

Use of Zwitterionic Buffer Additives to Improve the Separation of Proteins in Capillary Zone Electrophoresis

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Summary

In CZE, the adsorption of the proteins on the capillary wall is a common problem. This paper describes the simple method of utilizing zwitterionic buffer additives to improve the separation of proteins in untreated fused silica capillaries at neutral pH. Three kinds of zwitterion are evaluated in the separation of acidic, neutral, and basic proteins, including their effect on protein efficiency, mobility, separation, and resolution; the difference between the effects of the different additives are also highlighted. The method has proved to be a possible means of reducing protein adsorption, especially for basic proteins.

1 Introduction

It is commonly recognised that a key problem in capillary zone electrophoresis (CZE) separation of proteins is their inherent tendency to be adsorbed by the wall of fused silica capillary tubing. Such adsorption, which often leads to band broadening and irreproducible migration of the analytes, seriously impairs separation efficiency. Several efforts have been made to circumvent this problem. These include adjusting the pH of the operating buffer [1–3], coating the capillary surface [4–10], and using additives in the carrier medium [11–19].

Among these strategies, use of zwitterions as buffer additives to improve protein separation is most attractive. With this method, complicated capillary pretreatments are not needed and there is little effect on electroosmotic flow. Another important advantage is that, because zwitterions do not contribute to the conductivity of the operating buffer, high concentrations of the additives could be used to reduce adsorption without producing excessive Joule heat. This can, therefore, provide a protein separation system with fewer restrictions on the operating conditions investigated during optimization of the separation.

The utilization of zwitterionic additives was first proposed by Bushey and Jorgenson [12] and then adopted by other workers [17–19]. In the work reported herein we tried to develop a deeper understanding of this method. According to the principles of choosing zwitterions suggested by Bushey and Jorgenson [12], we synthesized three kinds of zwitterion: $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$ (trimethylammonium propylsulfonate, TMAPS, first developed by Waters [16]), $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$ (trimethylammonium butylsulfonate, TMABS), and $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{SO}_3^-$ (2-hydroxyl-3-trimethylammonium propylsulfonate, HTMAPS). These zwitterions were evaluated for the CZE separation of acidic, neutral and basic proteins. The results indicated that all have the capability to suppress protein – wall interactions, but to different extents, especially for the separation of basic proteins at neutral pH. The capability sequence is: TMABS > TMAPS > HTMAPS.

2 Experimental

2.1 Apparatus

CE was performed on a modular system comprising a PS/MJ30P0400-11 high voltage supply (Glassman High Voltage Inc., USA), a CV⁴ capillary electrophoresis detector (Isco, Lincoln, NE, USA), and an HP 3394A integrator (Hewlett-Packard, USA). Fused silica capillary tubing (75 μm i.d. $\times$ 375 μm o.d.) was purchased from Yongnian Optical Fiber Factory (Hebei 057150, P.R. China) and cut into 70 cm lengths for use as separation tube. The effective length was 50 cm.

2.2 Materials

Seven model proteins, albumin (chicken egg), myoglobin (horse heart), lysozyme (chicken egg white), cytochrome C (bovine heart), β -lactoglobulin A (bovine milk), β -lactoglobulin B (bovine milk), and α -chymotrypsinogen A (bovine pancreas) were obtained from Sigma (St Louis, MO, USA).

The two zwitterions TMABS and TMAPS were synthesized by a process described in the literature [20]. HTMAPS was synthesized with epichlorohydrin as raw material and glycidyltrimethylammonium chloride as intermediate, followed by reaction with sodium bisulfite; the method was a modification of that described in references 21 and 22.

Other chemicals used, e.g. sodium dihydrogen phosphate and disodium hydrogen phosphate, were of analytical grade except for formamide, which was of chemical reagent grade.

2.3 Method

Buffers were prepared using deionized water. Protein samples were prepared as 0.1 mg/mL and formamide (0.1 % solution in running buffer) was used as the neutral marker to measure electroosmotic flow rate. All buffers and samples were filtered through 0.45 μm pore size filters (Millipore, Milford MA, USA) and degassed before use.

Before use, or prior to the introduction of new buffers, capillaries were rinsed with potassium hydroxide solution (0.1 mol/L; 10 min), water (20 min), and buffer (20 min). Between repeated runs, they were rinsed for 10 min with fresh operating buffer.

3 Results and Discussion

In 0.1 mol/L phosphate running buffer at pH 6.9, three model proteins albumin (pI 4.7), myoglobin (pI 7.3), and lysozyme (pI 11.0) should be negatively charged, nearly neutral, and positively charged, respectively. The effects of zwitterion concentration on their separation efficiencies and electrophoretic mobilities are

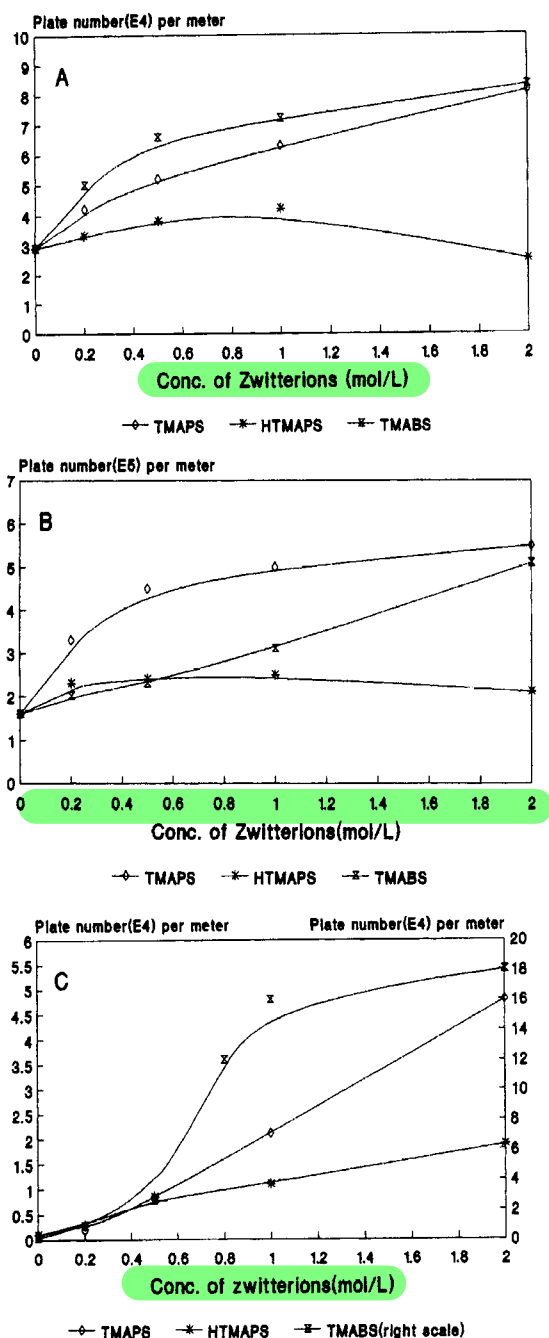


Figure 1
Effect of species of zwitterion additive on column efficiencies for the proteins albumin (A), myoglobin (B), and lysozyme (C). Buffer, 0.1 mol/L phosphate, pH 6.9; separating potential, 190 V/cm.

shown in **Figures 1** and **2** (data for the electrophoretic mobility of myoglobin are not given since its migration time was near to that of the EOF marker).

It is suggested that high concentrations of all three zwitterions can prevent the proteins from interacting with the capillary wall, thus enhancing separation efficiencies and migration velocities (the EOF changes with increasing zwitterion concentration were insignificant for the three zwitterions under investigation). On this occasion, the separation of the positively charged basic protein lysozyme was greatly improved. As the concentration of zwitterions was increased from 0 to 1 mol/L, the number of theoretical plates meas-

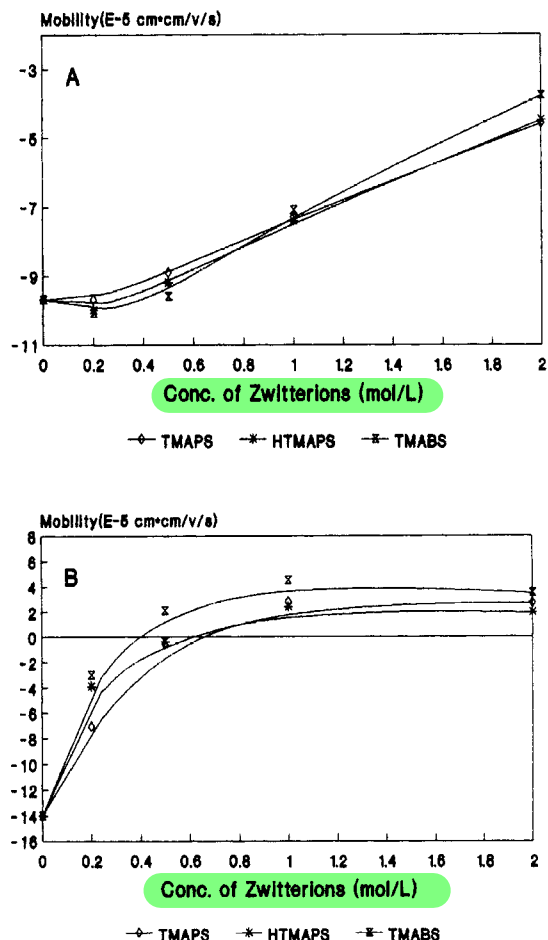


Figure 2
Effect of species of zwitterion additive on the mobilities of the proteins albumin (A) and lysozyme (B). Conditions as for Figure 1. '+' or '-' signs denote solute peak emerging before or after the neutral marker, respectively.

ured for lysozyme increased 20-fold with TMAPS, 10-fold with HTMAPS, and 200-fold with TMABS. They had no marked effects on the behavior of the acidic protein albumin or the neutral protein myoglobin, which do not show seriously adsorptive properties at this pH. This phenomenon was also observed for the other basic proteins such as cytochrome C and α -chymotrypsinogen A. **Figure 3** depicts the electropherograms obtained for cytochrome C with phosphate buffer only and with phosphate buffer containing zwitterions. As expected, although with phosphate buffer alone a symmetrical peak was not obtained for cytochrome C ($N < 100$) and the compound eluted after the neutral marker, use of the same buffer containing 1 mol/L TMABS resulted in the elution of cytochrome C as a narrow peak ($N = 60000$) before the neutral marker.

Because *Towns* and *Regnier* [20] have demonstrated that the adsorption of proteins (especially basic proteins) on the capillary wall is most pronounced near neutral pH, these three zwitterion additives have the favorable properties of providing a simple means of reducing protein adsorption under the harshest conditions.

Comparison of the three zwitterions (Figure 1) reveals that TMABS showed the best performance toward the basic proteins. The order of effectiveness of the three zwitterions was TMABS > TMAPS > HTMAPS.

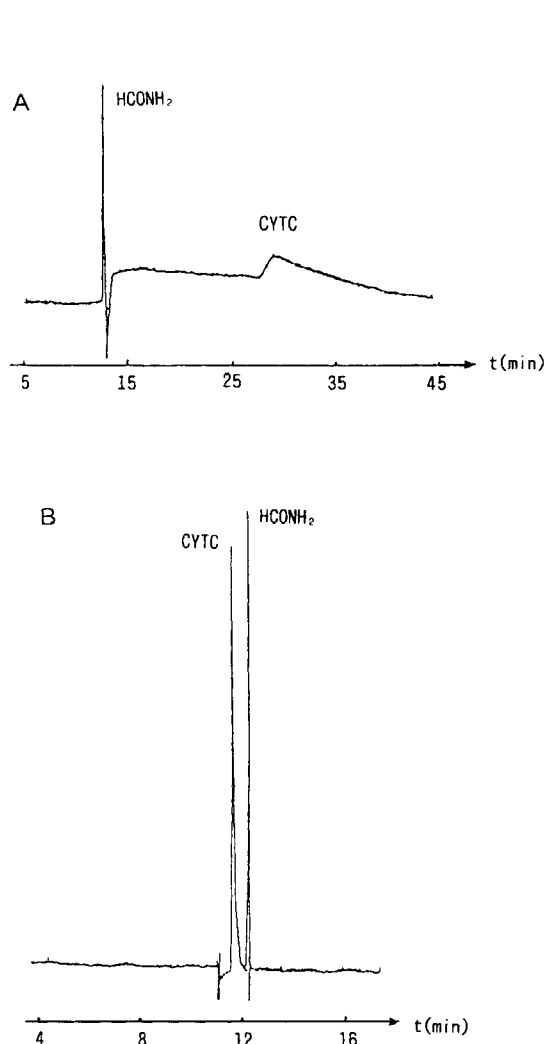


Figure 3
Electropherogram of cytochrome C. (A) Buffer, 0.1 mol/L phosphate, pH 6.9; injection, 10 kV for 6 s; separating potential, 190 V/cm; current, 66 μ A. (B) Buffer, 0.1 mol/L phosphate + 1 mol/L TMABS, pH 6.9; injection, 3 kV for 6 s; separating potential, 190 V/cm; current 42 μ A.

It was proposed by Jorgenson[16] that when choosing zwitterions the first consideration was to choose compounds which were zwitterionic over a wide pH range; the second was that they should have a high solubility in the buffer system employed; and the third was that as they increase in size, they should, ideally, not increase in hydrophobicity. The three zwitterions presented in this paper conform with the first two conditions. In this work no evidence was found in support of the third condition. For example, although, as a result of an additional methylene group in the molecule, the size and hydrophobicity of TMABS are greater than those of TMAPS, the separation efficiency, in terms of plate number, obtained for lysozyme with the former additive was ten times that for the former. Increasing the hydrophilicity of the TMAPS molecule by introduction of one hydroxyl group (giving HTMAPS) resulted, on the other hand, in a derivative which did not perform so well.

As shown in **Figure 4**, two similar proteins, β -lactoglobulin A and β -lactoglobulin B, which had the same molecular weights and differed only by 0.1 unit in pI, were eluted close to each other using pH 6.9 phosphate buffer. On addition of zwitterions to the buffer solution, even at high concentration, the separation could not be improved. This is because the increase in the column efficiencies

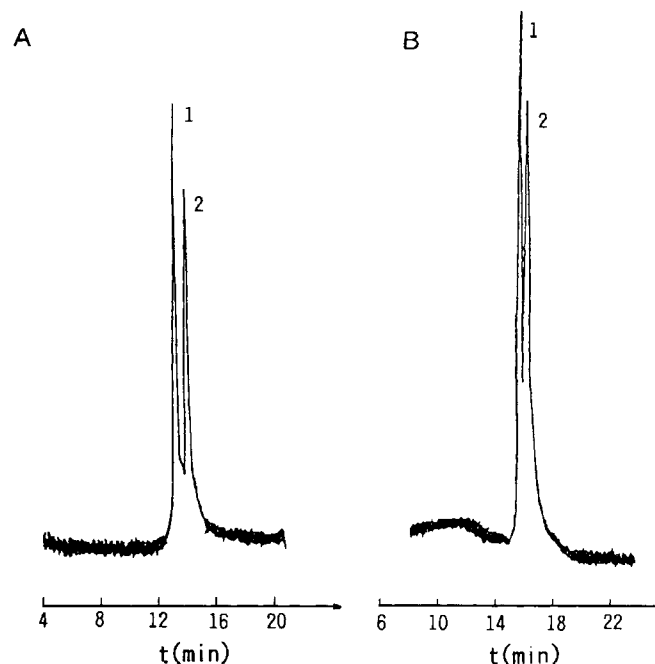


Figure 4
Electropherogram of β -lactoglobulin A (peak 1) and β -lactoglobulin B (peak 2). Buffer, (A) 0.1 mol/L phosphate, pH 6.9, (B) 0.1 mol/L phosphate + 2 mol/L TMAPS, pH 6.9; injection, 3 kV for 6 s; Separating potential, 190 V/cm.

and the electrophoretic mobilities of the two proteins was accompanied by a reduction in the difference between their electrophoretic mobilities. In other words, the atmosphere of the oriented zwitterionic ions surrounding the protein molecules might adversely reduce the individual characteristics of the solutes to be separated (as, in a similar fashion, do SDS molecules in conventional SDS-PAGE).

Nevertheless, when the sample mixture contains strongly basic protein components which cause deterioration of the separation of the components of interest, the zwitterionic additives can significantly improve the separation.

4 Conclusion

The zwitterionic buffer additives used above are effective in reducing the adsorptive interaction between proteins and the silanol groups on the capillary wall although, as demonstrated by the protein efficiencies measured in these experiments, they can not totally eliminate this interaction. Further attempts should be made to reduce protein adsorption as this still reduces separation efficiency, even in the presence of the zwitterionic additives.

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References

- [1] R.M. McCormick, *Anal. Chem.* **60** (1988) 2322.
- [2] H.H. Lauer and D. McManigill, *Anal. Chem.* **58** (1986) 166.
- [3] K.-J. Lee and G.S. Heo, *J. Chromatogr.* **559** (1991) 317.
- [4] S. Hjertén, *J. Chromatogr.* **347** (1985) 191.

- [5] J.K. Towns and F.E. Regnier, *J. Chromatogr.* **516** (1990) 69.
- [6] K.A. Cobb, V. Dolnik, and M. Novotny, *Anal. Chem.* **62** (1990) 2478.
- [7] G.J.M. Bruin, J.P. Chang, R.H. Kuhlman, K. Zegers, J.C. Kraak, and H. Poppe, *J. Chromatogr.* **471** (1989) 429.
- [8] G.J. Bruin, R. Huisden, J.C. Kraak, and H. Poppe, *J. Chromatogr.* **480** (1989) 339.
- [9] W. Nashabeh and Z.El Rassi, *J. Chromatogr.* **559** (1991) 367.
- [10] S.A. Swedberg, *Anal. Biochem.* **185** (1990) 51.
- [11] J.S. Green and J.W. Jorgenson, *J. Chromatogr.* **478** (1989) 63.
- [12] M.M. Bushey and J.W. Jorgenson, *J. Chromatogr.* **480** (1989) 301.
- [13] A. Emmer, M. Jansson, and J. Roeraade, *J. Chromatogr.* **547** (1991) 544.
- [14] M. Gilges, H. Husmann, M.-H. Kleemß, S.R. Motsch, and G. Schomburg, *J. High Resoln Chromatogr.* **15** (1992) 452.
- [15] J.A. Bullock and L.C. Yuan, *J. Microcol. Sep.* **3** (1991) 241.
- [16] M. Merion, B. Bell-Alden, E. Grover, U. Neue, and J. Peterson, Poster. No. PT-69, HPCE '91, San Diego, CA, USA (1991).
- [17] N.A. Guzman, J. Moschera, K. Iqbal, and A.W. Malick, *J. Chromatogr.* **608** (1992) 197.
- [18] M.A. Strega and A.L. Lagu, *J. Liq. Chromatogr.* **16** (1993) 51.
- [19] M.A. Strega and A.L. Lagu, *J. Chromatogr.* **630** (1993) 337.
- [20] P. Blumbergs, A.B. Ash, F.A. Daniher, C.L.C.L. Stevens, H.O. Michel, B.E. Hackley Jr, and J. Epstein, *J. Org. Chem.* **34** (1969) 4065.
- [21] J.D. McClure, *J. Org. Chem.* **35** (1970) 2059.
- [22] N. Parris, J.K. Weil, and W.M. Linfield, *JAOCS* **53** (1976) 60.
- [23] J.K. Towns and F.E. Regnier, *Anal. Chem.* **64** (1992) 2473.

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