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Analysis of synthetic peptides by capillary electrophoresis Effect of organic solvent modifiers and variable electrical potentials on separation efficiencies

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Abstract

In this study, procedures based on volatile ammonium acetate buffer electrolytes of high pH value containing different organic solvent modifiers have been developed to achieve very high efficiency separations of histidine-containing synthetic peptides by high-performance capillary electrophoresis (HPCE) employing untreated fused silica capillaries. Different organic solvents, including acetonitrile, methanol and ethanol, at high volume fractions were used to modify the composition of the background buffer electrolyte. With the peptides investigated, it was found that methanol had the greatest effect in terms of enhancement of separation efficiency, as determined from the evaluation of theoretical plate numbers, N, of these HPCE systems. On the other hand, separation selectivities, e.g. the α_{ij} values, did not change significantly as the volume fraction, ψ, of the organic solvents was increased up to $\psi = 60\%$ (v/v). Under these conditions, very rapid, e.g. 1–2 min, separation times could be still achieved. Compared to the effect of carrying out the separation of these peptides at constant voltage, a dramatic increase in the separation efficiency was also achieved by applying a linear voltage gradient during the HPCE experiment. Under optimal conditions of organic solvent composition and linear voltage gradient ramps, very high peak efficiencies for the studied set of synthetic peptides with N values of ∼2–3 million theoretical plates per meter could be routinely obtained with fast analysis times. Moreover, these buffer electrolyte conditions are compatible with direct interfacing of the HPCE effluent to electrospray ionisation and ion trap mass spectrometers, thus expanding the analytical capabilities of these HPCE systems.

Keywords: Buffer composition; Efficiency; Voltage gradients; Peptides

1. Introduction

High-performance capillary electrophoresis (HPCE) has attracted considerable interest as a separation technique over the past decade. Because of the plug flow characteristics of this electrically-driven mode of separation, efficient separation of complex mixtures of charged compounds [1–5] can be achieved with short analysis times. With appropriate choice of buffer electrolyte compositions, HPCE procedures can be interfaced with mass spectrometers [6,7], thus considerably expanding the analytical capabilities. Previously, many HPCE applications have been restricted to the use of aqueous background buffer electrolytes, although the application of fully organic or aqueous–organic solvent buffer electrolytes is attracting increased attention for the separation and quantitative analysis of anionic and cationic drug substances [8,9] and other classes of charged molecules. The addition of an organic solvent to modify the composition of the an aqueous buffer electrolyte often increases the solubility of organic analytes in the background buffer electrolyte and reduces either self–self association or interactions of hydrophobic compounds with the negatively charged capillary wall [9,10]. Moreover, when interfaced with electrospray ionisation mass spectrometry (ESI-MS), aquo–organic solvent buffer electrolytes in HPCE frequently generate an overall improvement in mass detection sensitivity since the lower surface tension and higher volatility favour electrospray formation [11].

Because charged analytes migrate in HPCE as a consequence of the additive effects of their intrinsic electromobility and the electroendosmotic flow, variations in the composition of the buffer electrolyte as well as the field

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strength are expected to have a profound impact on resolution. In fact, one of the most important parameters that can be experimentally manipulated in a straightforward manner to adjust the separation time in capillary electrophoresis is the applied voltage. The use of high electric field strengths is preferred as long as Joule heating can be properly controlled. Since longitudinal molecular diffusion and wall interactions are the major sources of peak broadening in HPCE, rapid electromigration processes under the plug flow conditions that apply in HPCE will tend to minimise zone dispersion. Also, the electrical conductivities of aquo–organic solvent buffer electrolytes are different from their corresponding fully aqueous buffers, and this feature allows application of higher electric field strengths, producing higher separation efficiencies with shorter analysis times.

In this study, we have systematically studied the effect of several different organic solvents on the separation efficiencies of a set of related histidine-containing peptides using volatile buffer systems of high pH values under conditions of constant or variable electrical field strength. We demonstrate that significant enhancement of separation efficiencies can be achieved with naked silica capillaries by the proper choice of the type and content of the organic modifier and electric field strength. The results provide insight into the influences of these factors, which can be utilised to optimise specific separations. Moreover, the described aquo–organic solvent buffer electrolytes are particularly suited to HPCE systems where subsequent in-line electrospray ionisation mass spectrometry analyses of the samples are intended.

2. Materials and methods

2.1. Chemicals and reagents for capillary electrophoresis

Ammonium acetate was purchased from BDH Australia (Kilsyth, Australia); sodium hydroxide and 28% (w/v) ammonia solution were obtained from Ajax (Sydney, Australia); acetonitrile, methanol, and ethanol (HPLC grade) were obtained from Biolab Scientific (Sydney, Australia). Unless otherwise stated, all other reagents were of analytical grade. All the peptides used in this study were synthesised and purified using procedures reported in a previous publication [12].

2.2. Capillary electrophoresis instrumentation

The investigations were performed with a HP^{3D}CE instrument from Agilent Technologies (Waldbronn, Germany) at ambient temperatures (the instrument was set up in an air-conditioned laboratory with the room temperature maintained at 22 ± 1 °C). The temperature of the cassette containing the capillary was controlled by a circulating air fan, and registered by a temperature monitor (U-lab, Melbourne, Australia) with temperature read-outs of 21–23 °C during the time that the experiments were carried out. The detection of peptides was performed at 214 nm.

2.3. Capillary electrophoresis

A stock solution of 100 mM ammonium acetate buffer, pH 10.0, was made by titrating 100 mM ammonium acetate with a 28% (w/v) ammonia solution, and was filtered through a 0.22 μm pore sized Millipore Aquapore filter. All of the aqueous–organic solvent buffer electrolytes were prepared by mixing appropriate proportions of the individual components in the ratio: 20% (v/v) 100 mM NH_4Ac–NH_4OH, pH 10.0, *x*% (v/v) of the organic solvent and (100%–*x*%–20%) (v/v) of Milli–Q water and degassing the mixture by ultra-sonication for 10 min before use. Bare fused silica capillaries, with an internal diameter of 50 μm and an outer diameter of 375 μm, were obtained from Beckman Instruments (Richmond, CA, USA). The total length of the capillary was 34.0 cm, and the effective length, from the inlet to the detection window, was 25.5 cm. About 2 mm of polyimide coating at the ends of the capillary was removed to prevent the possible weakening and swelling of the polyimide coating when exposed to buffer electrolytes of high organic solvent content [13]. For all separations the following method was routinely employed using these capillaries: (i) the samples were hydrodynamically injected at the cathodic inlet end of the capillary; (ii) the electric field was applied at the desired voltage to achieve separation within 2–15 min; (iii) a 10 min wash/regeneration step was carried out under a 50 mbar internal pressure with fresh background buffer electrolyte at the completion of each experiment. All electrophoretic separations were carried out at least in duplicate with *N*,*N*-dimethylformamide (DMF) (1 mg/ml) used as the internal electro-osmotic flow marker in all the experiments. Reproducibility and robustness of the separations were validated using procedures described previously [14].

3. Results and discussion

3.1. Separation of the synthetic peptides ***1***–***8*** with fully aqueous buffer electrolytes

All peptides used in this study were synthesised on either the Wang-resin with the first amino acid attached or the Glu-(rink amide MBHA) resin by 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase synthesis protocols [12], according to their specific amino acid sequence. These histidine-containing peptides are structurally related with peptides ***1***–***6*** and peptide ***8*** being fragments of the parent peptide ***7***. Their amino acid sequences, molecular masses, calculated effective charges, *q*, at pH 10.0 and charge–size ratio parameters, ξ_{fric}, are given in Table 1. Separation of this set of peptides by HPCE methods with fully aqueous buffer electrolytes of low to intermediate pH values, e.g.

Table 1
Amino acid sequence, molecular mass M_r, net-charge values q at pH 10.0, and charge–mass-ratio parameter $\xi_{fric} = q/M_r^{2/3}$ of peptides **1**–**8**

Peptide code	Amino acid sequence	M_r	q, pH 10.0	ξ_{fric} pH 10.0
1	DHDINR	768.78	−1.96	−0.0234
2	WDHDINR	955.00	−1.99	−0.0205
3	SWDHDINR	1042.08	−2.00	−0.0195
4	NSWDHDINR	1156.18	−2.00	−0.0182
5	HNSWDHDINR	1293.32	−1.99	−0.0167
6	HHNSWDHDINR	1430.46	−1.99	−0.0157
7	HHHNSWDHDINR	1567.60	−1.99	−0.0147
8	HHHNSW	816.83	−0.98	−0.0113

in the range of pH 2.0–7.5, has previously proven difficult because of strong interaction between positive charged side chain moieties of these peptides and the negatively charged capillary wall of the naked fused silica capillary. One strategy to suppress this adverse wall effect is to employ buffer electrolytes of high pH values. For example, with buffer electrolytes of pH > 9 both the capillary wall and the peptides are effectively anionic, with electrical repulsion anticipated between the negatively charged double layer at the wall surface and the ionised peptides. This effect is expected to lead to efficient separation of peptides, although selectivity may be reduced at these extreme pH conditions since the charge to mass ratios for peptides of similar sequence length may become in fully aqueous buffer electrolytes very similar.

Fig. 1 shows the separation of the histidine-containing peptides **1**–**8** with a 20 mM NH_4Ac buffer, pH 10.0. Under

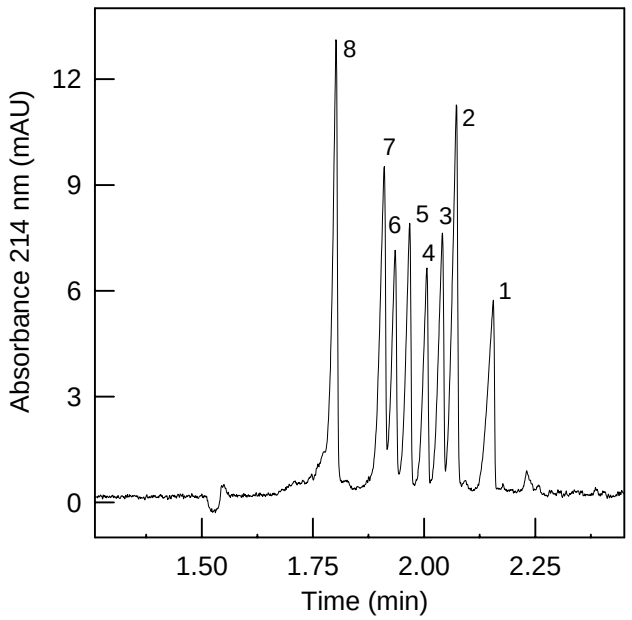

Fig. 1. Separation of synthetic peptides **1**–**8** with a 20 mM NH_4Ac buffer electrolyte, pH 10.0, The experimental conditions were: 50 μm i.d. unmodified fused silica capillary, total length L_t = 34.0 cm, effective length L_e = 25.5 cm; applied voltage, 13.6 kV; sample injection, 20 mbar, 3 s; UV detection at 214 nm. The peak efficiencies were in the range of 2.8 × 10^5 and 5.8 × 10^5 theoretical plates per meter.

Table 2
Reproducibility of the capillary electrophoretic separation of peptides **1**–**8** when the post-run condition included only a rinsing step with fresh buffer electrolyte (n = 5)

Peptide code	Average migration time (min)	R.S.D. (%)	Average peak height	R.S.D. (%)
1	2.156	0.5	5.5	4.5
2	2.074	0.5	10.8	5.7
3	2.042	0.5	7.6	4.4
4	2.007	0.5	6.5	5.6
5	1.968	0.5	8.0	4.3
6	1.936	0.5	7.1	4.3
7	1.911	0.4	9.1	4.2
8	1.803	0.4	13.1	6.5

these conditions, all of the peptides are negatively charged and are repelled from the electric double layer at the wall surface. Thus, the net charge of peptides **1**–**8** at pH encompass the range of −0.98 to −2.00 and the frictional ratio, ξ_{fric}, from −0.0113 to −0.0234. Moreover, the high electroosmotic flow with this buffer electrolyte results in fast separation of all peptides. As can be seen from Fig. 1, the eight peptides are nearly baseline-resolved, with separation efficiencies for most peptides, as measured from their N values, exceeding 500 000 theoretical plates/m. Since the adsorption of peptide samples on the capillary wall is significantly suppressed with this high pH buffer electrolyte, the commonly used regeneration procedure involving a sodium hydroxide solution wash between each sample injection is not needed. In fact, a 10-min rinse with fresh separation buffer enables highly reproducible separations of these peptides to be achieved, as illustrated in Table 2. This feature, coupled with the avoidance of a conventional sodium hydroxide wash step, provides a considerable practical advantage in terms of facilitating the rapid analysis of peptide mixtures with naked fused silica capillaries when the HPCE outlet/effluent is interfaced to an ESI-MS system (unpublished results). Therefore, we chose this aqueous background buffer electrolyte for further investigations.

*3.2. The effect of organic solvents on the selectivity and peak efficiencies of the synthetic peptides **1**–**8***

For the separation of peptides **1**–**8** a 20 mM NH_4Ac, pH 10.0, buffer electrolyte containing acetonitrile, methanol or ethanol as the organic solvent additive in the range of 0–60% (v/v) was investigated. Table 3 shows the extent of selectivity changes of peptides **1**–**8** upon addition of different concentrations of acetonitrile. As can be seen from Table 3, no significant change of separation selectivity, as measured from the α values, was induced. Similar observations were also found when methanol or ethanol with volume fractions up to 60% (v/v) was added to the aqueous buffer electrolyte. Taking into account the amino acid sequences of this set of peptides, the carboxyl groups of their C-terminal amino acids and aspartic acid side chains will be fully ionised at pH

Table 3
The influence of acetonitrile content in the buffer electrolyte on the separation selectivity of peptides **1**–**8**

Acetonitrile (%)	Selectivity variations for the peptides **1**–**8**						
	α_{7-8}	α_{6-7}	α_{5-6}	α_{4-5}	α_{3-4}	α_{2-3}	α_{1-2}
0	1.06	1.01	1.02	1.02	1.02	1.02	1.04
10	1.06	1.01	1.02	1.02	1.02	1.02	1.04
20	1.07	1.02	1.02	1.02	1.02	1.02	1.05
30	1.07	1.02	1.02	1.03	1.02	1.02	1.05
40	1.07	1.02	1.03	1.03	1.03	1.02	1.05
50	1.07	1.02	1.03	1.03	1.04	1.03	1.05
60	1.05	1.03	1.04	1.04	1.05	1.03	1.03

10.0, whilst the side chains of the histidine residue(s) should be essentially deprotonated because of their relatively low pK_a value(s). The guanidine side chain of arginine residues in peptides ***1***–***7*** will, however, be substantially protonated [15], because its very high pK_a value of 12.48 is still above the pH value of the buffer electrolyte buffer by 2.48 units. On the other hand, deprotonation of amino groups of the N-terminal amino acid will be nearly complete considering their pK_a values [16,17]. Because of their amino acid sequence similarities, the relative electrophoretic resolution of this set of related peptides should not be significantly influenced by addition of an organic solvent per se. The generalised effect of the organic solvent on protonic equilibria on the other hand needs to be factored into the electromigratory behaviour of peptides in the HPCE systems, although with sets of closely related peptides such as those employed in the present investigation, the individual differences are relatively small. As a consequence, the overall resolution trends of peptides ***1***–***7*** are expected to be similar with solvent induced, pH-dependent changes in migration times manifested but with no significant changes in migration order anticipated. Moreover, the impact upon peptide size in terms of the changes in the partial molar volume or Stokes' radius, due to dissociation and association of protons induced by the addition of organic solvents to the buffer electrolyte will be small [16].

Contrary to the minor change of separation selectivity discussed above (cf. data shown in Table 2), the addition of the various organic solvents to the ammonium acetate, pH 10.0, buffer electrolyte considerably increased the separation efficiency. Among the three organic solvents investigated, methanol had the greatest effect on separation efficiency. Fig. 2 demonstrates the influence of methanol content in the background buffer on the separation efficiencies (as N values) of peptides **1**–**8**. As illustrated in Fig. 2, the N values for all peptides increases upon addition of methanol, reaching a maximum when the volume fraction of methanol was about 20% (v/v). At higher volume fractions, i.e. $\psi >$ 0.3, of the organic solvent, the N values decline to varied extents for the different peptides. The greatest enhancement of separation efficiency was obtained for peptide ***7***. Compared to the corresponding fully aqueous buffer electrolyte

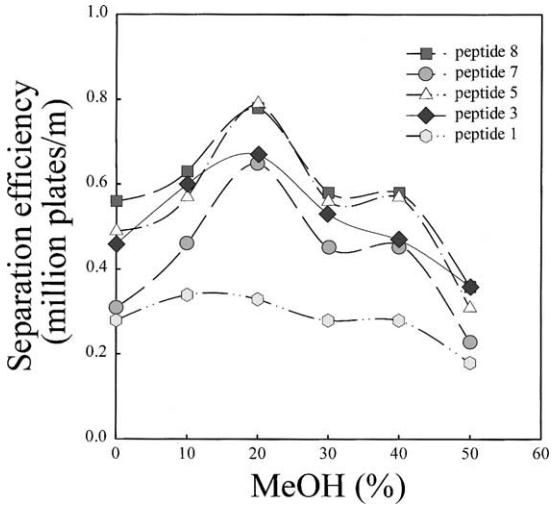

Fig. 2. The effect of methanol content in the background electrolyte on the separation efficiency of the synthetic peptides **1**–**8**. The background electrolyte was prepared by adding x% methanol to 20% 100 mM NH_4Ac buffer, pH 10.0, and (100%–x%–20%) water to achieve the desired volume fraction of methanol; other conditions are as given in Fig. 1.

condition, the N value for this peptide in the aquo-methanol buffer electrolyte with $\psi = 0.2$ more than doubled from ca. 300 000 theoretical plates per meter to ca. 620 000 plates per meter.

The mechanism for this effect leading to significantly enhanced peak efficiencies with charged peptides separated in naked fused silica capillaries has yet to be fully elucidated. Several contributory factors can, however, be identified. Firstly, because of their charge status, all of the peptides will migrate in the same direction as the electroendosmotic flow, and as a consequence their charge-to-size-ratio parameters, expressed as frictional ratio, will follow [17] the dependency $\xi_{fric} = q/M_r^{2/3}$ rather than $\xi_{fric} = q/M_r^{1/2}$. Secondly, offsetting this effect, differences in the intrinsic dielectric constants of the buffer electrolytes as the organic solvent content is changes will also impact on the electromobility through the dependencies:

$$u_{eo} = \frac{\varepsilon_0 \varepsilon_r \zeta E}{\eta} \quad (1)$$

$$\mu_{eo} = \frac{\varepsilon_r \zeta}{\eta} \quad (2)$$

$$\mu_e = \frac{q}{6\pi\eta r} \quad (3)$$

$$\mu_a = \mu_{eo} + \mu_e \quad (4)$$

where u_{eo} and μ_{eo} are the electrophoretic velocity and electrophoretic mobility of the EOF respectively; μ_a and μ_e are the apparent electrophoretic mobility and the intrinsic electrophoretic mobility of the charged peptide respectively; ε_0 is the permittivity in a vacuum, ε_r is the relative permittivity (dielectric constant) of the buffer electrolyte; ζ is the zeta

potential at the liquid-solid interface, η is the viscosity of the buffer electrolyte, E is the electric field strength (kV/m), q is the intrinsic charge of the peptide and R_s is the ion radius or Stokes' radius of the peptide respectively. As ε_r and η are varied in response to changes in the organic solvent content in the buffer electrolyte, μ_a will also change. Thirdly, as the organic solvent content in the buffer electrolyte is increased, this will also affect the protonic equilibrium leading to changes in the effective pH as well as the effective charge carried by the peptide. Changes in the buffer electrolyte composition will also influence the ζ value. Fourthly, it can be noted that changes in molecular diffusion of peptides will occur due to the decreased viscosity, η, of the buffer electrolyte upon addition of the organic solvent. Although superficially it could be expected that greater Brownian motion associated with decreased frictional drag can occur in buffer electrolytes of lower viscosity, this global picture does not take into account the influences of solvent stacking and preferential solvation phenomena that can occur between the peptide and the organic solvent molecules. Elsewhere, the importance of such solvent effects has been described for HPCE and HP capillary electrochromatography (CEC) separations of peptides [2,16–20]. The focusing of charged analytes due to more favourable poly-ion stacking dipole–dipole alignment changes to charge suppression and minimisation of isodesmic self-association of peptides in buffer electrolytes of higher organic solvent content would thus also contribute to the observed enhancement in peak efficiency. Such an effect would generate a "sweeping" phenomenon with the axial diffusion, aggregation and wall interactions minimised due to the choice of the organic solvent content, composition and pH of the buffer electrolyte. The participation of this "sweeping" phenomenon is consistent with the absence of strong peptide-wall interactions as evidenced from the lack of peak tailing (cf. Figs. 1 and 4). Several groups of investigators, including Hamrnikova et al. [21] and Towns and Regnier [22] have examined the impact on separation efficiency in capillary electrophoresis due to the adsorption of polycations to silica capillaries with buffer electrolytes of low pH values, e.g. pH 2.5. These findings indicate that the adsorption of positively charged species tend to occur preferentially at the capillary inlet and this leads to a non-uniform axial distribution of the zeta potential within the capillary and overall reduction in the separation efficiency due to these adverse wall effects. The choice of buffer electrolytes of high pH values, as used in the present investigations, appears to greatly minimise or avoid such double layer/wall effects, contributing to the significant enhancement in N values as seen in the present study.

Compared to liquid chromatographic systems, in the high performance capillary zonal electrophoresis of peptides plug flow conditions prevail and the contributions from band dispersion caused by eddy diffusion and mass transfer resistance are negligible [23]. As a consequence, the van Deemter equation [24], which in liquid chromatography takes the form:

$$H = A + \frac{B}{u} + Cu \tag{5}$$

where H is the height equivalent to a theoretical plate (HETP), u is the velocity of a solute through the column, and A, B and C are constants representing contributions to peak dispersion from eddy diffusion, molecular diffusion and resistance to mass transfer between the stationary and mobile phases, respectively. The corresponding peak efficiency relationship in HPCE can be represented by:

$$H = \frac{B}{u} \tag{6}$$

$$B = 2D_m \tag{7}$$

where D_m is the diffusion coefficient of the charged analyte. As the temperature of the system was well controlled in the present investigations, contributions to band dispersion from Joule heating effects can be largely excluded. As the main contribution to peak dispersing arises from the B-term of the Van Deemter equation, e.g. from molecular diffusion [23,25], then the shorter the peptide takes to migrate through a capillary, the smaller will be the band spreading, and consequently higher separation efficiencies will arise. The experimental findings indicated that enhanced peak efficiencies can be generally achieved under appropriate "sweeping" conditions using aquo–organic solvent buffer electrolytes of high pH value and intermediate to high organic solvent content.

3.3. The effect of variable separation voltage on peptide separation

Constant voltage, i.e. a fixed electric field strength, is the commonly used operational mode in capillary electrophoresis. In order to investigate the effect of varied electric field strengths on the separation efficiency and selectivity of these synthetic peptides, we adopted a new approach. We gradually increased in a linear fashion the applied electric field strength from a value near to 0 V at the beginning of a separation to a preset value over a defined period of time. No significant changes in the separation selectivity were found by employing this electric field strength gradient profile, but dramatic increases in the separation efficiency were achieved. Fig. 3 illustrates the separation of peptides **1**–**8** with a buffer electrolyte comprising methanol–100 mM NH_4Ac, pH 10.0, buffer–water (10:20:70, v/v/v) using different linear gradient potentials. After the injection of the peptide mixture, the voltage was linearly increased from 0 to 13.6 kV (corresponding to a field strength of 400 V/cm) over intervals of 2.0, 4.0, 6.0 and 8.0 min, and was then kept at a fixed value until the separation was complete. As can be seen from Fig. 3, a relatively slow increase in the electric field strength leads to a better separation performance. When the applied electric field strength was gradually increased from 0 to 400 V/cm over 6 min, the peak efficiency for most peptides exceeded 2×10^6 theoretical plates per

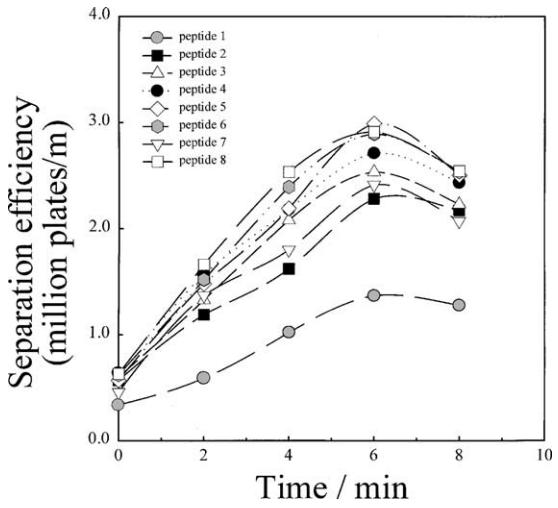

Fig. 3. The influence of variable electric field strengths on the separation efficiency of peptides **1**–**8**. The horizontal axis represents the time over which the voltage was increased linearly from 0 to 13.6 kV. The background electrolyte was MeOH–100 mM NH_4Ac buffer, pH 10.0, buffer–water (10:20:70, v/v/v). Other conditions are as given in Fig. 1.

meter and in several cases was above 3×10^6 million theoretical plates per meter. This corresponds outcome to a four- to five-fold enhancement of separation efficiency compared to the analogous separation carried out under constant voltage conditions of 400 V/cm.

It is well known [23] that the longitudinal molecular diffusion is the major source of peak dispersion in capillary electrophoresis, provided wall interaction effects are minimal or have been abolished through the appropriate choice of the buffer electrolyte. Consistent with the relationship given in Eq. (1), a higher separation voltage is therefore the preferred choice in order to achieve faster separations and better performance. According to theory, separation efficiency is predicted to increase linearly with the applied potential [16–20]. Compared to a constant potential of say 400 V/cm, linear gradient voltages, especially shallow linear gradient voltages, would considerably prolong the separation time for a peptide and, thus, on mass transport arguments larger electomigration times would lead to lower separation efficiencies. Under conditions of a linear voltage ramp, this conclusion is contradictory to the observed experimental findings. Again the involvement of a field strength driven "sweeping" effect as note above for the impact of organic solvent content on peak efficiencies may underpin this extraordinary enhancement of the N values. By combining the effect of organic solvents with gradient potential changes, extremely high efficiency separations of the peptides **1**–**8** can thus be achieved, as illustrated in Fig. 4.

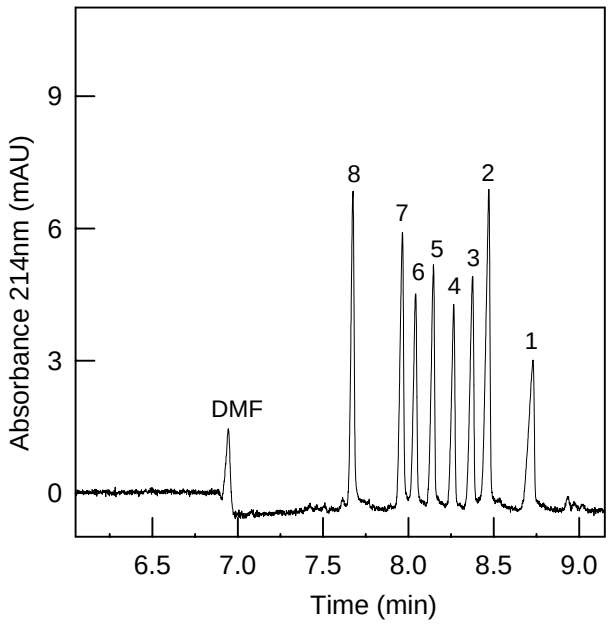

Fig. 4. Separation of peptides **1**–**8** using a buffer electrolyte of MeOH–100 mM NH_4Ac buffer, pH 10.0–water (20:20:60, v/v/v) with applied voltage increased linearly from 0 to 13.6 kV over 8 min. The experimental conditions were: 50 μm i.d. unmodified fused silica capillary, total length $L_t = 34.0$ cm, effective length $L_e = 25.5$ cm; applied voltage, 13.6 kV; sample injection, 20 mbar, 3 s; UV detection at 214 nm. The peak efficiencies were in the range of 2–3 $\times$ 10^6 theoretical plates per meter.

4. Conclusions

As demonstrated in these studies, the use of organic solvents as modifiers of high pH aqueous buffer electrolytes improves the separation efficiency of charged peptides in HPCE. The application of linear gradient electric field strength potentials can further significantly improve the separation performance. Although the mechanism behind this significant enhancement of separation efficiencies has not yet been fully elucidated, the combination of the above two strategies used in this study leads to highly efficient rapid separations of synthetic peptides. Theoretical plate numbers in excess of 3×10^6 per meter can be readily obtained under optimal conditions. To our knowledge, this is the first report to enhance separation performance of peptides in capillary electrophoresis by employing gradient potentials. Further work on the separation of synthetic peptides and tryptic protein digestions using this novel method in other buffer systems is now being carried out in our laboratory.

Acknowledgements

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