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# Triethylamine or diethylenetriamine as dynamic modifier for suppressing basic protein adsorption in capillary electrophoresis

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**Abstract:** Either triethylamine or diethylenetriamine can be conveniently used as a dynamic modifier to suppress the adsorption of basic proteins in capillary zone electrophoresis. Sufficiently high column efficiencies (> $2\times10^5$ plates/m for cytochrome C) were obtained with either of them along with the improvement of the peak shapes and repeatability of migration time. The relationship between the adsorption suppression effect of the modifier and its structure was discussed.

**Keywords:** Capillary zone electrophoresis, basic proteins, dynamic modifier, triethylamne, diethylenetriamine.

## Introduction

Capillary zone electrophoresis (CZE) is a powerful separation method for both small inorganic and organic ions as well as biomacromolecules. In order to fully exploit its potential in protein separation many efforts have been made to overcome the difficulties encountered with adsorption of basic proteins onto the capillary wall, such as adjusting the buffer pH to extreme values, manipulating buffer ionic strength, covalently modifying the silanol functionalities, and dynamically modifying the capillary surface with organic molecules[1-4]. Compared with the covalent coating method the dynamic modification is simpler. In this report we compared the use of several low molecular weight aliphatic amines, including two monoamines, two ethanolamines and two polyamines as the dynamic modifiers to enhance separation efficiency for protein by CZE. Among the six amines studied, triethylamine and diethylenetriamine are more effective in improving the column performance.

## Experimental

### *1. Materials*

Model proteins, lysozyme from egg white (pI 11.0), cytochrome C (pI 10.5) and α-chymotrysinogen A (pI 9.5) were obtained from Sigma Chemicals Co. (USA). Other chemicals used were of analytical grade. The water used was redistilled. The

concentration of buffer was based on that of the phosphoric acid taken before pH adjustment with NaOH or the amines under studies.

*2. Operation Conditions*

A modular CE system consisting of a laboratory-made high-voltage power supply (0~30 kV), a CV$^4$ detector (Isco, USA) and a HP 3394A integrator (Hewlett-Packard, USA) was used. The fused silica capillary tubing was purchased from Yongnian Optical Fiber Factory (Hebei). Protein samples were made up as 1mg/ml solution in the buffer. New columns were successively rinsed with 1 mol/L NaOH and water for 1 hr each, followed with the operating buffer for 5min. Between two runs, it was rinsed with 1mol/L NaOH, water and buffer for 2, 5 and 5 min, consecutively.

## Results and Discussion

It is well known that the adsorption of basic proteins on the inner wall of the capillary is due to the interaction between the positively charged basic protein at pH below its pI value and the negatively charged silanol anions on the wall surface at pH>2. The adsorption distorts the peak shape, impairs the column efficiency and worsens the repeatability of the migration time. Many amines were employed to overcome these difficulties, based on the fact that aliphatic amines can be protonated into cations at some appropriate pH, which can compete with the positively charged basic protein molecules for adsorption sites on the wall surface. The competence of the modifier depends on its structure, pKa value(s), concentration and the pH of the background electrolyte. The amine modifiers used in this study are all easily available, and are listed as follows with their pKa value(s) in parenthesis: diethylamine (10.8); triethylamine (10.72); ethanolamine (9.50); diethanolamine (~8.5); ethylenediamine (6.85, 9.92); diethylenetriamine (4.42, 9.21, 10.02).

It can be seen from **Table 1** that the substitution of sodium ion in the phosphate buffer with any amine cations listed above was effective, though in different extent, in improving the column efficiency of the test basic proteins. For cytochrome C and lysozyme, the presence of triethylamine or diethylenetriamine in the buffer at pH 7.0 increased the column efficiency up to $10^5$ plates/m. In fact, the analyte-wall interaction was not completely eliminated with the addition of the modifier, as can be seen by the asymmetrical peaks with tailing factors of around 2 for cytochrome C and lysozyme, compared with 3.56 to 8.46 for the rest modifiers. Except for ethanolamine, all the amines helped improving the repeatability of migration time of the test basic protein, see **Table 2**. Although diethylamine and triethylamine have nearly identical pKa values, the latter was much more effective in suppressing adsorption, while in diethylamine modified buffer tailing became even more pronounced, presumably due to the presence of secondary amine with the capability of hydrogen bond formation with the protein molecule. Moreover, ethylenediamine studied previously[2] was by no means more effective than some monoamines in suppressing adsorption. From the pKa values mentioned above it is obvious that at pH 7.0 ethylenediamine can only be protonated into

monovalent cations, just like the two monoamines tested. Moreover, the basicity of ethylenediamine is slightly weaker than that of triethylamine. So the latter is more effective as an adsorption suppressor at pH 7.0 than the former. As to the two ethanolamines they are less effective for use as adsorption suppressor, probably due to their higher hydrophilicity and less shielding power than those amines without hydroxyl groups. Diethylenetriamine seemed to be the most powerful modifier; as it is the only amine which can form divalent cations at pH 7.0, leading to most pronounced coulombic attraction with the capillary wall.

**Table 1.** Column efficiency (in plates/m) for basic proteins in the presence of various cations

| Additive | Separation voltage | $N_{Lys} \times 10^3$ | $N_{Cyt} \times 10^3$ |
|---|---|---|---|
| Sodium ion | 9kV | 2.84 | 0.614 |
| Diethylamine | 9kV | 17.9 | 18.5 |
| Ethylenediamine | 11kV | 27.9 | 49.1 |
| Triethylamine | 11kV | 487 | 285 |
| Diethylenetriamine | 10kV | 447 | 315 |
| Ethanolamine | 9kV | 11.7 | 54.0 |
| Diethanolamine | 10kV | 30.5 | 14.9 |

**Table 2.** Performance of dynamically coated column towards lysozyme and cytochrome C

| Additive | Tm(min)lys (RSD,n=5) | Tm(min)cyt (RSD,n=5) | Tf lys | Tf cyt |
|---|---|---|---|---|
| Sodium | 19.85 (5.20) | 25.39 (5.55) | 5.59 | 6.92 |
| Diethylamine | 7.88 (2.33) | 8.96 (1.58) | 7.25 | 8.46 |
| Triethylamine | 14.64 (2.54) | 16.27 (2.88) | 1.56 | 1.96 |
| Ethylenediamine | 6.61 (3.36) | 8.40 (2.16) | 5.60 | 6.37 |
| Diethylenetriamine | 11.49 (3.27) | 16.28 (2.82) | 1.80 | 2.07 |
| Ethanolamine | 6.50 (6.07) | 8.01 (6.63) | 2.96 | 6.00 |
| Diethanolamine | 5.97 (2.66) | 7.94 (2.83) | 2.52 | 3.56 |

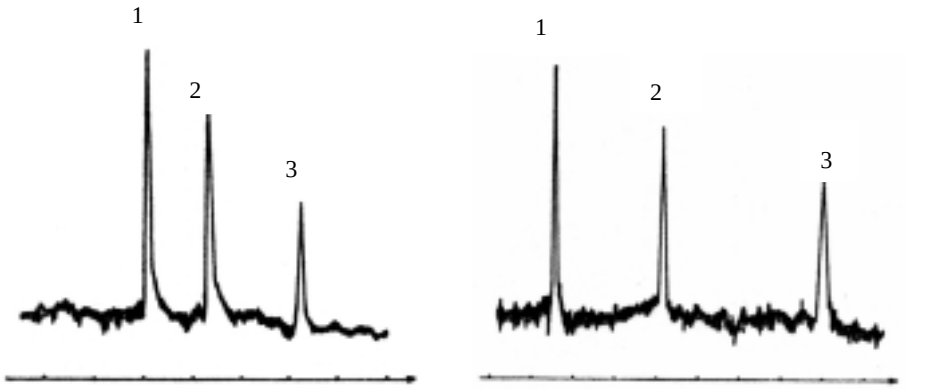
* Tm denotes migration time while Tf tailing factor.

Besides, with the decrease of adsorption of the test protein, the repeatability of migration time improved considerably, which is of utmost importance for the protein separation by capillary electrophoresis. Of the six amines tested triethylamine and triethylenetriamine gave baseline separation of the three tested proteins, see **Figure 1**.

Triethylamine phosphate at pH 2.5 was reported[3] to be effective in suppressing basic protein adsorption, however, based on our observation at this extreme low pH no adsorption of basic proteins occurs at all even in the absence of triethylamine.

Compared with ethylenediamine and proplylenediamine[1,2] the two modifiers here proposed seem more effective in suppressing basic protein adsorption.

**Figure 1** Electropherograms of typical basic proteins with an amine modifier




**(a) triethylamine** **(b) diethylenetriamine**

Condition: column: 50μm i.d.×375μm o.d., L=40cm, l=15cm; BGE: 100mmol/L phosphate buffer, pH=7.0; injection, electrokinetic, (a) 2kV/7s; (b) 3kV/7s; analysis: (a) 7kV/51.5 μA; (b) 10kV/52.0μA; analytes: 1: Lys, 2: Cyt, 3: Chy.

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