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Journal of Chromatography A, 772 (1997) 221–230

JOURNAL OF CHROMATOGRAPHY A

Capillary zone electrophoresis with external radial electric field control of electroosmotic flow and its application to the separation of synthetic oligopeptides

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Abstract

A new way of applying external radial electric field for the control of electroosmotic flow in capillary zone electrophoresis (CZE) has been developed. It is based on a new type of low-conductivity coating of the outer capillary surface by a polyaniline dispersion in hydroxypropylcellulose and on the application of a set of three high-voltage power supplies to form a constant radial electric field across the capillary wall as far as possible along the capillary length. Two power supplies are connected to the ends of the outer low-conductivity coating and the third one is applied to the ends of the inner capillary compartment filled with background electrolyte. The difference of electric potentials at the inner and outer capillary surface determines the voltage of radial electric field across the capillary wall and affects the electrokinetic potential at the solid–liquid interface inside the capillary. The effect of magnitude and polarity of external radial electric field on the flow-rate of electroosmotic flow, on the migration times of charged analytes (speed of analysis) and on the separation efficiency and resolution of CZE separations of synthetic oligopeptides, diglycine, triglycine, dalargin and dalargin–ethylamide has been evaluated. Application of external radial electric field has proved to be an efficient tool for regulation of electroosmotic flow in CZE and for optimization of migration times and resolution of oligopeptides in their CZE separations and analyses.

Keywords: Electric field, radial; Electroosmotic flow; Oligopeptides; Peptides

1. Introduction

Capillary zone electrophoresis (CZE) has developed into a highly efficient and highly sensitive separation technique which is now widely used for picoanalysis of synthetic and natural peptides and other ionogenic analytes [1–6]. However, many separations of peptide mixtures (as well as of other analytes) are not optimized from the standpoint of complete resolution in minimal time. An optimal separation in CZE can be defined as a baseline separation of all sample components in the shortest possible time. Both these parameters, i.e. resolution and migration time, are influenced by electroosmotic flow (EOF), which is often superimposed on electrophoretic separations of analytes in CZE. Consequently, control of EOF can significantly improve

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PII S0021-9673(97)00061-7

separability in CZE. In addition to the 'classical' ways of regulation of EOF by changes in composition of background electrolyte (pH, ionic strength, surfactant and polymer additives) and/or by modification of the inner capillary wall (dynamic or covalent coating), a new approach to EOF regulation was introduced almost simultaneously by Ghowsi and Gale [7–9] and by Lee et al. [10–12]. Although they realized this approach in experimentally different ways, the principle was the same, i.e. they applied an additional electric field from outside of the capillary. The potential gradient between the external and internal electric fields is perpendicular to the capillary wall and affects the magnitude and polarity of the electrokinetic potential, i.e. flow-rate and direction of EOF inside the capillary. In addition, these changes of electrokinetic potential influence the electrostatic interactions of analytes with capillary walls, which can be used to decrease the adsorption of peptides and proteins during their CZE separation [13].

Application of a radial electric field in CZE was realized in several ways, using different media surrounding the outside part of the capillary: low-conductivity buffer solution [10–12] or ionized air [14] placed in the tube or box coaxially situated with the separation fused-silica capillary or by coating of the outer surface of the capillary by a low-conductivity layer of polymeric conductor [15,16] or using the metal coating of the capillary [9,17,18].

The technologically simplest way of metal capillary coating has the disadvantage of non-uniform intensity of radial electric field along the capillary which results in non-uniform electrokinetic potential along the capillary wall and consequently additional local flows in the capillary are present, decreasing the separation efficiency of CZE [19–22]. A liquid solution and radioactively ionized gaseous medium were used for demonstration of the effect of an external electric field on EOF, but apparently they are not suitable for a broad application in the laboratory practice. The best way of applying an external electric field seems to be the low-conductivity coating of the capillary. This approach was used by Hayes and Ewing [14] who covered the outer surface of the polyimide coated fused-silica capillary by a layer of low-conductivity ionic polymer Nafion (fluorinated hydrocarbon with sulphonic groups). However, our experience with this type of capillary coating was not good, since its conductivity was shown to be strongly dependent on the air humidity and after some time its conductivity was lost completely.

The aim of this work was to overcome the shortcomings and drawbacks of the present ways of applying an external electric field in CZE by developing a new low-conductivity polymeric capillary coating and to demonstrate the usefulness of applying an external electric field in CZE as a means of direct and dynamic control of EOF, which is independent of the composition of the background electrolyte at a given pH. Our goal was to show the effect of external radial electric field on the CZE separation of synthetic oligopeptides, namely on the migration time (speed of analysis), separation efficiency and resolution of closely related synthetic peptides (oligoglycines and dalargins).

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade. Diglycine and triglycine were obtained from Reanal (Budapest, Hungary), phenol and acetic acid were from Lachema (Brno, Czech Republic). Dalargin (hexapeptide with the sequence H-Tyr-D-Ala-Gly-Phe-Leu-Arg-OH) and dalargin–ethylamide (H-Tyr-D-Ala-Gly-Phe-Leu-Arg-NH-C_2H_5), enkephaline type peptides with opiate activity were synthesized in the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences (Prague, Czech Republic).

2.2. Capillary zone electrophoresis (CZE)

CZE separations were performed in a laboratory-made device which was newly adapted to the application of external radial electric field. Since the development of such instrumentation was one of the goals of this paper, it is described in more detail in Section 3.

Peptide separations were carried out using acetic acid (0.5 mol/l, pH 2.5) as a background electrolyte (BGE) in the bare fused-silica capillaries. Peptide

samples were dissolved in BGE in a concentration range 0.3–0.8 mg/ml and they were introduced into the capillary by siphoning effect (0.005 bar, 5–15 s). Separations were performed at ambient temperature 23–25°C at low input power (voltage 8–10 kV, current 9–12 μA, 0.24–0.40 W per meter of capillary length) so that increase of temperature inside the capillary due to Joule heat can be neglected [23]. EOF was determined from the migration time of phenol, which can be used as an electroneutral marker at low pH of BGE.

3. Results and discussion

3.1. CZE device with external radial electric field

The developed device for CZE with external radial electric field control of EOF is based on our previously constructed laboratory made CZE apparatus [24] which has been adapted for the application of the external electric field to the part of the outer surface of the capillary [25]. The scheme of this adaptation is shown in Fig. 1. The core of the device is the fused-silica capillary, (FS) with outer polyimide (PI) coating, (supplied by the Institute of Glass and Ceramics Materials, Czech Academy of Sciences, Prague, Czech Republic) with the following dimensions: I.D. 0.056 mm, O.D.1 (fused-silica) 0.170 mm, O.D.2 (polyimide) 0.200 mm, total length, L, 300 mm, effective length, L_{ef}, 198 mm. An additional low-conductivity polymer coating was formed on the part of the outer surface of the capillary between coordinates $x1$ and $x2$. Of course, both low-conductivity and polyimide coatings are removed in the region of the detection window of the capillary. Low-conductivity coatings at both sides of the window are bridged by copper foil in which the slit of the detection window is made. For simplicity this 3-mm long uncoated part of the capillary is omitted in Fig. 1 and is neglected in the calculations presented below. The separation voltage, U_{sep}, provided by the high-voltage power supply HV1 (laboratory-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 filled with the background electrolyte, BGE. The external electric field is formed by the application of

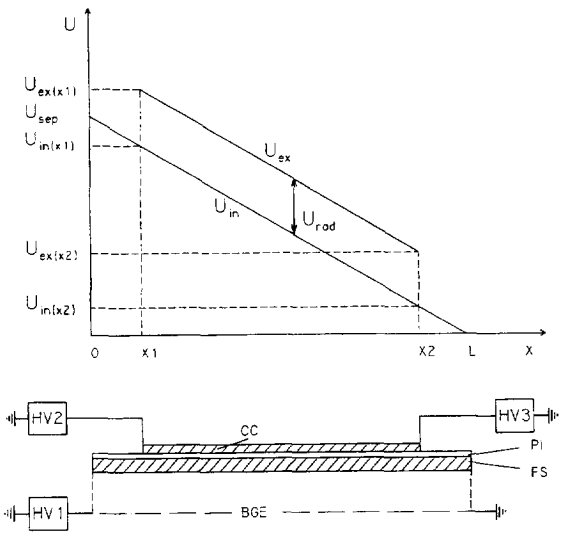

Fig. 1. Scheme of experimental setup of CZE with external electric field control of electroosmotic flow (bottom) and the course of voltage drop inside the capillary and on the external low-conductivity coating of the capillary (top). FS = fused-silica wall of the capillary, PI = outer polyimide coating of the capillary, CC = low-conductivity coating on the part of the capillary outer surface between coordinates $x1$ and $x2$, BGE = background electrolyte inside the capillary, HV1 = high-voltage power supply providing separation voltage U_{sep}, HV2, HV3 = high-voltage power supplies providing external voltages at positions $x1$ and $x2$, respectively. U = voltage, x = longitudinal coordinate, 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-conductive coating of the capillary), U_{rad} = radial voltage (across the capillary wall), U_{sep} = separation voltage.

two different voltages to the ends of the outer low-conductivity coating of the capillary from the high-voltage power supplies HV2 and HV3 (high-voltage modules CZE R1000 with reversible polarity, 30 kV, 300 μA, Spellman, Plainview, NY, USA), respectively. The difference of applied voltages at external and internal capillary surface forms an additional electric field which is perpendicular to the capillary wall and influences the electrokinetic potential at the interface between the inner capillary wall and BGE, and consequently, the flow-rate and direction of EOF inside the capillary. Common grounding of all three power supplies HV1, HV2 and HV3 (see Fig. 1) ensures that well defined voltage differences on the capillary system are obtained.

3.2. Preparation of low-conductivity capillary coating

A new low-conductivity capillary coating is formed from polyaniline (PANI) dispersion in hydroxypropylcellulose (HPC).

Basic dispersion of PANI, stabilized by a small amount of HPC, was prepared in aqueous solution and low-molecular-mass substances were removed by dialysis against water. The polymers have been then converted into alcoholic solution by dialysis against ethanol. The particle size of PANI in dispersions was close to below one micrometer.

Dispersion solutions of PANI in ethanol were mixed with ethanol solutions of HPC. PANI was changed to conductive protonized form by increasing acidity of the solution to pH 2 by the addition of hydrochloric acid. Through changes in the content of PANI in the solution (20–5%, m/m) the different conductivities were achieved in the range 10^{-2}–10^{-10} S cm^{-1}.

Capillary tubes were coated by these polymer solutions by drawing through the coating jet filled with the polymer solution. After drying, capillaries with low-conductivity coatings with resistivity 10^{7}–10^{12} Ω/cm were obtained. For CZE experiments the capillaries with the resistivity 7 GΩ/cm were used.

The coating procedure and the total instrumentation description will be given in more detail elsewhere [26].

3.3. 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 [22]. With this knowledge and from the fact that the resulting electrokinetic potential is equal to the sum of electrokinetic potential originating from the dissociation of the silanol groups of the fused-silica and of the electrokinetic potential induced by external radial electric field, it follows that the external electric field should be applied at a maximum length of the capillary and should be constant along the longitudinal axis of the capillary. The demand for constant voltage of radial electric field, U_{rad}, along the capillary, which is given as the difference of voltages on the external and internal part of the capillary wall,

$$U_{rad} = U_{ex} - U_{in} \quad (1)$$

means that the voltage drop on the external and internal part of the capillary should be the same, i.e. the lines of voltage drops along the capillary length inside and outside of the capillary should be parallel. However, because of practical reasons—the impossibility to apply the external voltage to the part of the capillary which is dipped into the solution of BGE in the electrode vessel—it is not possible to apply the external voltage along the whole length of the capillary, but only to that part where the conductive coating can be formed, i.e. between the coordinates $x1$ and $x2$ in Fig. 1. For this reason it is convenient to use two power supplies to set the voltages at these positions with constant shift with respect to the internal voltages in the same longitudinal positions. Furthermore, the procedure for deriving the voltages at the ends of the outer low-conductive capillary coating is described.

Let us consider the capillary of the length L. Separation voltage U_{sep} is applied to the inner capillary compartment. Specific resistance of BGE inside the capillary is constant, i.e. internal voltage, U_{in}, is linearly increased from zero at the grounded end of the capillary (coordinate L) to the value U_{sep} at the high potential end of the capillary (see Fig. 1). Internal voltage at position x between the coordinates 0 and L is given by the equation:

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

From this equation and from the demand for a constant difference between U_{in} and U_{ex} along the capillary (see eq. 1), we obtain the equations for external voltages at positions $x1$ and $x2$:

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

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

The examples of values U_{sep}, $U_{in(x1)}$, $U_{in(x2)}$, $U_{ex(x1)}$, $U_{ex(x2)}$ and U_{rad} (see Fig. 1) in practical applications of external electric field in CZE sepa-

Table 1
Effect of external radial electric field on EOF in CZE

$U_{in(x1)}$ (kV)	$U_{in(x2)}$ (kV)	$U_{ex(x1)}$ (kV)	$U_{ex(x2)}$ (kV)	U_{rad} (kV)	t_{eo} (s)
+7	+1	off	off	off	392
+7	+1	+10	+4	+3	1400
+7	+1	+9	+3	+2	1100
+7	+1	+8	+2	+1	923
+7	+1	+7	+1	0	785
+7	+1	+5	−1	−2	590
+7	+1	+3	−3	−4	399
+7	+1	+1	−5	−6	318
+7	+1	−1	−7	−8	292

$U_{in(x1)}$, $U_{in(x2)}$ = internal voltages at positions $x1$, $x2$, respectively; $U_{ex(x1)}$, $U_{ex(x2)}$ = external voltages at positions $x1$, $x2$, respectively; $x1$, $x2$ = positions to which external voltages are connected; U_{rad} = voltage of radial electric field (see Fig. 1), t_{eo} = migration time of EOF marker (phenol). Average values of t_{eo} from two experiments with stabilized EOF flow-rate are presented. Data obtained from CZE separations of diglycine, triglycine and phenol (see Figs. 2 and 3). Separation voltage U_{sep} = 8.0 kV, total capillary length L = 300 mm, coordinates of the positions to which external voltages are connected: $x1$ = 38 mm, $x2$ = 262 mm. For other experimental data see Section 2.

rations of a test mixture of diglycine, triglycine and phenol are given in Table 1.

3.4. Influence of external electric field on CZE separation

Influence of external radial electric field on the flow-rate of EOF, migration time, resolution and efficiency of CZE was tested by separation of a model mixture of oligopeptides diglycine and triglycine and using phenol as an EOF marker. The examples of separation of this mixture in the absence of external radial electric field and at different voltages of external radial electric field are shown in Figs. 2 and 3. Significant influence of external electric field on the migration time of phenol (i.e. on EOF velocity) and on the migration times of charged peptide analytes is apparent. Depending on the magnitude and polarity of the external radial electric field, the EOF velocity can be decreased (see Fig. 2b,c,d), increased (see Fig. 3c,d) or it can remain more or less the same (see Fig. 3b) in comparison with the state when the external field is not applied (U_{rad} = off, see Fig. 2a Fig. 3a, respectively).

The results of these measurements are summarized in Table 1, where the appropriate values of internal and external voltages, their differences (radial voltages, U_{rad}) and the measured migration times of EOF marker (phenol) are given. Comparison of these migration times in the absence and in the presence of an external electric field shows, that even in the absence of an 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 field with voltage $U_{rad} = -4$ kV (see line 1 and line 7 of Table 1).

From the migration times of EOF marker and from the other experimental data (separation voltage, U_{sep}, capillary effective length, L_{ef}, and total length, L) the more illustrative and comparable data, electroosmotic mobility (velocity of EOF related to unit intensity of internal electric field), m_{eo}, were calculated according to the following equation:

$$m_{eo} = \frac{L_{ef} L}{t_{eo} U_{sep}} \quad (5)$$

The dependence of EOF mobility, m_{eo}, on the applied radial electric field, U_{rad}, is graphically presented in Fig. 4. The course of the measured dependence is in good qualitative agreement with the previously published data and with the theoretical prediction of the course of this dependence [15,16,27].

The influence of external radial electric field on the separation efficiency and resolution in CZE is demonstrated by the separation of closely related

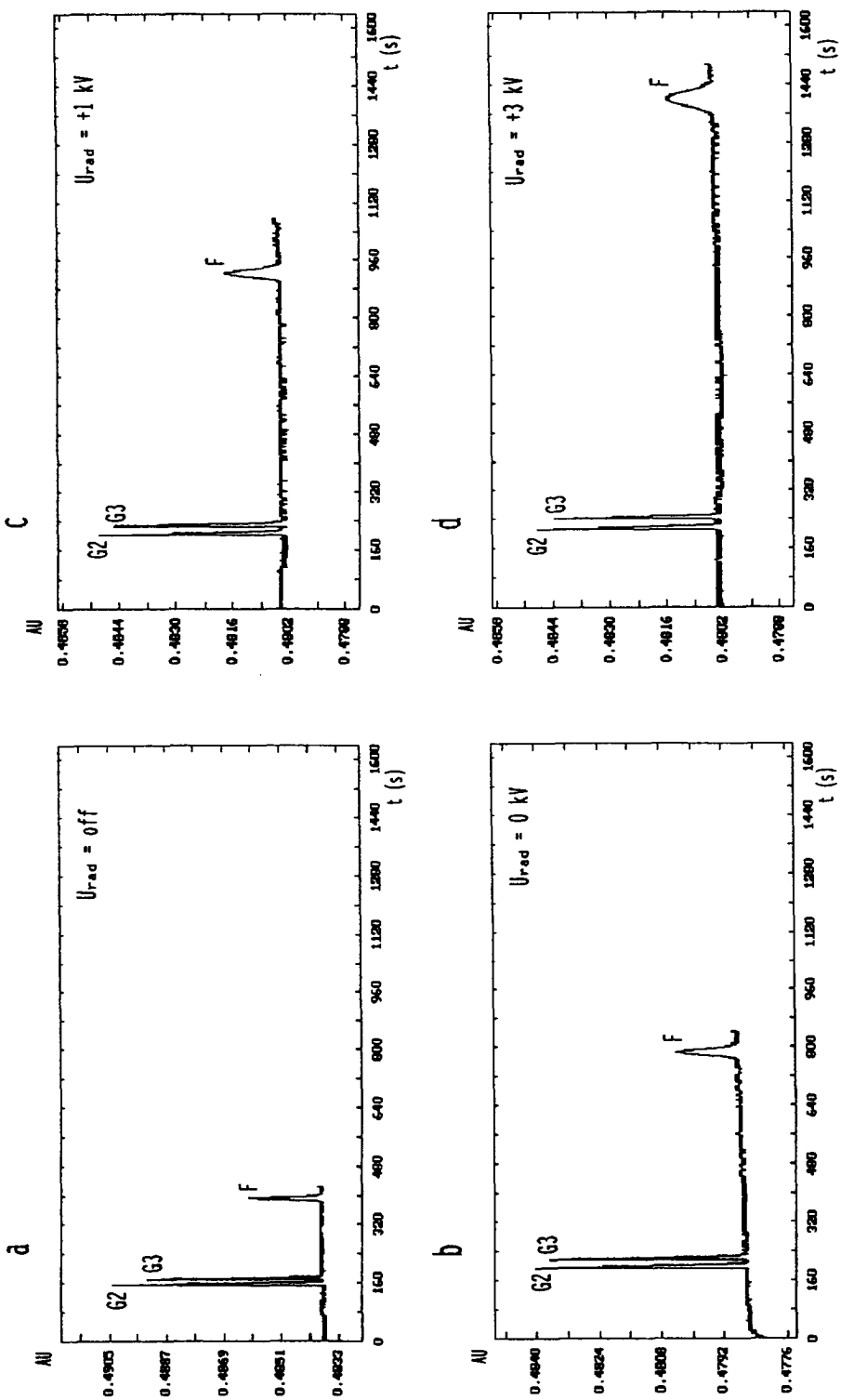

Fig. 2. CZE separation of diglycine (G2), triglycine (G3) and phenol (F) in the absence of external radial electric field (a) and in the presence of zero and positive external electric fields of different voltages: $U_{rad}=0$ (b), $U_{rad}=+1$ kV (c), $U_{rad}=+3$ kV (d). For other experimental conditions see Section 2. AU-absorption at 206 nm, t = time.

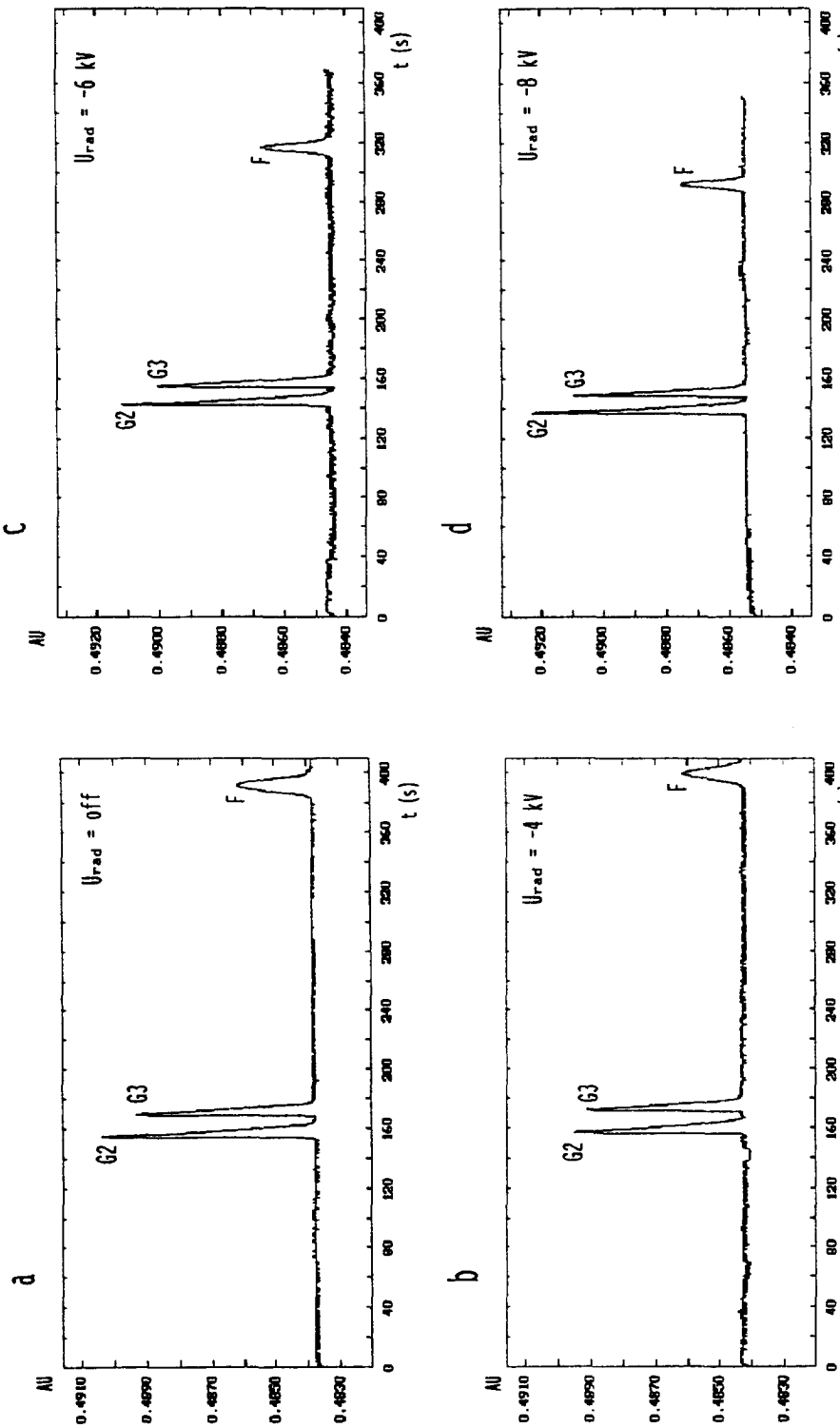

Fig. 3. CZE separation of diglycine (G2), triglycine (G3) and phenol (F) in the absence of external radial electric field (a) and in the presence of negative external radial electric fields of different voltages: $U_{rad} = -4$ kV (b), $U_{rad} = -6$ kV (c), $U_{rad} = -8$ kV (d). For other experimental conditions see Section 2. AU = absorption at 206 nm, t = time.

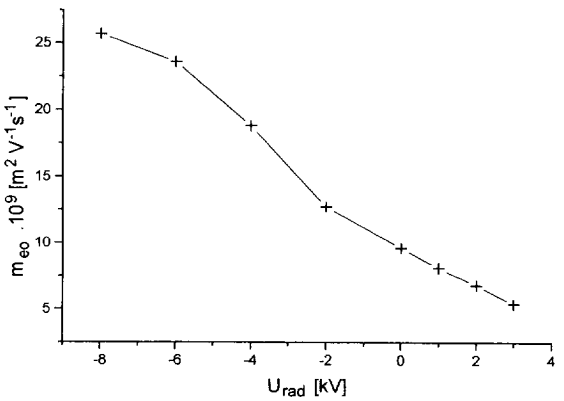

Fig. 4. Experimentally determined dependence of electroosmotic mobility, m_{eo}, on the external radial electric field, U_{rad}. For experimental details see the Section 2 and Table 1.

Table 2
Effect of external radial electric field on efficiency, resolution and analysis time of CZE separation of dalargins

U_{rad} (kV)	Dalargin–NH–Et $N/1000$	Dalargin $N/1000$	R	t (s)
off	49.8	34.8	1.2	154
0	66.5	55.6	1.9	207
0	74.4	71.4	2.0	214
−3	72.0	57.9	1.7	173
−6	64.4	34.4	1.2	130
−9	70.1	37.5	1.0	125

U_{rad} = voltage of radial electric field, Dalargin–NH–Et–dalargin–ethylamide, R = resolution, N = number of theoretical plates, t = minimal time of analysis [migration time of the end of the second (last) analyte zone].

synthetic peptides, dalargin and dalargin–ethylamide. The incomplete separation of these two peptides in the absence of external electric field is shown in Fig. 5a. If the external voltages on the ends of the outer low-conductivity coating were set to be equal to the inner voltages at the corresponding positions $x1$ and $x2$, respectively, i.e. the radial voltage across the capillary wall was zero, the EOF was reduced substantially, the migration times of analytes were increased and complete separation of dalargins was achieved (see Fig. 5b). On the other side at negative values of external radial field, the EOF was increased, i.e. migration times reduced, and the separation was even more incomplete than in the case of absence of external field (see Fig. 5c,d).

The influence of external radial electric field on the separation efficiency and resolution of CZE separations of dalargins is quantitatively demonstrated in Table 2, where the numbers of theoretical plates and resolution are given.

The increased efficiency of CZE separation, i.e. the higher number of theoretical plates in the presence of an external electric field can be assigned to the changes of electrokinetic potential induced by the external electric field which reduce the electrostatic interactions of positively charged peptides, at acid pH 2.5, of BGE with the negatively charged inner surface of the bare fused-silica capillary wall.

The migration times of the ends of peaks of the second (last) analyte (see the last column of Table 2) which represent the minimum necessary time of separation and detection of a given analyte mixture, show that the time of CZE analysis can also be substantially influenced by the external radial electric field. An optimal CZE separation can then be chosen as one which provides complete resolution in minimum time.

4. Conclusions

Our experiments confirmed that the application of external radial electric field represents an efficient tool for the control of electroosmotic flow and migration time in CZE. By changes in polarity and in magnitude of the external radial electric field, the electroosmotic flow can be either reduced or increased in comparison with the state when an external field is not applied. Through the external EOF control, a dynamic effective length of the capillary is obtained and the separation time can be optimized according to the complexity of peptide mixtures and according to the differences in their effective electrophoretic mobilities. In addition to EOF control, the induced changes of electrokinetic potential influence the electrostatic interactions (reduce adsorption) of separated peptides with the capillary wall and in this way increase the separation power of CZE.

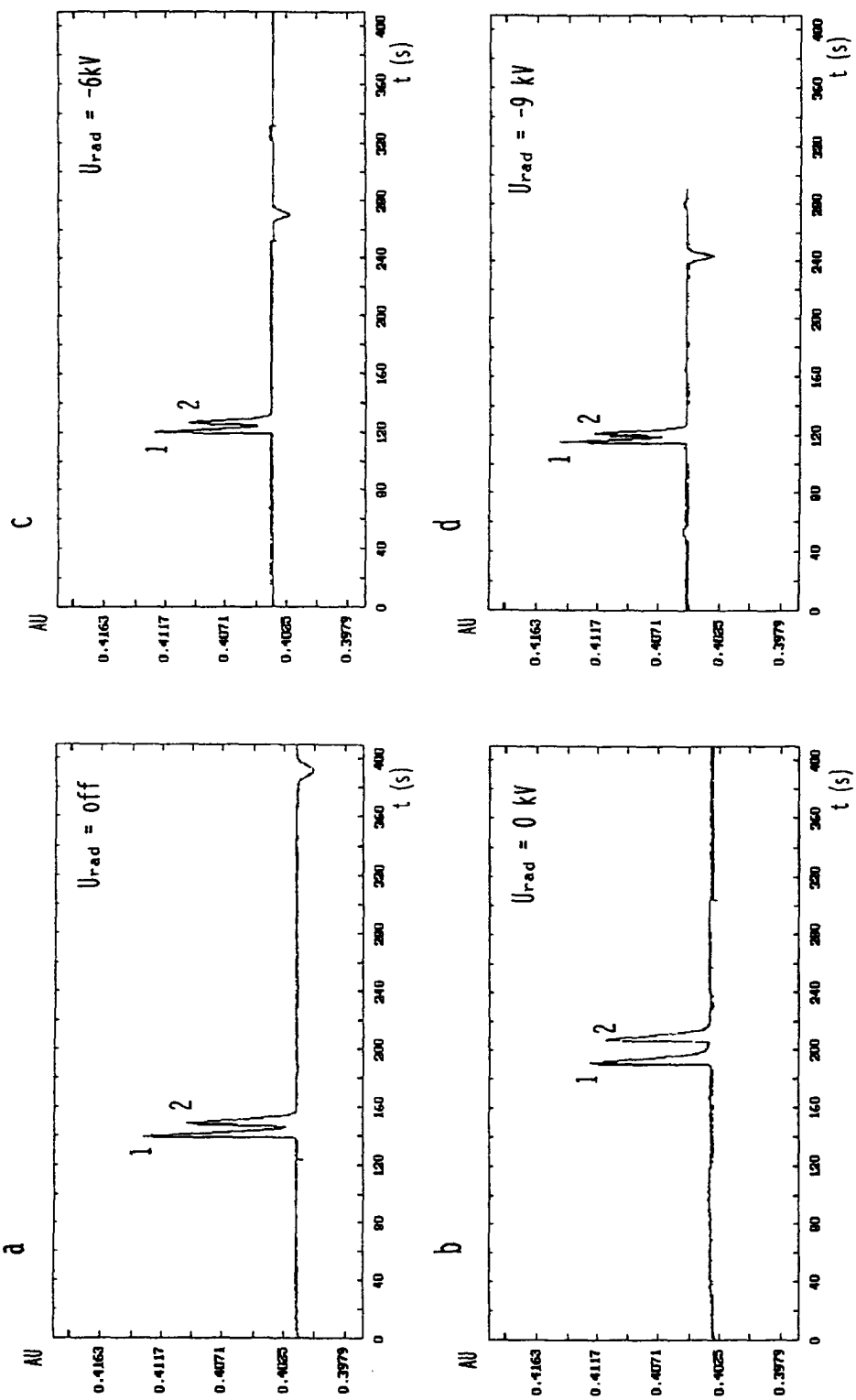

Fig. 5. CZE separation of dalargin and dalargin–ethylamide in the absence of external radial electric field (a) and in the presence of external electric fields of different voltages: $U_{rad} = 0$ kV (b), $U_{rad} = -6$ kV (c), $U_{rad} = -9$ kV (d). For other experimental conditions see the Section 2. AU-absorption at 206 nm. t = time.

Acknowledgments

The financial support of this work by the Grant Agency of the Czech Republic, grants no. 203/93/0718, 505/94//0940 and 203/96/K128 is acknowledged. Mr. J. Štěpánek and Mrs. V. Lišková are thanked for technical assistance and for the help with preparation of the manuscript. Ing. V. Pokorný is thanked for help in figure drafting.

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