEC 300 and MHEC 30K) and PVA. PEG and PVP suppressed electroosmosis less efficiently. We can see that the effects of these polymers depended on their chain lengths. Triton X-100 forming under our working conditions [a 0.2% (w/v) concentration in the carrier electrolyte] micelles [28] was slightly less efficient than MHEC and PVA but it was more efficient than PEG and PVP.

PVA, Triton X-100 and water soluble cellulose derivatives are traditionally applied in ITP to suppress electroosmosis in the capillary tubes made of fluoroplastics [8,9]. From the electrokinetic measurements [25] and recent CZE works [29,30] it is apparent that PVA is effective in combinations with various tube materials. It is also known that this polymer has a strong memory effect on PTFE [8]. Methylhydroxyethyl derivatives of cellulose were

shown to exhibit similar behaviours in ITP [31]. Here, we investigated in details performances of the FEP capillary tubes dynamically coated with MHEC 30K (the coating procedure is described in Section 2.4) in the CZE separations using the carrier electrolyte solutions with and without the EOF suppressors. The data obtained from these experiments are given in Table 3. A comparison of these data with those obtained in the separation compartment without the MHEC coating show that the coating led to dramatic improvements of the separation efficiencies when PEG and PVP polymers were used as the additives in the carrier electrolyte solutions (see also Fig. 4). The data in Table 3 indicate that also the chain lengths of both PVP and PEG contributed to the improvements of the separation efficiencies. In this context we should stress that the MHEC 30K coated separation compartment with PVP 750K in

Table 3
Separation efficiencies for model analytes in the presence of the additives in the carrier electrolyte at pH=5.2

Acid	MHEC 300		MHEC 30K		PEG 4K		PEG 5M		PVP 40K		PVP 750K		PVA 80K		Triton X-100	
	H_{tot}^{a}	H_{tot}^{b}	H_{tot}^{a}	H_{tot}^{b}	H_{tot}^{a}	H_{tot}^{b}	H_{tot}^{a}	H_{tot}^{b}	H_{tot}^{a}	H_{tot}^{b}	H_{tot}^{a}	H_{tot}^{b}	H_{tot}^{a}	H_{tot}^{b}	H_{tot}^{a}	H_{tot}^{b}
1	5.99	6.37	6.12	6.50	–	5.86	10.19	6.29	–	5.43	23.95	5.02	5.72	6.05	6.14	6.45
2	5.87	6.54	6.25	6.37	–	5.89	10.27	6.39	–	5.41	27.56	4.98	5.63	6.07	6.09	6.46
3	4.23	4.20	4.22	4.11	71.72	4.95	11.10	4.40	138.78	4.19	31.43	4.07	4.05	4.05	5.28	4.80
4	4.65	4.64	4.59	4.41	87.23	5.67	13.97	5.07	182.98	4.84	47.31	4.00	4.62	4.64	6.51	5.69
5	5.62	5.76	5.55	5.25	108.50	6.97	17.10	5.59	106.40	5.43	53.97	4.91	5.14	5.17	7.59	6.65
6	6.57	6.52	6.23	5.81	–	7.91	14.09	6.27	–	5.92	–	4.75	5.92	5.85	8.61	7.55
7	6.46	6.48	6.31	5.89	–	8.05	17.56	6.51	–	5.72	–	4.88	6.02	5.95	8.31	7.23
8	8.03	8.40	7.91	7.64	–	9.30	21.06	8.04	–	7.63	38.66	7.36	7.16	7.35	10.36	9.13
9	6.72	6.62	6.61	6.23	–	8.71	23.70	7.07	–	6.46	53.22	6.07	6.37	6.34	8.26	7.27

H_{tot}, height equivalent to a theoretical plate (μm); [a] additive present in the carrier electrolyte at a 0.2% (w/v) concentration; [b] as in (a) with the capillary tube coated by MHEC 30K (for details see Section 2.4).
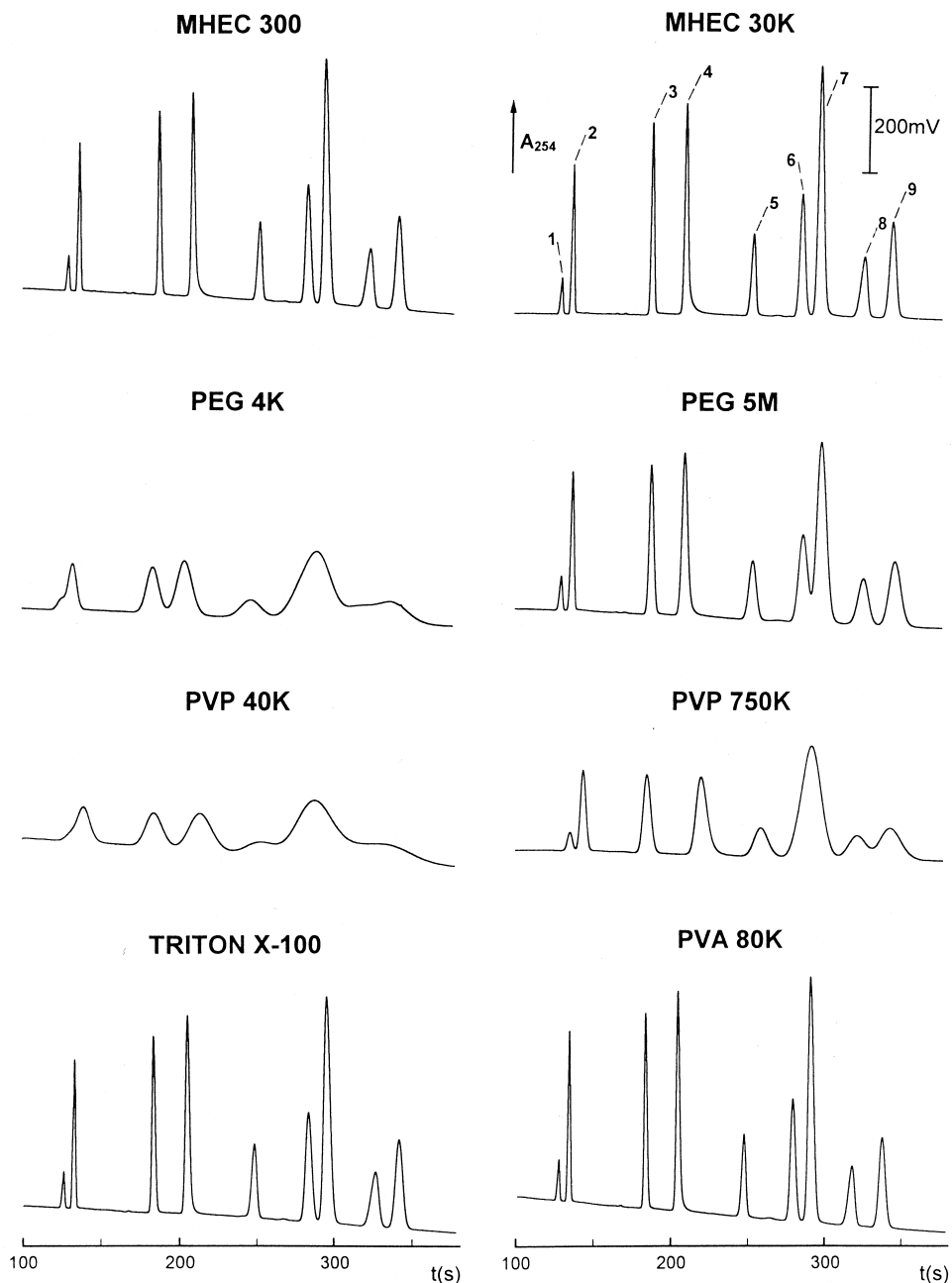
Fig. 3. Electropherograms from the separations of a model mixture of anions at pH=5.2 in the carrier electrolyte solutions containing various EOF suppressors. The concentrations of the anions in the model mixture are given in Section 2.2. For the assignments of their peaks see Table 2. The driving current was in all instances set at 140 μA (the voltage between the driving electrodes was 8 kV).

the carrier electrolyte solution provided the highest separation efficiencies for the model analytes among the all alternatives investigated in this work. The

effect was reproducible as can be seen from the data obtained for the same capillary tube at various times (Table 4). PVP interacts in aqueous solutions with
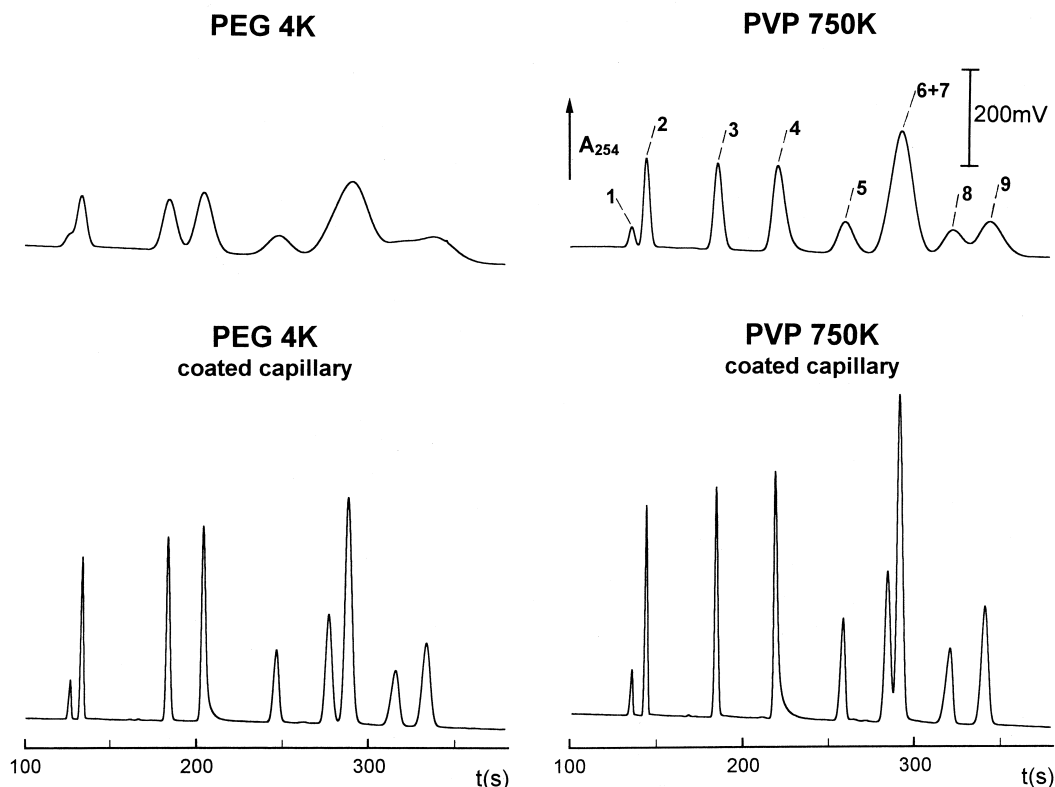
Fig. 4. Electropherograms from the separations of the same model mixture of anions as in Fig. 3 in the MHEC 30K coated capillary tube. The coating procedure is described in Section 2.4. The carrier electrolyte solutions contained PEG 4K (left) and PVP 750K (right) additives at 0.2% (w/v) concentrations, respectively. For the peak assignments see Table 2. The composition of the carrier electrolyte is given in Table 1 (system No. 1). The driving current was set at 140 µA in the all experiments (the voltage between the driving electrodes was 8 kV).

Table 4
Reproducibilities of the separation efficiencies for the model analytes in the capillary tubes coated with MHEC 30K at pH=5.2

Acid	MHEC 30K				PEG 5M				PVP 750K			
	H_{tot}^a	S.D.^{a}	H_{tot}^b	S.D.^{b}	H_{tot}^a	S.D.^{a}	H_{tot}^b	S.D.^{b}	H_{tot}^a	S.D.^{a}	H_{tot}^b	S.D.^{b}
1	6.50	0.19	6.62	0.37	6.30	0.45	6.08	0.82	5.02	0.32	4.78	0.18
2	6.37	0.34	6.49	0.35	6.39	0.59	5.96	0.68	4.99	0.30	4.86	0.29
3	4.11	0.08	4.00	0.17	4.40	0.32	3.85	0.15	4.07	0.14	3.64	0.12
4	4.41	0.15	4.43	0.18	5.07	0.24	4.22	0.39	4.01	0.28	3.33	0.08
5	5.25	0.13	5.08	0.13	5.61	0.71	4.73	0.34	4.91	0.37	4.18	0.16
6	5.81	0.07	5.73	0.13	6.28	0.63	5.12	0.22	4.75	0.12	4.42	0.10
7	5.89	0.13	5.81	0.12	6.52	0.66	5.33	0.22	4.88	0.14	4.59	0.09
8	7.64	0.06	7.51	0.24	8.05	0.73	7.26	0.74	7.37	0.39	6.68	0.33
9	6.23	0.07	6.26	0.16	7.08	0.73	5.95	0.16	6.07	0.16	5.49	0.10

H_{tot} (µm), height equivalent to a theoretical plate; [a,b] original data and data obtained with a one week delay, respectively; S.D., standard deviation (for details of the coating see Section 2.4).

some aromatic anions and these interactions influ-ence the effective mobilities of the anions involved in these interactions [22,32]. Undoubtedly, differ-ences in the migration times for some of the model analytes can be ascribed to these interactions (see Figs. 3 and 4).

In the separations performed in the MHEC 30K coated separation compartment with the carrier elec-trolyte solution containing no EOF suppressor the plate height values for the analytes (Table 5) were close to those in which MHEC was present in the carrier electrolyte (Table 3). This is a somewhat unexpected result when we realize that the addition of water soluble cellulose derivative into the solution in which the electrophoretic separations were per-formed several times increased the bulk viscosity of this solution [25]. On the other hand, this is in an agreement with the behaviours of other EOF sup-pressors (PVA, Triton X-100) which changed the bulk viscosities only negligibly while strongly reduc-ing electroosmosis [25]. Therefore, the presence of MHEC in the carrier electrolyte solution served, mainly, to maintain the surface layer of the additive intact.

The MHEC 30K coating was found to be stable on the FEP surface, at least, for 20 CZE runs as is apparent from the reproducibilities of the plate height data in Table 5. We observed that for a higher number of runs in some instances they increased abruptly by about 30–50%. Such changes were probably linked with a partial removal of the MHEC

Table 5
Reproducibilities of efficiencies and migration times of model analytes for 20 runs in the capillary tube coated with MHEC 30K without additive at pH=5.2

No.	H_{tot} (µm)	S.D.	t_m (s)	S.D.
1	6.48	0.34	141.79	1.40
2	5.82	0.17	149.96	1.59
3	4.11	0.11	205.60	2.06
4	4.30	0.19	226.96	2.27
5	4.71	0.14	272.37	2.25
6	5.29	0.10	304.23	1.70
7	5.47	0.17	317.00	1.60
8	6.60	0.21	346.64	2.59
9	5.91	0.17	363.35	1.57

H_{tot}, average values of height plate; S.D., standard deviation; t_m, migration time of the analytes; the coating procedure is described in Section 2.4.

coating and its renewal in the way described in Section 2.4 restored the original separation perform-ance. Similar observations for water soluble cellulose derivatives in quartz tubings were also reported [10].

Our experimental data (Tables 3–5) provided total values of the plate heights. As such they summed up changes in the separation efficiencies attributable to the presence of the EOF suppressors in the carrier electrolyte solutions. Therefore, the experimental data could not provide in a straightforward way estimates of the electroosmotic dispersion attribut-able to actual CZE working conditions.

Simple models of CZE separations [6,12] show that an electroosmotic contribution to the plate height (H_{EO}) of the CZE analyte migrating in a hydro-dynamically closed separation compartment is de-termined by the following equation:

$$H_{EO} = \frac{r^2 \zeta^2 \varepsilon^2 zFE}{24RT\eta^2 m^2} \qquad (1)$$

where r is the inner radius of the capillary tube, ε the dielectric constant, ζ the effective charge number of the analyte, F the Faraday constant, R the gas constant, η the viscosity of the solution, T the average temperature in the capillary tube, m the effective mobility of the analyte and E the electric field strength. When numerical values of the parame-ters in Eq. (1) are known (or they can be estimated) this equation enables to calculate a contribution of the electroosmotic dispersion term (H_{EO}) to the total plate height value of the analyte, e.g., in the way described by Reijenga and Kenndler [12]. Then a comparison of the calculated and experimental data provides a means of estimating electrokinetic param-eters of the separation compartment under actual working conditions (see below). The calculations (using the HPCESIM program described in Ref. [12]) revealed that under our working conditions realistic estimates of the plate heights for the ana-lytes can be obtained only when the viscosity coefficient at the wall (η_w) is assumed to be less than 10 mPa·s (see also Fig. 5). These calculations also showed that the electroosmotic and diffusion disper-sions were main band broadening contributors in our experiments. The calculated and experimental plate height data for the model analytes are given in Table 6. The data calculated for $\zeta = 0$ mV correspond to the
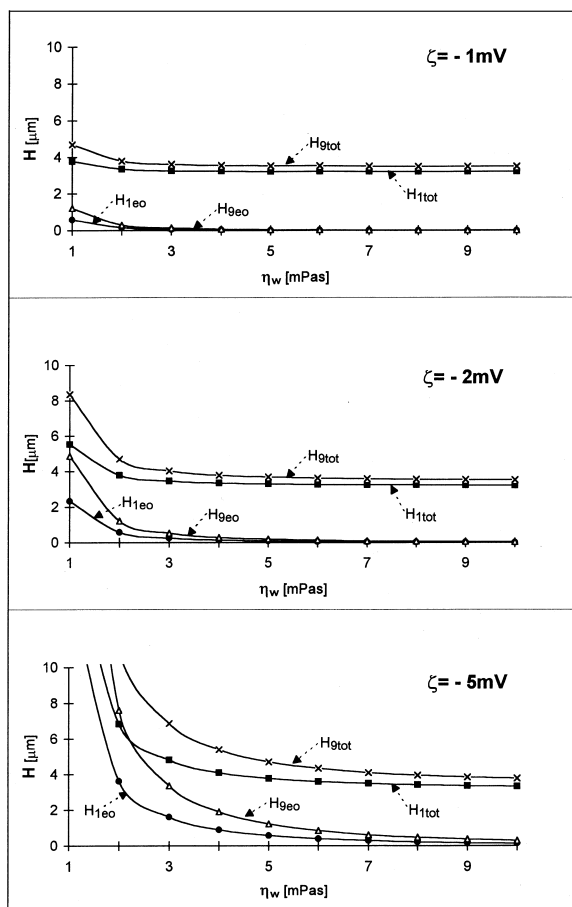
Fig. 5. Dependence of the plate heights of the 2,6-naphtha-lenedisulfonate (1) and *p*-aminobenzoate (9) on the wall viscosity (η_w) for various ζ-potential values. H_{tot} = a total value of the plate height, H_{eo} = a contribution of the electroosmosis dispersion term to the plate height value. Parameters used in the calculations [12]: (1) *Equipment*: mode—closed; stabilization mode—current; voltage = −8 kV; current = 140 μA; thermostatting temperature = 20°C; cooling medium—circulating liquid. (2) *Capillary*: material—PTFE; length to the detector = 190 mm; total length = 230 mm; I.D. = 300 mm; O.D. = 700 mm. (3) *Injection*: mode—sample loop; injection volume = 400 nl. (4) *Detection*: mode—universal response; slit width = 200 μm; time constant = 0.4 s; relative noise = 10. (5) *Buffer*: MES: $m_0 = 0$, $m_1 = -26.8$, $pK_1 = 6.13$; histidine: $m_0 = 29.6$, $m_1 = 0$, $m_2 = 28.8$, $pK_1 = 6.04$, $pK_2 = 9.34$; bulk viscosity = 1 mPa/s.

estimates of the separation efficiency limits attainable under our working conditions while those for $\zeta = -2$ mV and $\zeta = -5$ mV take into account residual EOFs. From the plate height data we can see that electroosmotic dispersions for the ζ-potential

values in the range of 0–(−)2 mV and for $\eta_w = 5$ mPa/s differ only within the frame of random errors characterizing the CZE experiments (see Table 4). The plots in Fig. 5 based on the calculated data indicate that the role of the ζ-potential values in this range is more important for lower η_w values. They also show that in an electrokinetic characterization of the separation compartment some uncertainty may be essential as we have to cope with two parameters (ζ-potential and η_w) which are apparently in some extent interrelated.

When the calculated and experimental data in Table 6 are compared we can conclude that MHEC 30K alone did not eliminate the electroosmotic dispersions completely (it is apparent that this statement can be extended also to other EOF suppressors studied in this work). On the other hand, some data for the MHEC 30K coated tube with PVP 750K in the carrier electrolyte solution suggest that using this combination we approached much closer to the working conditions without electroosmotic dispersion. However, assuming only electroosmotic and diffusive band broadening the plate height values for anthranilate (8) and *p*-aminobenzoate (9) may indicate a change in the ζ-potential and/or η_w during the separation. Such an explanation of the higher plate height values cannot be excluded. If this were the case it should lead to lowered separation efficiencies in the subsequent runs performed in the separation compartment without replenishing the carrier electrolyte solution (in this way no step is taken to change the electrokinetic state of the separation compartment). Our experiments performed under such working conditions, however, showed no changes in the plate heights of the model analytes. Therefore, it seems more appropriate to assume that for anthranilate and *p*-aminobenzoate other dispersive phenomena, e.g., such as adsorption [33] and/or electrodiffusion [34] play a role.

3.2. Influence of EOF supressor on the separation efficiencies at pH = 9.2

In CZE separations carried out at pH = 9.2 (system No. 2, Table 1) maximum ζ-potential values for the tubes made of fluoroplastics are typical [25,26]. Under these acid–base conditions we investigated only the EOF suppressors giving maximum sepa-

Table 6
Comparison of calculated and experimental values of the plate heights

Acid	Calculated[c]				Experimental		
	t_m (s)	H_{tot} (μm)			t_m (s)	H_{tot} (μm)	
		$\zeta = 0$ mV	$\zeta = -2$ mV	$\zeta = -5$ mV		MHEC 30K[a]	PVP 750K[b]
1	138.0	3.20	3.29	3.78	131.8	6.62	4.78
2	–	–	–	–	139.3	6.49	4.86
3	184.8	3.52	3.61	4.05	189.9	4.00	3.64
4	210.0	3.25	3.35	3.90	211.2	4.43	3.33
5	253.8	3.10	3.24	3.97	254.5	5.08	4.18
6	283.8	3.37	3.52	4.28	286.2	5.73	4.42
7	295.8	3.37	3.53	4.34	298.3	5.81	4.59
8	330.0	3.37	3.55	4.51	326.4	7.51	6.68
9	352.8	3.48	3.68	4.70	345.4	6.26	5.49

t_m, migration time of the analytes; H_{tot}, height equivalent to a theoretical plate; ζ, zeta potential; [a] additive present in the carrier electrolyte at a 0.2% (w/v) concentration; [b] as in (a) with the capillary tube coated by MHEC 30K (for details see Section 2.4); [c] calculated for $\eta_w = 5$ mPa s, $\eta_{bulk} = 1$ mPa/s and for the rest of the parameters see Fig. 5.

ration efficiencies at pH=5.2. The plate height data obtained for a group of the model analytes are summarized in Table 7. Behaviours of the studied EOF suppressors were analogous to those observed at pH=5.2 with the MHEC 30K coated separation compartment and PVP 750K present in the carrier electrolyte giving the highest separation efficiencies. A comparison of the plate heights obtained for the analytes at pH=5.2 and 9.2 (Tables 3 and 7) shows a significant change for 2,4-dihydroxybenzoate (4). From typical frontings of the peaks of this constituent in the electropherograms in Fig. 6 we can probably ascribe this change to electromigration dispersion [11,12]. A detailed investigation of this

Table 7
Separation efficiencies for model analytes in the presence of the additives in the carrier electrolyte at pH=9.2

No.	MHEC 30K		PEG 5M		PVP 750K	
	H_{tot}^a	H_{tot}^b	H_{tot}^a	H_{tot}^b	H_{tot}^a	H_{tot}^b
1	6.43	5.80	–	6.05	–	4.24
2	5.71	5.74	37.48	6.02	52.22	3.85
3	5.20	5.18	–	5.18	–	5.20
4	8.68	8.73	50.66	8.92	56.84	7.41
5	6.46	6.51	–	6.77	–	–
6	5.56	5.39	–	5.54	–	5.23
7	4.88	4.98	–	4.98	–	–

H_{tot} (μm), height equivalent to a theoretical plate; [a] additive present in the carrier electrolyte at a 0.2% (w/v) concentration; [b] as in (a) with the capillary tube coated by MHEC 30K (for details see Section 2.4).

behaviour of 2,4-dihydroxybenzoate was not carried out in this work.

Proteins are known to adsorb on the walls of the CE separation compartment with a subsequent change of the ζ-potential [33,35]. In our arrangement of the CZE separation system these disturbances should be manifested via decreased separation efficiencies of the analytes. Albumin and γ-globulin were investigated in this context to asses an extent to which the presence of proteinous matrix in the sample can influence the CZE separations of the analytes. Experiments with albumin were carried out in the electrolyte system No. 2 (Table 1) containing MHEC 30K at a 0.2% (w/v) concentration using the following sequence of the CZE runs:

(i) The sample containing a seven component model mixture was separated. (ii) Without replenishing the separation compartment with a fresh solution of the carrier electrolyte three runs with a 1% (w/v) aqueous solution of albumin were carried out to "contaminate" the surface of the separation compartment. (iii) Immediately (without replenishing the separation compartment with a fresh solution of the carrier electrolyte), the CZE run with the sample as in (i) was carried out. A comparison of the plate heights as obtained for the model analytes in the run (i) and (iii) revealed that the runs with albumin did not influence these performance parameters in the run (iii). This implies that the presence of EOF suppressor in the carrier electrolyte was in this
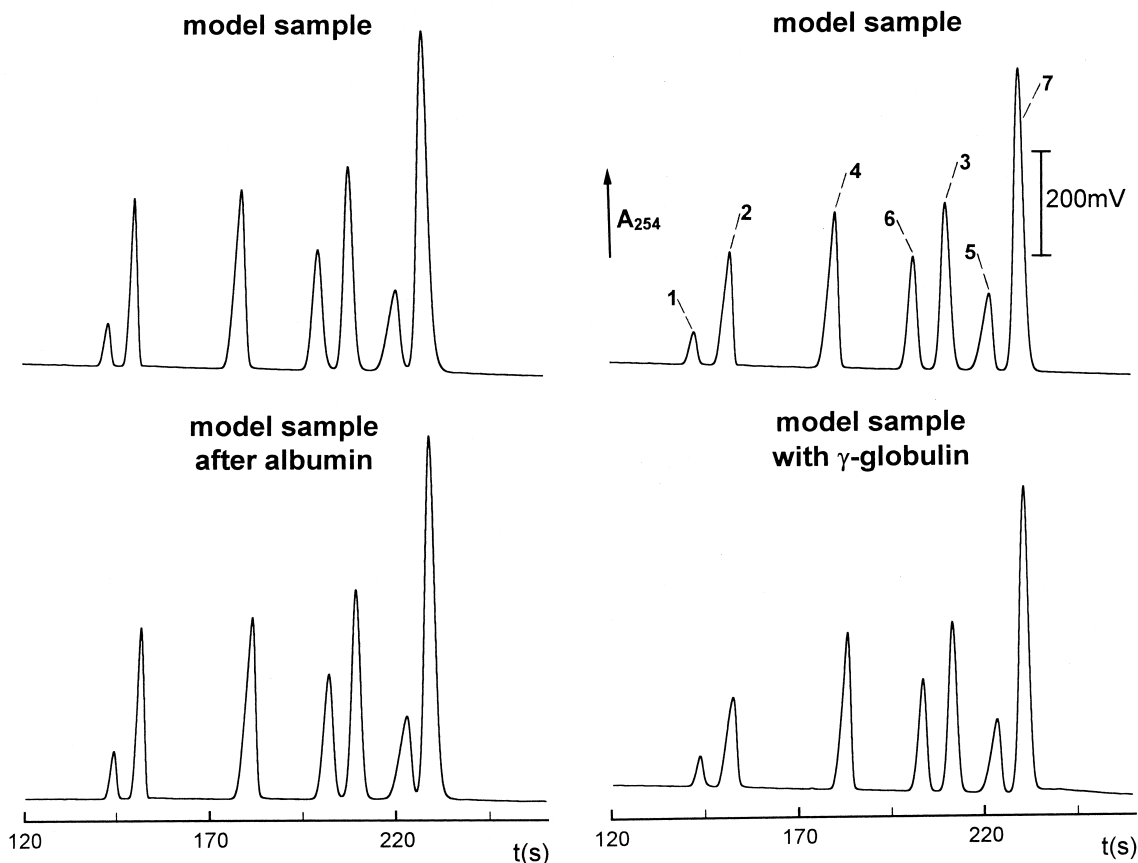
Fig. 6. Influences of albumin and γ-globulin on the CZE separations of a model mixture of anions at pH=9.2. The carrier electrolyte solution No. 2 (Table 1) containing MHEC 30K at a 0.2% (w/v) concentration was used in the separations. For the peak assignments see Table 2. The concentrations of the model anions are given in Section 2.2. The separations were carried out with a 140 μA driving current (the voltage was 8 kV). Further details are given in Section 3.2.

instance effective in preventing electroosmotic disturbances due to the CZE runs with albumin.

Experiments with γ-globulin were carried out in the same way as with albumin. Also in this instance no disturbances in the separation efficiencies of the model analytes were detected. In addition, we performed CZE separations of the same model mixture containing besides the model analytes a 1% (w/v) concentration of γ-globulin. Typical electropherograms as obtained from the separations of the model mixture without and with γ-globulin are given in Fig. 6. From these electropherograms we can see that also in this instance no disturbances were detected.

Our experiments with proteins suggest that the use of EOF suppressors in the hydrodynamically closed

separation compartment eliminates their negative disturbances. However, this cannot be accepted as conclusive in a general sense since the proteins taken into our experiments did not represent a full scale of adsorptivities with respect to FEP. Nevertheless, the CZE approach studied in this work is worth to be investigated in details in a context with matrices causing some problems in CZE [35].

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