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Optimization of the capillary zone electrophoresis loading limit and resolution of proteins, using triethylamine, ammonium formate and acidic pH

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Abstract

Capillary zone electrophoresis (CZE) of five model proteins (lysozyme, myoglobin, ribonuclease A, α-lactalbumin, and trypsinogen), using ammonium formate as the electrophoretic buffer and triethylamine (TEA) as a buffer additive at pH 2.5, was used for protein separation. The electrophoretic behavior of these proteins was examined with respect to various concentrations (10–40 m*M*) of TEA and of ammonium formate. Based on the experimental parameters of electrophoretic resolution, current, and peak separation time, an electrolyte (30 m*M* each of TEA and ammonium formate) was empirically derived as the optimum for scale-up separation. The loading limit for proteins, covering a wide range of injection volumes (60–990 nl) and amount of protein (1–21 pmol of each protein), was investigated on 75 and 100 μm I.D. untreated fused-silica capillaries. Protein adsorption (average <15%) was experimentally determined using this volatile buffer system.

Keywords: Proteins; Triethylamine; Ammonium formate; Lysozyme; Myoglobin; Ribonuclease A; α-Lactalbumin; Trypsinogen

1. Introduction

In CZE, narrow zones are expected for the separation of large biomolecules such as proteins because they have relatively lower diffusion coefficients compared to peptides and other small molecules [1]. Unfortunately, the strong interaction between proteins and the silanol groups (ionized and non-ionized) on the inner wall of a fused-silica capillary contributes to zone-broadening, poor resolution, low protein recovery, and a migration time shift due to the modified electroosmotic mobility (μ_{eo}). Several strategies have been used to overcome this adsorption problem. Free silanols on the inner surface can be chemically modified to produce a coated non-ionic layer to minimize protein–wall interactions [1–9]; however, column stability remains a challenge. Buffers of basic pH (e.g., pH 11) increase the number of negative charges and thus enhance the repulsion of negatively charged proteins from the deprotonated silanols [10]; buffers of acidic pH (e.g., pH 1.5) protonate and thus neutralize the acidic silanols (pK_a~3) [11]. The use of high buffer concentration is applicable to protein separation, but is

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limited to a small I.D. (e.g., 25 μm) capillary due to the high current that is generated [12,13]. Alternatively, the charge characteristics of the fused-silica inner surface can be modified by buffer additives such as zwitterions [14,15], non-ionic surfactants [16], ionic surfactants [17,18], neutral hydrophilic polymers [19], sugar amines [20,21], and alkylamines [21,22]. Besides the chemistry of the fused-silica inner wall, the variety and complex structural nature [23,24] of proteins are also largely responsible for the numerous investigations of protein separations using fused-silica capillaries.

In recent years, we have published a series of papers that describe the analytical and preparative CZE of neuropeptides using ammonium formate [20 m*M*; titrated to pH 2.5 with trifluoroacetic acid (TFA)] [25–28]. In one study, we performed preparative CZE, and isolated a fraction from a bovine pituitary homogenate without any high-performance liquid chromatography (HPLC) [29]. The analysis of that collected fraction by liquid secondary ion mass spectrometry (LSIMS) revealed a compound with a protonated molecule ion [$(M+H)^+$, *m/z* 556] that corresponds to that of leucine enkephalin (LE= YGGFL). We have also analyzed opioid peptide-containing proteins (OPCPs) in bovine pituitary, using reversed-phase HPLC, radioimmunoassay (RIA), and mass spectrometry (MS) [30–32].

The preparative CZE of proteins will provide an additional dimension for the separation of these endogenous proteins from complex biological materials prior to their MS analysis. Because the buffer system used for our peptide separation is not suitable for protein separation due to the aforementioned factors, a different volatile buffer system had to be developed. A volatile electrolyte is necessary for the MS analysis of a lyophilized fraction. Although on-line CZE-electrospray ionization (ESI) MS offers a high sensitivity for MW analysis of CZE-separated proteins, only a few laboratories are equipped with such a capability [33–37].

Accordingly, in this present study, a volatile buffer system, consisting of ammonium formate as the electrophoretic buffer at pH 2.5 and TEA as buffer additive, was examined for the separation of one neutral, one acidic, and three basic model proteins. TEA was chosen because it is volatile and it is cationic at that pH. Recent publications have reported that TEA and many other similar amine-containing monocationic compounds (e.g., triethanolamine, propylamine, glucosamine, etc.) were effective in controlling and stabilizing μ_{eo} by electrostatically interacting with silanols [38] and in improving protein separation [21]. However, in order to perform protein separation with an optimal loading limit to maximize sample collection, concentrations (30 m*M* each) of ammonium formate and TEA were empirically optimized for the important CZE factors of the electrophoretic current, stabilization of separation time, resolution of proteins, and peak separation time. Afterwards, using this optimized electrolyte, loadability was examined on 75 μm and 100 μm I.D. capillaries. The protein adsorption within a defined volume of the capillary was also investigated.

2. Experimental

2.1. Reagents and materials

All of the model proteins (Table 1) studied here were purchased from Sigma (St. Louis, MO, USA), and were used without any further purification. CZE buffers were prepared from ammonium formate (J.T. Baker, Phillipsburg, NJ, USA) alone or with neat TEA (Pierce, Rockford, IL, USA), and were titrated to pH 2.5 with neat TFA (Pierce). Fused-silica capillaries with 75 and 100 μm I.D. (both 360 μm O.D.) were purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Methods

2.2.1. CZE

CZE was performed on an ISCO Model 3140 electropherograph (ISCO, Lincoln, NE, USA) that was outfitted with an IBM Personal System/2 Model 30 286 computer (IBM, Armonk, NY, USA). The peptide bond absorption of the protein was monitored at 200 nm, using the built-in variable-wavelength UV detector. The operation of the instrument and of the data collection/analysis were controlled by the ISCO ICE 3.1.0 level software.

The fused-silica capillaries (75 and 100 μm I.D.) were 1 m long, with 60 cm from the capillary inlet to

Table 1
Proteins investigated

Peak No.	Proteins	K,R,H[a]	C[b]	p*I*	Molecular mass (Da)[c]
1	Lysozyme (chicken egg white)	18	8	11.0	14 314
2	Myoglobin (horse heart)	32	0	7.3	16 951
3	Ribonuclease A (bovine pancreas)	18	8	9.4	13 690
4	α-Lactalbumin (bovine milk)	15	8	4.8	14 183
5	Trypsinogen (bovine pancreas)	20	12	9.3	23 990

[a] The total number of these three protonated (at pH 2.5) amino acid residues lysine (K), arginine (R), and histidine (H).
[b] *C*=the number of cysteines.
[c] Molecular mass calculated from the amino acid composition [39].

the detector; those column volumes were 4.4 and 7.9 μl, respectively. Capillaries were preconditioned with the following sequence of solvents for approximately two column volumes for each solvent: H_2O, 1 *M* NaOH, H_2O, 0.1 *M* HCl, H_2O, and the appropriate buffer as specified for each experiment.

Salomon et al. [40] have estimated that the number of negative charges within a fused-silica capillary corresponding to the number of sites for cation adsorption above pH 6 in a 50 μm I.D. capillary, is within the range of $0.9–2.9\times10^{16}$ charges/m^2. That range corresponds to 3.5–11.3 pmol and to 4.7–15.1 pmol charges/m^2 for our 1 m by 75 and 100 μm I.D. capillaries, respectively. The two column volumes of 0.1 *M* HCl (corresponding to 0.9 and 1.6 μmol H^+ for the 75 and 100 μm I.D. capillaries, respectively) suffice to fully protonate the inner surface silanols; the pH of the separation buffer maintains that level of protonation.

A mixture of the five model proteins was prepared (1 mg/ml of each protein in water), and several portions of that mixture were sampled and frozen (−20°C) for use on different days. Injection was performed by applying the instrument's injection vacuum from the outlet beaker [25,26], and injection volumes (15–990 nl, corresponding to 0.3–21 pmol of each protein) were calculated [26]. Protein identity in an electropherogram was confirmed by the CZE of each individual protein. The operating voltage was 27 and 22 kV for the 75 and 100 μm I.D. capillaries, respectively, and the temperature was regulated to within 30±0.5°C by the electropherograph's built-in air-circulating system.

All electropherograms were acquired in triplicate, and the migration time reproducibilities (0.6–1.1%, R.S.D., $n=5$) were obtained under analytical conditions.

2.2.2. Protein adsorption

The adsorption of a protein onto a portion (40 cm) of the inner surface of a capillary was evaluated, based on a modification of the procedure described by Towns and Regnier [16]. Because we do not have two separate in-line CZE UV detectors on our instrument, separations (in triplicate) were first performed at the detector window that was located 37 cm from the capillary inlet of a 1 m long capillary; the capillary was shifted 40 cm, and measurements (triplicate) were made at the second detector window located 77 cm from the capillary inlet. The capillary was rinsed and conditioned as described above prior to each adsorption experiment. Injection volumes of 30 nl (0.7% of column volume; 0.6 pmol of each protein) and 60 nl (0.8% of column volume; 1.2 pmol) were used for the 75 and 100 μm I.D. capillaries, respectively. Protein adsorption was calculated by comparing the computer-calculated peak areas obtained at these two windows.

3. Results and discussion

Table 1 lists the five model proteins that were used in this study; the sum of the lysine (K), arginine (R), and histidine (H) amino acid residues; the number of cysteine (C) residues; the p*I*; and the molecular mass that is calculated from the amino acid composition. These proteins (numbered in the order of their correspondingly increasing migration time in Fig. 1) have a molecular mass that ranges

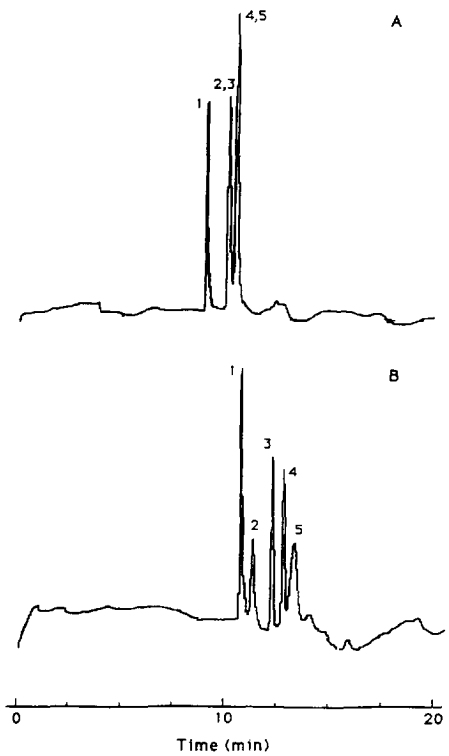

Fig. 1. Electropherograms of the mixture of five model proteins separated using 20 (A) and 40 (B) m*M* ammonium formate pH 2.5. The separation used a 75 μm I.D. capillary; injection volume was 15 nl; AUFS was 0.05 at 200 nm. The peak numbers (1–5) correspond to lysozyme (370 fmol), myoglobin (320 fmol), ribonuclease A (260 fmol), α-lactalbumin (250 fmol), and trypsinogen (350 fmol), respectively. Monitored currents were 51 and 96 μA for Fig. 1A and B, respectively.

from 14 000 to 24 000. With the exception of α-lactalbumin (p*I* 4.8) and myoglobin (p*I* 7.3), the other three proteins (lysozyme, ribonuclease A, and trypsinogen) have a p*I* value much greater than 7. Nevertheless, based on their p*I* values and on the number of positively-charged amino acid residues, each one of these five proteins would have a net positive charge at pH 2.5, and would migrate toward the cathode.

Fig. 1A shows the electropherogram when the mixture of five model proteins was separated, using the ammonium formate buffer (pH 2.5) that has been published for peptide separation [25]. The two pairs of proteins 2,3 and 4,5 co-migrated. In Fig. 1B, the buffer concentration was doubled, and each protein was separated. This improvement in separation and in the increase in each migration time was expected when using a buffer of increased concentration because of the increase in the ionic strength [41]. However, the combination of the narrow time-frame (ca. 3 min.) of the migration time between lysozyme and trypsinogen, which are the first- and last-migrating proteins, and the relatively high current (96 μA) precluded any further increase in the buffer concentration to resolve the co-migrating proteins. Because our main objective was to develop an electrolyte that would be suitable for the preparative separation of proteins with a capillary with I.D. ≥75 μm, we investigated the use of TEA to improve the separation without generating any excessive current.

Thus, the next set of experiments was performed to analyze the effects on CZE of fixing the ammonium formate concentration at 20 m*M*, and varying the TEA concentration over the range 10–40 m*M*. Likewise, the TEA concentration was fixed at 20 m*M*, and the ammonium formate concentration was varied from 10–40 m*M*.

The electropherograms in Fig. 2 show the effect of increasing the concentration of TEA (10, 20, 30, 40 m*M*) on the separation of these five model proteins in the presence of a fixed amount of ammonium formate (20 m*M*) at pH 2.5. With the exception of the relatively constant resolution observed between peaks 1 (lysozyme) and 2 (myoglobin), Fig. 2A–D illustrate that the separation generally increases as the TEA concentration increases. Another effect of increasing the TEA concentration is the increased migration time of all five proteins; however, the migration time of trypsinogen (peak 5) increased even more rapidly than the other four protein peaks. Furthermore, the migration time difference between myoglobin (peak 2) and lysozyme (peak 1) decreased, but the difference between myoglobin (peak 2) vs. ribonuclease A (peak 3) increased. These data indicate that, not only does TEA have an effect on the μ_{eo} [21,38], but also on the electrophoretic mobilities (μ) and thus the unique behaviors of trypsinogen and myoglobin under these experimental CZE conditions. The nature of the behavior observed under these experimental conditions with myoglobin and trypsinogen is not fully understood, but their tertiary structures, charges [23,24], degree of denaturation, and the degree of their interactions (e.g., electrostatic and/or hydrophobic) with the capillary

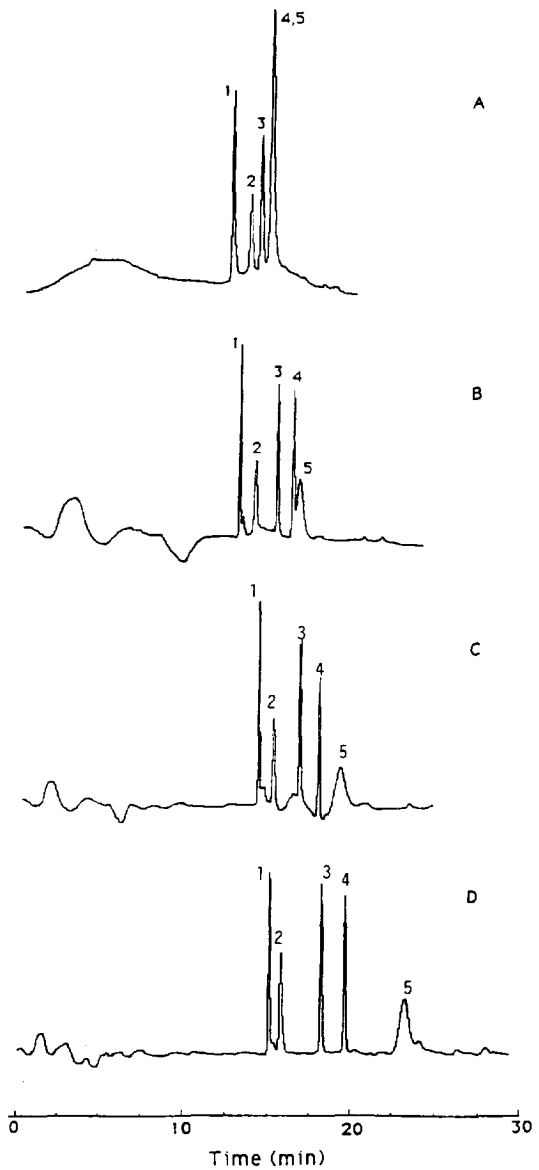

Fig. 2. Electropherograms of the mixture of five model proteins at four different TEA concentrations. Ammonium formate concentration (20 m*M*) was constant, and the electrolyte pH was 2.5. A 75 μm I.D. capillary was used. Monitored currents were 56, 72, 80 and 96 μA for A–D, respectively. The other experimental conditions are the same as in Fig. 1. The TEA concentrations were 10, 20, 30, and 40 m*M* in A–D, respectively.

inner wall might all be involved. For example, myoglobin is the only protein investigated here that has no disulfide bonds (no cysteine residues; see Table 2) [40], and the probability that its structure

Table 2
Protein adsorption

Proteins (No.)	100%−% Adsorbed (±S.D., n=3)	
	75 μm	100 μm
Lysozyme (1)	86±8	91±7
Myoglobin (2)	65±5	67±9
Ribonuclease A (3)	99±8	98±6
α-Lactalbumin (4)	96±5	93±10
Trypsinogen (5)	86±12	91±7
Average value[a]	86±8	88±8

[a] Average value determined for all five model proteins. Difference in peak area, as measured at the 37 cm vs. the 77 cm window (see Section 2).

and charge distribution would be perturbed is higher vs. the other four proteins studied. The broad trypsinogen peak might be the result of longitudinal diffusion [42] due to trypsinogen's relatively long migration time, its analyte–wall surface interactions [43], and because it is the largest protein investigated in this study. Other thermal factors (i.e., degradation) may play a role.

The electrophoretic currents were 56, 72, 80 and 96 μA in Fig. 2A–D, respectively. A comparison of the current (96 μA) between Fig. 1B and Fig. 2D indicates that TEA improves the separation of these five proteins versus ammonium formate without increasing the current. That comparison indicates that the relatively bulkier TEA^+ cation improves the electrophoretic resolution vs. the NH_4^+ cation. This relationship among current, resolution, and cation identity will become more important below when a larger I.D. (100 μm) capillary is used for scale-up separation.

The experimental data in Fig. 3 demonstrate the effect of increasing the ammonium formate concentration (10–40 m*M*) in the presence of a constant 20 m*M* TEA. The migration time difference between myoglobin (peak 2) and lysozyme (peak 1) decreases, and increases for myoglobin vs. ribonuclease A (peak 3), as the ammonium formate increases from 10 to 40 m*M*. The migration time of trypsinogen (peak 5) increases, but less so compared to Fig. 2. The migration times of peaks 1, 3 and 4 only shifted by an average maximum of 4% (vs. 22% in Fig. 2), suggesting that the TEA (20 m*M*) was more effective than ammonium formate in affecting (Fig. 2) and stabilizing μ_{eo} (Fig. 3). The resolution

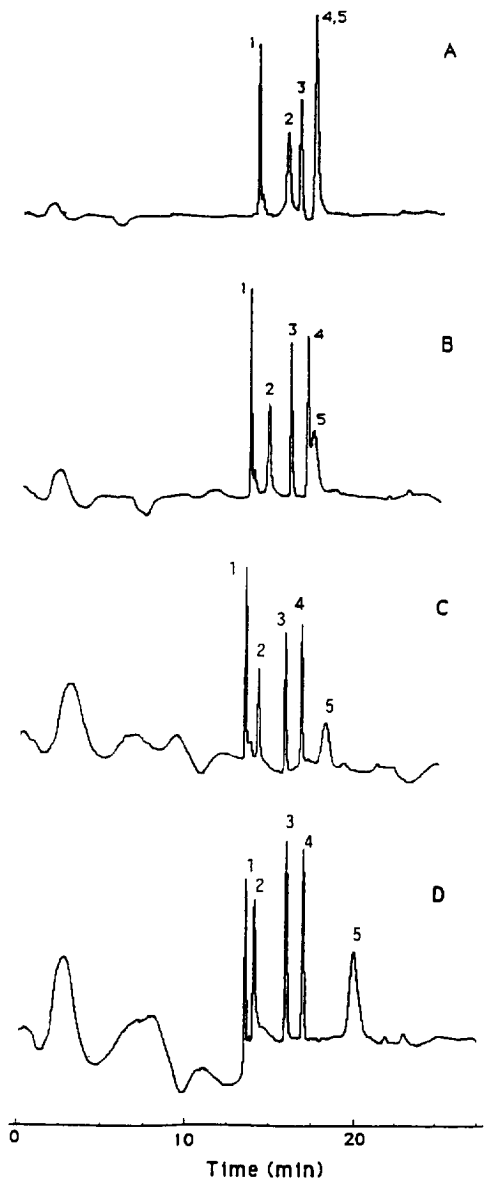

Fig. 3. Electropherograms of the mixture of five model proteins at four different ammonium formate concentrations. TEA concentration (20 m*M*) was constant, and the electrolyte pH was adjusted to 2.5. A 75 μm I.D. capillary was used. Monitored currents were 53, 70, 87, and 111 μA for A–D, respectively. The other experimental conditions are the same as in Fig. 1. The ammonium formate concentrations were 10, 20, 30, and 40 m*M* in A-D, respectively.

among lysozyme (peak 1), ribonuclease A (peak 3), and α-lactalbumin (peak 4) remains relatively unchanged. Finally, the currents were 53, 70, 87, and 111 μA for Fig. 3A–D, respectively. Compared to the electropherograms shown in Fig. 2C and D (80 and 96 μA, respectively), the currents are higher in the data shown in Fig. 3C and D.

In general, the data in Figs. 2 and 3 indicate that TEA at different concentrations modifies μ_{eo} (Fig. 2), but at a constant concentration controls and stabilizes μ_{eo} (Fig. 3), improves the resolution, and generates less current, whereas an increased ammonium formate concentration improves the peak height for trypsinogen, maintains a shorter separation time in the presence of TEA, but generates higher currents (Fig. 3C and D).

Collectively, these empirical data suggest that an equal concentration of the two electrolyte components at 30 m*M* would optimize the resolution, peak efficiency, current and separation time of these five proteins. To test that postulate, the following experiments were done. Under well-defined analytical conditions [using a 75 μm I.D. capillary with an injection volume of 30 nl (0.7% of column volume; 0.6 pmol of each protein)], migration time reproducibilities [1.0, 0.6, 0.7, 0.9 and 1.1% (R.S.D., $n=5$) for peaks 1–5, respectively] were obtained with this optimal buffer (data not shown). Therefore, the loading limit of this protein mixture was investigated by using this optimal buffer.

The injection volume (60, 230, and 470 nl) and injected amount (1.2, 4.8, and 9.7 pmol of each protein, respectively) were also both studied in a 75 μm I.D. capillary. As expected, the optimal buffer also gave a well-behaved electropherogram (Fig. 4A) when 60 nl (1.4% of capillary volume), corresponding to an average of 1.2 pmol of each protein, was injected. At an injection volume of 230 nl (5.2% of capillary volume; 4.8 pmol of each protein) (Fig. 4B), the separation was maintained. However, the separation deteriorated when 470 nl (10.7% of capillary volume), corresponding to 9.7 pmol of each protein, was injected (Fig. 4C). If we consider that the electrophoretic separation deteriorates somewhere between the amounts of protein used in Fig. 4B and C, then the maximal loading limit is in the range of 5–10 pmol of each protein for the 1 m long 75 μm I.D. capillary. These results agree with our previous study [27] that indicated that an injection volume much greater than 2% of the capillary volume may be used to increase loadability. It is also

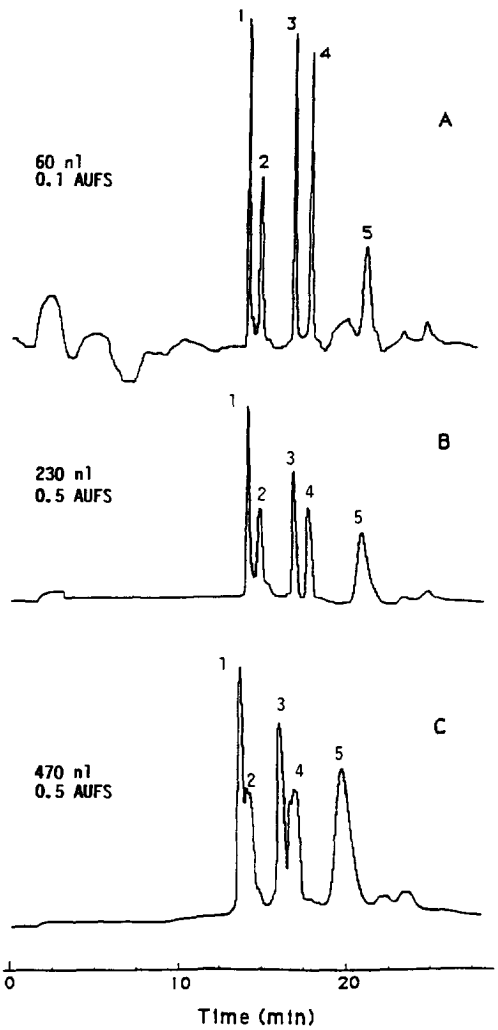

Fig. 4. Electropherograms of the mixture of five model proteins on a 75 μm I.D. capillary at three different injection volumes. Injection volumes (nl) and AUFS values (0.1, 0.5) are shown. The electrolyte was 30 m*M* each of TEA and ammonium formate, at pH 2.5. The amount of each protein is in the range of 1–1.5 (average, 1.2), 3.8–5.7 (4.8), and 7.8–11.6 (9.7) pmol for A–C, respectively. The monitored current was ca. 98 μA.

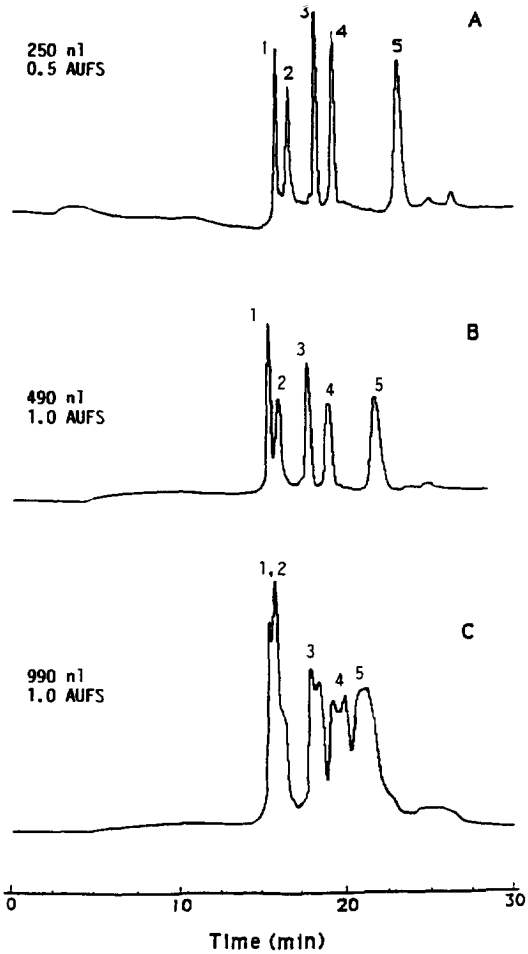

Fig. 5. Electropherograms of the mixture of five model proteins on a 100 μm I.D. capillary at three different injection volumes. Injection volumes (nl) and AUFS values (0.1, 0.5) are shown. The electrolyte is the same as that used in Fig. 4. The amount of each protein is in the range of 4.2–6.2 (average, 5.2), 8.2–12.1 (10.1), and 16.5–24.4 (20.5) pmol for A–C, respectively. The monitored current was ca. 154 μA.

observed that, in Fig. 4A–C, the peak height and area of trypsinogen varied much more compared to the other four proteins, suggesting that perhaps denaturation and interactions with the inner capillary wall existed for this protein under these experimental conditions (see also Table 2, below).

Fig. 5 contains the corresponding experiment for the 100 μm I.D. capillary. When an injection volume (250 nl; 3.2% of column volume) and an injected amount (5.2 pmol of each protein) comparable to Fig. 4B were used, a well-behaved electropherogram was also obtained on a 100 μm I.D. capillary (see data in Fig. 5A). When 490 nl (6.2% of column volume), corresponding to 10.1 pmol of each protein, was injected (Fig. 5B), separation was maintained, whereas separation at a comparable injection amount (Fig. 4C) in a 75 μm I.D. capillary was not. (Note also the two-fold difference in the AUFS between

Fig. 4C and Fig. 5B). If the separation deteriorates between the amounts shown in Fig. 5B and C (990 nl, 21 pmol each protein), then an upper loading limit in the range of 10–21 pmol of each protein is possible with the 100 μm I.D. capillary. This loadability is a two-fold improvement over the 75 μm I.D. capillary (which has a 4.4 μl column volume) in Fig. 4, in good agreement with the ca. two-fold increase in column volume (7.9 μl) of the 100 μm I.D. capillary.

In addition to the differences in the injection volumes used in Fig. 4 and Fig. 5, the different amounts of injected proteins that adsorbed onto the wall would modify μ_{eo}, and cause the observed shift of migration times. This factor was tested by the protein adsorption experiment (Table 2). The data in Table 2 demonstrated that the average amount of protein adsorbed onto the inner wall of the 75 μm I.D. capillary was 14%, and onto the 100 μm I.D. capillary was 12%. However, due to the relatively high coefficients of variation in these measurements, no firm conclusions can be drawn from that difference. It is interesting to note that myoglobin, which is the highest adsorbed protein, has the highest ratio of protonated (R, K, and H) amino acids-to-molecular mass, and also that isoforms of this protein are present (see below).

The data in Fig. 6 were used to test whether the deterioration of the separation observed in Fig. 4C and Fig. 5C was due to the large injection volume (ca. 10% of the column volume) or to the large amount of protein (9.7 and 20.5 pmol, respectively, of each protein) injected. The protein mixture was diluted (2×), and an injection volume of 470 nl (similar to Fig. 4C) was applied to a 75 μm I.D. capillary. Unlike Fig. 4C, this same injection volume gave the separation shown in Fig. 6, where proteins 3 and 4 were now completely separated, and 1 and 2 were somewhat more-separated compared to that separation seen in Fig. 4C. These results indicate that the decreased resolution observed in Fig. 4C was largely due to the large amount of protein injected and not due to the injection volume (as high as 10% of the capillary volume) under these experimental conditions. Also, the slower EOF observed in Fig. 6 may play a role.

Table 2 summarizes the protein adsorption study that was performed on the 75 and 100 μm I.D.

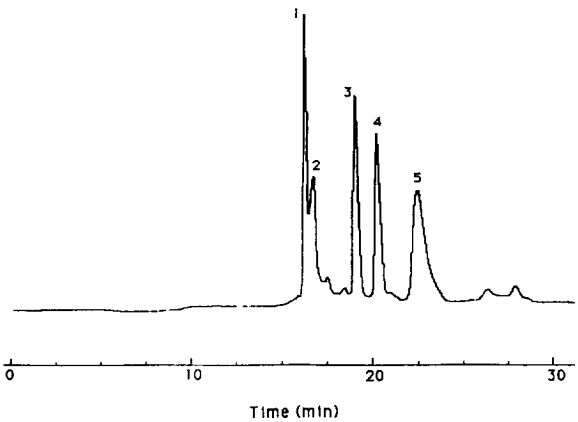

Fig. 6. Electropherogram of the mixture of five model proteins on a 75 μm I.D. capillary at an injection volume of 470 nl. The protein mixture shown in the legend of Fig. 4C was diluted (2×). AUFS is 0.5. The other experimental conditions are the same as shown in Fig. 4.

capillaries, using the optimized buffer. In addition to the well-recognized problem of reproducibility in CZE for quantitative analysis [44], the experimental manipulation that was performed with only one detector (see Section 2) contributed further to the large errors observed. For example, the coefficients of variation averaged 8.9% for the 75 μm, and 9.1% for the 100 μm columns. Nevertheless, Table 2 gives the average of the (triplicate) data, and indicates an overall adsorption of these five model proteins (average <15%) under these experimental conditions. The average adsorption (12%) obtained with the 100 μm I.D. capillary is slightly lower than that (14%) obtained with the 75 μm I.D. capillary. The cause of the high myoglobin adsorption (34%; average of the 75 and 100 μm I.D. data) is not fully understood; however, the potential differential electrophoretic separation of myoglobin's sub-types with different p*I* values (2–7 sub-types have been reported) [45] may play a role.

Acknowledgments

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References

[1] J.W. Jorgenson and K.D. Lukacs, Science, 222 (1983) 266–272.
[2] S. Hjertén, J. Chromatogr., 347 (1985) 191–198.
[3] S.A. Swedberg, Anal. Biochem., 185 (1990) 51–56.
[4] K.A. Cobb, V. Dolnik and M. Novotny, Anal. Chem., 62 (1990) 2478–2483.
[5] W. Nashabeh and Z.E. Rassi, J. Chromatogr, 559 (1991) 367–383.
[6] J.K. Towns, J. Bao and F.E. Regnier, J. Chromatogr., 599 (1992) 227–237.
[7] Z. Zhao, A. Malik and M.L. Lee, Anal. Chem., 65 (1993) 2747–2752.
[8] D. Schmalzing, C.A. Piggee, F. Foret, E. Carrilho and B.L. Karger, J. Chromatogr. A, 652 (1993) 149–159.
[9] T.-L. Huang, P.C.H. Shieh, E.V. Koh and N. Cooke, J. Chromatogr. A, 685 (1994) 313–320.
[10] H.H. Lauer and D. McManigill, Anal. Chem., 58 (1986) 166–170.
[11] R.M. McCormick, Anal. Chem., 60 (1988) 2322–2328.
[12] J.S. Green and J.W. Jorgenson, J. Chromatogr., 478 (1989) 63–70.
[13] F.-T.A. Chen, J. Chromatogr., 559 (1991) 445–453.
[14] M.M. Bushey and J.W. Jorgenson, J. Chromatogr., 480 (1989) 301–310.
[15] F.-T.A. Chen, L. Kelly, R. Palmieri, R. Biehler and H.E. Schwartz, J. Liq. Chromatogr., 15 (1992) 1143–1161.
[16] J.K. Towns and F.E. Regnier, Anal. Chem., 63 (1991) 1126–1132.
[17] Å. Emmer, M. Jansson and J. Roeraade, J. Chromatogr., 547 (1991) 544–550.
[18] J.E. Wiktorowicz and J.C. Colburn, Electrophoresis, 11 (1990) 769–773.
[19] H. Lindner, W. Helliger, A. Dirschlmayer, M. Jacquemar and B. Puschendorf, Biochem. J., 283 (1992) 467–471.
[20] Y.J. Yao and S.F.Y. Li, J. Chromatogr. A, 663 (1994) 97–104.
[21] D. Corradini, A. Rhomberg and C. Corradini, J. Chromatogr. A, 661 (1994) 305–313.
[22] N.A. Guzman, J. Moschera, K. Iqbal and A.W. Malick, J. Chromatogr., 608 (1992) 197–204.
[23] W. Kopaciewicz, M.A. Rounds, J. Fausnaugh and F.E. Regnier, J. Chromatogr., 266 (1983) 3–21.
[24] F.E. Regnier, Science, 238 (1987) 319–323.
[25] H.G. Lee and D.M. Desiderio, J. Chromatogr. A, 666 (1994) 271–283.
[26] H.G. Lee and D.M. Desiderio, J. Chromatogr. B, 655 (1994) 9–19.
[27] H.G. Lee and D.M. Desiderio, J. Chromatogr. B, 662 (1994) 35–45.
[28] H.G. Lee and D.M. Desiderio, J. Chromatogr. A, 686 (1994) 309–317.
[29] H.G. Lee, J.-L. Tseng, R.R. Becklin and D.M. Desiderio, Anal. Biochem., 229 (1995) 188–197.
[30] L. Yan, G. Fridland, J.-L. Tseng and D.M. Desiderio, Biochem. Biophys. Res. Commun., 196 (1993) 521–526.
[31] L. Yan, J.-L. Tseng, G.H. Fridland and D.M. Desiderio, Am. Soc. Mass Spectrom., 5 (1994) 377–386.
[32] G.H. Fridland and D.M. Desiderio, Anal. Lett., 27 (1994) 2117–2125.
[33] J.H. Wahl, D.R. Goodlett, H.R. Udseth and R.D. Smith, Anal. Chem., 64 (1992) 3194–3196.
[34] J.H. Wahl, D.C. Gale and R.D. Smith, J. Chromatogr. A, 659 (1994) 217–222.
[35] D.F. Hunt, J. Shabanowitz, M.A. Moseley, A.L. McCormack, H. Michel, P.A. Martino, K.B. Tomer and J.W. Jorgenson, in Jörnvall, Höög and Gustavsson (Editors), Methods in Protein Sequence Analysis, Birkhäuser, Basel, 1991, pp. 257–266.
[36] T.J. Thompson, F. Foret, P. Vouros and B.L. Karger, Anal. Chem., 65 (1993) 900–906.
[37] R.B. Cole, J. Varghese, R.M. McCormick and D. Kadlecek, J. Chromatogr. A, 680 (1994) 363–373.
[38] N. Cohen and E. Grushka, J. Chromatogr. A, 678 (1994) 167–175.
[39] M.O. Dayhoff, Atlas of Protein Sequence and Structure; National Biomedical Research Foundation Washington, DC, 1972, pp. D-82, 105, 130, 136, and 138.
[40] K. Salomon, D.S. Burgi and J.C. Helmer, J. Chromatogr., 559 (1991) 69–80.
[41] H.J. Issaq, I.Z. Atamma, G.M. Muschik and G.M. Janini, Chromatographia, 32 (1991) 155–161.
[42] X. Huang, W.F. Coleman and R.N. Zare, J. Chromatogr., 480 (1989) 95–110.
[43] M.R. Schure and A.M. Lenhoff, Anal. Chem., 65 (1993) 3024–3037.
[44] E.V. Dose and G. Guiochon, Anal. Chem., 64 (1992) 123–128.
[45] P.G. Righetti and T. Caravaggio, J. Chromatogr., 127 (1976) 1–28.