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Journal of Chromatography A, 772 (1997) 103–114

JOURNAL OF CHROMATOGRAPHY A

Capillary zone electrophoresis in a hydrodynamically closed separation system with enhanced sample loadability

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Abstract

Some phenomena linked with capillary zone electrophoresis (CZE) performed in a hydrodynamically closed separation system with enhanced sample load capacity via the use of capillary tubes of larger I.D.s were studied. Calculations of the plate heights for varying amounts of the analytes loaded onto 50 and 300 μm I.D. columns under identical CZE separating conditions revealed that the analytical advantages of using columns of larger I.D. include significantly reduced contributions of electromigration dispersion. At higher concentrations of the carrier electrolytes this gain, however, can be partially lost due to increased thermal dispersive effects. Calculated resolutions for a varying ratio of a pair of the analytes loaded onto 50 and 300 μm I.D. columns favoured the use of the latter I.D. in situations when the ratio of the analytes was higher than ca. 10^2:1. CZE experiments were carried out in a 300 μm I.D. capillary tube made of fluorinated ethylene–propylene copolymer (FEP) with a porous cellophane membrane serving as a hydrodynamic barrier to prevent a flow of the solution in the separation compartment due to a pressure difference between the electrode vessels. Movement of the membrane was found to be a source of undesired flows in the separation compartment, which adversely affected both the reproducibilities of migration times of the analytes and their separation efficiencies. Mechanical support eliminated these problems. Dispersive phenomena associated with electroosmosis in the closed separation compartment were effectively suppressed by using high molecular weight derivatives of water soluble polymers (methylhydroxyethylcellulose and polyethyleneglycol) in the carrier electrolyte solutions. Examples from the separations of various groups of analytes (synthetic food colourants, some inorganic anions, alkali and alkaline earth metal cations and glycoforms of erythropoietin) are given to illustrate a practical utility of the studied CZE approach.

Keywords: Capillary columns; Sample loadability; Hydrodynamically closed separation system

1. Introduction

Sensitive detectors and columns of adequate sample load capacities are generally effective in achieving low concentration limits of detection (LODs) in capillary zone electrophoresis (CZE). At present, detection performance parameters of some CZE detectors expressed via their mass limits of detection are such (see, e.g., Refs. [1–6]) that requirements related to the sample load capacities of the CZE columns can be marginal also for low ppb–ppt concentrations of the analytes. Unfortunately, this is not general and, currently, CZE may be considered as an analytical technique of moderate concentration sensitivity, e.g., when combined with preferred photometric absorbance detectors [2,4,7].

The columns of 20–75 μm I.D.s are favoured in CZE to achieve very high separation efficiencies for the analytes [4,8,9]. Undoubtedly, this is mainly due to minimized thermal dispersive effects in the col-

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PII S0021-9673(97)00110-6

umns of such I.D.s (see, e.g., Refs. [4,8,9]). On the other hand, the separations in the columns of such dimensions are a priori more amenable to the electromigration dispersion [4,10] and/or to inherently generated isotachophoretic (ITP) stacking of the separated sample constituents [11–13]. In fact, these two phenomena may be considered to set the sample load limits in CZE. These limits can be to some extent manipulated and concentration adaptations of the separated constituents (ruled by the concentration of the carrier electrolyte) and/or the geometrical dimensions of the columns provide relevant means for doing this.

The use of the capillary tubes of larger I.D.s (200–3000 μm) was typical in some pioneering works on CZE [10,14,15]. At present, however, such an approach is used very seldom and, in fact, it is considered only in some works dealing with preparative CZE separations (see, e.g., Refs. [16–18]). Nevertheless, reports dealing with analytical separations in capillary tubes of 200–300 μm I.D.s clearly show their analytical benefits, especially in terms of the attainable LOD values [10,19–25].

Although not essential (see, e.g., Refs. [17,26]) CZE separations in tubes of larger I.D.s are usually carried out with hydrodynamically closed separation compartments to prevent any movement of the solution in which the separation is performed. This mode of the CZE separation requires that electroosmosis is suppressed as otherwise the electroosmotic dispersion can very significantly decrease the separation efficiency [4,10,15]. The use of suitable additives in the carrier electrolyte solutions was shown [10,19–25,27] to provide effective means in suppressing electroosmosis. It was also shown [27] that under such conditions the electroosmotic dispersion can be kept on a level not deviating from that characterizing hydrodynamically opened CZE separation systems.

CZE separations in hydrodynamically closed separation compartments provided with 200–300 μm I.D. capillary tubes are a subject of our current interest in both the single column [19–25] and coupled-column [28–31] separation systems. Our interest in this CZE approach has been stimulated by the following reasons:

(i) Favourable features of the use of the tubes of larger I.D.s in the CZE analysis due to their enhanced sample load capacities linked with lower LODs.

(ii) Fluctuations in the electroosmotic flow are not reflected (or only marginally) in the migration velocities of the separated constituents. This is favourable, e.g., in the quantitations requiring that the separated constituents migrate with constant velocities through the detector [4].

(iii) CZE performed in a hydrodynamically closed separation compartment can be easily implemented into advanced capillary electrophoresis separation systems, e.g., into those operating with coupled columns [28–31].

The present work was aimed at investigating some factors which contribute to analytical benefits associated with the use of capillary tubes of larger I.D.s in CZE with a hydrodynamically closed separation compartment or which determine limits of such an approach. Dependencies of the plate heights of the CZE separands on the amounts loaded onto the column and on the concentration of the carrier electrolyte were calculated to estimate contributions of various dispersive phenomena in 50 and 300 μm I.D. capillary tubes. Resolutions for a pair of the CZE separands in tubes of these I.D.s, depending on their concentration ratios in the loaded sample, were calculated for the carrier electrolytes of different concentrations. This gave a possibility of estimating a role of the I.D. of the column in instances when the concentration ratio of the analytes present in the loaded sample is very high. Various factors which can adversely affect the migration velocities and separation efficiencies in hydrodynamically closed CZE separation systems were studied experimentally. Examples of applications are given to illustrate a practical use of the studied CZE approach for various groups of analytes.

2. Experimental

2.1. Instrumentation

A CS Isotachophoretic Analyzer (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used in the single-column configuration of the separation unit. The separation unit itself is schematically shown in Fig. 1. All CZE separations were carried out in a 300

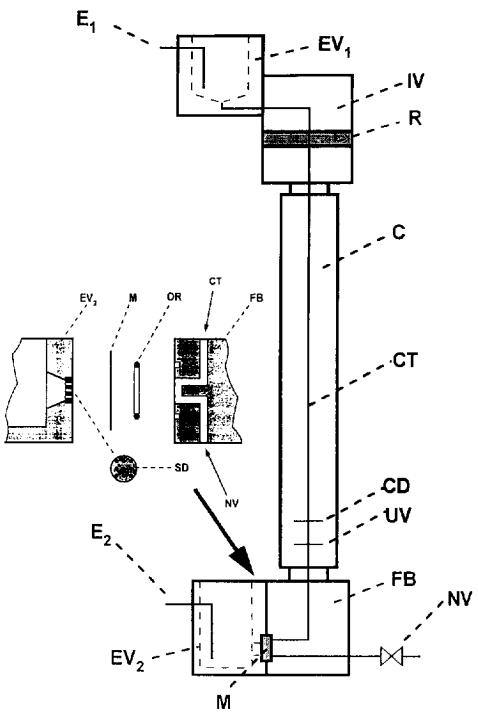

Fig. 1. CZE separation unit with a hydrodynamically closed separation compartment. EV_1 EV_2 = electrode vessels with the driving electrodes (E_1, E_2); IV, injection valve with an internal sample loop drilled in a rotating disc (R); CT, capillary tube assembled in a plexiglass cartridge (C); CD, UV, on-column cells for the conductivity and photometric absorbance detectors, respectively; FB, filling block; M, porous membrane made of cellophane; SD, supporting disc made of plexiglass with capillary channels for an electrically conductive connection of the solutions in the electrode vessel (EV_2) and in the capillary tube; OR, O-ring for a liquid connection of the filling block (FB) with the electrode vessel (EV_2).

μm I.D. capillary tube (O.D.≈700 μm) made of fluorinated ethylene–propylene (FEP) copolymer. The lengths of the tubes changed in the experiments and the relevant data are given in the corresponding legends to the figures. UV photometric detectors from the following manufactures were used in this work:

(i) UVD 2 on-column photometric detector from Villa-Labeco;

(ii) Spectra 100 photometric detector (Thermo Separation Products, San Jose, CA, USA);

(iii) UV-M II photometric detector (Pharmacia-LKB, Uppsala, Sweden).

For the conductimetric detection a conductivity detection unit of the CS Isotachophoretic Analyzer (Villa-Labeco) was used.

The detectors were connected to a 486 DX computer via a Unilab data acquisition unit (Fitek, Šal'a, Slovak Republic). Software ITP.Win (versions 2.15 and 2.17) from KasComp (Bratislava, Slovak Republic) was used for the acquisition and processing of the data.

2.2. Chemicals

The electrolyte components of the carrier electrolytes were obtained from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA), Merck (Darmstadt, Germany), Lachema (Brno, Czech Republic), Aldrich (Steinheim, Germany) and Reanal (Budapest, Hungary). Methylhydroxyethylcellulose 30 000 (m-HEC), polyvinylpyrrolidone K90 and polyethyleneglycol 5 000 000 (PEG) obtained from Serva were used as additives to the carrier electrolyte solutions. Water demineralized by a Pro-PS water purification system (Labconco, Kansas City, KS, USA) was used for the preparations of the solutions.

2.3. Samples

Synthetic food colourants were obtained from Aldrich and their stock solutions were prepared as described elsewhere [21]. An erythropoietin sample (800 μg/ml) was kindly provided by Professor J.F.K. Huber (University of Vienna, Vienna, Austria). The rest of the samples was prepared from currently available chemicals delivered by the above suppliers.

3. Results and discussion

3.1. I.D. of the capillary tube and the sample load

To estimate contributions of various dispersive phenomena to separation efficiencies in the capillary tubes of different I.D.s we employed a calculation procedure as elaborated by Reijenga and Kenndler for simulations of CZE separations [32]. Although based on a simple model this procedure was shown to provide data which are in reasonable agreement with experiments carried out in a 75 μm I.D. capillary tube [33]. Its applicability for a 300 μm

I.D. capillary tube in the closed separation compartment was shown for a group of nitrophenols [23]. Therefore, these CZE analytes and identical working conditions were chosen for our calculations.

To assess sample loadabilities of 50 and 300 μm I.D. tubes under realistically chosen working conditions (Table 1) dependencies of the plate heights (H) on the amounts of the loaded analytes were calculated. These data for *o*-nitrophenol are plotted in Fig. 2 (they represent typical results as obtained also for other nitrophenols). From the plots it is apparent that for the 50 μm tube the electromigration dispersion (H_{cm}) was critical as far as the sample load is concerned. In the 300 μm tube at lower sample loads the thermal dispersion (H_t) mainly contributed to the H values while at a ca. 1000 fmol sample load H_{cm} started to dominate. Other dispersive phenomena were in both instances less significant.

A role of the concentrating effect of the carrier electrolyte is illustrated by plots in Fig. 3. Here, to achieve close migration times for the analytes the same current densities in both tubes for a given concentration of the carrier electrolyte were used in the calculations. For the same reasons the current density values were proportional to the concentration of the carrier electrolyte. The sample loads used in these calculations were in the ratio of the cross-sections of the tubes. The plotted dependencies show that in the tube of 50 μm I.D. (a 100 fmol sample

Table 1
Physico–chemical constants and parameters used in the calculations

Parameters of instrument		
Equipment	Mode:	Closed
	Stabilization mode:	Current
	Thermostating temperature (°C):	25
	Cooling medium:	Circulated liquid
Capillary	Material:	PTFE
	Length to detector (mm):	300
	Total length (mm):	350
	I.D. (mm):	300 (50)[a]
	O.D. (mm):	700 (450)[a]
	Zeta potential (mV):	0
Injection	Mode:	Sample loop
	Injection volume (nl):	100(2.5)[a]
	Concentration (mmol/l):	0.05–50(2–2000)[a]
Detection	Mode:	UNIVresponse
	Slit width (mm):	100
	Time constant (s):	0.01
	Relative noise:	10
Parameters of buffer		
Buffer	Conc. of glycine (mmol/l)	25–300
	pH	9.1
	Viscosity at wall (mPas):	100
	Bulk viscosity (mPas):	1
Constants taken for calculation		
Buffer		
Glycine	pK	9.78
	m	−37.4
Ammediol	pK	8.78
Sample		
o-Nitrophenol	pK	7.23
	m	−33.4
2,5-Dinitrophenol	pK	5.22
	m	−31.3

[a] The values in the parentheses were used for the 50 μm I.D. tube.

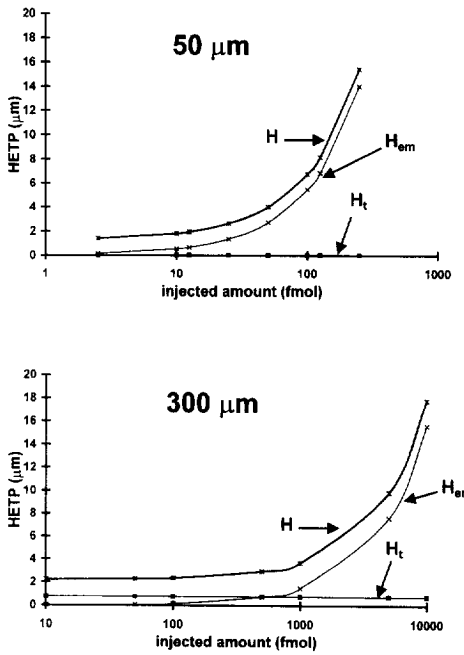

Fig. 2. Dependence of the plate heights calculated for varying amounts of *o*-nitrophenol loaded onto 50 and 300 μm I.D. columns. Parameters used for the calculations are given in Table 1. HETP, height equivalent to the theoretical plate (plate height); *H*, total values of the plate heights; H_{em}, H_t, contributions of the electromigration and thermal dispersions to the plate height values, respectively.

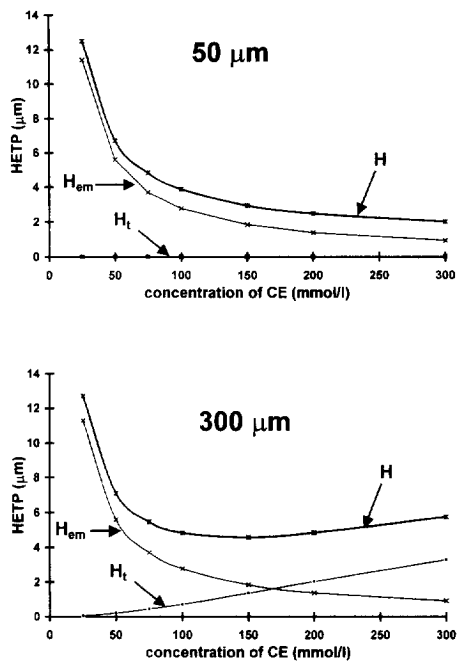

Fig. 3. Dependence of the plate heights for 100 (50 μm I.D.) and 3600 fmol (300 μm I.D.) loads of *o*-nitrophenol on the concentration of the carrier electrolyte. Values of the parameters used in the calculations of the dependence are given in Table 1. The current densities for different concentrations of the carrier electrolytes (expressed for a 1 mmol/l concentration) were 20.37 μA l/mm^2 mmol.

load) the concentrating effect improved the *H* values. However, a comparison with the corresponding dependence in Fig. 2 also indicates a degree of column overloading at a 300 mmol/l concentration of the carrier electrolyte. On the other hand, an increase of H_t with the concentration of the carrier electrolyte overwhelmed to some extent a positive role of the concentration process in the 300 μm tube.

Resolutions for a pair of nitrophenols loaded onto the columns at various concentration ratios were calculated for two concentrations of the carrier electrolytes (Fig. 4). In these calculations we assumed that 5 fmol amounts of the analytes represent minimum loadable amounts for which relevant analytical information could be obtained. The plots in Fig. 4 show that for lower concentration ratios of nitrophenols the 50 μm tube offers more favourable resolutions while for higher concentration ratios the use of the 300 μm tube may have significant advantages. These plots also indicate that a higher concentration of the carrier electrolyte is more convenient for the 50 μm tube, especially when the analytes present in the loaded sample differ by a factor of 100 or more. Undoubtedly, a main benefit of the 300 μm tube is linked with its higher cross-section. The data plotted in Fig. 4 indicate that a higher concentration of the carrier electrolyte in this tube may probably be beneficial only for very high ratios of the analytes.

Although the above calculations of the plate heights and the resolutions were performed for a simplified model (e.g., ITP stacking which in some instances can be generated in the CZE separations, [11–13] was not taken into account, convective effects in the separation compartment [34] which can occur in the tubes of larger I.D.s could not be considered) they provide reasonable estimates of advantages and limitations of the CZE separations performed in the capillary tubes of larger I.D.s.

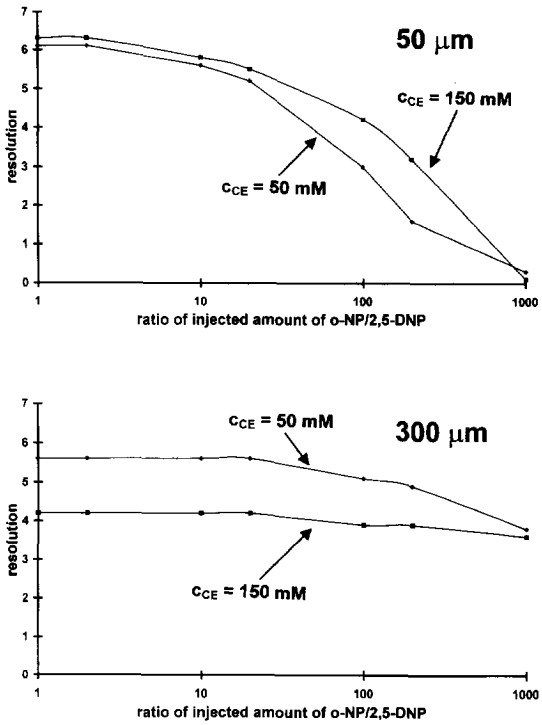

Fig. 4. Plots of the calculated resolutions for varying concentration ratios of *o*-nitrophenol and 2,5-dinitrophenol loaded onto 50 and 300 μm I.D.s columns. The amounts of 2,5-dinitrophenol in the loaded samples were constant (5 fmol).

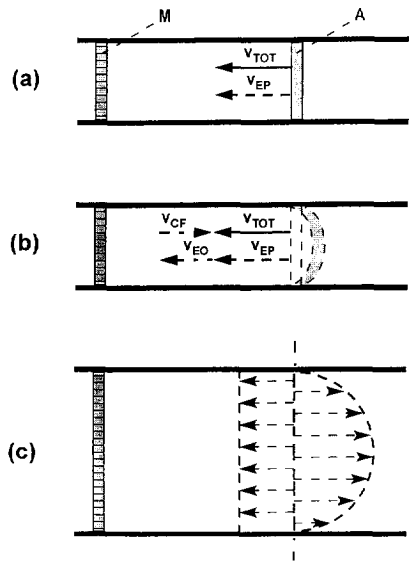

Fig. 5. An electroosmotic dispersion of the analyte band in the CZE separation compartment hydrodynamically closed by a porous membrane. a, b, profiles of the analyte band (a) without and (b) with an electroosmotic transport of the solution in the separation compartment, respectively; c, superpositions of the velocity profiles of the electroosmotic flow (directed to the left) and the hydrodynamic counter-flow (directed to the right) in a plane perpendicular to the axis of the capillary tube. M, membrane; A, the analyte band; v_{TOT}, total linear migration velocity of the analyte; v_{EP}, linear electrophoretic migration velocity; v_{EO}, linear electroosmotic velocity; v_{CF}, linear velocity of the hydrodynamic counter-flow in the separation compartment.

3.2. Electroosmotic dispersion in a hydrodynamically closed CZE separation compartment

When the CZE separation compartment is closed by a membrane to prevent a hydrodynamic flow of the solution of the carrier electrolyte in which the separation is performed electroosmosis is responsible for significant dispersions of the bands of the analytes [4,15,34]. A mechanism of this dispersive process is schematically illustrated in Fig. 5. Here, it is assumed that the electroosmotic volume velocity of the solution through the membrane is negligible relative to that in the separation compartment. Then the electroosmotically transported solution in the separation compartment is accumulated at the membrane (Fig. 5b) and a pressure difference between the membrane and the opposite end of the separation compartment is the result. This pressure difference is associated with a counter-flow of the solution. Its velocity (v_{CF}, in Fig. 5) rapidly attains a steady-state value corresponding to the velocity of the electroosmotic flow (v_{EO}, in Fig. 5) with a zero nett flow of the solution through any plane in the separation compartment (Fig. 5c). A parabolic velocity profile of the hydrodynamic counter-flow adversely affects the profiles of the analyte bands as schematically shown in Fig. 5b.

An electropherogram in Fig. 6a clearly demonstrates an impact of such a dispersive process on the shapes of the CZE zones under a non-zero electroosmotic flow in the separation compartment. As already mentioned above electrophoretic separations in hydrodynamically dosed separation compartments require that the electroosmotic flow is suppressed to a zero value in order to approach an idealized situation as shown in Fig. 5a. This can be achieved, e.g., by using suitable additives in the solutions of

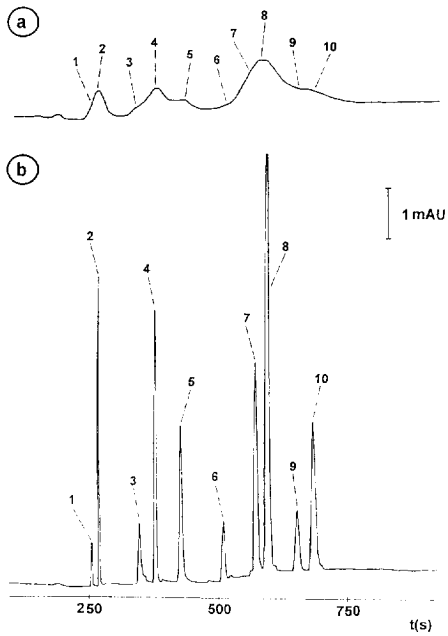

Fig. 6. Electropherograms from the separations of a model mixture of anions in a 300 μm I.D. capillary tube (a) without and (b) with an additive (m-HEC) in the carrier electrolyte solution (system No. 1, Table 2). The length of the FEP capillary tube was 350 mm (300 mm to the detector (Spectra 100, set at 254 nm)). 1, 2,6-dihydroxybenzoate; 2, 2-naphtylamine-4,8-disulfonate; 3, 2,6-naphtalenedisulfonate; 4, sulfanilate; 5, 2,4-dinitrophenol; 6, 3,5-dihydroxybenzoate; 7, 4-hydroxybenzoate; 8, sorbate; 9, anthranilate; 10, 4-aminobenzoate. The driving current in both instances was 100 μA.

the carrier electrolytes [10,19–25,27–31]. An electropherogram in Fig. 6b clearly illustrates a positive effect of such an additive (see Table 2 for electrolyte systems).

3.3. Some phenomena influencing migration velocities in a hydrodynamically closed separation compartment

The migration velocities of the analytes in the CZE separation compartment closed by a membrane are determined by their electrophoretic velocities (v_{EP}, in Fig. 5). Therefore, we can see very good agreements of the migration times of the analytes also in instances when the electroosmotic flows differed considerably (see Fig. 6). This indicates that the fluctuations of the parameters directly linked with the migration velocities of the analytes (e.g., migration times and peak areas as provided by the detection systems) should mainly be caused by the fluctuations in the v_{EP} values. This is a potential analytical advantage of the closed separation systems in comparison to their opened counterparts in which the fluctuations may originate in both the electrophoretic and electroosmotic transport velocities (for the fluctuations of the migration velocities (Δv), in the opened system it is reasonable to assume: $\Delta v = \sqrt{(\Delta v_{EO})^2 + (\Delta v_{EP})^2}$)

Table 2
Electrolyte systems

Parameter	1	2	3	4	5
Solvent	H_2O	H_2O	H_2O	H_2O	H_2O
Anion	MES	TES	SUCC	BENZ	HEPPSO
Concentration (mmol/l)	100	30	7	5	10
Counter-ion	HIS	IMID	BPT	TART	BTP
pH	5.2	6.85	3.55	5.2	7.25
Additive	m-HEC	PEG	m-HEC	m-HEC	m-HEC
Concentration (%, w/v)	0.2	0.2	0.2	0.2	0.2
Complexing agent	β-CD	PVP	CROWN	–	
Concentration (mmol/l)	–	6	–	40	–
Concentration (%, w/v)	–	–	5	–	–

MES, 2-(N-morpholino)ethanesulfonic acid; HIS, histidine; m-HEC, methylhydroxyethylcellulose 30 000; TES, N-[tris(hydroxymethyl)-2-aminoethane]sulfonic acid; PEG, polyethyleneglycol 5 000 000; β-CD, β-cyclodextrin; SUCC, succinic acid; BTP, 1,3-bis[tris(hydroxymethyl)-methylamino]propane; IMID, imidazole; PVP, polyvinylpyrrolidone; BENZ, benzimidazole; TART, tartaric acid; CROWN, 18-crown-6-ether; HEPPSO, N-(hydroxyethyl)piperazin-N′-3-(2-hydroxy)propanesulfonic acid.

The membrane in the separation compartment may be a source of significant fluctuations in the migration velocities. Here, the following phenomena are to be taken into consideration:

(i) Osmosis associated with transport of the solvent through the membrane when the solutions on both sides of the membrane have different compositions. Therefore, to minimize this source of errors solutions of identical compositions need to be placed in both the separation and electrode compartments (see also Fig. 1).

(ii) In filling the separation compartment with the solution of the carrier electrolyte via a needle valve (Fig. 1) the membrane is exposed to a pressure difference. This results in its mechanical deformation as shown in a diagram in Fig. 7b. After closing the filling valve the membrane is slowly relaxed to its original position. This movement of the membrane is accompanied by a flow of the solution in the separation compartment leading to both the deformations of the zone profiles and changes of the migration times of the analytes. These disturbances are illustrated by electropherograms in Fig. 8. For example, we found that depending on the time interval left for the relaxation of the membrane before the application of the sample the migration times of the analytes deviated by more than 5% under otherwise identical working conditions. Also large deviations in the peak widths of the analytes (up to 50%) were typical in these experiments. These undesired effects we eliminated (minimized) in the way described by Koval' for ITP [35]. Details of this solution based on mechanical support of the membrane are shown in Fig. 1. Its impact on the separation performance is apparent when the electropherograms in Fig. 8 are compared to that in Fig. 6b.

(iii) Leaks of the solution from the separation compartment through a leaky membrane and/or through an leaky filling valve (see Fig. 1) can also

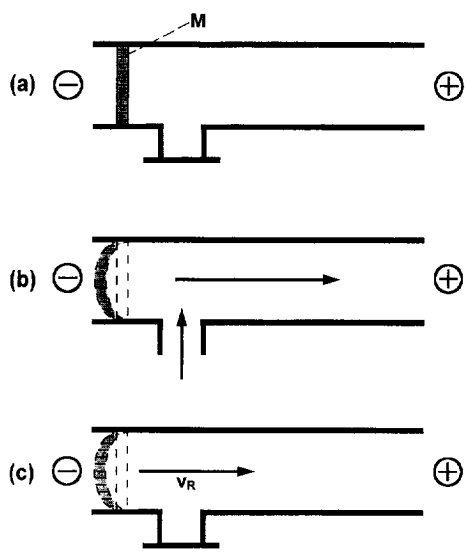

Fig. 7. Behaviour of the membrane (M) hydrodynamically closing the separation compartment when a pressure difference is applied to fill the capillary tube with a solution of the carrier electrolyte. a, situation before the pressure difference is applied; b, filling the capillary tube; c, a relaxation of the membrane into its original position (a) in the closed separation compartment.

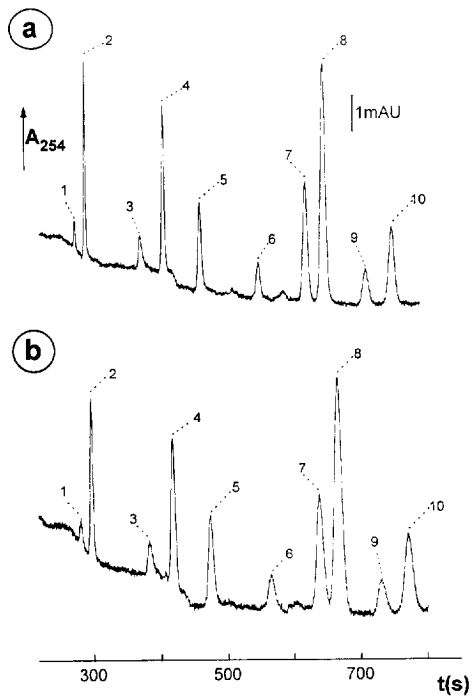

Fig. 8. Influence of the counter-flow of the carrier electrolyte solution generated by a movement of the membrane (Fig. 7) on the migration times and widths of the analyte peaks. a, the separation compartment (Fig. 1) was opened via the outlet channel in the injection valve (not shown in Fig. 1) for 10 s after it was filled with the solution of the carrier electrolyte; b, the same as in (a) only no time was left for a relaxation of the membrane before the application of the sample onto the column. The working conditions and the peak assignments were the same as in Fig. 6b.

contribute to errors in the migration velocities. While a wide choice of high quality membranes is available we found that needle valves [36] provide the most reliable means in preventing leaks through the filling valves of the closed separation compartments (see Fig. 1).

(iv) Ionogenic products of the electrode reactions at the driving electrodes (E1 and E2 in Fig. 1) can enter the separation compartment. The changes in the compositions of the carrier electrolyte formed in this way can seriously affect the migration velocities of the analytes. Problems of this type we solved in the way currently used in ITP, i.e., by increasing the volumes of the solution present between the electrodes and the separation compartment. Such a solution enables the carrying out of many repeated CZE runs without refilling the separation compartment between the runs while highly reproducible migration times are typical [24].

3.4. CZE separations of various groups of the analytes in a 300 μm I.D. capillary tube

Synthetic food colourants permitted for application to food products can be conveniently analyzed by CZE in the separation compartment of enhanced load capacity with LOD values mostly at low ppb concentrations using a photometric absorbance detector operating at a 254 nm wavelength [21,22]. In this way very sensitive analytical procedures for the determination of the colourants in various food matrices can be elaborated. An electropherogram in Fig. 9 illustrates limiting possibilities of the present CZE approach in the determination of a group of five colourants when injected in a 100 nl volume. In comparison to the other CZE alternatives (see Ref. [22] and references given therein) the values in Fig. 9 represent very significant improvements (10–100 times) in the limits of quantitation [37]. Undoubtedly, using the capillary tube of a 300 μm I.D. not only an enhanced sample loadability but also a higher effective pathlength of the on-column photometric detector was effective [2].

Recently, we showed [24] that using CZE in the arrangement as discussed in this work, inorganic anions which currently need to be determined in various samples could be resolved within 4–5 min with high selectivities at a low pH of the carrier

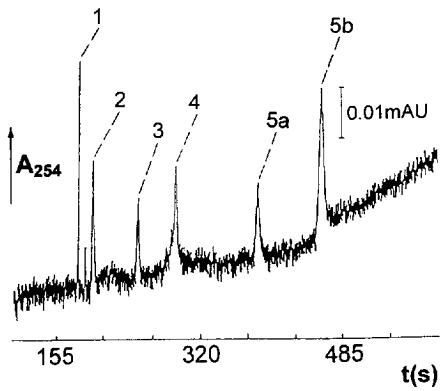

Fig. 9. An electropherogram from the separation of synthetic food colourants present in the sample at concentrations approaching their limits of quantitations. The separation was carried out in a 300 μm I.D. capillary tube made of FEP. The length of the tube was 300 mm (240 mm to the detector (UV-M II, set at 254 nm)). The electrolyte system No. 2 (Table 2) was used for the separation with a 150 μA driving current. 1, amaranth (190 ppb); 2, brilliant black BN (380 ppb); 3, sunset yellow FCF (190 ppb); 4, chromotrope FB (380 ppb); 5, two constituents of quinoline yellow (750 ppb-total concentration).

electrolyte. This procedure was found convenient in detection of trace anionic constituents (fluoride, nitrite and phosphate) in water samples in the presence of excesses of sulfate, chloride and nitrate. In this work we investigated possibilities of CZE in a 300 μm I.D. capillary tube provided with on-column conductivity detection in resolving these anions when present at very differing concentrations in the injected sample. Electropherograms in Fig. 10 were obtained from these investigations. The electropherograms clearly show that under the employed working conditions that anions of close effective mobilities could also be rapidly resolved when present in the samples at 10^3:1 molar concentration ratios. From the dependencies presented in Fig. 4 it can be deduced that the I.D. of the tube played an important role in achieving such a separation performance. From the electropherogram in Fig. 10b, however, we can see that the ITP stacking [11–13] of the analytes was significant. This focusing effect, in fact, set the limits of our CZE approach in terms of a maximum concentration ratio of the analytes loadable onto the column.

Plots presented in Figs. 2 and 3 show that in the separations carried out in a 300 μm I.D. tube

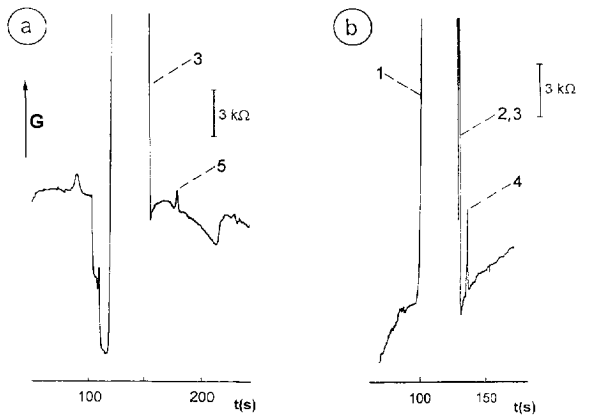

Fig. 10. Electropherograms from the separations of inorganic anions present in the samples at 10^{-3}:1 molar concentration ratios. The separations were carried out in the electrolyte system No. 3 (Table 2) with a 30 μA driving current. a, a model mixture containing nitrate (3) at a 62 ppm concentration and nitrite (5) at a 46 ppb concentration; b, a dietary salt sample containing chloride (1) at a 36 ppm concentration and spiked with iodide (4) at a 125 ppb concentration (nitrate (3) and sulfate (2) originated from the salt sample).

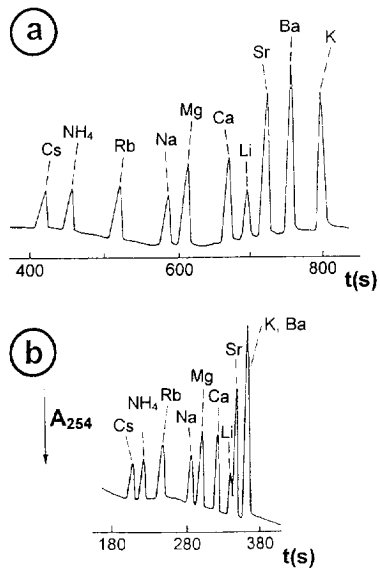

Fig. 11. CZE separations of alkali and alkaline earth metal cations and ammonium in the electrolyte system No. 4 (Table 2). a, 50 μA driving current; b, 100 μA driving current. The separations were carried out in a 300 μm I.D. FEP capillary tube of a 300 mm length (250 mm to the detector (UVD 2, set at 254 nm).

significant thermal effects can occur. These influence not only the plate heights of the analytes but also their effective mobilities. The changes of the effective mobilities reflect both the changes of the actual mobilities of the anionic forms of the analytes and acid–base and complex equilibria involved [34]. Electropherograms in Fig. 11 obtained from the separations of alkali and alkaline earth metal cations via their complexing equilibria with tartrate and 18-crown-6-ether illustrate such an effect. The thermal effects are apparent, especially, for the cations forming strong complexes with 18-crow-6-ether (K^+ and Sr^{2+}). In this context, however, it is to be noted that performing the separations with a stabilized driving current highly reproducible migration times of the cations were typical using this temperature sensitive electrolyte system [20].

Erythropoietin (EPO) is glycoprotein hormone of molecular mass in the range of 34 000–38 000 with 40% of its weight being attributable to its carbohydrate structure. CZE carried out in a hydrodynamically opened separation compartment was investigated by the separation of the EPO glycoforms present in recombinant EPO preparatives [38,39]. Recently, we paid attention to the use of various capillary electrophoresis techniques for the separations and fractionations of the EPO glycoforms [40]. Electropherograms in Fig. 12 show typical reproducibilities achieved in the CZE profiling of the glycoprotein as obtained in a hydrodynamically closed separation system as discussed in this work. We can see that both the within day (Fig. 12a vs. Fig. 12b) and day-to-day (Fig. 12a vs. Fig. 12c) reproducibilities were very good. To achieve such reproducibilities it was essential to pay special attention to the preparation of the carrier electrolyte solutions with respect to the concentration of carbonate and, especially, by performing the separations under conditions preventing entrance of ambient CO_2 into the carrier electrolyte solutions. Such precautions are necessary in order to keep the electric conductivity (ionic strength) and pH of the carrier electrolyte solution constant within tolerable limits. Effective means of achieving this for our configura-

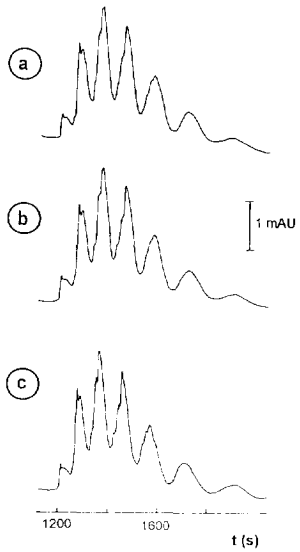

Fig. 12. Electropherograms obtained from the CZE separations of glycoforms of erythropoietin in the electrolyte system No. 5 (Table 2) in a 300 μm I.D. capillary tube made of FEP. a, Run immediately after the sample kept at −60°C was thawed; b, the tenth run with the same sample as in (a); c, a different aliquot of the same sample as in (a) separated in the same electrolyte system with a one month delay. The driving current in the runs was stabilized at 10 μA. The length of the tube was 400 mm (350 mm to the detector (Spectra 100, set at 225 nm)).

tion of the CZE separation system are discussed in more detail elsewhere [25].

Acknowledgments

This work was supported by a grant provided by the Slovak Grant Agency for Science No. 1/1461/94 and by the USA–Slovak Scientific and Technological Program under project No. 007-95. The authors thank Dr. Jetse Reijenga (Eindhoven University of Technology, Netherlands) for the generous gift of a copy of the HPCE.SIM program.

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