External electric field control of electroosmotic flow in non-coated and coated fused-silica capillaries and its application for capillary electrophoretic separations of peptides

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Abstract

The influence of an external electric field on the electroosmotic flow in the noncoated (bare) fused-silica capillaries and in the fused-silica capillaries with covalent coating of the inner surface by the polymer of a new acrylamido derivative, *N*-(acryloylaminoethoxy)ethyl-β-ᴅ-glucopyranose, has been tested in the capillary electrophoretic separations of peptide analytes. The effect of magnitude and polarity of the external electric field on the flow-rate of the electroosmotic flow, the migration times of charged analytes and the separation efficiency and resolution of separations of synthetic oligopeptides, diglycine, triglycine, glycyl–proline and prolyl–glycine, by capillary zone electrophoresis has been evaluated. The effect of the external electric field on the velocity of the electroosmotic flow was much higher in the bare fused-silica capillaries than in the coated capillaries. Better separation of the analyzed peptides was achieved in the coated fused-silica capillaries. An external electric field proved to be an effective tool for control of the electroosmotic flow and for optimization of the speed and resolution of capillary electrophoretic separations of synthetic peptides. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Regulation of electroosmotic flow (EOF) in capillary zone electrophoresis (CZE) is extremely important, since the velocity of the EOF codetermines the resulting migration velocity of the analytes in CZE, and in this way it affects not only speed of analysis, but also the separation efficiency, selectivity and resolution [1–3]. EOF velocity is given by the product of electroosmotic mobility (EOF velocity normalized to unit intensity of the electric field) and the intensity of the electric field. Apparently, the simplest EOF regulation can be achieved by changes of electric field strength. The disadvantage of this way of regulation is the same proportional change of electroosmotic and electrophoretic velocities, since also electrophoretic velocity is given by the product of electrophoretic mobility and intensity of the

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electric field. In addition, increasing the EOF by the higher intensity of the electric field is limited by the Joule heat effects, i.e., the intensity of the electric field can be increased only to the level at which the input electric power does not cause too high an increase of temperature inside the separation compartment.

The other ways of EOF regulation in CZE, and in other capillary electromigration methods, are based on direct proportionality of the electroosmotic mobility to the electrokinetic (zeta) potential (potential drop in the diffusion part of the double layer at the inner capillary wall) and permittivity of the solution of the background electrolyte (BGE) and on indirect proportionality of EOF mobility to viscosity of the solution of BGE. These magnitudes are governed both by the composition of the BGE inside the capillary and by the quality of the inner capillary surface. Consequently, EOF regulation may be achieved by changes in composition of the BGE, e.g., EOF is increasing with decreasing ionic strength and increasing pH, EOF is reduced by the addition of polymer additives increasing the viscosity of BGE, and/or by the dynamic or covalent coating of the inner surface of the capillary. The disadvantage of these ''chemical'' ways of EOF regulation is that they are not independent and change of one magnitude also influences the others.

Fundamentally, a new way of EOF regulation which overcomes the above disadvantages was theoretically introduced by Ghowsi and Gale [4,5] and experimentally performed by Lee et al. [6,7]. Their approach consists of the application of an additional electric field on the outer surface of the capillary. The formed potential gradient between this externally applied electric field and the internal electric field (driving force of electrophoresis and EOF) acts across the capillary wall as a radial electric field and affects the density of the electric charge on the inner capillary wall, i.e., the magnitude and polarity of the electrokinetic potential and the flow-rate and direction of EOF inside the capillary.

The external electric field was applied in CZE in several ways using different media surrounding the outside part of the capillary: low-conductivity buffer solution [6–8] or ionized air [9] placed in the tube or box coaxially situated with the separation fused-silica capillary, by metal coating [10–12] or by coating the capillary by a layer of low-conductivity

polymeric conductor, e.g., ionic polymer Nafion [13,14] or polyaniline [15,16]. From them the last mentioned mode, i.e., low-conductivity polymeric coating seems to be the most suitable thanks to its technical compatibility with CZE instrumentation and mainly due to the fact that the course of the external filed can be easily manipulated and allows formation of uniform electrokinetic potential along most of the capillary length.

In addition to the effect on EOF, the changes of electrokinetic potential induced by an external electric field may also influence the electrostatic interactions of charged analytes with capillary walls and can contribute to the lower sorption of the analytes. This effect was confirmed by higher efficiency and better resolution of CZE separations of some proteins [17] and peptides [15]. However, the peaks of some peptides and proteins in their CZE separations remained relatively broad indicating other types of interactions than electrostatic to be responsible for adsorption of these analytes to the capillary wall and for broadening of their zones. For suppression of these types of interactions many different types of capillary coatings have been developed as can be seen in the recent reviews [18–20].

In order to have both the possibility of EOF regulation by an external electric field and to suppress the hydrophobic and other interactions of the analytes with the inner capillary wall the aim of the presented paper was to test the application of an external electric field to the capillary with a hydrophilic inner coating and to compare the effect of the external electric field on the EOF regulation and on the peptide analytes' interactions with the capillary wall in this internally coated capillary and in the noncoated (bare) fused-silica capillary. For these purposes the home-made CZE device had to be adapted for the application of an external electric field, and the procedures for capillary coating by outer low conductivity coating and for inner new acrylamido derivative based inert coating had to be developed.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent

grade. Diglycine and triglycine were obtained from Reanal (Budapest, Hungary), glycyl–proline and prolyl–glycine were from Sigma (St. Louis, MO, USA) and acetic acid was from Lachema (Brno, Czech Republic). Buffer and sample solutions were prepared from the deionized and redestilled water and filtered by 0.45-μm filter (Millipore, Bedford, USA) prior use in CZE.

2.2. CZE device adapted for application of external electric field

CZE separations were performed in a home-made device [21] which was adapted for the application of the external electric field. The basic scheme of the device is shown in Fig. 1a. The separation compartment consists of the fused-silica capillary, FS, with usual outer polyimide (PI) coating and with a newly developed low-conductivity polymer outer coating (OC) on a part of the outer capillary surface allowing the application of the external electric field, and with the polyacrylamido-derivative-based inner coating (IC) suppressing the adsorption of analytes to the inner fused-silica capillary wall. The outer low-conductivity coating is formed on the part of the outer surface of the capillary, between coordinates $x1$ and $x2$ (see Fig. 1b) with the exception of detection window of the UV absorption detector operating at 206 nm, where both low-conductivity and polyimide coatings were removed. Low-conductivity coatings at both sides of the detection window are bridged by the brass flat cylindric component with a groove in which the slit of the detection window (0.050×0.600 mm) is made and in which the capillary is placed. For simplicity this 3 mm long uncoated part of the capillary is omitted in Fig. 1a and it is neglected also in the calculations presented in Section 3. The separation voltage, U_{sep}, provided by the high-voltage power supply HV1 (home-made power supply with reversible polarity, 20 kV, 500 μA), is applied as an internal voltage, U_{in}, to the ends of the inner capillary compartment dipped into the electrode vessels and filled with the BGE. The external longitudinal electric field is formed by the connection of two different voltages to the ends of the outer low-conductivity coating of the capillary (at coordinates $x1$ and $x2$) from the high-voltage power supplies HV2 and HV3 (high-voltage modules CZE R1000 with reversible polarity, 30 kV, 300 μA,
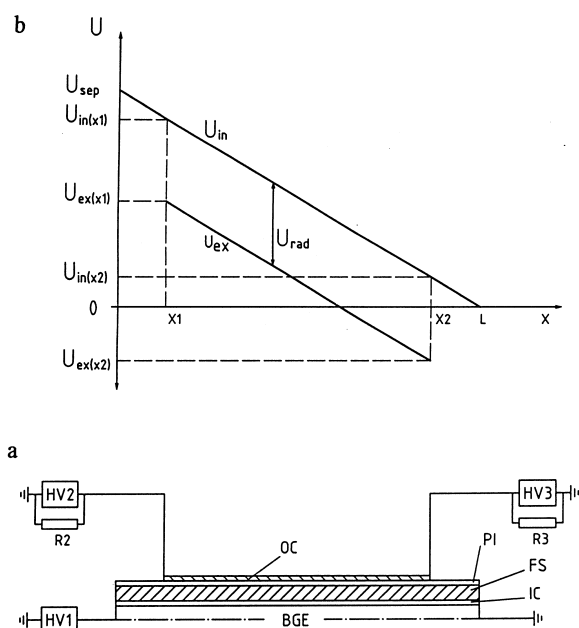

Fig. 1. (a) Scheme of the experimental CZE device adapted for application of external electric field. (b) Course of internal, external and radial voltage along the longitudinal axis of the coated fused-silica capillary. FS = Fused-silica capillary wall; PI = polyimide outer capillary coating; OC = outer coating: low-conductivity layer of copolymer of polyaniline and *p*-phenylendiamine (applied between capillary coordinates $x1$ and $x2$) to which the external electric field is connected; IC = inner coating of the capillary: poly(AEG), BGE-background electrolyte inside the capillary; HV1 = high-voltage power supply providing the internal separation voltage, U_{sep}; HV2, HV3 = high-voltage power supplies providing the external voltages at positions $x1$ and $x2$, respectively; R2, R3 = load resistances of power supplies HV2, HV3, respectively; U = voltage; x = longitudinal coordinate of the capillary; L = total length of the capillary; $x1$, $x2$ = positions to which the external electric field is connected via power supplies HV2 and HV3; U_{in} = internal voltage (inside the capillary); U_{ex} = external voltage (on the outer low-conductivity coating of the capillary); U_{rad} = radial voltage (across the capillary wall); U_{sep} = separation voltage.

Spellman, Plainview, NY, USA), respectively. The difference of electric potentials applied outside and inside the capillary forms an additional electric field which is oriented perpendicularly to the capillary wall and influences the electrokinetic potential at the interface between inner capillary wall and BGE, and consequently, flow-rate and direction of EOF inside the capillary. Load resistances R2 and R3 (each of them 300 Mohm) of high voltage power suplies HV2 and HV3, respectively, and common grounding of all

three power supplies HV1, HV2 and HV3 (see Fig. 1a) ensures the application of well defined voltages at positions $x1$ and $x2$. Two power supplies HV2 and HV3 are used to allow the application of constant voltage difference (radial voltage, U_{rad}) between the inner and outer capillary surfaces along the capillary between the positions $x1$ and $x2$ (see Fig. 1b).

2.3. CZE separation conditions

CZE peptide separations were carried out in the bare and in the polyacrylamide derivative-coated fused-silica capillaries of the following dimensions: I.D.=0.056 mm, OD1 (fused-silica)=0.180 mm, O.D.2 (polyimide)=0.200 mm, total length (L)=315 mm, effective length (from injection end to the detector) (L_{ef})=200 mm. The bare fused-silica capillaries with polyimide coating were supplied by the Institute of Glass and Ceramics Materials (Czech Academy of Sciences, Prague, Czech Republic), the inner and outer coating procedures are described in Section 3, since their development represents a part of the achieved results of this work.

A solution of 0.5 mol/l acetic acid, pH 2.5, was used as the BGE. Peptide samples were dissolved in water or in the BGE in the concentration range 0.3–0.8 mg/ml and they were introduced into the capillary manually forming a hydrostatic pressure (0.005 bar) for 5–25s. Separations were performed at ambient temperature, 23–25°C, at the constant voltage of 8.0 kV with the electric current in the range 7.8–8.2 μA. The EOF velocity was determined from the migration time of water.

3. Results and discussion

3.1. Preparation of outer and inner capillary coatings

The outer low conductivity polymeric capillary coating with a specific longitudinal resistivity of 4 Gohm/cm was prepared from the dispersion of nonsoluble particles of the conductive material (copolymer of aniline and p-phenylenediamine) in the polystyrene matrix. Suitable adhesion of the coating solution to the outer polyimide capillary coating was achieved by the application of N-methylpyrollidone as a polystyrene solvent which was able both to dissolve the matrix polymer and to swell the polyimide coating. The conductive filling material of the polystyrene matrix was prepared by fine grinding of nonsoluble copolymers of aniline and p-phenylene-diamine (p-PDA). The copolymers were prepared by chemical oxidation of aniline and p-PDA by ammonium peroxodisulphate in the medium of 1 mol/l HCl according to the early developed procedure [22]. The advantage of these materials is the possibility to control their conductivity in the range of several orders by the relative content of nonconductive p-PDA. The copolymer was in the form of micropartices of the dimension 2–4 μm and their aggregates.

The inner capillary coating by a new polyacrylamido-derivative was prepared according to the following procedure: Fused silica capillary columns were coated with a polymer obtained by radical polymerization of a novel acrylamido-derivative, N-(acryloylaminoethoxy)ethyl-β-D-glucopyranose (AE-G) [23]. Capillaries coated with poly(AEG) were prepared by covalently linking the polymer to a surface activated with γ-methacryloxypropyl functionalities bonded to the wall through a direct silicon–carbon linkage, prepared as described in Ref. [24]. Briefly, a hydride-modified support was formed by reacting triethoxysilane with a silica substrate in the presence of water and hydrochloric acid, using dioxane as the solvent. The γ-methacryloxypropyl group residue was linked to the hydride-modified substrate by hydrosilylation of allyl methacrylate in the presence of a Pt catalyst. γ-Methacryloxypropyl activated capillaries were filled with a solution of N-(acryloylaminoethoxy)ethyl-β-D-glucopyranose (15%, w/v). The four monomer solutions contained the appropriate amount of catalyst (1 μl of N,N,N′,N′-tetramethylethylenediamine (TEMED) and 1 μl of 40% ammonium peroxydisulfate per ml of gelling solution) and was degassed under vacuum (20 mm Hg) for 40 min. Polymerization was allowed to proceed overnight at room temperature and then the capillaries were emptied with an HPLC pump. The stability of these capillaries is generally very good, they can be used for more than 300 runs at pH 8.5, and at low pH the stability is even better [23].

3.2. Application of constant radial electric field along the capillary

Theoretical description of the influence of EOF on

the efficiency of CZE separation shows that the disturbing effect of EOF in CZE is minimized if the electrokinetic potential is constant along the capillary length [25–27]. From this theoretical conclusion and from the fact, that the resulting electrokinetic potential is equal to the sum of the electrokinetic potential originating from the dissociation of the silanol groups of the fused-silica and of the electrokinetic potential induced by the radial electric field, it follows, that the radial electric field should be applied at the maximal length of the capillary and should be constant along the longitudinal axis of the capillary. However, for the practical reasons, i.e., prevention of shortcircuit between external and internal electric fields, the former can be applied only to the part of the capillary which is not dipped into the BGE in the electrode vessels, i.e., to the outer polymeric coating between positions $x1$ and $x2$ (see Fig. 1). The voltage of the radial electric field, U_{rad}, is given as the difference of voltages (electric potentials) on the external and internal side of the capillary wall, U_{ex} and U_{in}, respectively:

$$U_{rad} = U_{ex} - U_{in} \tag{1}$$

The internal voltage, U_{in}, is linearly increasing from zero at the grounded capillary end (coordinate L) to the value of separation voltage, U_{sep}, at the high-potential capillary end (coordinate 0 (zero)) (see Fig. 1b). Internal voltage, U_{in}, at position x between the end coordinates 0 and L is given by the equation:

$$U_{in(x)} = U_{sep} \frac{L - x}{L} \tag{2}$$

To achieve the constant shift of optional external and internal voltages in the positions $x1$ and $x2$, two power supplies have to be used to form the external voltage. The values of external voltages in the positions $x1$ and $x2$, $U_{ex(x1)}$, $U_{ex(x2)}$, respectively, are dependent both on the driving separation voltage of CZE, U_{sep}, and on the requested radial voltage, U_{rad}:

$$U_{ex(x1)} = U_{in(x1)} + U_{rad} = U_{sep} \frac{L - x1}{L} + U_{rad} \tag{3}$$

$$U_{ex(x2)} = U_{in(x2)} + U_{rad} = U_{sep} \frac{L - x2}{L} + U_{rad} \tag{4}$$

The examples of applied voltages (separation, internal, external and the resulting radial), i.e., the values of U_{sep}, U_{in}, U_{ex} and U_{rad} (see Fig. 1b) in the practical applications of the external electric field in CZE separations of a test mixture of diglycine and triglycine in the noncoated and coated fused-silica capillaries are given in Table 1.

Table 1
Effect of external and radial electric field on EOF in CZE peptide separations performed in noncoated and poly(AEG)-coated fused-silica capillaries[a]

$U_{in(x1)}$ (kV)	$U_{in(x2)}$ (kV)	$U_{ex(x1)}$ (kV)	$U_{ex(x2)}$ (kV)	U_{rad} (kV)	$(t_{eo})_{nc}$ (s)	$(v_{eo})_{nc}$ (mm/s)	$(t_{eo})_c$ (s)	$(v_{eo})_c$ (mm/s)
+7	+1	off	off	off	276	0.73	546	0.37
+7	+1	+11	+5	+4	1253	0.16	630	0.32
+7	+1	+9	+3	+2	827	0.24	610	0.33
+7	+1	+7	+1	0	511	0.39	590	0.34
+7	+1	+5	−1	−2	365	0.55	576	0.35
+7	+1	+3	−3	−4	278	0.72	560	0.36
+7	+1	+1	−5	−6	230	0.87	538	0.37
+7	+1	−1	−7	−8	195	1.03	520	0.38
+7	+1	−3	−9	−10	174	1.15	498	0.39

[a] $U_{in(x1)}$, $U_{in(x2)}$ = Internal voltages at positions $x1$ and $x2$, respectively (see Fig. 1b); $U_{ex(x1)}$, $U_{ex(x2)}$ = external voltages at positions $x1$ and $x2$, respectively; $x1$, $x2$ = positions to which external voltages are connected; U_{rad} = voltage of radial electric field; $(t_{eo})_{nc}$, $(t_{eo})_c$ = migration times of EOF marker (water) in noncoated and coated fused-silica capillaries, respectively; $(v_{eo})_{nc}$, $(v_{eo})_c$ = linear velocities of EOF in noncoated and coated fused-silica capillaries, respectively. Average values of EOF migration times and velocities from two experiments with stabilized EOF flow-rate are presented. Data obtained from CZE separations of diglycine and triglycine dissolved in water using 0.5 mol/l acetic acid, pH 2.5 as the BGE. Separation voltage, U_{sep} = 8.0 kV; total capillary length, L = 315 mm; coordinates of the positions to which external voltages are connected: $x1$ = 40 mm, $x2$ = 275 mm. For other experimental data see Sections 2.2 and 2.3.

3.3. Effect of external electric field on EOF in noncoated and coated fused-silica capillaries

The effect of the external electric field on the flow-rate of the EOF in the noncoated (bare) fused-silica capillaries and in the capillaries with inner poly(AEG) coating was tested by separation of model mixture of oligopeptides, diglycine and triglycine, and using the sample solvent (water) as an EOF marker in these two types of capillaries. Examples of the separation of this mixture in the absence of the external electric field and at some values of enforced radial electric field in the non-coated capillary are shown in Fig. 2 and in the coated capillary in Figs. 3 and 4. The significant influence of the external electric field on the migration time of the EOF marker (water) and on the migration times of charged peptide analytes in the bare fused-silica capillary is apparent. Depending on the magnitude and polarity of the radial electric field, the EOF velocity can be decreased (see Fig. 2b), increased (see Fig. 2d) or it can remain moreorless the same (see Fig. 2c) in comparison with the state when the external field is not applied (U_{rad}=off, see Fig. 2a). A special case is the state when the external and internal voltages are equal, i.e., radial voltage is zero. In this case the EOF is reduced in comparison with absence of the external field.

Unlike the noncoated fused-silica capillaries, the effect of the external electric field on the EOF in the capillaries with the inner poly(AEG) coating was substantially smaller as demonstrated in Figs. 3 and 4 where the records of CZE separations at some different values of the radial electric fields are presented. The general tendency, i.e., reduction of EOF at positive values of radial field and increased EOF at negative radial voltages, is the same as in the noncoated capillary, but both the absolute and relative changes of EOF are much smaller than in the noncoated capillaries. The lower effect of the radial field on EOF in the coated capillaries is probably caused by the lower polarizability of the poly(AEG)-coated fused-silica capillaries than the bare fused-silica capillaries and for that reason the relative portion of the charge induced by the external field on the inner capillary surface and contributing to the resulting electrokinetic potential is smaller.

Some experimental data and results of the CZE separations of diglycine and triglycine in the non-coated and coated fused-silica capillaries are summarized in Table 1, where the appropriate values of internal and external voltages at positions $x1$ and $x2$, their differences (radial voltages, U_{rad}) and the measured migration times of the EOF marker (water) in the noncoated capillary, $(t_{eo})_{nc}$, and in the coated capillaries, $(t_{eo})_c$, are given. The EOF velocities in noncoated capillaries, $(v_{eo})_{nc}$, and in coated capillaries, $(v_{eo})_c$, presented also in Table 1, were calculated from the effective length of the capillary, L_{ef}, and from the migration times of the EOF marker, t_{eo}:

$$v_{eo} = L_{ef}/t_{eo} \qquad (5)$$

A comparison of migration times and EOF velocities in the absence and presence of the external electric field shows that even in the absence of the enforced external field by power supplies HV2 and HV3, a voltage gradient across the capillary wall is present, the effect of which is approximately equivalent to the enforced radial voltage, $U_{rad}=-4$ kV (see line 1 and 6 in Table 1 and CZE records in Fig. 2a and c and Fig. 4a and b). The data presented in Table 1 document strong effect of external radial field on the EOF in the noncoated capillaries. The EOF velocity at radial voltage -10 kV is about 1.5 times higher and at radial voltage $+4$ kV, it is more than four times lower than the EOF in the absence of radial voltage.

From the EOF velocities, v_{eo}; the separation voltage, U_{sep}, and total capillary length, L, the more representative and comparable data, electroosmotic mobilities, m_{eo}, were calculated:

$$m_{eo} = \frac{v_{eo}L}{U_{sep}} \qquad (6)$$

The graph of the dependence of the EOF mobility, m_{eo}, on the applied radial electric field, U_{rad}, in the noncoated and coated fused-silica capillaries is presented in Fig. 5. Again, a much higher effect of the external electric field on the EOF mobility in the noncoated than in the coated capillaries is apparent.

The external electric field influences not only the EOF velocity but also efficiency, resolution and migration time of CZE analysis as can be seen from the separations shown in Figs. 2–4 and from the data presented in Tables 2 and 3. As expected, at positive
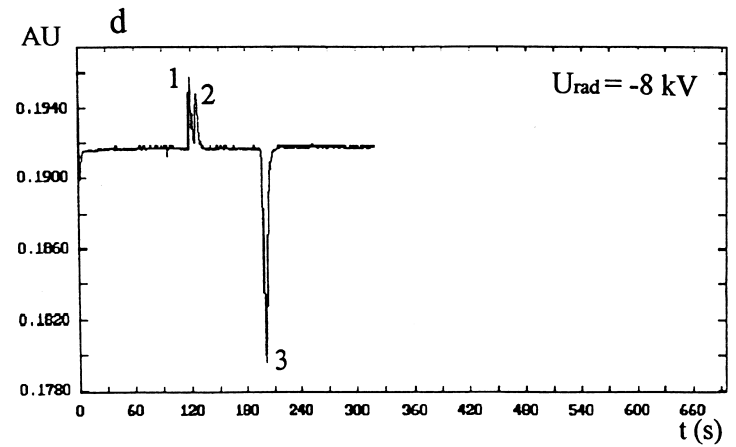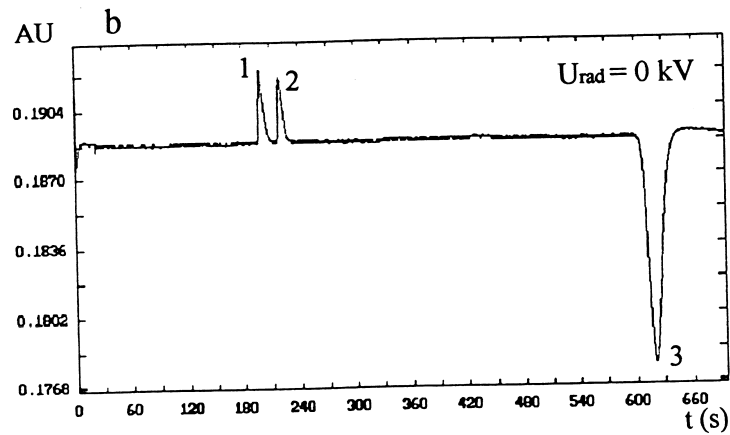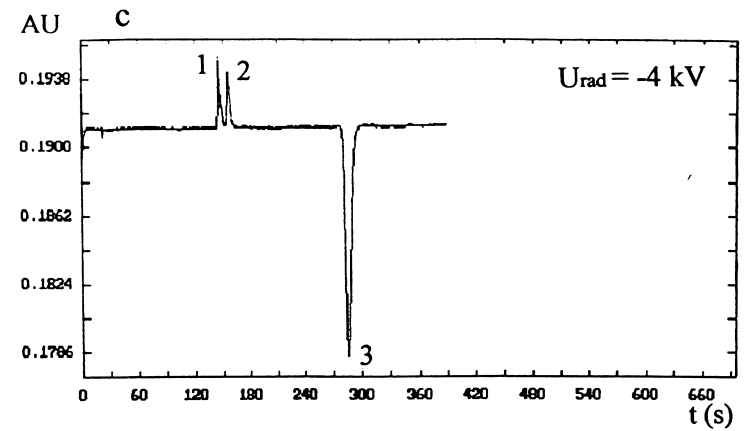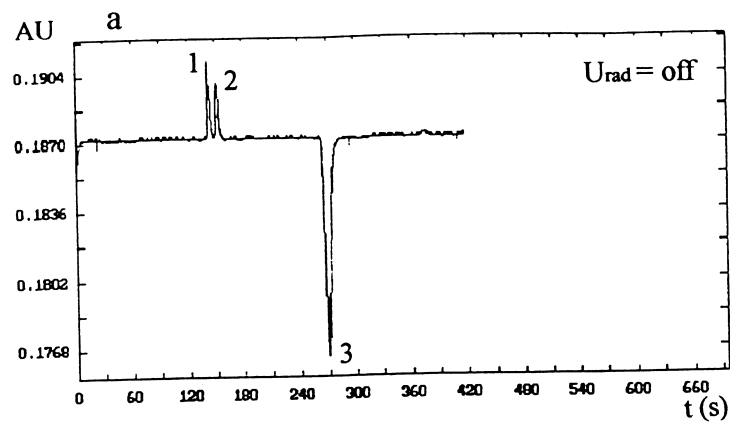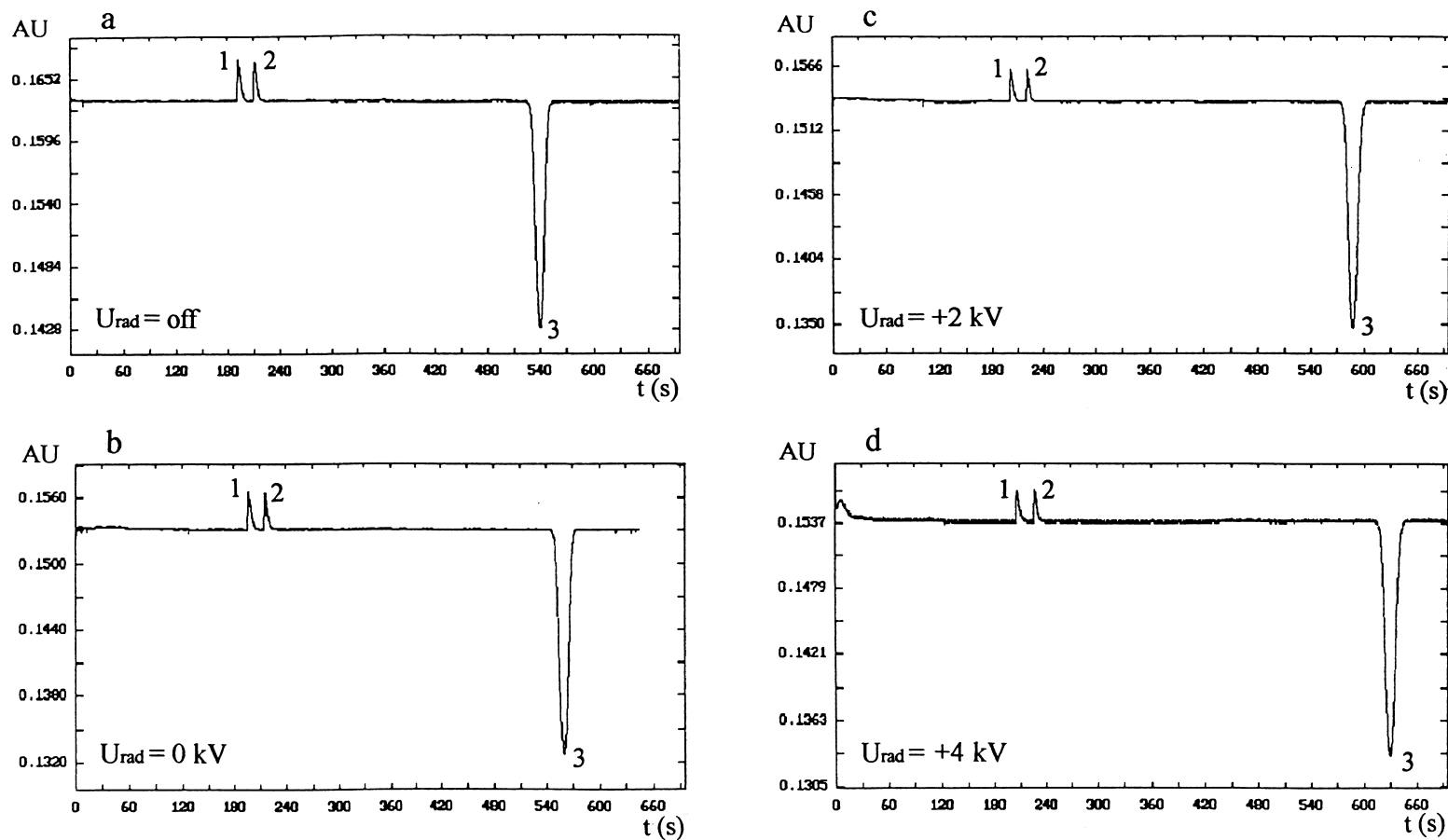
Fig. 2. CZE separation of diglycine (peak 1) and triglycine (peak 2) dissolved in water (peak 3) in the noncoated fused-silica capillary using 0.5 mol/l acetic acid as the BGE in (a) the absence of radial electric field and the presence of different radial electric fields: (b) $U_{rad} = 0$, (c) $U_{rad} = -4$ kV, (d) $U_{rad} = -8$ kV. For other experimental conditions see the text. AU = Absorption at 206 nm, t = migration time.

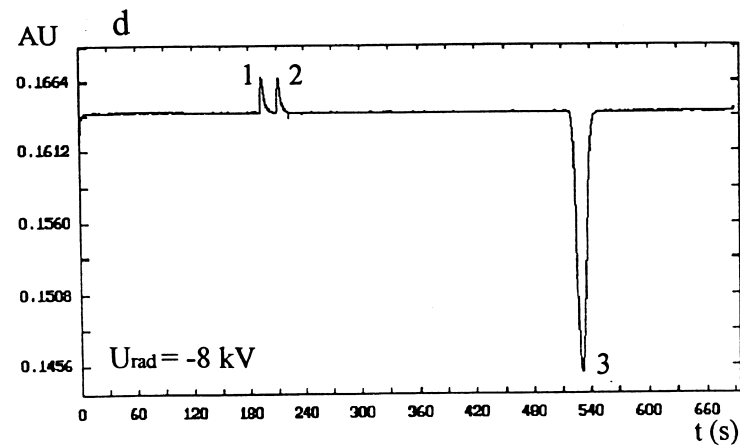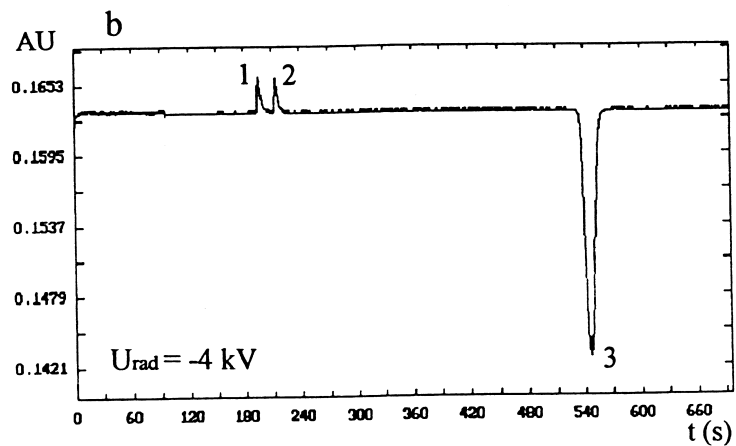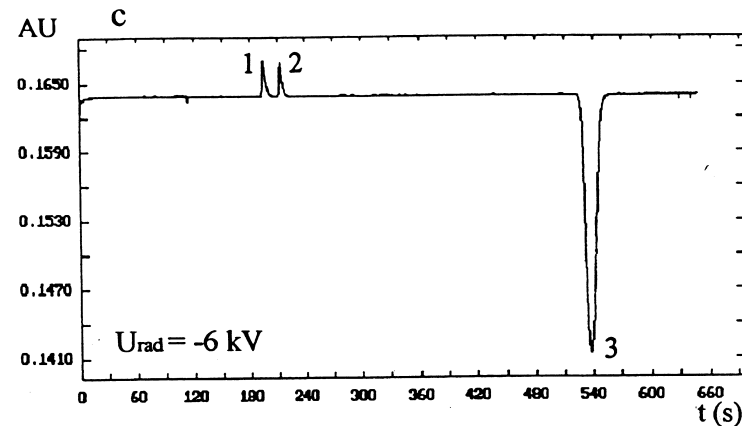V. Kašička et al. / J. Chromatogr. B 741 (2000) 43–54

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Fig. 3. CZE separation of diglycine (peak 1) and triglycine (peak 2) dissolved in water (peak 3) in poly(AEG)-coated fused-silica capillary in (a) the absence of radial electric field and the presence of zero and positive radial voltages: (b) $U_{rad} = 0$ kV, (c) $U_{rad} = +2$ kV, (d) $U_{rad} = +4$ kV. The other conditions as in Fig. 2. AU = Absorption at 206 nm, t = migration time.
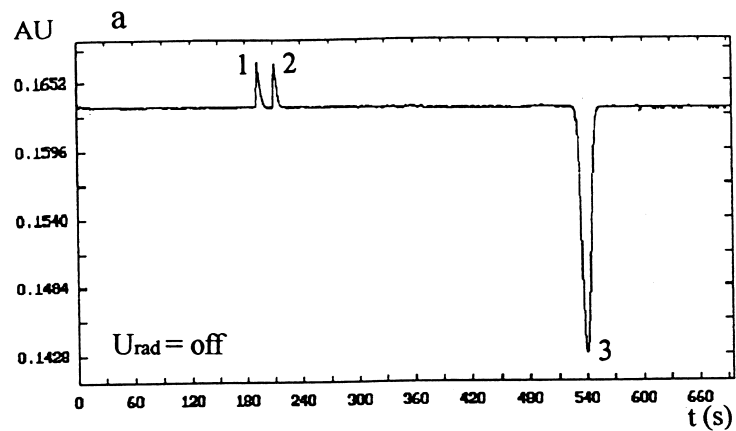
Fig. 4. CZE separation of diglycine (peak 1) and triglycine (peak 2) dissolved in water (peak 3) in poly(AEG)-coated fused-silica capillary in (a) the absence of radial electric field and the presence of negative radial voltages: (b) $U_{rad} = \equiv 4$ kV, (c) $U_{rad} = -6$ kV, (d) $U_{rad} = -8$ kV. The other conditions as in Fig. 2. AU=Absorption at 206 nm, t=migration time.
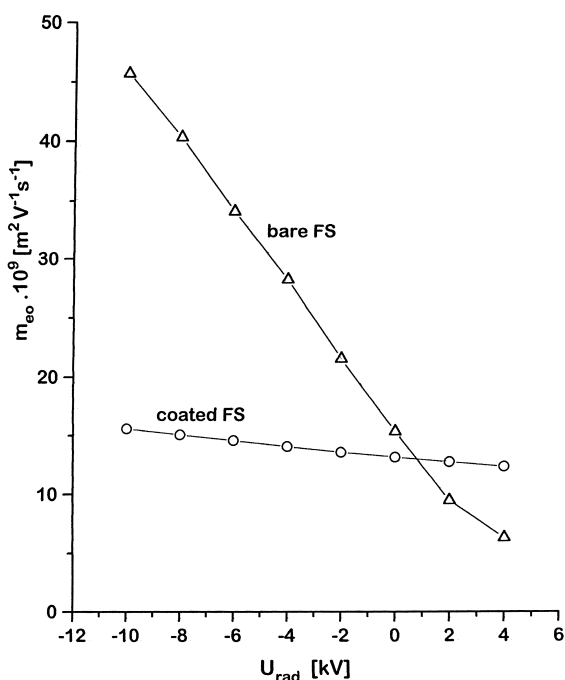
Fig. 5. Dependence of electroosmotic mobility, m_{eo}, on the radial electric field, U_{rad}, in the noncoated (bare) fused-silica capillary and in the poly(AEG)-coated capillary.

values of radial voltage, the resolution is increased but the price for that is the prolonged separation time due to lower EOF. At negative values of radial voltage, the time of analysis (represented by the migration time of the end of the second (last) analyte zone) is lower, but the resolution is also smaller.

Separation efficiency (expressed by the number of theoretical plates) does not change significantly at

Table 2
Effect of radial electric field on efficiency, resolution and analysis time of CZE separation of diglycine and triglycine in noncoated fused-silica capillary[a]

U_{rad} (kV)	Diglycine ($N/1000$)	Triglycine ($N/1000$)	R	t (s)
off	38.5	36.6	1.7	156
+4.0	35.7	38.2	2.8	257
0.0	32.7	39.3	2.4	220
−4.0	33.8	32.6	1.8	160
−8.0	39.9	33.1	1.5	130

[a] U_{rad} =Voltage of radial electric field, N=number of theoretical plates, R=resolution, t=time of analysis (rounded migration time of the end of the second (last) analyte zone).

Table 3
Effect of radial electric field on efficiency, resolution and analysis time of CZE separation of diglycine and triglycine in poly(AEG)-coated fused-silica capillary[a]

U_{rad} (kV)	Diglycine ($N/1000$)	Triglycine ($N/1000$)	R	t (s)
off	42.2	44.6	2.6	217
+4.0	44.7	74.3	3.2	233
0.0	46.1	65.1	3.0	225
−4.0	40.0	55.9	2.8	220
−8.0	41.1	64.3	2.9	216

[a] U_{rad} =Voltage of radial electric field, N=number of theoretical plates, R=resolution, t=time of analysis (rounded migration time of the end of the second (last) analyte zone).

different voltages of radial electric field in noncoated capillaries (see Table 2). This can be explained by the fact that adsorption of the analytes due to their electrostatic interactions with the inner capillary wall, which can be potentially influenced by the external electric field, is not the main source of zone broadening. Higher efficiency of CZE peptide separations in the coated capillaries is caused by the lower adsorption of peptide analytes to the hydrophilic inner coating than to the bare fused-silica. This is apparent especially in the case of triglycine which is more hydrophobic than diglycine. The changes in the number of theoretical plates are not correlated with the changes of migration times due to the application of an external electric field, which indicates that the peak width is not limited by diffusion, but the other dispersion factors, such as electromigration dispersion and/or real initial sample zone length are predominant.

The practical example of the application of poly-(AEG)-coated fused-silica capillaries and the external electric field control of the EOF to a better peptide separation is demonstrated in Fig. 6. Dipeptides, Pro–Gly and Gly–Pro as representatives of peptides with high proline and glycine amino acid residues contents, exhibit relatively strong adsorption to the bare fused-silica [28]. This was confirmed also by our experience and due to their adsorption to the capillary wall and small differences in electrophoretic mobilities in the given experimental conditions no separation of these two peptides was achieved in the noncoated fused-silica capillary (see Fig. 6a). Although tailing peaks of these peptides occurred also in the poly(AEG)-coated capillaries, their partial
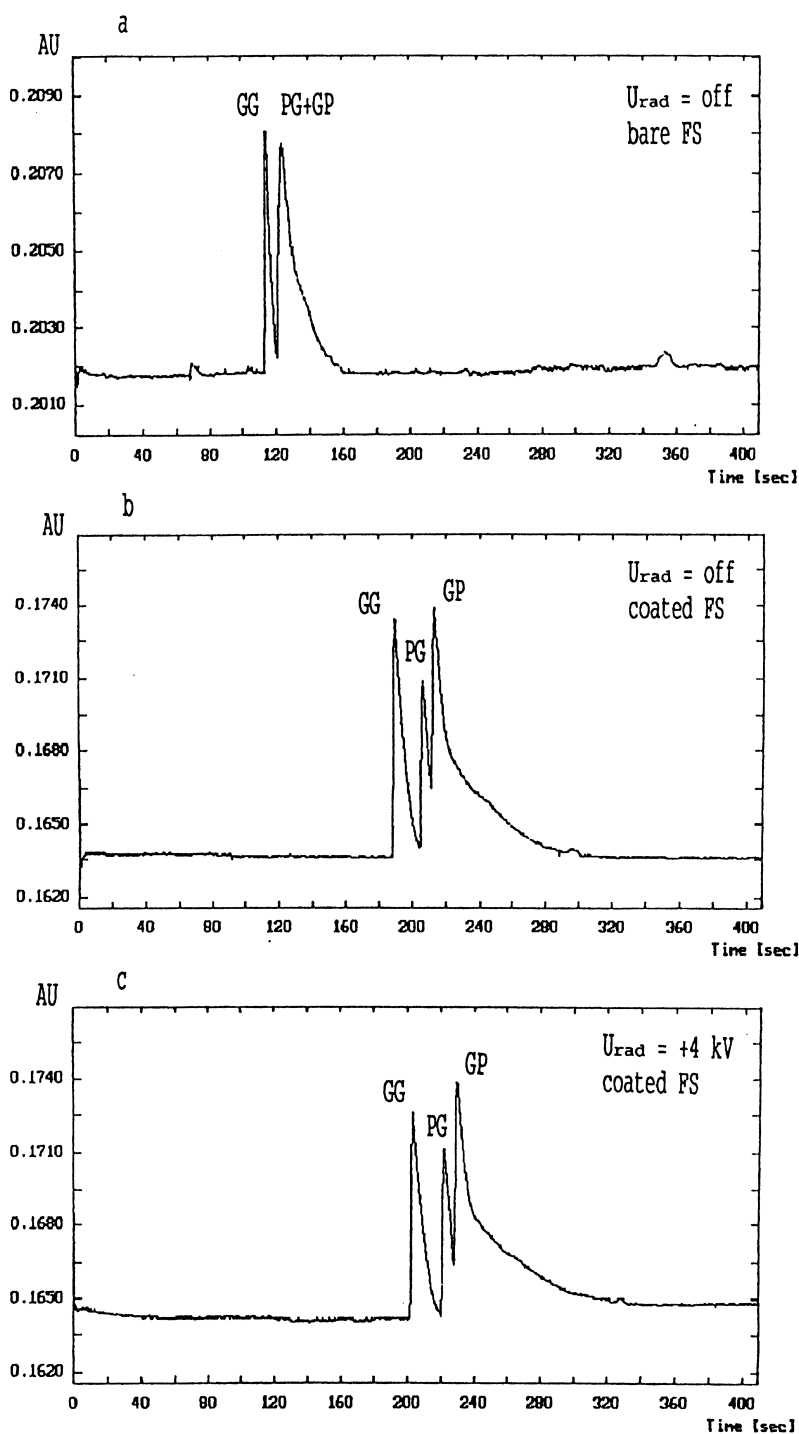
Fig. 6. CZE separations of diglycine (GG), prolyl–glycine (PG) and glycyl–proline (GP) in the absence of radial electric field in (a) the bare fused-silica capillary and (b) the poly(AEG)-coated capillary and (c) in the presence of radial voltage, $U_{rad} = +4$ kV, in the poly(AEG)-coated capillary. The other conditions as in Fig. 2. AU=Absorption at 206 nm.

separation was achieved in this capillary. Further enlargement of the resolution of these two peptides (from 0.6 to 0.8) as well as the resolution of Gly–Gly and Pro–Gly dipeptides (from 1.6 to 1.9) was achieved by the application of an external electric field with a positive value of radial voltage, $U_{rad} = +4$ kV (see Fig. 6c). However, for a complete, base line separation of all dipeptides a stronger effect of the external electric field on the EOF in the coated capillaries would be necessary. The other types of inner capillary coating will be tested for the application of the external electric field since the recent papers [29,30] indicate that the effect of an external electric field in the coated capillaries allows EOF control even at alkaline BGE, which is not possible due to the high content of dissociated silanol groups in the bare fused-silica capillaries.

4. Conclusions

External electric field has been shown as an effective tool for the regulation of EOF in CZE. By changes in polarity and magnitude of the external electric field, the EOF can be substantially reduced or increased in comparison with the state of absence of the external field, which is equivalent of having a dynamic capillary length. In this way the speed of analysis and resolution may be optimized according to the complexity of analyte mixtures and according to the differences in their effective electrophoretic mobilities.

A much stronger effect of the external field on EOF was observed in the bare fused-silica capillary than in the fused-silica capillary with an inner coating by hydrophilic derivative of polyacrylamide. A better resolution of proline-containing peptides was achieved in the coated capillary due to their lower adsorption to the inner capillary wall and also due to the lower EOF in the coated capillary.

Acknowledgements

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