Journal of Chromatography, 608 (1992) 13–22
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 399

Conversion of capillary zone electrophoresis to free-flow zone electrophoresis using a simple model of their correlation

Application to synthetic enkephalin-type peptide analysis and preparation

Václav Kašička, Zdeněk Prusík and Jan Pospíšek

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingovo 2, 166 10 Prague 6 (Czechoslovakia)

ABSTRACT

A simple mathematical model of the correlation between capillary zone electrophoresis (CZE) and continuous free-flow zone electrophoresis (FFZE) was developed. The model results from the fact that both methods are based on the same separation principle (zone electrophoresis) and both are performed in a free solution of the same composition. Based on this model, a procedure for the conversion of an analytical microscale CZE separation into a preparative FFZE separation was developed. The applicability of this procedure is demonstrated by CZE analysis at the picomole level and FFZE preparative fractionation (60 mg/h throughput) of crude synthetic biologically active peptide [D-Tle^{2,5}]-dalargin. The combination of CZE and FFZE provides an efficient and economical tool for synthetic peptide analysis and preparation.

INTRODUCTION

Capillary electromigration methods, generally called high-performance capillary electrophoresis (HPCE), have undergone a period of rapid development [1–5] and they are becoming a powerful tool for the separation of both low- and high-molecular-mass charged and, in the case of electrokinetic chromatography, also uncharged substances and particles. Nowadays HPCE is mostly used for analytical purposes. However, attempts to use HPCE in a micropreparative mode have been reported and this trend will probably become more pronounced in the future especially in cases where separation problems are better solved by HPCE than by other, *e.g.*, chromatographic, methods.

The reason for the less frequent application of the micropreparative mode of HPCE is not only an inevitable compromise between capacity and separability but also the more complicated conversion from an analytical HPCE mode to a micropreparative mode than in chromatography. This is due to the fact that in HPCE both ends of the capillary are dipped into the electrode vessels and an electric field is applied for the whole duration of the experiment. Although several devices have been developed [6–9] and some micropreparations were succesfully achieved, one substantial disadvantage remains: because of the miniature dimensions of the capillary separation compartment, the capacity of the preparation is very low, in the range of nanograms to micrograms depending on the capillary dimensions

Correspondence to: Dr. Z. Prusík, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingovo 2, 166 10 Prague 6, Czechoslovakia.

0021-9673/92/$05.00

and initial sample concentration. The possibilities of increasing the capacity by increasing the capillary diameter are limited owing to Joule heat problems in capillaries of larger diameter, especially under non-steady-state conditions as in capillary zone electrophoresis (CZE).

A different way to increase the capacity of a preparation is to convert the capillary separation into a continuous thin-layer free-flow system in a flow-through electrophoretic chamber [10,11]. In such a type of separation the capacity can be increased by several orders of magnitude up to several hundred milligrams per hour.

The principle of free-flow zone electrophoresis (FFZE) [10–13] is that an electric field is oriented perpendicularly to the laminar flow of the carrier electrolyte and of the sample solution. Different sample components are deflected from the direction of the laminar flow depending on their electrophoretic mobilities at the different angles and at the outlet side of the chamber they are collected in the fraction collector. Provided that both types of separation, *i.e.*, capillary and free-flow, are performed in the same separation mode (zone electrophoresis in our case), a direct correlation can be found between these two methods [14,15]. Based on this correlation, CZE can be used for the development of suitable separation conditions, especially with regard to pH and carrier electrolyte composition, which can then be used directly in preparative FFZE. However, the different migration times of various components in CZE must then be converted into optimized deflection angles in FFZE.

The aim of this work was to develop a simple mathematical model of the correlation between CZE and FFZE, to develop a procedure for the conversion of analytical CZE separation into the preparative FFZE process and to demonstrate the application of the procedure to the analysis and preparation of the synthetic peptide [D-Tle^{2,5}]-dalargin.

THEORY

In this section a simple mathematical model of the electrophoretic and electroosmotic movements in the capillary and in the flow-through chamber is presented and their correlation is shown.

The resulting velocity, v_r, of the movement of a charged substance in a d.c. electric field in the capillary (see Fig. 1a) is given by the sum of the electrophoretic velocity, v_{ep}, and the electroosmotic flow velocity, v_{eo}:

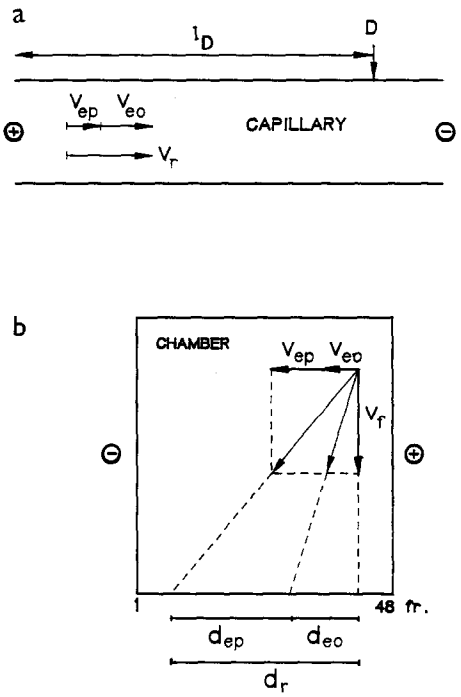

Fig. 1. Vector sum of velocities in (a) CZE and (b) FFZE. v_{ep} = Electrophoretic velocity; v_{eo} = electroosmotic velocity; v_r = resulting migration velocity; l_D = effective length of the capillary; D = detector position on the capillary; d_{ep} = electrophoretically migrated distance; d_{eo} = electroosmotically migrated distance; d_r = resulting migrated distance.

$$v_r = v_{ep} + v_{eo} \quad (1)$$

In CZE performed in a capillary with an effective length l_D (l_D is a capillary length from the sample injection end to the detector), from eqn. 1 it follows that

$$\frac{l_D}{t_r} = \frac{l_D}{t_{ep}} + \frac{l_D}{t_{eo}} \quad (2)$$

where t_r, t_{ep} and t_{eo} are the resulting, electrophoretic and electroosmotic migration times, respectively.

By combination of eqns. 1 and 2, the following relationship is obtained for the electrophoretic velocity, $v_{ep,c}$, in the capillary:

$$v_{ep,c} = \frac{l_D}{t_{ep}} = \frac{l_D(t_{eo} - t_r)}{t_{eo}t_r} \quad (3)$$

From this relationship, $v_{ep,c}$ can be calculated from the experimentally available data: t_r is the resulting measurable migration time of a charged substance which is moved in the capillary by both electrophoretic movement and electroosmotic flow and t_{eo} is measured as the migration time of an uncharged substance which is moved by the electroosmotic flow only.

The electroosmotic velocity in the capillary, $v_{eo,c}$, is given as the ratio of l_D and t_{eo}:

$$v_{eo,c} = l_D/t_{eo} \quad (4)$$

The superposition of velocities in the flow-through electrophoretic chamber and the distances migrated by both charged and uncharged substances are shown in Fig. 1b.

In FFZE the resulting migrated distance, d_r, is given as the sum of the electrophoretically migrated distance, d_{ep}, and the electrosmotically moved distance, d_{eo}:

$$d_r = d_{ep} + d_{eo} \quad (5)$$

The distance d_{ep} is given as the product of electroosmotic velocity in the flow-through chamber, $v_{ep,f}$, and the mean flow-through time, t_f:

$$d_{ep} = v_{ep,f}t_f \quad (6)$$

Substituting eqn. 6 into eqn. 5, the following relationship can be derived:

$$v_{ep,f} = (d_r - d_{eo})/t_f \quad (7)$$

Eqn. 7 makes it possible to calculate the electrophoretic velocity from the experimentally available data, *i.e.*, from the resulting deviation distance, d_r, of a charged substance, from the deviation distance, d_{eo}, of an uncharged substance and from the known mean flow-through time, t_f. The electroosmotic velocity in the flow-through chamber, $v_{eo,f}$, is obtained as the ratio of the electroosmotically moved distance, d_{eo}, and the flow-through time, t_{eo}:

$$v_{eo,f} = d_{eo}/t_f \quad (8)$$

From eqns. 3 and 7, the ratio, p, of electrophoretic velocities in the flow-through chamber and in the capillary can be expressed as

$$p = \frac{v_{ep,f}}{v_{ep,c}} = \frac{(d_r - d_{eo})t_r t_{eo}}{t_f l_D (t_{eo} - t_r)} \quad (9)$$

In most instances the ratio p will be < 1 because the electrophoretic velocity in the flow-through chamber is usually lower than that in the capillary because of the lower electric field intensity and lower temperature in the efficiently cooled flow-through chamber, with a usual gap width of 0.5–0.3 mm, than in the capillary with a typical I.D. one order of magnitude lower (0.025–0.075 mm). It is reasonable to assume that p is approximately constant for different charged components separated by CZE and FFZE under the same separation conditions. In other words, this assumption means that if the electrophoretic velocity of the substance 1 is p times lower in FFZE than in CZE, then the electrophoretic velocity of substance 2 will also be p times lower in FFZE than in CZE. This is a realistic assumption if we realize that the deviations of p may be caused by (a) the differences in the temperature dependences of the electrophoretic mobilities of different substances and (b) adsorption of the sample components on the inner surface of the separation compartments.

As the coefficients of the temperature dependence of electrophoretic mobilities for most substances are approximately the same (2.5–2.8%/°C), the deviations caused by (a) will be negligible in most instances. More critical may be case (b), because adsorption of some sample components on the different wall materials of the separation compartments (fused-silica capillary and glass–glass or glass–plastic flow-through chamber) with different volume/surface ratios sometimes occurs. This phenomenon may be more pronounced for very small I.D. fused-silica capillaries without an inner coating. However, the symptoms of adsorption (*e.g.*, peak tailing) can be recognized and the separation conditions can be changed in order to suppress the adsorption. Henceforth in this paper the adsorption of the sample components on the inner surface of the separation compartments is neglected. Provided that p can be assumed to be constant for different sample components, p determined for standard component 1 can be used also for other substances (*e.g.*, components 2,3,...) that are separated under the same conditions as component 1 in CZE and FFZE. Based on the above-mentioned relationships and assumptions, the procedure for the conversion of an analytical CZE separation into a preparative FFZE separation can be formulated.

Let us consider a peptide sample for which suitable conditions for CZE separation have been developed. From the analysis of this sample, which we may call CZE_2, the resulting migration times, t_{r2}, of a charged sample component and the electroosmotic time, t_{eo2}, of an uncharged sample component can be obtained. Substituting these values into eqn. 3, the electrophoretic velocity of the components in the capillary, $v_{ep,c2}$, can be calculated.

Then the CZE analysis of a mixture of standard charged components and of an uncharged electroosmotic flow marker is performed under the same separation conditions as in CZE_2. From this analysis, which we may call CZE_1, the resulting migration time of a charged standard component, t_{r1}, and the electroosmotic time of a zero-charged component, t_{eo1}, are obtained. Substituting them into eqn. 3, the electrophoretic velocity of the standard components in the capillary, $v_{ep,c1}$, can be calculated.

The standard mixture is then separated in the free-flow zone electrophoresis regimen ($FFZE_1$) under estimated non-optimized conditions, *i.e.*, usually at a lower than optimum deflection angle. From this experiment the magnitudes of the resulting migrated distance of charged components, d_{r1}, and the migrated distance of an uncharged component, d_{eo1}, are obtained. The electrophoretic velocity of the charged standard components in the flow-through chamber, $v_{ep,f1}$, is then calculated according to eqn. 7.

From these experiments, the ratio p of electrophoretic velocities of the standard components in the free-flow chamber and in the capillary can be determined:

$$p = v_{ep,f1}/v_{ep,c1} \tag{10}$$

and the electrophoretic velocity of the sample components in FFZE, $v_{ep,f2}$, can be calculated:

$$v_{ep,f2} = v_{ep,c2}p \tag{11}$$

The resulting distance, d_{r2}, of the sample components in the chamber can be predicted:

$$d_{r2} = d_{eo1} + v_{ep,f2}t_f \tag{13}$$

Based on these predicted resulting migration distances of different sample components, their separability by FFZE can be estimated.

In addition to the prediction of separability, the prediction of the resulting migrating distance of the fastest sample is important for practical conversion of CZE into FFZE, as this distance should be shorter than the maximum safety distance, d_{max}. Such a prediction allows the prevention of the migration of the fastest sample component in the close vicinity of ion-exchange or other membranes separating electrode vessels from the separation chamber. At the lateral sides of the FFZE chamber in homogeneities of electric field and carrier electrolyte composition, pH and conductivity changes often occur and fast sample components can be damaged or even lost on reaching this region. Also, electric breakthrough of membranes in contact with the precipitating protein zone may thus be avoided.

Based on the predicted values of the migration distances of the sample components, the separation conditions of FFZE can be optimized, *i.e.*, the clamp voltage and/or flow-through time can be either increased if the migrated distances are too small and the separation capability of the FFZE chamber is not exploited effectively, or these parameters have to be decreased if the predicted distances indicate that sample components might migrate outside the collector region of the FFZE chamber.

EXPERIMENTAL

Instrumentation and methods

CZE was carried out in an apparatus developed in our laboratory [14]. It consists of a fused-silica capillary (I.D. 0.050 mm, O.D. 0.150 mm, with an outer polyimide coating, total length 310 mm, effective length to the detector 200 mm), supplied by the Institute of Chemistry of Glass and Ceramic Materials, Czechoslovak Academy of Sciences (Prague, Czechoslovakia), a single-wavelength on-column UV detector set at 206 nm and a 20-kV high-voltage power supply, with a current or voltage stabilization regimen. The sample was introduced manually by hydrostatic pressure formed by a height difference of 50 mm between the sample solution and electrode vessel solution level for 5–15 s. All experiments were performed under the following conditions: carrier electrolyte, 0.5 mol/l acetic acid; constant current, 10.0 μA; voltage, 9.2–9.5 kV; and temperature, ambient (22–23°C).

FFZE experiments were carried out in an apparatus developed in our Institute [12,16]. Separation was realized in a glass flow-through chamber

(500 × 500 × 0.5 mm, with an effective length from sample inlet to fraction collector outlets, *i.e.*, sample hydrodynamic flow trajectory, of 440 mm), cooled on both sides, under the following conditions: carrier electrolyte, 0.5 mol/l acetic acid (pH 2.6); electrode electrolyte, 1.0 mol/l acetic acid; mean flow-through time, 31 min; number of collected fractions, 48; temperature of outer cooling media at the chamber outlet, −3°C; constant voltage, 3000 V; current, 118–121 mA; and off-line fraction evaluation (absorbance at 280 nm). Sample solution was introduced continuously at a flow-rate of 1.5 ml/h.

Chemicals

All chemicals were of analytical-reagent grade. Diglycine and triglycine were purchased by Reanal (Budapest, Hungary) and phenol and acetic acid from Lachema (Brno, Czechoslovakia); the acetic acid was distilled before use. [D-Tle^{2,5}]-Dalargin was synthesized in our Institute (for more details, see Results and Discussion).

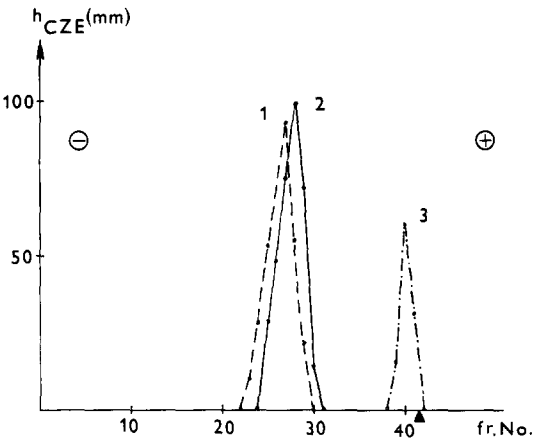

Fig. 3. FFZE separation of standard mixture evaluated by CZE analysis of individual FFZE fractions. 1 = Diglycine (15 mg/ml); 2 = triglycine (15 mg/ml); 3 = phenol (5 mg/ml). h_{CZE} = Peak height of the sample component present in the FFZE fractions; ▲ = coordinate of the sample inlet in the chamber; fr. No. = fraction number.

RESULTS AND DISCUSSION

From the above theory, it follows that for practical utilization of the correlation between CZE and FFZE, *i.e.*, for conversion of analytical CZE into preparative FFZE, the ratio p of the electrophoretic velocities of standard components in the flow-through chamber and in the capillary must be known. Its value was determined from the CZE and FFZE separations of a standard mixture containing diglycine, triglycine and phenol (see Figs. 2 and 3). Both separations were performed in the same medium (0.5 mol/l acetic acid, pH 2.6) which was earlier found suitable for the CZE analysis of the crude synthetic peptide [D-Tle^{2,5}]-dalargin. The peptides diglycine and triglycine were used as standards of

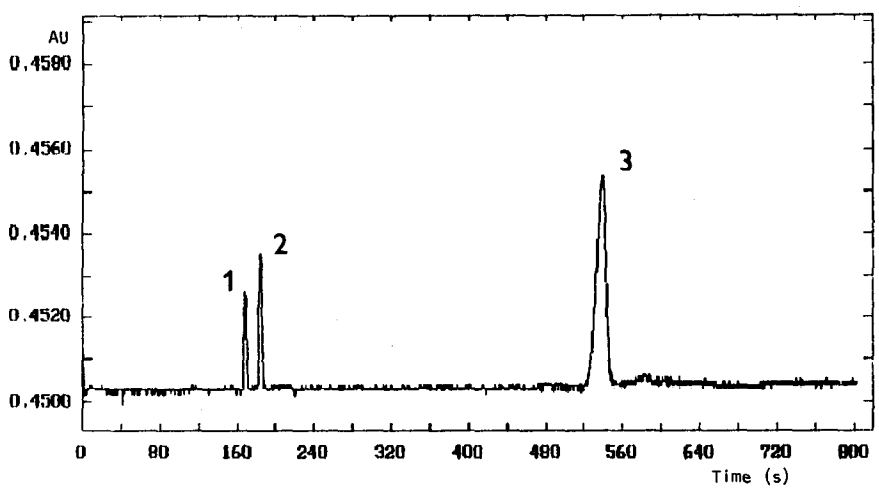

Fig. 2. CZE analysis of standard mixture. 1 = Diglycine (0.3 mg/ml); 2 = triglycine (0.3 mg/ml); 3 = phenol (0.1 mg/ml). Absorbance at 206 nm; AU = absorbance.

TABLE I

EXPERIMENTAL DATA FOR CZE AND FFZE SEPARATIONS OF STANDARD COMPONENT MIXTURE

t_r = Resulting migration time; t_{eo} = migration time of electroosmotic marker; d_r = resulting migration distance; d_{eo} = distance migrated by electroosmotic flow. Details of experimental conditions are given in the text.

Standard component	CZE		FFZE	
	t_r (s)	t_{eo} (s)	d_r (mm)	d_{eo} (mm)
Diglycine	168	–	145	–
Triglycine	184	–	135	–
Phenol	515	510	15	15

charged substances and phenol, non-dissociated in the low-pH medium, served as an uncharged electroosmotic flow marker.

The experimental data, namely migration times and the migration distances of standard mixture components obtained from their CZE and FFZE separations, are given in Table I.

From these data, the electrophoretic and electroosmotic velocities, v_{ep} and v_{eo}, both in the capillary and in the flow-through chamber were calculated using eqns. 3 and 4 for CZE data and eqns. 7 and 8 for the FFZE data. The ratio p was determined from the calculated velocities using eqn. 10. The results are summarized in Table II.

The value of p, determined for standard components, can be used for the prediction of the migration distances of other sample components. This fact was utilized in the analysis and preparation of [D-Tle^{2,5}]-dalargin. First CZE analysis of the crude synthetic peptide was performed (see Fig. 4) and the migration times of the fastest component A, of the main synthetic product M, of the slow component S and of the uncharged component N were determined from this analysis (see t_r in Table III).

Substituting these data and the average value of p from Table II into eqns. 3 and 11 sequentially, the electrophoretic velocities of these components, $v_{ep,c}$ in the capillary and $v_{ep,f}$ in the flow-through chamber, were calculated (see Table III). Using these calculated values and eqn. 12, the predicted migration distances of the selected sample components were obtained (see Table III). These predicted values of migration distances (15–221 mm) indicated a relatively suitable distribution of the sample components at the fraction outlet side of the FFZE chamber. For this reason, the same separation conditions of FFZE that were used for the standard mixture (clamp voltage 3000 V, flow-through time 31 min) were applied also for the separation of [D-Tle^{2,5}]-dalargin.

[D-Tle^{2,5}]-Dalargin, a synthetic hexapeptide with the sequence H–Tyr–D-Tle–Gly–Phe–D-Tle–Arg–OH, represents an analogue of dalargin (H–Tyr–D-Ala–Gly–Phe–Leu–Arg–OH), an enkephalin-type peptide with opiate activity. The abbreviation D-Tle indicates an amino acid residue of tertiary leucine D-configuration; the other amino acid residues are present in the L-configuration.

The crude product of this peptide was synthetized by the usual Merrifield solid-phase method. With the exception of D-Tle, all amino acids were added to

TABLE II

CALCULATED ELECTROPHORETIC AND ELECTROOSMOTIC VELOCITIES OF STANDARD COMPONENTS IN CZE AND FFZE

v_{ep} = Electrophoretic velocity; v_{eo} = electroosmotic velocity; p = ratio of electrophoretic velocities in FFZE and CZE. For further details, see text.

Standard component	CZE		FFZE		p(FFZE/CZE)
	v_{ep} (mm/s)	v_{eo} (mm/s)	v_{ep} (mm/s)	v_{eo} (mm/s)	
Diglycine	0.805	–	0.0699	–	0.087
Triglycine	0.707	–	0.0645	–	0.091
Phenol	0	0.396	0	0.0081	–

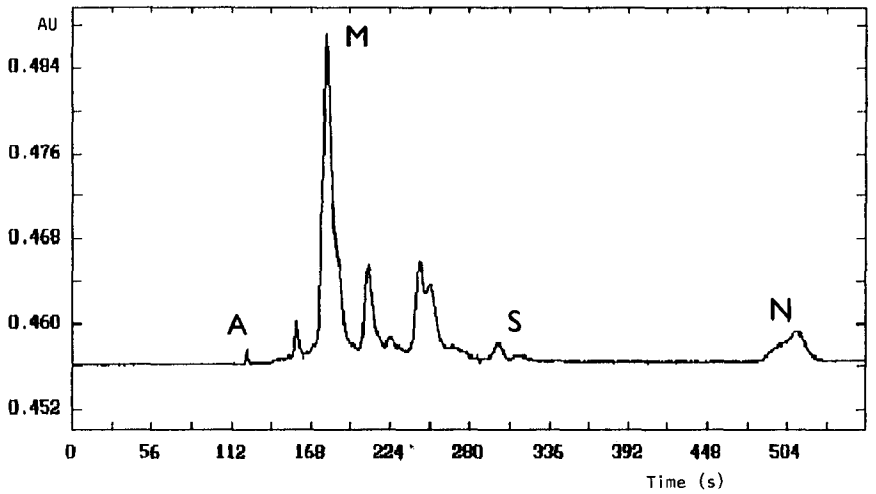

Fig. 4. CZE analysis of the crude synthetic product of [D-Tle^{2,5}]-dalargin. 0.6 mg of the lyophilizate dissolved in 0.4 ml of 0.5 mol/l acetic acid. A = Fastest component; M = main component of the synthetic product; S = slow component; N = uncharged component(s). Absorbance at 206 nm; AU = absorbance.

the reaction in the form of hydroxybenzotriazole esters; D-Tle was condensed directly in the reaction mixture in the presence of N,N′-dicyclohexylcarbodiimide and hydroxybenzotriazole. Coupling of all amino acids was checked by the Kaiser test (to the loss of blue colour). The peptide was cleaved from the resin (simultaneously with the cleavage of the protective group) by liquid hydrogen fluoride (10 ml per gram of resin) containing 5% of ethanedithiol and 20% of anisole. After hydrogen fluoride distillation the peptide was eluted from the resin with 50% acetic acid. The solution was diluted with water to 15% (v/v) concentration of acetic acid and lyophilized. The lyophilizate of the crude synthetic product (190 mg) was dissolved in 5 ml of 0.5 mol/l acetic acid, centrifuged and applied to the FFZW separation. The pattern of UV absorbance at 280 nm of FFZE fractions is shown in Fig. 5.

Comparing Figs. 4 and 5, the qualitative similarity of the CZE and FFZE separation patterns can be observed. The differences in relative peak heights are caused by the different wavelengths used for detection in CZE (206 nm) and FFZE (280 nm). It is naturally evident that the separation power of FFZE

TABLE III

MIGRATION TIMES, ELECTROPHORETIC VELOCITIES AND PREDICTED AND EXPERIMENTAL MIGRATION DISTANCES OF SELECTED COMPONENTS OF CRUDE PRODUCT OF [D-Tle^{2,5}]-DALARGIN

t_r = Resulting migration time obtained from CZE analysis in Fig. 4; $v_{ep,c}$ = electrophoretic velocity in the capillary; $v_{ep,f}$ = electrophoretic velocity in the chamber; $d_{r,pred}$ = predicted migration distance; $d_{r,real}$ = experimental migration distance obtained from FFZE separation in Fig. 5. Details of experimental conditions are given in the text.

Sample component[a]	CZE		FFZE		
	t_r (s)	$v_{ep,c}$ (mm/s)	$v_{ep,f}$ (mm/s)	$d_{r,pred}$ (mm)	$d_{r,real}$ (mm)
A	123	1.234	0.1111	221	235
M	175	0.747	0.0665	139	155
S	310	0.250	0.022	56	55
N	510	0	0	15	15

[a] A = Fastest component; M = main component of the synthetic product; S = slow component; N = uncharged component(s).

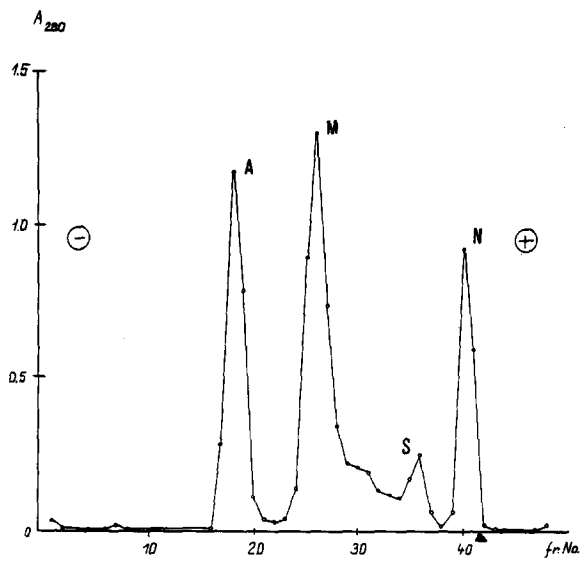

Fig. 5. FFZE separation of crude preparation of [D-Tle^{2,5}]-dalargin. A = Fastest component; M = main synthetic product ([D-Tle^{2,5}]-dalargin); S = slow component; N = uncharged component(s). Experimental conditions are given in the text. A_{280} = absorbance at 280 nm; fr. No. = fraction number; ▲ = coordinate of the sample inlet in the chamber.

is lower than that of CZE. This is understandable when one considers all the differences in these two experimental arrangements of the same zone electrophoresis principle: better anticonvective stabilisation and Joule heat dissipation in the 0.05 mm I.D. capillary than in the 0.5-mm gap flow-through chamber, about one order of magnitude lower migration time in CZE for the main product, one order of magnitude lower sample concentration in CZE than in FFZE, absence of hydrodynamic flow in the capillary and the relatively large width of the collected fraction (10.4 mm) in FFZE. However, although the FFZE separation is not complete, its usefullness for peptide purification is obvious from Fig. 5. The purification effect of FFZE was quantitatively evaluated by CZE analysis of individual FFZE fractions. Examples of these analyses for FFZE fractions 18, 26, 31 and 36 are shown in Fig. 6.

Fig. 6a and b represent examples of fractions containing electrophoretically homogeneous material; single peaks were obtained in CZE analyses. Fig. 6c and d show CZE analyses of FFZE fractions 31 and 36 containing mixtures of several sample components that remained unresolved after FFZE. Nevertheless, these side-product components of peptide synthesis were not the subject of further interest and their further separation was not pursued. The main product of the solid-phase peptide synthesis, [D-Tle^{2,5}]-dalargin, is completely free from the fastest component and relatively well separated also from the slower components. Some fractions of the main product were obtained in a pure form, that can be isolated as an acetate and/or acetic acid adduct after lyophilization, *i.e.*, directly applicable in a physiologically tolerable form for biological tests.

The high degree of purity of the main peak component was checked also by high-performance liquid chromatographic analysis and its sequence was confirmed by amino acid analysis: Tyr (0.93), D-Tle (1.80), Gly (0.98), Phe (0.96), Arg (1.02). The opiate activity of [D-Tle^{2,5}]-dalargin is substantially decreased in comparison with dalargin, probably because of the great changes in configuration and conformation of peptide chain caused by the presence of two voluminous D-Tle residues, which results in weaker interaction with the receptor [17].

Comparison of the experimentally determined migration distances of selected components with the predicted migration distances (see Fig. 5 and Table III) shows relatively good agreement. The relatively small discrepancy between the predicted and experimental distances of the fastest sample component (*ca.* 6%) confirms the quantitative correlation between CZE and FFZE. The larger discrepancy with the main peak component (*ca.* 10%) is probably caused by its relatively high concentration in the FFZE experiment. The accuracy of the prediction is also unfavourably influenced by the relatively large fraction width in our apparatus (10.4 mm).

CONCLUSIONS

The procedure developed, involving consecutive utilization of CZE, FFZE and CZE methods in peptide analysis and preparation, represensts a rapid and economical approach to the decision as to whether and how to use effectively a carrier-free and almost loss-free continuous electrophoretic mode of preparation and what the optimized separation conditions are.

First CZE is used for microanalyses of the synthetic peptide at the nanogram level. Suitable separa-

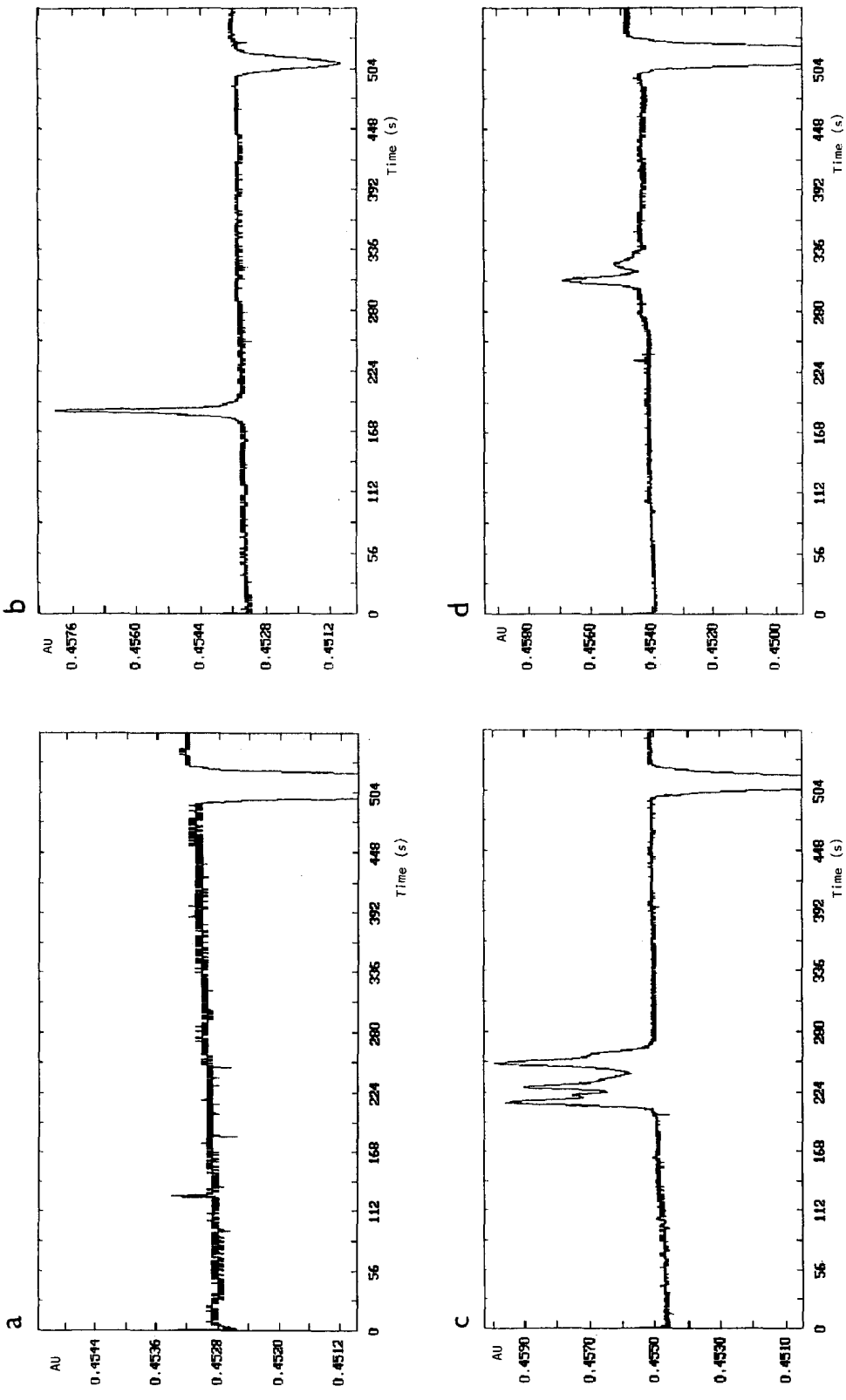

Fig. 6. CZE analyses of selected FFZE fractions: (a) fraction 18; (b) fraction 26; (c) fraction 31; (d) fraction 36. Aliquots of these fractions were analysed directly as obtained by FFZE. For experimental details, see text. Absorbance at 206 nm; AU = absorbance.

tion conditions are developed with minimum sample and electrolyte consumption. These data obtained by optimized CZE are used for conversion into preparative FFZE without loss of the sample material for FFZE tests. The purity of FFZE fractions is then examined by CZE and the purification effect of FFZE can thus be quantitatively evaluated.

ACKNOWLEDGEMENTS

P. Mudra, J. Štěpánek, J. Weisgerber, K. Danihelka and K. Ženíšek are thanked for their help in the development and the construction of the experimental devices used in this work. The skilful assistance of V. Lišková in the FFZE experiments is appreciated.

REFERENCES

1 B. L. Karger (Guest Editor), *1st International Symposium on High Performance Capillary Electrophoresis, Boston, MA, April 10–12, 1989*; *J. Chromatogr.*, 480 (1989).
2 B. L. Karger (Guest Editor), *2nd International Symposium on High Performance Capillary Electrophoresis, San Francisco, CA, January 29–31, 1990*; *J. Chromatogr.*, 516, No. 1 (1990).
3 J. W. Jorgenson (Guest Editor), *3rd International Symposium on High Performance Capillary Electrophoresis, San Diego, CA, February 3–6, 1991*; *J. Chromatogr.*, 559 (1991).
4 W. G. Kuhr, *Anal. Chem.*, 62 (1990) 403R.
5 Z. Deyl and R. Struzinský, *J. Chromatogr.*, 569 (1991) 63.
6 S. Hjertén and M. D. Zhu, *J. Chromatogr.*, 327 (1985) 157.
7 D. J. Rose and J. W. Jorgenson, *J. Chromatogr.*, 438 (1988) 23.
8 X. Huang and R. N. Zare, *J. Chromatogr.*, 516 (1990) 185.
9 N. Banke, K. Hansen and I. Diers, *J. Chromatogr.*, 559 (1991) 325.
10 Z. Prusik, in Z. Deyl (Editor), *Electrophoresis. Part A: Techniques*, Elsevier, Amsterdam, 1979, p. 229.
11 H. Wagner, V. Mang, R. Kessler and W. Speer, in C. J. Holloway (Editor), *Analytical and Preparative Isotachophoresis*, Walter de Gruyter, Berlin, 1984, p. 347.
12 Z. Prusík, *J. Chromatogr.*, 91 (1974) 867.
13 H. Wagner and J. Heinrich, in H. Tschesche (Editor), *Modern Methods in Protein and Nucleic Acid Research*, Walter de Gruyter, Berlin, 1990, p. 69.
14 Z. Prusík, V. Kašička, P. Mudra, J. Štěpánek, O. Smékal and J. Hlaváček, *Electrophoresis*, 11 (1990) 932.
15 Z. Prusík, V. Kašička, G. Weber and J. Pospíšek, in B. J. Radola (Editor), *Electrophorese Forum '91*, Technische Universität München, Munich, 1991, p. 201.
16 Z. Prusík, J. Štěpánek and V. Kašička, in B. J. Radola (Editor), *Electrophoresis '79*, Walter de Gruyter, Berlin, 1980, p. 287.
17 J. Pospíšek, T. Barth, Z. D. Bespalova, M. I. Titov, H. P. Mašková, A. S. Molokoedov and N. F. Septov, *Collect. Czech. Chem. Commun.*, 54 (1989) 22.