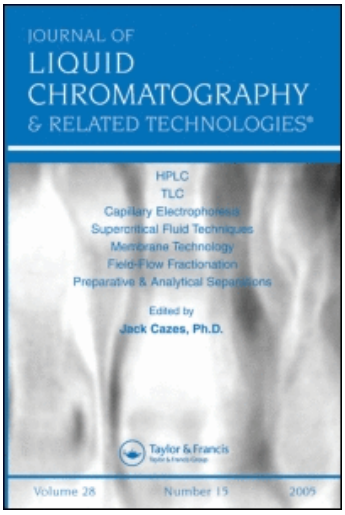


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# IMPROVED PEPTIDE MAPPING BY CAPILLARY ZONE ELECTROPHORESIS USING TRIETHYLENETETRAMINE PHOSPHATE BUFFER AS THE ELECTROLYTE SOLUTION

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## ABSTRACT

An acidic separation buffer for peptide mapping by capillary zone electrophoresis (CZE) in both bare fused-silica and acrylamide-coated capillaries is evaluated. The buffer system consists of a mixture of the aliphatic polyamine triethylenetetramine and phosphoric acid in aqueous media. The study has been performed at pH 3.0, which is within the buffering capacity range in the acidic domain determined by the $pK_{a1}$ values of both the tetramine (3.2) and the polyprotic inorganic acid (2.1). Consequently, the solution resulting by combining phosphoric acid and triethylenetetramine at pH 3.0 is a buffering system exhibiting two conjugate acid-base pairs, both effective at controlling the protonic equilibra at this pH value.

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The suitability of the triethylenetetramine phosphate buffer at performing efficient peptide mapping by CZE has been evaluated on a tryptic digest of the protein cytochrome c from horse heart. The study is based on the comparison of the tryptic peptide separation performed by CZE with either triethylentetramine phosphate or sodium phosphate buffers, both containing the same concentration of phosphate ions. The results show that both separation performance and electroosmotic flow are greatly influenced by the incorporation of triethylenetetramine in the buffer system. With the triethylenetetramine phosphate buffer, the electroosmotic flow results in being either suppressed or reversed from cathodic to anodic in bare fused-silica and in acrylamide-coated capillaries, respectively. This effect is indicative of the specific adsorption of positively charged molecules of triethylenetetramine into the immobilized region of the electric double layer at the interface between the capillary wall and the electrolyte solution.

The improvement of peak shape observed with the triethylenetetramine phosphate buffer is also related to the adsorption of the tetramine evidenced by the variation of the electroosmotic flow. The specific adsorption of cationic triethylenetetramine results in masking the silanol groups and other active adsorption sites on the capillary wall, that are believed to be responsible for the poorer performance obtained with sodium phosphate buffer. Thus, the results demonstrate that besides being an effective component of the buffering system, triethylenetetramine acts as a dynamic coating reagent influencing, significantly, efficiency and resolution of the tryptic peptides.



## INTRODUCTION

Peptide mapping is one of the most powerful methods for identification and characterization of proteins. The procedure involves the enzymatic or chemical cleavage of a protein and the subsequent separation of the generated peptides by a suitable chromatographic or electrophoretic technique.(1) The proteolytic enzymes commonly employed for the selective fragmentation of the protein include trypsin, chymotrypsin, V8 proteinase from *Staphylococcus aureus*, thermolysin, clostripain, and endoproteinase lys-C.(2)

The proteolytic digestion by trypsin is generally quantitative under proper conditions and generates peptides, averaging 7-12 amino acids, by cleaving proteins at the C terminal side of the basic residues lysine or arginine. The tryptic peptides may provide structural information on the protein and their size is com-

patible with the most common separation techniques used to yield the peptide map. Electrophoresis,(3) ion-exchange chromatography,(4) thin layer chromatography,(5) reversed-phase high performance liquid chromatography (RP-HPLC),(6) and, more recently, capillary zone electrophoresis (CZE),(7) also in combination with chromatographic techniques,(8,9) have successfully been used for peptide mapping.

Owing to their excellent resolving power and flexibility, RP-HPLC and CZE are today the primary separation modes employed for peptide mapping.(7) These two techniques are complementary to each other.(10). This is because, whereas the separation in RP-HPLC takes place according to differences in hydrophobicity,(11) separations in CZE are based on differences in the mass and charge of the peptides.(12) Thus, CZE is the technique of choice when a second analytical method is required to detect coelutions in RP-HPLC of complex tryptic digests.(13)



The proper selection of the composition and pH of the electrolyte solution is essential to achieve the selective and efficient CZE separation of the complex mixture of peptides generated by the tryptic cleavage of a protein. In most cases, buffers in the range of pH 2-3 are selected in order to ensure that the majority of peptides are positively charged, and that the dissociation of the free silanol at the inner wall of bare fused-silica capillaries is suppressed. Under these conditions, the positively charged peptides migrate toward the cathode and their interactions with the capillary wall are reduced. In addition, the low or negligible electroosmotic flow associated with the use of acidic electrolyte solutions enhances resolution and peak capacity.(14) In accordance with these considerations, acidic buffers are frequently employed as the electrolyte solution for peptide mapping by CZE.

Whilst all buffers can maintain the pH of the electrolyte solution constant and can serve as background electrolytes, they are not equally meritorious in CZE. The chemical nature of the buffer system can be responsible for poor efficiency, asymmetric peaks, and other untoward phenomena arising from the interactions of its components with the sample. In addition, the composition of the electrolyte solution can strongly influence sample solubility and detection, native conformation, molecular aggregation, electrophoretic mobility, and electroosmotic flow. Consequently, selecting the proper composition of the electrolyte solution is of paramount importance in optimizing the separation of tryptic peptides in CZE.

In this study, we examine the suitability of an acidic buffering system consisting of triethylentetramine and phosphoric acid in improving the separation of tryptic peptides by CZE in both bare fused-silica and acrylamide-coated capillaries. Triethylentetramine is an aliphatic tetramine having $pK_a$ values in the acidic domain, which has proven to be effective at masking the silanol adsorption sites for proteins in bare fused-silica capillaries.(15) The buffer is prepared, by titrat-

***Table 1.*** Theoretical Tryptic Peptides of Cytochrome C from Horse Heart

| Fragment Position | Mass | Sequence of Tryptic Fragments |
|---|---|---|
| 1–5 | 546.578 | GDVEK |
| 6–7 | 203.241 | GK |
| 8–8 | 146.189 | K |
| 9–13 | 633.789 | IFVQK |
| 14–22 | 1018.171 | CAQCHTVEK |
| 23–25 | 260.293 | GGK |
| 26–27 | 283.330 | HK |
| 28–38 | 1168.321 | TGPNLHGLFGR |
| 39–39 | 146.189 | K |
| 40–53 | 1470.558 | TGQAPGFTYTDANK |
| 54–55 | 260.293 | NK |
| 56–60 | 603.719 | GITWK |
| 61–72 | 1495.664 | EETLMEYLENPK |
| 73–73 | 146.189 | K |
| 74–79 | 677.798 | YIPGTK |
| 80–86 | 779.008 | MIFAGIK |
| 87–87 | 146.189 | K |
| 88–88 | 146.189 | K |
| 89–91 | 404.423 | TER |
| 92–99 | 964.126 | EDLIAYLK |
| 100–100 | 146.189 | K |
| 101–104 | 433.418 | ATNE |



ing to pH 3.0 with phosphoric acid, a 20 mM triethylentetramine aqueous solution. The paper describes the use of this buffer in CZE for the efficient separation of the tryptic digest of the protein cytochrome c from horse heart.

## EXPERIMENTAL

### Chemicals and Samples

Triethylenetetramine (TETA) was supplied by Fluka (Milan, Italy) and was used without further purification. Mesityl oxide and γ-methacryloxypropyltrimethoxysilane were purchased from Aldrich (Milan, Italy). Cytochrome c from horse heart, TPCK-treated trypsin, and tris-(hydroxymethyl)-aminomethane (Tris) were obtained from Sigma (Milan, Italy). Reagent-grade phosphoric acid, citric acid, acetic acid, hydrochloric acid, potassium hydroxide, calcium chloride,

sodium acetate, and HPLC-grade water were supplied by Carlo Erba (Milan, Italy). Acrylamide, potassium persulfate, N,N,N'N'-tetramethylethylendiamine (TEMED), all of electrophoresis-grade, were purchased from Bio-Rad Laboratories (Milan, Italy).

## Electrophoresis

The experiments were performed using an HP $^{3D}$Capillary Electrophoresis System, equipped with a diode array UV-Vis detector, and an air-cooling device for thermostating the capillary cartridge (Agilent, Waldbronn, Germany). The instrument was interfaced with an HP Vectra XM 5 166 MHz personal computer utilizing the HP $^{3D}$CE ChemStation software for system control and data acquisition and evaluation. Either bare fused-silica or acrylamide-coated capillary tubes of 0.050 mm I.D., 0.375 mm O.D., and total length of 41.5 cm (33 cm to the detector) were mounted in the cartridge. The capillary tube was purchased from Quadrex (New Haven, CT, USA). Samples were injected hydrodynamically at 50 mbar for 2.0 s and were detected at 214 nm.

To prepare the TETA phosphate buffer, the volume of the amine required for the concentration stated was mixed in a beaker with at least 90% (v/v) of the total water volume, and the solution was titrated to pH 3.0 with phosphoric acid under stirring at 25°C. Thereafter, the solution was transferred to a volumetric flask and its final volume was adjusted with water. The running electrolyte solutions containing phosphate buffer were prepared in a similar manner by titrating the requested amount of phosphoric acid to pH 3.0 with 0.1 M sodium hydroxide solution. The pH was measured with a glass electrode Model HI 1131 and Model HI 9017 microprocessor pH-meter, both from Hanna Instruments (Woonsocket, RI, USA). All solutions were filtered through a type HA 0.22 μm membrane filter (Millipore, Vimodrome, Italy) and degassed by sonication before use.

The running electrolyte in the electrode compartments was renewed after five to six runs, and before each run the capillary was rinsed with the running electrolyte for 3 min. Each time a running electrolyte solution of new composition was employed, this was flushed trough the capillary for 30 min. All experiments were carried out at a constant voltage of 15 kV, with the temperature of the capillary cartridge set at 25°C. The electroosmotic flow was determined by measuring the migration time of mesityl oxide. It was detected at the cathodic end of the capillary tube with running electrolytes consisting of phosphate buffer at pH 3.0 and either at the cathodic or the anodic end, upon reversing the polarity of the power supply, with the TETA phosphate buffer at pH 3.0. The electroosmotic flow was considered to be virtually suppressed when the neutral marker did not appear at the detector 300 min after sample injection had taken place at both the

cathodic and anodic end. All measurements of electroosmotic flow were made in triplicate.

## Capillaries

Prior to use for the first time, the new fused-silica capillary was flushed successively with 0.5 M sodium hydroxide (30 min), water (10 min), and 0.5M hydrochloric acid (30 min), followed by a second treatment with water (10 min), 0.5 M sodium hydroxide (30 min), water (10 min). Chemical coating of the inner surface of the capillary tube with non-crosslinked acrylamide was performed according to the method described by Hjerten(16) with the following minor modifications. A volume of 150-μL of γ-methacryloxypropyl-trimethoxysilane was mixed with 5.0-mL of a 1:1 (v/v) mixture of water and methanol adjusted to pH 3.5 (apparent pH) with acetic acid. The capillary was flushed successively with 0.5 M sodium hydroxide; water, 0.5 M hydrochloric acid and water as described above, and then rinsed with the γ-methacryloxypropyltrimethoxysilane solution. After reaction at room temperature for 60.0 min, the γ-methacryloxypropyltrimethoxysilane solution was withdrawn and the capillary was flushed with fifty capillary volume of water. The treatment with the γ-methacryloxypropyltrimethoxysilane solution was performed twice. The capillary was then rinsed with a deareated solution containing 0.4 g of acrylamide, 8 μL TEMED and 20 mg potassium persulfate in 10 mL of water. After reaction at room temperature for 30.0 min, the excess polymerized non-bonded acrylamide was withdrawn and the capillary was washed with fifty capillary volumes of water.



## Tryptic Digest

Horse heart cytochrome c (2.5 mg/mL) was digested at 37°C with TPCK-treated trypsin at an enzyme to substrate ratio of 1:100 in a solution containing 100 mM sodium acetate, 10 mM Tris hydrochloride, and 0.1 mM calcium chloride and adjusted to pH 8.3. The reaction was stopped after 4 hours by addition of a tenth volume of concentrated phosphoric acid and the mixture was stored at –20°C.

## RESULTS AND DISCUSSION

Peptide mapping by CZE at acidic pH values are traditionally carried out with phosphate or citrate buffers in aqueous media as the background electrolyte solution. As the most popular detection mode in CZE is photometric, the UV

absorbance detection at low wavelength requested for high-sensitivity peptide mapping, limits the choices to phosphate buffers because citrate absorbs strongly at wavelengths ≤ 260 nm.

The pKa value of phosphoric acid in acidic domain is 2.1.(17) Therefore, alkali phosphates have the highest buffering capacity, around pH 2.1. Nevertheless, the buffering capacity is considered effective within a range taken as one pH unit below and above the $pK_{a1}$ of phosphoric acid, i.e. from pH 1.2 to pH 3.1. This implies that the ratio of the concentration of phosphoric acid to its conjugate base, which comprises the buffer system, varies between 0.1 and 10.

The $pK_{a1}$ values of the tetramine TETA are 3.25, 6.56, 9.08, and 9,74(18). Therefore, in acidic domain, TETA has effective buffering capacity from pH 2.25 to pH 4.25. All experiments were carried out at pH 3.0, which is within the buffering capacity range in the acidic domain determined by the $pK_{a1}$ values of both phosphoric acid (2.1) and TETA (3.25). Consequently, at pH 3.0 the TETA phosphate solution exhibits two conjugate acid-base pairs that are both effective at controlling the protonic equilibria and, therefore, it can be considered as a dual buffering system.

The TETA phosphate solution was prepared from an aqueous solution of TETA, which was titrated to pH 3.0 with phosphoric acid. The final solution contained 20 mM TETA and 82 mM phosphate ions. The sodium phosphate buffer was prepared, by titrating to pH 3.0, with sodium hydroxide an aqueous solution containing the same molar concentration of phosphoric acid that was employed to prepare the TETA phosphate pH 3.0 buffer.

The study on the suitability of TETA for improving the separation of tryptic peptides by CZE with the phosphate salt of this tetramine as the background electrolyte, was carried out in comparison to the separation performance obtained with sodium phosphate buffer containing the same concentration of phosphate ions (82.0 mM). At an electric field strength of 361.5 V/cm, the current associated with the use of TETA phosphate and sodium phosphate buffer was 30.1 and 44.5 μA, respectively. The significant lower running current associated with the use of TETA phosphate buffer is related to the mobility of the TETA ion, which is expected to be lower than sodium.(19) Thus, besides the high buffering capacity due to the simultaneous action of two conjugate acid-base pairs, the TETA phosphate pH 3.0 buffer also exhibits low conductance that implies low Joule heating.

Cytochrome c from horse heart contains 104 amino acid residues, with a total of 19 lysine and 2 arginine residues.(20) Digestion of horse heart cytochrome c with trypsin results in the formation of 22 fragments, 17 of which are peptides that vary in size from 2 to 14 amino acids. The sequences and molecular masses of the expected tryptic fragments for cytochrome c are reported in Table 1. These data were determined by the program "PEPTIDE-MASS,"(21) which is freely accessible via ExPAsy World Wide Web server, at the URL address: http:/www.expasy.ch/www/tools.htmL.

Almost all the expected tryptic peptides were resolved in a bare fused-silica capillary using the 20 mM TETA phosphate pH 3.0 buffer as the electrolyte solution. The peptide map obtained with this buffer is displayed in Figure 1. The separation of the tryptic digest of cytochrome c carried out in the same bare fused-silica capillary with sodium phosphate buffer, under otherwise identical pH value, phosphate ion concentration (82 mM), and experimental conditions, is displayed in Figure 2.

Comparison of Figures 1 and 2 reveals that better resolution and peak shape was obtained with TETA phosphate buffer. It can also be observed that the tryptic peptides migrated faster in sodium phosphate than in TETA phosphate buffer. These differences in the migration velocity were related to the electroosmotic flow, which was cathodic with sodium phosphate ($\mu_{eo} = 5.7 \times 10^{-9}$ $m^2V^{-1}s^{-1}$), whereas with TETA phosphate buffer no electroosmotic flow was measured at either the cathodic or the anodic end, upon reversing the polarity of the power supply.



The suppression of the electroosmotic flow observed with TETA phosphate buffer, is indicative of the specific adsorption of the positively charged molecules of the aliphatic tetramine into the immobilized region of the electric double layer at the interface between the capillary wall and the electrolyte solution.(22) The adsorption of the amine results in a drastic variation of the positive charge density in the immobilized region of the electric double layer, which reduces the zeta potential and, hence, the electroosmotic flow. The adsorbed amine also has the effect of masking the silanol groups on the capillary wall, whose interactions with the tryptic peptides are believed to be responsible for the poor peak shape observed with sodium phosphate buffer.

Chemical coating of the inner surface of the capillary tube with non-crosslinked acrylamide, sensibly reduced the detrimental effects of these undesirable interactions and almost suppressed the electroosmotic flow measured with phosphate buffer, which decreased from $5.7 \times 10^{-9}$ to $8.5 \times 10^{-10}$ $m^2V^{-1}s^{-1}$. Such effects are related to the deactivation of the silanol groups, which reacted with the γ-methacryloxypropyltrimethoxysilane and were subsequently converted to neutral hydrophilic moieties. However, the electropherogram displayed in Figure 3 shows that performing the separation with phosphate buffer, efficiency and peak symmetry were only partially improved by the chemical treatment of the inner surface of the capillary tube. This can be ascribed either to the partial coating of the capillary tube, which is evidenced by the measurement of a residual cathodic electroosmotic flow, or to the peptide interactions with the coated capillary wall of other than electrostatic nature.

Further improvement of both efficiency and resolution was obtained separating the tryptic peptides in the acrylamide-coated capillary with TETA phosphate buffer as the electrolyte solution, as is evidenced by the electropherogram displayed in Figure 4. The lower migration velocity of the tryptic peptides

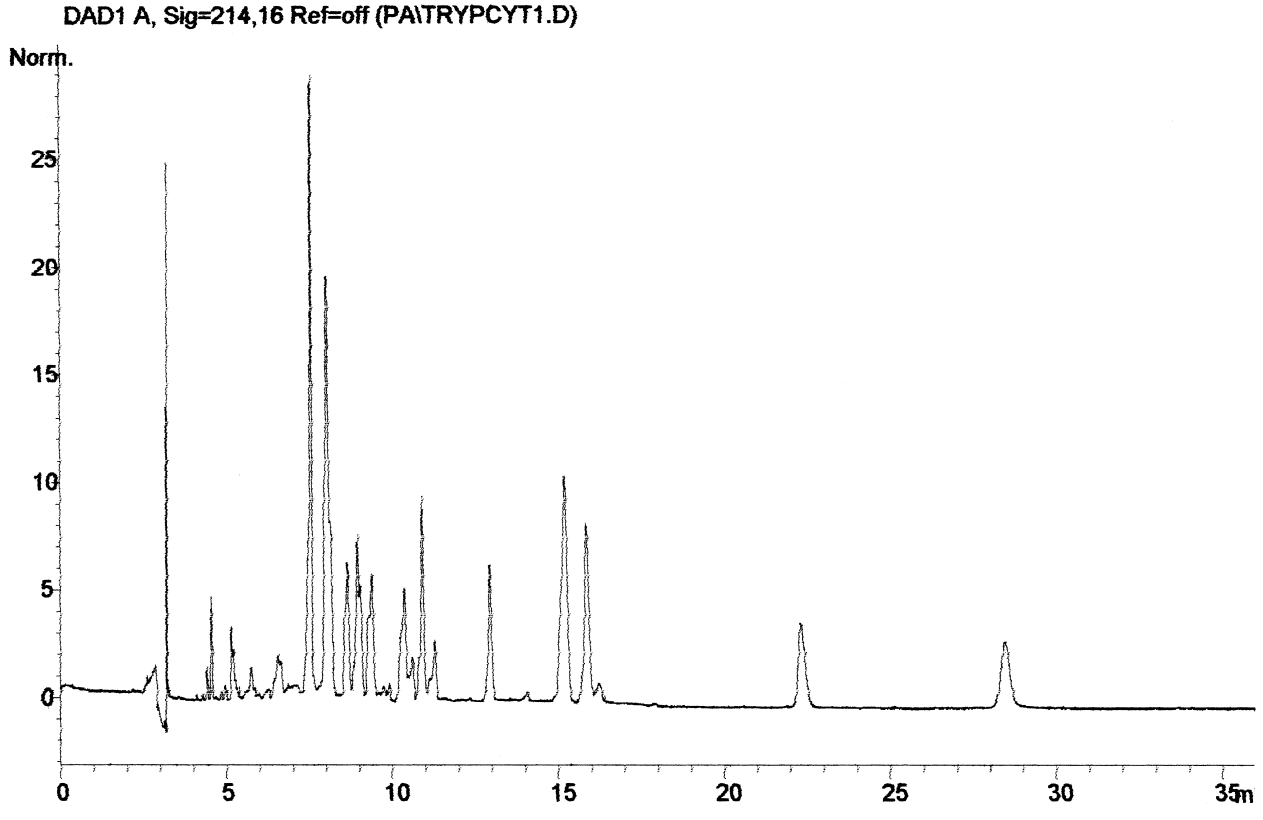


*Figure 1.* Separation of tryptic peptides of equine cytochrome c in bare fused-silica capillary with TETA phosphate buffer (pH 3.0) as the electrolyte solution. Buffer composition as it is described in the text; capillary, bare fused-silica 0.050 mm I.D, 0.375 mm O.D., total length 41.5 cm (33.0 cm to the detector); applied voltage, 15 kV; detection wavelength, 214 nm at the cathodic end; temperature, 25°C.

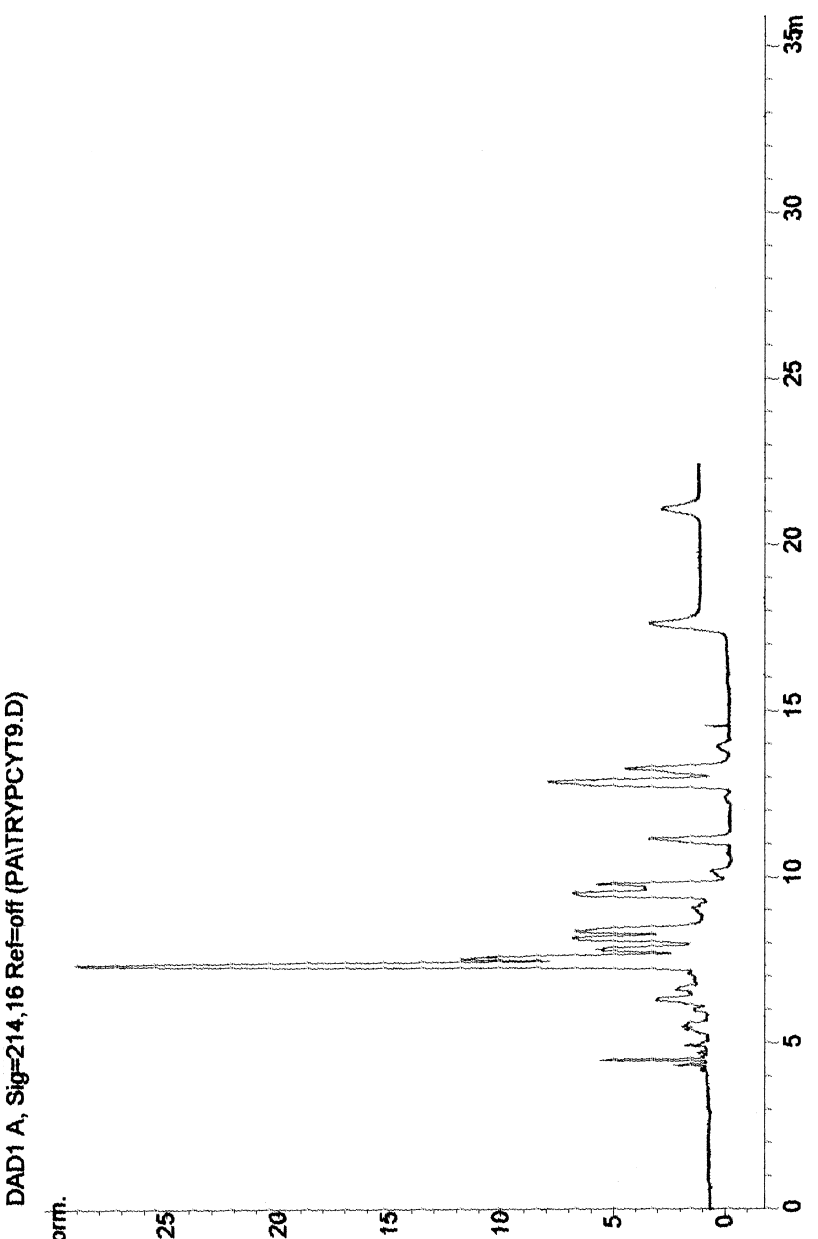


*Figure 2.* Separation of tryptic peptides of equine cytochrome c in bare fused-silica capillary with sodium phosphate buffer (pH 3.0) as the electrolyte solution. Buffer composition as it is described in the text; other conditions as in Fig. 1.

observed with TETA phosphate buffer (see Figs. 3 and 4), is the consequence of the anodic electroosmotic flow exhibited by the acrylamide-coated capillary with this electrolyte solution ($\mu_{eo}$ = - 4.0 x $10^{-10}$ $m^2V^{-1}s^{-1}$). The measurement of an anodic electroosmotic flow indicates that the positive charge density in the Stern region of the electric double layer, determined by the specific adsorption of cationic TETA, exceeds the negative charge density on the capillary wall. This is expected to be lower than the negative charge density on the capillary wall of the bare fused-silica capillary, because it has been reduced by the chemical deactivation of the majority of the silanol groups on the inner surface of the capillary tube. Therefore, in the acrylamide-coated capillary, the same concentration of TETA determining the suppression of the cathodic electroosmotic flow in the bare fused silica capillary, is sufficient to change the zeta potential from negative to positive with the result of reversing the direction of the electroosmotic flow from cathodic to anodic.

The positive zeta potential at the interface between the capillary wall and the electrolyte solution is expected to induce repulsive coulombic forces between the positively charged tryptic peptides and the capillary surface, with the consequence of precluding other possible undesirable interactions. This results in the enhanced efficiency obtained with the combined use of the acrylamide-coated capillary and TETA phosphate buffer.

The experimental data prove that TETA acts as a dynamic coating reagent of the capillary wall of both bare fused silica and acrylamide chemically coated capillaries. The dynamic coating was determined by the adsorption of the aliphatic tetramine in the compact region of the electric double layer as a combination of multi-site electrostatic and hydrophobic interactions. The adsorption results in masking the silanol groups and the other adsorption active sites on the capillary wall and in altering the electroosmotic flow. This property is common to a variety of other amino compounds that has been proposed as additives of the electrolyte solutions employed in CZE of peptides and proteins.(23)

The comparison of the four electropherograms displayed in Figures 1 to 4 reveals that both in bare fused silica (Figs. 1 and 2) and in acrylamide-coated capillary (Figs 3 and 4), better resolution of adjacent peaks is obtained with TETA phosphate than with sodium phosphate buffer. This experimental evidence can also be related to the influence of TETA on efficiency and electroosmotic flow, from which the specific adsorption of the cationic tetramine into the immobilized region of the electric double layer at the interface between the capillary wall and the electrolyte solution can be inferred.

Recently, Rathore and Horváth have introduced the concept of "virtual migration distances," that separates the real extent of the sample migration, i.e. the appropriate length of the capillary, into two virtual parts, $l_s$ and $l_o$.(24) The first virtual migration distance, $l_s$, represents the separation component of the overall migration process, and it is considered as the virtual distance that the ana-

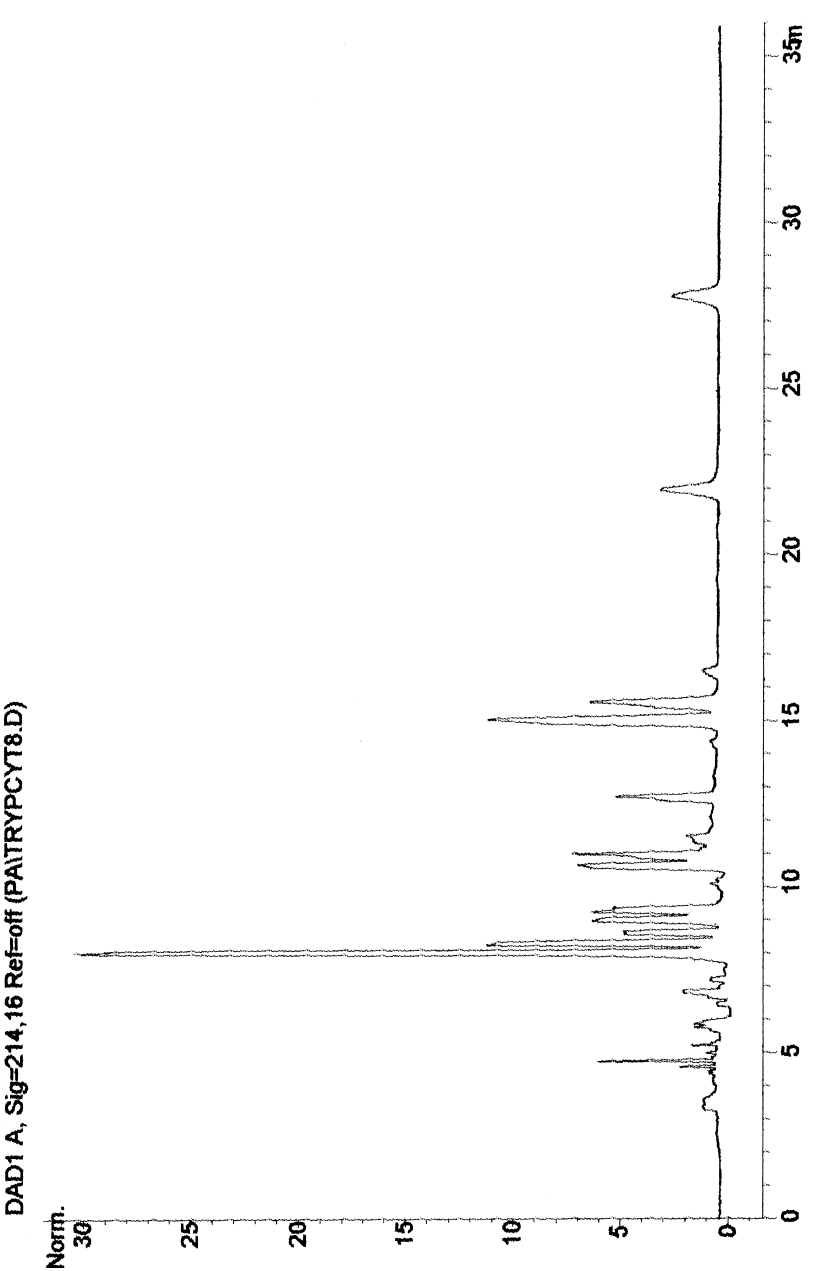


*Figure 3.* Separation of tryptic peptides of equine cytochrome c in acrylamide-coated capillary with sodium phosphate buffer (pH 3.0) as the electrolyte solution. Capillary, acrylamide-coated fused-silica 0.050 mm I.D, 0.375 mm O.D., total length 41.5 cm (33 cm to the detector); buffer composition and other conditions as in Fig. 2.

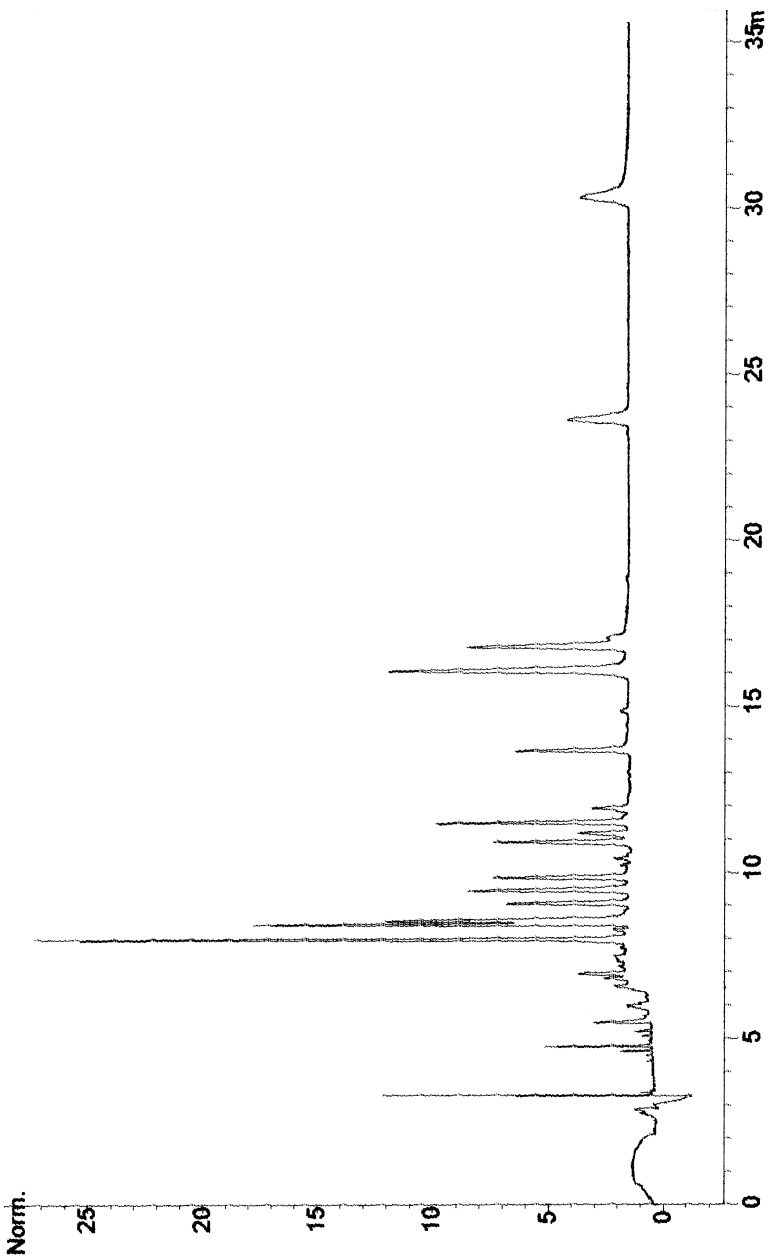


*Figure 4*. Separation of tryptic peptides of equine cytochrome c in acrylamide-coated capillary with TETA phosphate buffer (pH 3.0) as the electrolyte solution. Capillary, acrylamide-coated fused-silica 0.050 mm I.D, 0.375 mm O.D., total length 41.5 cm (33 cm to the detector); buffer composition and other conditions as in Fig. 1.

lytes move under the direct influence of the electric field. The second virtual migration distance, $l_o$, represents a non-separative component of the overall migration process, and it expresses the virtual distance that the analytes move with the electroosmotic flow. By definition, the sum of these two virtual migration distances always equals the real effective length of the capillary column, $L_m$. Therefore, if one of the virtual distances is negative the other must be positive and larger than $L_m$.

According to these concepts, lowering the electroosmotic flow or reversing its direction from cathodic to anodic has the effect of increasing the virtual distance associated with the separative migration, $l_s$, with the result of increasing resolution. In other words, lowering the electroosmotic flow, or reversing its direction, results in improving resolution because a longer column with unvaried electric field strength is mimicked.

Our experimental data show that the use of TETA phosphate buffer determined improved peptide resolution, which is accompanied by a drastic variation of the electroosmotic flow, both in bare fused-silica and in acrylamide-coated capillary. In either cases, the electroosmotic flow is lower than that measured with sodium phosphate buffer. This means that the virtual distances, $l_o$, that the tryptic peptides move with the electroosmotic flow decrease with using TETA phosphate buffer. Since the sum of the two virtual migration distances $l_s$ and $l_o$ has to be equal to the real migration distance, which is expressed by the effective length of the capillary, the virtual distance associated with the separative migration, $l_s$, increases. This has the same effect of using a longer capillary without varying the electric field strength. The result is the improved resolution that has been experimentally observed.



## CONCLUSIONS

The buffer system consisting of triethylenetetramine and phosphoric acid has proven to be superior to sodium phosphate buffer in separating the tryptic peptides of cytochrome c by CZE at acidic pH, both in bare fused-silica and in acrylamide-coated capillaries. The improvement in the separation performance is ascribed to the capability of TETA in acting as a dynamic coating reagent of the capillary wall by adsorbing into the immobilized region of the electric double layer at the solid-liquid interface. Such behavior results in altering the electroosmotic flow, which is suppressed in the bare fused-silica capillary, and reversed from cathodic to anodic in the acrylamide coated capillary. Suppressing the electroosmotic flow, or reversing its direction, results in improving resolution by mimicking a longer capillary with unvaried electric field strength. The dynamic coating of the capillary wall with TETA also has the effect of masking the silanol groups and the other adsorption active sites on the capillary wall, whose interac-

tions with the tryptic peptides are believed to be responsible for the poor efficiency and peak symmetry observed with sodium phosphate buffer. The positive influence of TETA on peak shape also contributes to improving resolution that is directly proportional to the square root of efficiency.(14)

## REFERENCES


1. Ingram, V.M.; Biochem. Biophys. Acta **1958**, *28*, 539-545.
2. Fragment of Polypeptides by Enzymic Methods. In *Practical Protein Chemistry–A Handbook*; Darbre, A., Ed.; John Wiley & Sons: New York, 1986; 122-148.
3. Bennett, C. Methods Enzymol. **1967**, *11*, 330-339.
4. Hill, R.L.; Delaney, R. Methods Enzymol. **1967**, 339-351.
5. Stephens, R.E. Anal. Biochem. **1978**, *84*, 116-126.
6. Dong, M.W. Tryptic Peptide Mapping by Reversed Phase Liquid Chromatography. In *Advances in Chromatograph*; Giddings, J.C., Gruska, E., Brown, P.R., Eds.; Marcel Dekker: New York, 1992; Vol. 32, 21-51.
7. Ganzler, K.; Warne, N.W.; Hancock, W.S. Analysis of rDNA-Derived Proteins and their Posttranslational Modifications, in Capillary Electrophoresis. In *Analytical Biotechnology*, Righetti, P.G., Ed.; CRC Press: Boca Raton, 1996; 183-238.
8. Issaq, H.J.; Chan, K.C.; Janini, G.M.; Muschik, G.M. Electrophoresis **1999**, *20*, 1533-1537.
9. Behnke, B.; Metzger, J.W. Electrophoresis **1999**, *20*, 80-83.
10. Frenz, J.; Wu, S.; Hancock, W.S. J. Chromatogr. **1989**, *480*, 379-391.
11. Melander, W.R.; Horváth, C.S. Reversed-Phase Chromatography. In *High-Performance Liquid Chromatography–Advances and Perspectives*; Horváth, Cs., Ed.; Academic Press: New York, 1980; Vol. 2, 113-319.
12. Grossman, R.D.; Colburn, J.C.; Lauer, H.H. Anal. Biochem. **1989**, *179*, 28-33.
13. Larmann, J.P.; Lemmo, A.V.; Moore, A.W.; Jorgenson, J.W. Electrophoresis **1993**, *14*, 439-447.
14. Giddings, J.C. Sep. Sci. **1969**, *4*, 181-189.
15. Corradini, D.; Cannarsa, G. LC-GC **1996**, *14*, 326-332.
16. Hjerten, S. J. Chromatogr. **1985**, *347*, 191-198.
17. *CRC Handbook of Chemistry and Physics*; Weast, R.C., Ed.; CRC Press: Boca Raton, 1981; D-144.
18. Martell, A.E.; Smith, R.M. *Critical Stability Constants*, Plenum Press: New York, 1974; 32-141.
19. Hiemenz, P.C. *Principles of Colloid and Surface Chemistry*, 2nd Ed.; Marcel Dekker: New York, 1986; 737-757.

20. Margoliash, E.; Smith, E.L.; Kreil, G.; Tuppy, H. Nature **1961**, *192*, 1125-1127.
21. Wilkins, M.R.; Lindskorg, I.; Gasteiger, E.; Bairoch, A.; Sanchez, J.-C.; Hochstrasser, D.F.; Appel, R.D. Electrophoresis **1997**, *18*, 403-408.
22. Corradini, D.; Rhomberg, A.; Corradini, C. J. Chromatogr.**1994**, *661*, 305-313.
23. Corradini, D. J. Chromatogr B **1997**, *699*, 221-257.
24. Rathore, A.S.; Horváth, C.S. J. Chromatogr. A **1996**, *743*, 231-146.

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