ELSEVIER

Journal of Chromatography A, 680 (1994) 363–373

JOURNAL OF CHROMATOGRAPHY A

Evaluation of a novel hydrophilic derivatized capillary for protein analysis by capillary electrophoresis–electrospray mass spectrometry

Richard B. Cole*[,a], Johnson Varghese[a], Randy M. McCormick[b], Douglas Kadlecek[b]

[a]*Department of Chemistry, University of New Orleans, New Orleans, LA 70148, USA*
[b]*Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94088-3603, USA*

Abstract

A new type of hydrophilic derivatized capillary has been used to enable the on-line capillary electrophoresis separation and electrospray mass spectrometric detection of a mixture of proteins containing bovine cytochrome *c*, tuna cytochrome *c* and horse heart myoglobin. Less than 40 fmol of each compound were loaded into the capillary. Baseline resolution of components was achieved, as were accurate assignments of molecular masses. The hydrophilic derivatized capillaries were taken through extensive testing procedures to characterize their performance and capabilities for protein analysis. A mixture of six proteins (cytochrome *c*, ribonuclease A, α-chymotrypsinogen, myoglobin, carbonic anhydrase II and α-lactalbumin) in acetic acid–sodium acetate buffer was used to delineate the relationships between migration time and pH, along with migration time and buffer concentration for each protein. The variations in capillary efficiency as a function of pH and as a function of buffer concentration were also characterized for the same six proteins in the acetic acid–sodium acetate system. A pH of 4.8 was found to offer an excellent compromise between separation efficiency (up to 500 000 theoretical plates) and analysis time. Capillary efficiencies were also found to be very good when employing a Tris · HCl electrolyte adjusted to pH 4.8. Lastly, electropherogram reproducibility and capillary durability were examined with the finding that little deterioration of the capillary occurred over the course of 400 injections (200 h run time). This represents a notable improvement over previously documented derivatization procedures designed to reduce protein adsorption to fused-silica capillary walls.

1. Introduction

Simultaneous biotechnological advances in the fields of separation science and mass spectrometry (MS) have led to bright new prospects for direct analyses of complex biological sample mixtures. The development of microcapillary separation techniques such as capillary electrophoresis (CE), and the acceptance of electrospray ionization (ES) MS as a highly sensitive method of choice for the identification of molecular masses of unknown biopolymers have opened new possibilities for trace detection and identification of biological species, particularly polypeptides and proteins [1,2]. ES-MS produces a series of multiply charged ions corresponding to intact protein species with different numbers of protons attached. Unknown molecular masses

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00405-X

and charge states are readily determined by ES-MS provided that the peaks corresponding to multiply charged ions from a particular species can be distinguished from extraneous peaks in the mass spectrum. The ability to do this is aided by the fact that consecutive, integral charge states are always present in the "envelope" of peaks arising from biopolymers which give good ES-MS responses.

In general, as a detection method for CE, ES-MS imposes some restrictions on CE operation. For example, the choice of buffers is limited to those of high volatility so as to improve sensitivity and to avoid problems of fouling of the mass spectrometer ion source. Furthermore, the overall buffer composition must be of relatively low ionic strength to minimize conductance down the capillary. This serves both to diminish the negative effects of Joule heating on capillary efficiency, and to ensure that voltage fluctuations do not occur at the electrospray needle. Such fluctuations will inevitably result in severe noise "spikes" on the generated ion current electropherograms.

Approaches to interfacing CE systems to electrospray mass spectrometers include the "liquid sheath" electrode [3] where a "make-up" flow of liquid is mixed with the CE effluent immediately prior to ES-MS analysis. The additional volume increases the total solution flow to a level of a few μl/min which is compatible with the attainment of a stable spray condition. An alternative means of linking CE to MS is via the liquid junction interface [4]. This interface offers the advantage of decoupling, to some degree, the CE voltage from the ES voltage. This can somewhat alleviate the aforementioned problem of voltage variations at the two high-voltage sources. A disadvantage of having a "break" in the capillary, however, is that some band broadening occurs at the liquid junction, and some sample is inevitably lost.

The development of CE, and thus also CE–ES-MS, for protein analysis has been hampered by the tendency for highly charged proteins to adsorb onto the walls of the fused-silica capillaries commonly employed in CE analyses. To circumvent this problem, analysts have performed separations at pH values above the isoelectric points of analyte proteins, so as to create a net coulombic repulsion between the proteins and the capillary wall [5]. Another method was to use a phosphoric acid buffer whereby available phosphate moieties would bind to silanol sites, thus improving the capacity of these sites to accept a proton at low pH [6]. The magnitude of the negative charge on the capillary wall was significantly reduced, as was the electroosmotic flow. The use of buffers containing high salt concentrations has also been shown to minimize adsorption of proteins by a cation-exchange mechanism at sites on the silica surface [7]. Rapid and high-efficiency separation of proteins by free solution CE is also possible via the use of high-concentration borate buffers at elevated pH values [8,9].

Each of the above approaches relies upon the manipulation of the CE buffer composition to reduce protein adsorption. The fact that each employs high concentrations of non-volatile buffers renders these methods rather unsuitable for on-line CE–ES-MS analyses. Alternative approaches to overcoming the problem of CE wall adsorption include the development of coated capillaries employing bonded phases such as non-cross-linked polyacrylamide [10], silanes [6], polyethylene glycol [11] and a pentafluorinated arene [12]. Coated capillaries, in particular hydrophilic types, offer the advantage of more flexibility with respect to the choice of buffer. Cationic functional groups [e.g., aminopropylsilyl (APS) substituents] have also been employed in the fabrication of capillary coatings [13,14]. The cationic bonded phase, attached to what were previously silanol sites, effectively reverses the overall polarity of the capillary wall. This changes the direction of electroosmotic flow, necessitating "reversed polarity" CE operation (i.e., negative potential at the source vial). At pH values below the isoelectric point of a particular protein, the protein will experience net coulombic repulsion from the positively charged capillary wall. While representing a significant advance for CE–MS protein analyses,

the APS capillaries suffered from rather rapid decomposition of attached cationic sites. This resulted in variable migration times as the capillary surface could change substantially, even between consecutive runs. While this is not always important for CE–MS analyses, i.e., often only resolution of neighboring bands and mass spectral response are critical, the overall lifetimes of the APS capillaries were rather short.

Another approach to overcoming the problem of adsorption to the silica wall in CE analysis was to add a cationic surfactant to the buffer. At surfactant concentrations above the "critical micelle concentration", analyses enter into the domain of micellar electrokinetic capillary chromatography [15]. Tetraalkylammonium salts have been used frequently in this capacity. Added surfactant adsorbs to the inside surface of the fused-silica capillary to effectively create a positively charged capillary wall. As in the case of the positively charged bonded phase, the direction of electroosmotic flow is opposite to that of bare capillaries, and reversed polarity operation must be employed. Recent work by Varghese and Cole [16] demonstrated the validity of the surfactant buffer additive approach for CE–ES-MS analysis of peptides having molecular masses up to 1645. In that study, cetyltrimethylammonium chloride was the employed surfactant. The main limitation of the surfactant additive approach to CE–ES-MS analysis was that the surfactant was constantly infusing into the mass spectrometer ion source, thus suppressing analyte ion formation and limiting sensitivity. In addition, build-up of the surfactant in the ES ion source necessitated daily counterelectrode cleaning.

In this paper, a new approach to CE–ES-MS analysis has been taken. Capillaries coated with a hydrophilic polymer have been employed for protein analyses. This paper is a report on the performance characteristics of the novel derivatized capillaries including relative protein migration times as a function of pH and buffer concentration, capillary efficiencies as a function of pH and buffer concentration, and capillary lifetimes as measured by the constancy of retention times after repeated injections. Examples of CE–UV and CE–ES-MS separations of protein mixtures are also presented.

2. Experimental

2.1. CE–UV absorbance detection

For CE–UV separations, a Model CES-I capillary electrophoresis system (Dionex, Sunnyvale, CA, USA) was used. Samples were introduced into the 50 cm (45 cm anode to detector) × 75 μm I.D. × 375 μm O.D. capillary via hydrodynamic injections of protein mixtures for 10 s at a 50 mm height differential. Starting protein sample concentrations were 80–250 μg/ml. Separation voltage was typically 20 kV (positive polarity at source vial) and the absorbance detection wavelength was 215 nm. The capillary was automatically rinsed with fresh buffer for 3 min before each run. Capillary cooling during the run was implemented by surrounding the capillary with a forced-air cooling jacket. Electropherograms were recorded on an AI-450 data system (Dionex) at a digitization rate of 5 or 10 Hz.

2.2. CE–ES-MS

On-line CE–ES-MS was performed using a Dionex Model CES-I capillary electrophoresis system coupled to a Vestec 201 quadrupole mass spectrometer (Vestec, Houston, TX, USA) equipped with an electrospray ionization source [17]. The mass spectrometer data system was a Vector 2 system (Teknivent Corp., Maryland Heights, MO, USA). The CE–ES-MS interface employed in this study has been described previously [18]. One instrumental modification relative to the design used in prior CE–ES-MS studies was that the "nozzle" normally used as a counterelectrode for the electrospray "needle" was replaced by a flat stainless-steel plate with an aperture of 0.4 mm. To maintain adequate pumping in the first stage of the ion source, and

to enable an appropriate needle-to-counterelectrode distance for electrospray operation, the spacer which guides the movement of the counterelectrode on the interior of the source was modified according to manufacturer specifications to accommodate the stainless-steel plate. The stainless-steel electrospray "needle" used for CE–ES-MS analyses had an I.D. of 400 μm and an O.D. of 800 μm. This rather large needle was needed to accommodate the 100 cm × 375 μm O.D. × 75 μm I.D. derivatized CE capillary. All CE–UV and CE–ES-MS data represent raw data which have not been subjected to post-acquisition processing. Conditions for each acquisition are given in figure legends.

2.3. Capillaries and chemicals

Capillaries (Protophor B1) were prepared at Dionex; the capillaries consist of a coating of high-molecular-mass charge-neutral polymer attached to the walls of a fused-silica capillary. Details of capillary preparation are the subject of a separate report [19]. Buffers were prepared from reagent-grade salts and acids in 18 MΩ cm deionized water; buffers were filtered through 0.22-μm filtration membranes and degassed briefly via ultrasonification before use. All proteins used in this study were purchased from Sigma (St. Louis, MO, USA). Proteins were dissolved in deionized water and then centrifuged briefly to remove residual particulate materials.

3. Results and discussion

Initial phases of this investigation were centered around evaluation of the performance, utility and long-term stability of the coated capillaries. A number of factors such as buffer pH and ionic strength, buffer salts and separation voltage were optimized before testing the utility of the capillaries for on-line CE–ES-MS analyses. During the initial phase of this testing, UV absorbance detection was utilized to expedite the evaluation process and to avoid the complexities of on-line MS detection.

The effect of buffer pH on the migration times of six proteins comprising a test mixture is shown in Fig. 1. In the hydrophilic derivatized capillaries, adsorption of highly protonated species to the capillary wall is not severe, resulting in rapid elution with symmetrical peak shapes. In the pH range of 2.1 to 3.8, migration times do not vary significantly, indicating that overall, the degree of protonation and the electroosmotic flow are rather constant in this range. As the pH is raised above 3.8, the migration times of all proteins increase, indicative of the lower degree of protonation of protein species, while separation of the proteins improves dramatically. At the higher pH values employed (e.g., pH 5 to 7), increasing migration times correlate roughly with decreasing isoelectric point (pI) values of the proteins, i.e., those proteins with higher pI values are observed to migrate faster. The order of elution in the pH range of 5 to 7 was cytochrome c (pI = 10.7), ribonuclease A (pI = 9.3), α-chymotrypsinogen (pI = 9.2), myoglobin (pI = 7.1), carbonic anhydrase II (pI = 6.6) and α-lactalbumin (pI = 4.8). The relatively long migration times at higher pH and the upward slopes of the migration time vs. pH curves are indicative of a high dependence of migration time on electrophoretic mobility, and a reduced electroosmotic flow compared to non-derivatized fused-silica capillaries.

Electrophoretic resolution of the various test proteins comprising the mixture did not improve significantly as the buffer pH was raised from 2.1 to 3.8. Above pH 3.8, the lower degree of protonation lengthened migration times and resolution improved substantially. As will be shown in more detail below, peak efficiencies for the proteins generally decreased above pH 4.8. An operating buffer of pH 4.8 thus offers the best compromise of resolution and analysis time for separation of the test proteins. Using acetic acid–sodium acetate buffers at pH 4.8 (25 and 50 mM), electropherograms of the mixture of test proteins are shown in Fig. 2. The efficiency and peak shape for each of the proteins are excellent. Migration times increased significantly for the buffer of elevated ionic strength (Fig. 2b). More detail describing the relationship between migra-

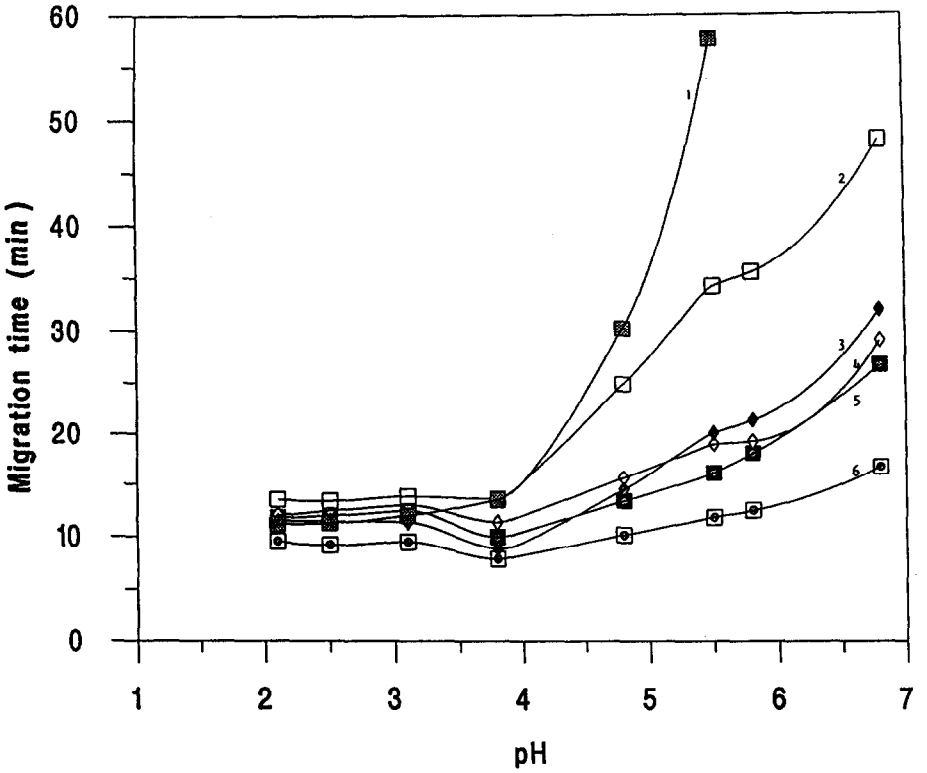

Fig. 1. Effect of buffer pH on the migration time of six standard proteins [cytochrome *c* (bovine) (6), ribonuclease A (bovine) (5), α-chymotrypsinogen (bovine) (4), myoglobin (horse heart) (3), carbonic anhydrase II (bovine) (2), and α-lactalbumin (bovine) (1)] using the hydrophilic derivatized capillary. The buffers employed were as follows: pH 2.1–3.1, 50 m*M* phosphoric acid–sodium dihydrogenphosphate; pH 3.8–5.5, 50 m*M* acetic acid–sodium acetate; pH 5.8–6.8, 50 m*M* sodium dihydrogenphosphate–disodium hydrogenphosphate.

tion time and log buffer concentration is shown in Fig. 3. The data displayed in this figure were obtained on a separate occasion from data shown in Fig. 2, using a hydrophilic derivatized capillary from a different lot. Migration times for proteins in acetic acid–sodium acetate buffers at three concentration levels (25, 50 and 100 m*M*) readily increase with buffer ionic strength, which is consistent with a decreased electrophoretic mobility and a decreased electroosmotic flow. The dependence of mobility on buffer concentration (also ionic strength) in electrophoresis has been studied by several researchers [11,20,21]. These reports document an inverse relationship between mobility and buffer concentration. Experiments by Altria and Simpson [20] determined that a plot of electroosmotic flow versus log surfactant concentration was linear (negative slope). The influence of buffer concentration on the rate of electroosmotic flow was also examined by VanOrman et al. [22]. That study demonstrated that electroosmotic flow decreases linearly with increasing log buffer concentration for inorganic buffers such as phosphate and borate. Electrophoretic mobility in CE has been described as being proportional to $C^{-1/2}$ where C is the buffer concentration [23]. Electroosmotic flow in the capillaries used in the current study is rather low because the capillary wall is derivatized with a neutral polymer and the buffer pH (4.8) is relatively low. Changes in ionic strength of the buffer are thus likely to principally affect the electrophoretic mobility of the proteins under examination.

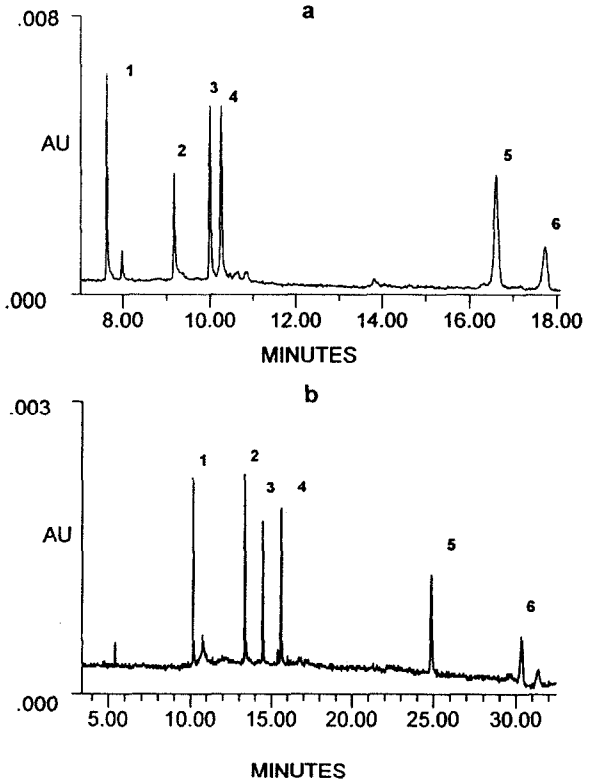

Fig. 2. Separation of six standard proteins: (a) 25 m*M* acetic acid–sodium acetate pH 4.8 buffer; (b) 50 m*M* acetic acid–sodium acetate pH 4.8 buffer. The following conditions pertain to both a and b. Sample: 83 ng/μl of each protein: 1 = cytochrome *c*; 2 = ribonuclease A; 3 = myoglobin; 4 = α-chymotrypsinogen; 5 = carbonic anhydrase II; 6 = α-lactalbumin. CE voltage: 15 kV, positive polarity; detection: 215 nm UV absorbance; injection: hydrodynamic, 5 s at 25 mm.

The mixture of the six proteins was also used to evaluate capillary efficiency as a function of pH. The relationship between pH and efficiency for each protein is shown as a three-dimensional plot in Fig. 4. As demonstrated in the figure, efficiency is quite variable from one protein to another. Most of the proteins, however, exhibit efficiency maxima in the pH range of 3.1 to 5.5, supporting the choice of this as the optimum region to perform analyses of these compounds. Separation efficiencies in excess of 300 000 theoretical plates were routinely achieved for all proteins in this study. α-Lactalbumin, which is an acidic protein (p*I* = 4.8), generally yielded lower efficiencies than the other model proteins which are characterized by neutral or basic p*I* values.

Further evaluation of capillary efficiency was undertaken by varying buffer concentration while maintaining a constant pH of 4.8. Fig. 5 is a bar plot of capillary efficiency for five of the test proteins as a function of acetic acid–sodium acetate buffer concentration (25, 50 and 100 m*M*). Efficiencies up to 500 000 theoretical plates were obtained for certain proteins. For four of the five proteins, efficiency was lowest when the buffer concentration was highest. It is likely that Joule heating is a major factor contributing to the loss of efficiency at higher buffer concentrations, as the problem of Joule heating is expected to increase with increasing ionic strength of the solution. Capillary efficiencies were also evaluated using a tris(hydroxymethyl)aminomethane hydrochloride–tris(hydroxymethyl)aminomethane (Tris · HCl) electrolyte adjusted to pH 4.8 (Table 1). Although still impressive, efficiencies were lower using Tris · HCl as compared to the acetic acid–sodium acetate buffer. In general, lower-molecular-mass, higher-volatility buffer constituents are more suitable for use in on-line CE–ES-MS because they are readily pumped away by the vacuum system of the mass spectrometer. Acetic acid and ammonium acetate are often used in this capacity.

Three of the test proteins previously employed (cytochrome *c*, ribonuclease A and α-chymotrypsinogen) were used to evaluate capillary lifetime. Repetitive injections of the three-component mixture were run for 400 analyses using a single capillary which was subjected to the rinse procedure outlined in the Experimental section. Migration times for each protein were measured in a running buffer of acetic acid–sodium acetate at pH 4.8. Fig. 6 shows excellent reproducibility of migration times observed for the 400 injections, representing 200 h of actual run time. The variation in peak efficiency over the course of this evaluation was about 15%. Deterioration of the coating in the derivatized capillary thus appears to be quite minor over the duration of data acquisition.

Relative to the UV absorbance detection used

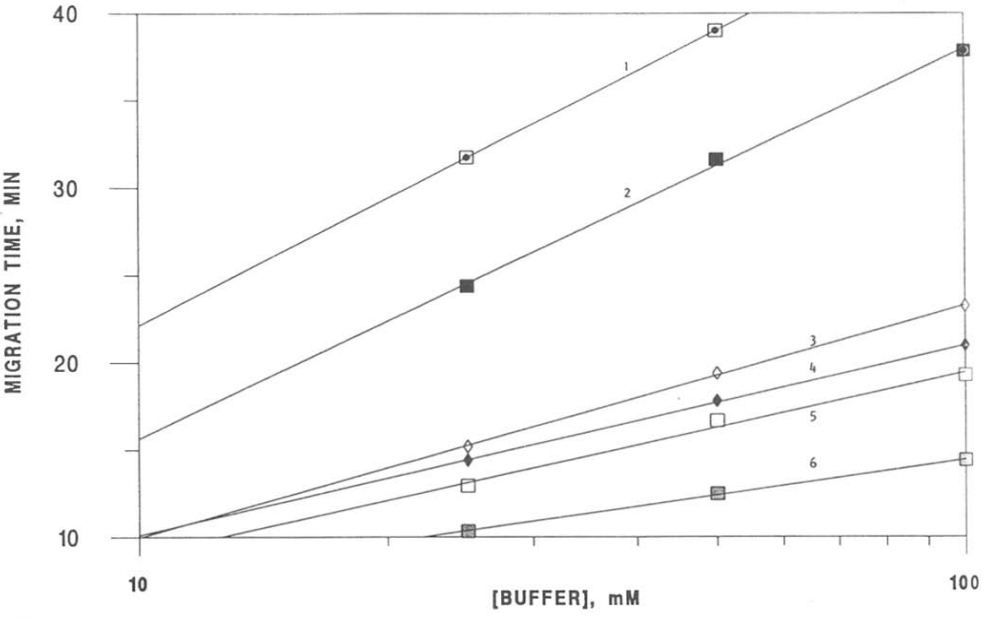

Fig. 3. Effect of buffer concentration on the migration times of six standard proteins. All buffers were acetic acid–sodium acetate pH 4.8. Concentration is plotted using a log scale with points at 25, 50 and 100 m*M*. 1 = α-Lactalbumin; 2 = carbonic anhydrase II; 3 = myoglobin; 4 = α-chymotrypsinogen; 5 = ribonuclease A; 6 = cytochrome *c*.

in the previous experiments, much higher specificity in detection, of course, will be offered by MS. For most compounds subjected to positive-ion ES-MS, cation attachment to neutral molecules will occur to form ionic species. Under adjustable conditions, fragmentation via collision-induced dissociation can be promoted,

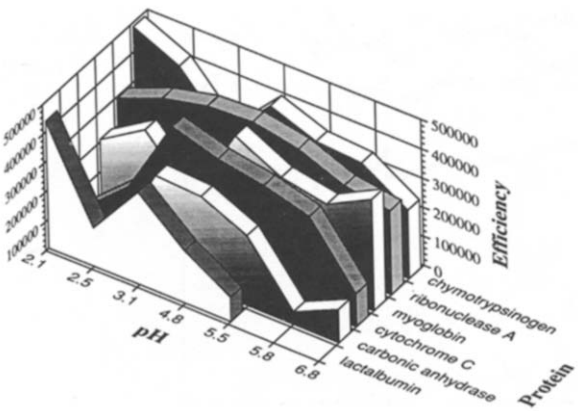

Fig. 4. Effect of buffer pH on capillary efficiency (theoretical plates) for six standard proteins. The buffers employed were identical to those used in Fig. 1. Each point represents the average efficiency value obtained from six injections of the mixed protein standard.

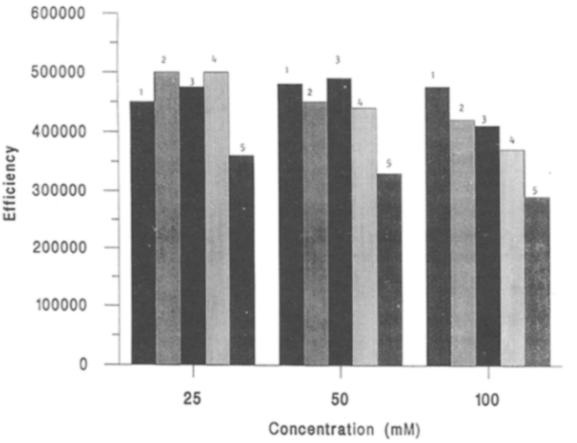

Fig. 5. Effect of buffer concentration on capillary efficiency (theoretical plates) for five standard proteins. The buffer was acetic acid–sodium acetate pH 4.8, ranging in concentration from 25 to 100 m*M*. 1 = Cytochrome *c*; 2 = ribonuclease A; 3 = α-chymotrypsinogen; 4 = myoglobin; 5 = carbonic anhydrase II.

Table 1
Average migration times and capillary efficiencies of basic protein standards analyzed with a hydrophilic derivatized capillary using a 50 m*M* Tris · HCl electrolyte adjusted to pH 4.8

Protein	Migration time (min)	Efficiency (number of plates)
Cytochrome *c*	11.2 ± 3.49%	297 000 ± 40.2%
Ribonuclease A	14.8 ± 2.64%	228 000 ± 51.6%
α-Chymotrypsinogen	17.0 ± 2.99%	210 000 ± 85.9%

CE voltage 20 kV.

thus producing a mass spectrum for each individual compound which elutes from the CE capillary. Optimal CE conditions, however, are not always compatible with satisfactory ES-MS conditions. For example, extremely sharp peaks which have widths narrower than a few seconds will not always permit the acquisition of multiple quadrupole mass spectrometer scans to enable verification of the presence of ion signals and to allow signal averaging. In extreme cases, the entire peak may elute before a single full MS scan can be obtained, leading to an incomplete mass spectrum which may be missing the major peak(s) of interest. For this and possibly other reasons related to the highly specific nature of MS detection, analysts are often less demanding in terms of separation performance for CE–MS analyses than for cases where CE–absorbance, CE–fluorescence or CE–electrochemical detection systems are used.

Shown in Figs. 7 and 8 is the on-line CE–ES-MS analysis of a mixture of three proteins, i.e., cytochrome *c* from bovine, cytochrome *c* from tuna, and myoglobin from horse heart. These compounds were present in the original sample solution at 50, 50 and 100 ng/μl, respectively, and they range in molecular mass from about 12 000 to 17 000. Electrokinetic injection was employed (+ 10 kV for 7 s), and the quantity of each compound loaded into the capillary was derived from the apparent solute mobilities of each component which were determined after

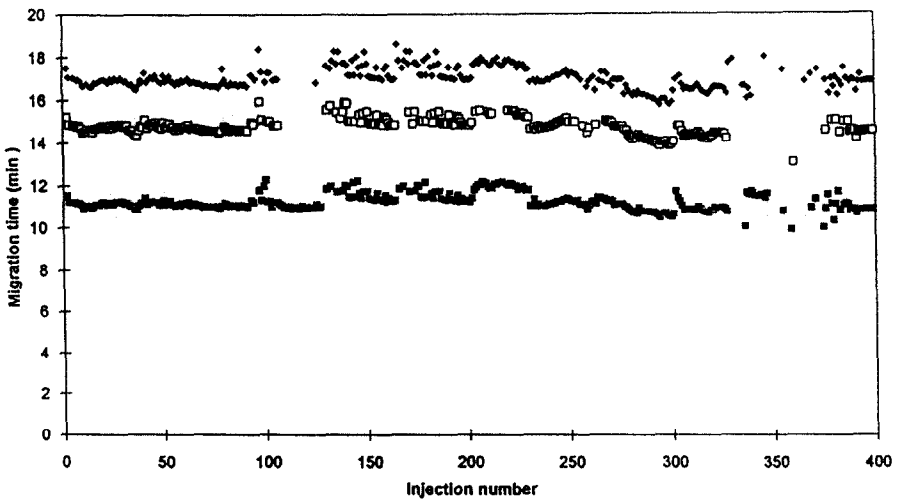

Fig. 6. Lifetime testing of the hydrophilic derivatized capillary via consecutive injections of a protein standard solution consisting of cytochrome *c* (bovine) (■), ribonuclease A (bovine) (□) and α-chymotrypsinogen (bovine) (◆). The concentration of each protein was 170 ng/μl; the buffer was pH 4.8 acetic acid–sodium acetate.

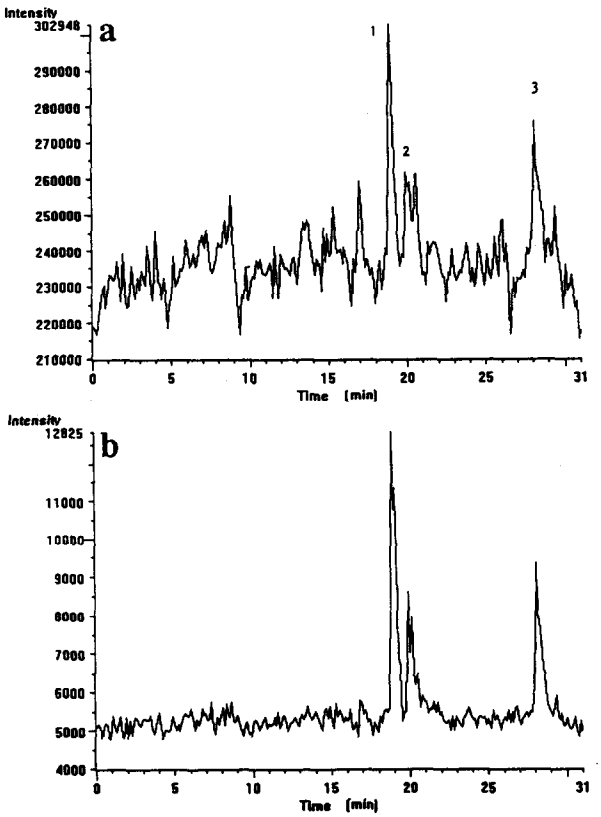

Fig. 7. On-line CE–ES-MS of a protein mixture containing cytochrome *c* (bovine) (1, M_r = 12 231), cytochrome *c* (tuna) (2, M_r = 12 029) and myoglobin (horse heart) (3, M_r = 16 951). CE voltage: + 30 kV; buffer: acetic acid–ammonium acetate pH 4.8; capillary length: 100 cm; electrokinetic injection: 10 kV, 7 s. (a) Reconstructed ion electropherogram showing ion current from *m/z* 850 to 1250; (b) selected ion electropherogram from the same run showing sum of ion current from the three most abundant peaks (see Fig. 8) in the mass spectrum from each protein (sum of nine peak intensities in total).

the run had elapsed. Calculation estimated that 37 fmol, 36 fmol and 36 fmol of cytochrome *c* (bovine), cytochrome *c* (tuna) and myoglobin, respectively, had been loaded into the capillary.

Fig. 7a displays the reconstructed ion electropherogram of mass spectrometer scans from *m/z* 850 to 1250. From the same run, the selected ion electropherogram composed of the three most abundant ions from each protein is shown in Fig. 7b. The two cytochrome *c* variants were separated from one another with baseline

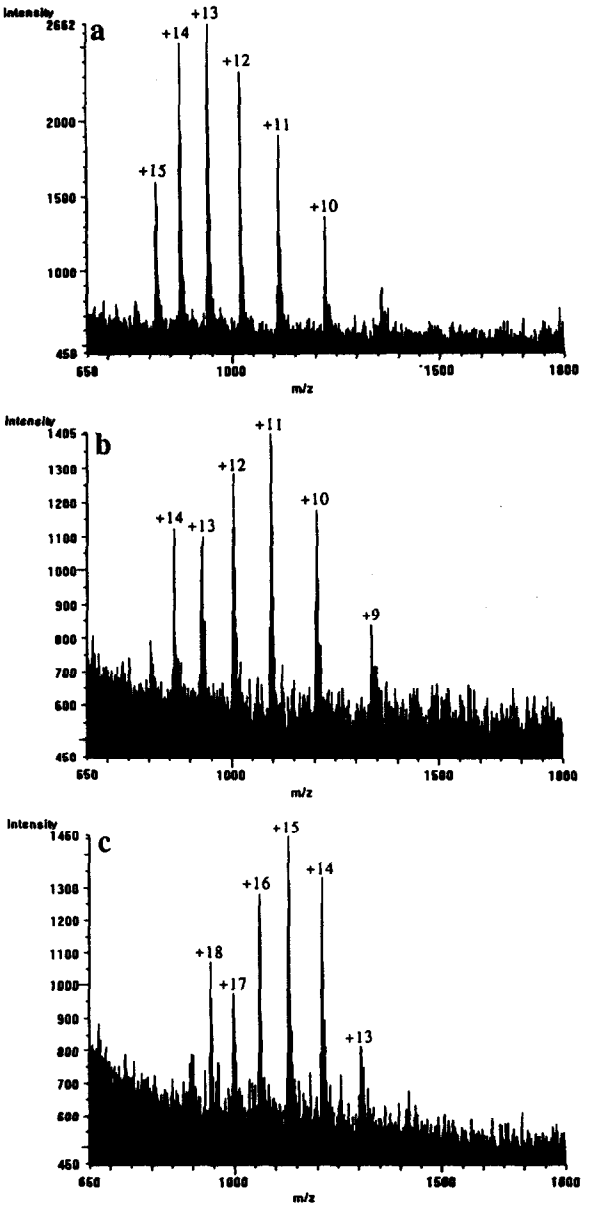

Fig. 8. Electrospray mass spectra from same on-line CE–ES-MS run as shown in Fig. 7. Electrospray needle voltage: 2.69 kV; counterelectrode plate: 315 V; sheath flow: methanol–water–acetic acid (60:37:3) at 11 μl/min. (a) On-line electrospray mass spectrum of cytochrome *c* (bovine) comprised of four scans from 18.83 to 19.19 min (peak 1); (b) on-line electrospray mass spectrum of cytochrome *c* (tuna) comprised of four scans from 19.90 to 20.26 min (peak 2); (c) on-line electrospray mass spectrum of myoglobin (horse heart) comprised of four scans from 28.07 to 28.43 min (peak 3).

resolution, and each eluted well before myoglobin, which has a lower p*I* value. As each protein exited the CE capillary, mass spectra were obtained (Fig. 8a–c) revealing the characteristic "envelope" of peaks corresponding to intact protein molecules in various charge states, i.e., molecules which have different numbers of protons attached. Each protein could be detected during four consecutive 5-s scans, which is quite adequate for statistical purposes of ensuring that ions are indeed representative of eluting species (usually a minimum of three MS scans is desired). As in most CE–MS work, the peak widths (Fig. 7) are somewhat wider (e.g., 15–20 s) than what would be expected under optimal efficiency CE conditions. Deconvolution of the labeled mass spectral peaks corresponding to multiply charged intact protein molecules allowed assignment of charge states for individual peaks and calculation of the molecular masses of each protein. The molecular mass of cytochrome *c* from bovine (Fig. 8a) was determined to be 12 231, cytochrome *c* from tuna (Fig. 8b) was found to be 12 030, and myoglobin from horse heart (Fig. 8c) was calculated as 16 951. Literature values [24] for the molecular masses of the three proteins are 12 231, 12 029 and 16 951, respectively. Although the sensitivity of detection was quite adequate for many applications (< 40 fmol of each component was injected onto the capillary), it is possible that even better sensitivity may be achieved by moving to smaller inner diameter capillaries [25].

4. Conclusions

The hydrophilic derivatized capillaries used in this study were found to be quite effective at alleviating the problems of adsorption associated with standard fused-silica capillaries while also allowing reasonable migration times for proteins having p*I* values in the range of 4.8–11.5. A buffer pH of about 4.8 was shown to provide excellent separations and capillary efficiencies for a mixture of six proteins. The reproducibility of migration times was determined to be excellent over the course of 400 repeat injections, indicating that decomposition of the derivatized capillaries was quite minor. These capillary characteristics are highly desirable for on-line CE–ES-MS coupling where a high degree of specificity in detection is gained over other CE detection systems. The CE–ES-MS separation of a mixture of three proteins present at 50–100 ng/μl concentration levels (original solution) from which less than 40 fmol of each component was loaded into the capillary was demonstrated. Compared to CE–ES-MS experiments employing other types of derivatized fused-silica capillaries also designed to reduce protein adsorption to the capillary wall, the reproducibility of electropherograms obtained using the hydrophilic derivatized capillary is vastly improved, and the lifetime of the capillary is greatly extended. In addition, no cationic additives constantly enter the mass spectrometer source as happens in CE–ES-MS systems when employing a positively charged surfactant buffer additive to reduce analyte adsorption to the capillary wall. The absence of buffer additives and capillary decomposition products infusing into the mass spectrometer improves sensitivities for all analytes, and diminishes the frequency of mass spectrometer source cleaning.

Acknowledgement

R.B.C. and J.V. thank the NIH for financial support through grant P01-DC00379.

References

[1] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong and C.M. Whitehouse, *Mass Spectrom. Rev.*, 9 (1990) 37.
[2] R.D. Smith, J.A. Loo, C.G. Edmonds, C.J. Barinaga and H.R. Udseth, *Anal. Chem.*, 62 (1990) 882.
[3] R.D. Smith, C.J. Barinaga and H.R. Udseth, *Anal. Chem.*, 60 (1988) 1948.
[4] E.D. Lee, W. Muck, J.D. Henion and T.R. Covey, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 253.
[5] H.H. Lauer and D. McManigill, *Anal. Chem.*, 58 (1986) 166.
[6] R.M. McCormick, *Anal. Chem.*, 60 (1988) 2322.
[7] J.S. Green and J.W. Jorgenson, *J. Chromatogr.*, 478 (1989) 63.

[8] K.-J. Lee and G.S. Heo, *J. Chromatogr.*, 559 (1991) 317.
[9] F.-T.A. Chen, *J. Chromatogr.*, 559 (1991) 445.
[10] S. Hjertén, *J. Chromatogr.*, 347 (1985) 191.
[11] G.J.M. Bruin, J. Chang, R. Kuhlman, K. Zegers, J. Kraak and H. Poppe, *J. Chromatogr.*, 471 (1989) 429.
[12] S.A. Swedberg, *Anal. Biochem.*, 185 (1990) 51.
[13] M.A. Moseley, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, *Anal. Chem.*, 63 (1991) 109.
[14] M.A. Moseley, J.W. Jorgenson, J. Shabanowitz, D.F. Hunt and K.B. Tomer, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 289.
[15] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
[16] J. Varghese and R.B. Cole, *J. Chromatogr. A*, 652 (1993) 369.
[17] M.H. Allen and M.L. Vestal, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 18.
[18] J. Varghese and R.B. Cole, *J. Chromatogr.*, 639 (1993) 303.
[19] R.M. McCormick, in preparation.
[20] K.D. Altria and C.F. Simpson, *Chromatographia*, 24 (1987) 527.
[21] W. Nashabeh and Z. El Rassi, *J. Chromatogr.*, 514 (1990) 57.
[22] B.B. VanOrman, G.G. Liversidge and G.L. McIntire, *J. Microcol. Sep.*, 2 (1991) 176.
[23] H.J. Issaq, I.Z. Atamna, G.M. Muschik and G.M. Janini, *Chromatographia*, 32 (1991) 155.
[24] R.D. Smith, J.A. Loo, R.R. Ogorzalek Loo, M. Busman and H.R. Udseth, *Mass Spectrom. Rev.*, 10 (1991) 359.
[25] J.H. Wahl, D.R. Goodlett, H.R. Udseth and R.D. Smith, *Anal. Chem.*, 64 (1992) 3194.