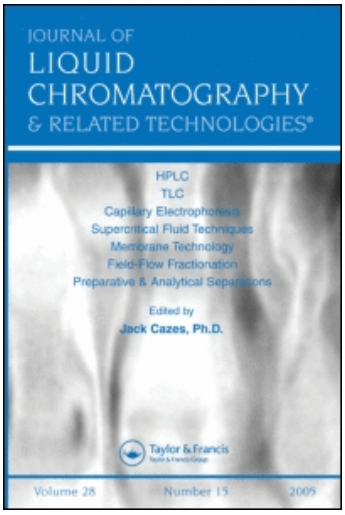

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STUDY ON PHOSPHATE OF ETHYLENE-DIAMINE, 1,3-DIAMINOPROPANE AND 1,4-DIAMINOBUTANE AS CARRYING ELECTROLYTE IN OPEN-TUBULAR CAPILLARY ELECTROPHORESIS

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ABSTRACT

The use of ethylenediamine, 1,3-diaminopropane and 1,4 -diminobutane phosphate as carrying electrolyte in open- tubular capillary electrophoresis is proposed. The advantage of them as carrying electrolyte was found in the ability to slow electroosmotic mobility because of adsorption of their ions to the capillary inner wall which caused decrease of ζ potential of the wall. Difference among ethylenediamine, 1,3-diminopropane and 1,4-diaminobutane lies in their adsorption, and enthylene diamine gave higher electroosmotic mobility than other two at same concentration maybe due to its weak adsorption. The dependence of electroosmotic mobility on diamine concentration, pH, neutral salt concentration and applied voltage was determined. Increased diamine concentration caused decrease of electroosmotic mobility, but increased pH, neutral salt concentration and applied voltage gave reversed effects. The

addition of neutral salt caused decreased adsorption of diamine ions, and the increased applied voltage resulted in temperature rise. The usefulness of diamine phosphate as carrying electrolyte was demonstrated in the improved separation of basic proteins in CZE .

INTRODUCTION

The heart of any separation technique is found in the ability to adjust experimental parameters to optimize a specific analysis. With respect to efficiency, selectivity, and time, electrophoretic separation in open-tubular can be optimized by adjusting operation voltage, capillary dimension and buffer system. Much of early work employed phosphate buffers. Zwitterionic buffer systems developed by Good have become popular now because of their low conductivity.

Buffer type, buffer concentration, buffer ionic strength and buffer pH must be considered in buffer selection. The concentration of the buffer ions should be appromately 1000 times larger than that of the solute ions in order to minimize distortion of the solute zone in the applied electric field(1,2). The tailing of cationic solutes can be minimized through the addition of neutral and zwitterionic salts to the operating buffer to compete for the adsorption sites(3, 4, 5) . Excessive Jeale heat which can not dissipated sufficiently by the capillary tube precludes the use of buffer systems with high concentration and high ionic strength.

Separation of special compounds such as neutral species, chiral compounds, and proteins needs special buffer systems which often have special buffer additives. Various compounds such as organic solvents(6,7) , ionic surfactants, nonionic surfactants, chiral surfctants, metal complexes, cyclodextrins, modified cyclodextrins and bile salts were used as buffer additives to achieve desired separation (8).

Amines such as spermine(9), morpholine(10), ethylenediamine (11), 1,3-diaminopropane(12), 1,4 -diaminobutane(13, 14) , 1, 5 -diaminopentane(15) have been used as buffer additives, but no systematic investigations on their application as carrying electrolyte were published. In this report, the use of ethylenediamine, 1, 3- diminopropane and 1, 4- diminobutane phosphate as carrying electrolyte in open- tubular capillary electrophoresis is proposed. The advantage of them as carrying electrolyte was found in the ability to slow electroosmotic mobility. The dependence of electroosmotic mobility on experimental parameters such as diamine concentration, pH, neutral salt and appllied voltage was determined. The usefulness of diamine phosphate as carrying electrolyte was demonstrated in the improved separation of basic proteins in capillary zone electrophoresis (CZE).

EXPERIMENTAL

Apparatus

The capillary electrophoresis apparatus used in this study resembles that reported earlier by Jorgenson and Lukacs(1). It was constructed by Peking Institute of Technology Application (Peking, China). It consists of a high voltage d. c. power supply delivering up to +/-30kV, and a UV detector which has several wavelengths optional with a fixed and removable device for on column detection, a plexiglass box with a safety interlock and a syringe installation used to flush capillaries. The electrophoregrams were recorded with a hp3390A integrator (Hewlett-Packard, Avondale, PA, USA).

Reagents and materials

Ethylenediamine anhydrous, 1, 3- diaminopropane, sodium sulfate anhydrous, phosphoric acid, dimethyl sulphoxide were

purchased in China. 1,4-diaminobutane was purchased from Fluka (CH-9470 Buchs, Switzerland).

Carrying solutions were prepared by using the correspondent amount of diamine, then adjusting the pH value with phosphoric acid. These solutions were used to determine electroosmotic mobility of the capillary at different conditions. In some cases, sodium sulphate anhydrous was added to them in order to examine the effect of neutral salt on electroosmotic mobility. 1% dimethyl sulphoxide (DMSO) was prepared as neutral marker of the electroosmotic mobility.

Lysozyme from egg white (pI11.0) was purchased from Fluka (CH-9470 Buchs, Switzerland). Cytochrome C (pI10.5) from horse heart, trypsinogen from bovine pancreas (pI9. 3) and α -chymotrypsinogen A from bovine pancreas (pI9.1) were purchased from Sigma (St.Louis, MO, USA) . Stock protein mixture with individual concentration of about 2.0 mg/ml were prepared and separated in indicated carrying solutions.

Sample injection was accomplished by syphoning for 5s- 10s at 8cm. In all experiments, deionized water was used.

Electrophoresis

Fused silica capillary tubes (Yongnian Photoconductive Fibre Factory, Hebei, China) of 50um i. d. and 375um o.d. were used with a total length 70cm or 64cm in which the detector was placed at 20cm from the capillary outlet. Detection was monitored at 214nm. Before using, a new capillary was flushed with 1M KOH for 1 hour and then equilibrated with carrying solution overnight using a syringe to force the solution through it. When change of carrying solution was needed, the same step was followed. Between two runs, the capillary was first flushed with one capillary volume of 1M KOH, then with carrying solution for 5 minutes. All analyses were run at ambient temperature without temperature control.

RESULTS AND DISCUSSION

Electric double layer and electroosmotic mobility of the capillary

Because of the ionization of silanol groups, the inner wall of fused silica capillary always carries negative charge when filled with aqueous solution. At the interface between the wall and the solution, negative charges are balanced by positive ions in the solution. A part of counterions will be absorbed by the wall, giving rise to an immobilized compact layer. The remaining counterions are distributed in a diffuse layer, their concentration approaching the bulk value as the distance from the wall increased to infinity. Then an electric double layer adjacent to the capillary inner wall is generated, which is represented in Figure 1. The potential drop is linear in the compact layer, but decreases exponentially within the diffuse layer. When an electric field is applied to the capillary, positive counterions in the diffuse layer migrate toward the cathode and pull solvent molecules with them, giving rise to a flow of the carrying solution through the capillary which is called electroosmotic flow. On the other hand, the compact layer will not move towards the cathode. The corresponding potential in the plane between the compact layer and the diffuse layer is called ζ potential. The relation between the electroosmotic mobility μ_{eosm} and the ζ potential is given by(16)

$$\mu_{eosm} = \frac{\varepsilon_o \varepsilon \zeta}{\eta} \quad \cdots\cdots\cdots\cdots (1)$$

where ε_o is the permittivity of free space, ε is the permittivity of the carrying solution, and η is the viscosity of the carrying solution. The relation between the ζ potential and the thickness of diffusion layer δ is given by(17):

$$\zeta = \frac{4\pi\delta e}{D} \quad \cdots\cdots\cdots\cdots (2)$$

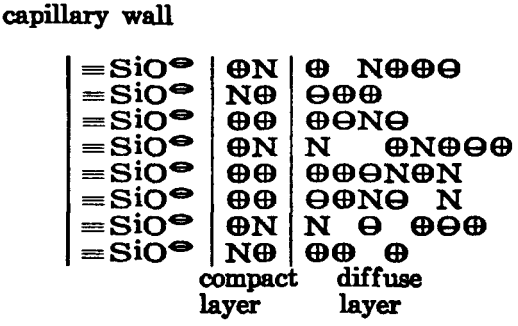

FIGURE 1. Representation of electric double layer, N represent water molecule.

$$\delta = [3\times10^{7}\,|Z|\,C^{1/2}]^{-1} \quad \ldots \quad (3)$$

where e is the amount of charge per unit surface area in the plane between the compact layer and the diffuse layer, and D is the diffusion coefficent, Z is the number of valence electrons, C is concentration of the counterion in carrying solution respectively.

In our study, the electroosmotic mobility was caculated from the migration time of an electrically neutral marker substance from equation (4)

$$\mu_{eom} = lL/t_o V \quad \ldots \quad (4)$$

where l is the distance between the inlet of the capillary tube and the detector, L in the total length of the capillary tube, t_o is the retention time of the neutral marker and V is the applied voltage, respectively. In CZE mode, dimethyl suphoxide was used as neutral marker, whereas in MECC mode, methanol was used instead.

Dependence of electroosmotic mobility on diamine concentration

At a constant pH8. 5, the dependence of electroosmotic mobility on diamine concentration was determined and shown in

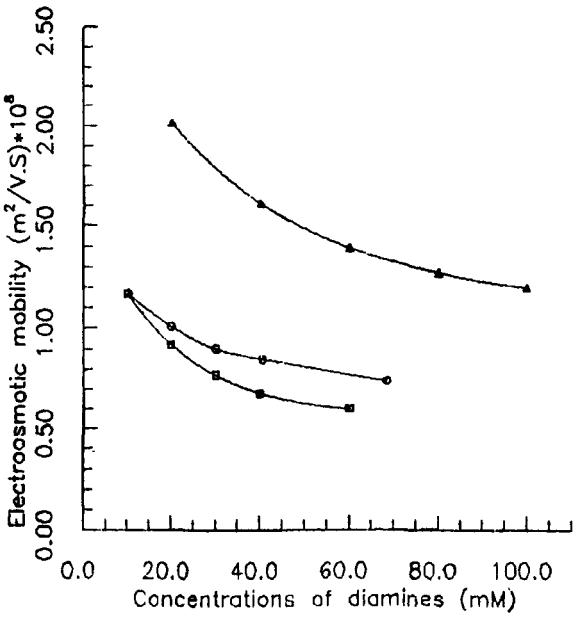

FIGURE 2. Dependence of electroosmotic mobility on diamine concentration with diamine phosphate as carrying electrolyte. Conditions: applied voltage, 13.5kV; capillary, 70cm total length, 50cm to the detector, 50um i. d. ; buffer, pH8. 5; ambient temperature, 14-16℃. △ ethylenediamine, □ 1,3- diaminopropane, ○ 1,4-diaminobutane.

Figure 2. It is observed that diamine phosphate made slow electroosmotic mobility compared with common carrying electrolyte(18), and increased diamine concentration resulted in decrease of electroosmotic mobility.

Change on diamine concentration caused two effects on the electric double layer. First, incrasing the concentration decreased thickness of the diffusion layer δ according to equation (3), so decreased ζ potential of the electric double layer and the eletroosmotic mobility μ_{eos} according to equation

(2) and equation (1). Second, increasing the concentration decreased the amount of charge per unit surface area in the plane between the compact layer and diffuse layer, that is e in equation (2). Unlike metal ions, positively charged diamine ions were absorbed by the capillary inner wall much stronger, and caused reduction of e, then caused reduction in ζ potential and μ_{eom} according to equation (2) and equation (1).

Difference among ethylenediamine, 1,3-diminopropane and 1,4-diminobutane lies in their adsorption to the capillary inner wall. It seemed that ethylenediamine ions were absorbed weaker than other two maybe because of its short carbon chain, so the electroosmotic mobility of the capillary with it was the highest at same concentration. There was not very much difference between 1,3-diminopropane and 1,4-diminobutane.

Dependence of electroosmotic mobility on pH

At constant diamine concentration, the dependence of electroosmotic mobility on pH from 5.5 to 10. 5 with diamine phosphate as carrying electrolyte was determined and shown in Figure 3. It is observed that increasing pH caused increase in electroosmotic mobility as a half of titration curve in the experimental pH range.

The dependence of electroosmotic mobility on pH with common carrying electrolyte is already known from the literature(18) , and it resembles a titration curve in which there is a 10- fold increase over the pH range from 3 to 11 with the largest increase being from 4 to 8. The reason for this curve is ionization of silanol groups on the capillary inner wall which can give a silmilar curve between $\equiv SiO^{\ominus}$ concentration and pH, and the amount of charge per unit surface area e is determined by $\equiv SiO^{\ominus}$ concentration. When diamine phosphate was used as carrying electrolyte, e was determined not only by the concentration of $\equiv SiO^{\ominus}$ group, but also by diamine ions absorbed by the capillary inner wall. At low pH, increased pH

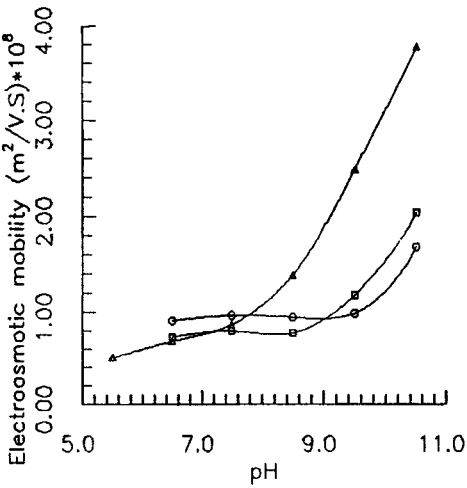

FIGURE 3. Dependence of electroosmotic mobility on pH with diamine phosphate as carrying electrolyte. △ 60mM ethylenediamine and □ 30mM 1, 3- diaminopropane, coditions: applied voltage, 13.5kV; capillary, 70cm total length, 50cm to the detector, 50um i.d.. ○ 30mM 1,4-diaminobutane, conditions: applied voltage, 12kV; capillary, 64cm total length, 44cm to the detector, 50um i.d.. Ambient temperature, 14-16℃.

caused increase of $\equiv SiO^{\ominus}$ concentration, but increased concentration of $\equiv SiO^{\ominus}$ also caused more absorbed diamine ions which made e almost unchanged, so there were pH ranges with almost constant electroosmotic mobility in Figure 3. With increasing pH, absorbed diamine ions may not be large enough to make e unchanged anymore, then a titration curve relation between electroosmotic mobility and pH was observed. From ethylenediamine, 1,3-diaminopropane to 1,4-diaminobutane, the pH range with almost constant electroosmotic mobility value became wider and wider, maybe attributed to the adsorption becoming stronger and stronger.

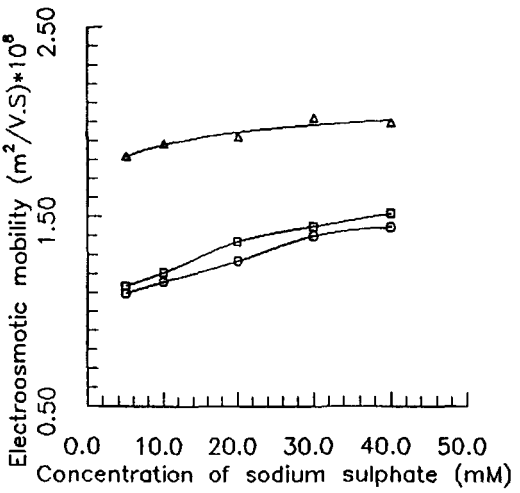

FIGURE 4. Dependence of electroosmotic mobility on sodium sulphate concentration with diamine phosphate as carrying electrolyte. Conditions: applied voltage, 9kV; capillary, 64cm total length, 44cm to the detector, 50um i.d.; buffer, pH8. 5; ambient temperature, 23-24℃. △ ethylenediamine; □ 1, 3 -diaminopropane; o 1,4-diaminobutane.

Effect of neutral salt on electroosmotic mobility

As shown above, the advantage of diamine phosphate as carrying electrolyte was found in the ability to slow electroosmotic mobility. More results showed that the adddition of neutral salt can speed the electroosmotic mobility, as demonstrated in Figure 4.

As predicted above, incrasing the concentration of carrying electrolyte decrease thickness of the diffusion layer δ according to equation (3), so decrease ζ potential of the electric double layer and the eletroosmotic mobility μ_{eom} according to equation (2) and equation (1). So there seemed to be some conflict between theory and experimental results. This

may be also ascribed to the adsorption of diamine ions to the capillary inner wall. Because neutral salt ions were absorbed by the capillary inner wall much looser than diamine ions, they maybe served to increase the amount of charge per unit urface area e through entering the compact layer. In addition, increasing its concentration caused increase of current through the capillary tube which maybe also caused temperature rise of the carrying solution, and then increase of the electroosmotic mobility.

Effect of applied voltage on electroosmotic mobility

The effect of applied voltage on electroosmotic mobility is shown in Figure 5. It was not anticipated that increasing the applied voltage caused increase in eletroosmotic mobility.

It was supposed that increasing applied voltage maybe increased the amount of charge per unit surface area in the plane between the compact layer and diffuse layer. That was because positively charged diamine ions were absorbed by negatively charged capillary inner wall through electric attraction, and increasing applied voltage maybe weakened this attraction, therefore increased e, ζ, μ_{eom}. But more results denied this hypothesis.

Although increased applied voltage caused increase of the electroosmotic mobility, linear relationship between electroosmotic flow and current through the capillary tube was obtained, as shown in Figure 6. This linear relationhip was well recognized theoretically by Terabe et al(19) and Tsuda et al(20) with common carrying eletrolyte. Their works also revealed nonlinear relationship between electroosmotic flow and applied voltage, and similiar relationship between electroosmotic mobility and applied voltage just like ours can be derived. So there seemed no difference between diamine phosphate carrying electrolyte solution and commmmon carrying electrolyte solution in this respect, and the effect of applied voltage on

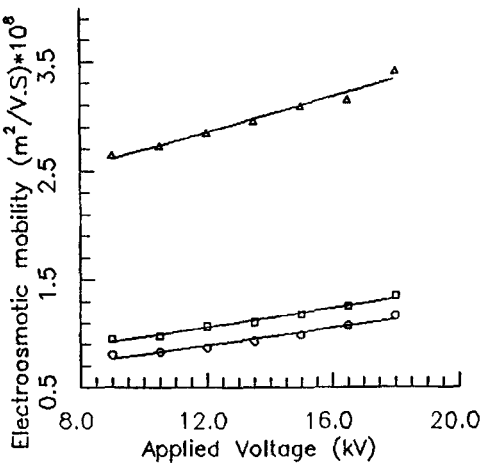

FIGURE 5. Dependence of electroosmotic mobility on applied voltage with diamine phosphate as carrying electrolyte. △ ethylenediamine, □ 1,3-diaminopropane, ○ 1, 4- diaminobutane. Conditions: capillary, 70cm total length, 50cm to the detector, 50um i.d.; buffer, pH9.5 60mM diamine + 30mM Na_2SO_4 ; ambient temperature, about 17℃.

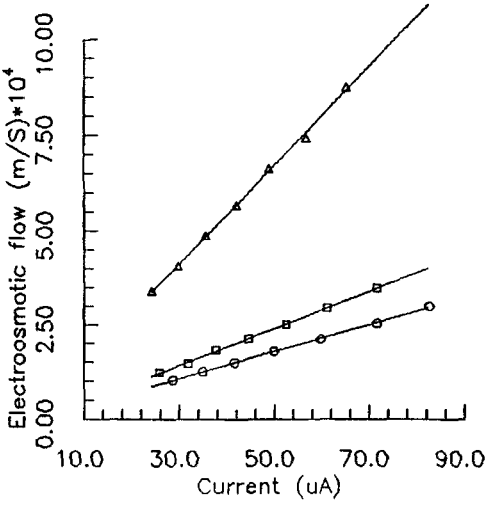

FIGURE 6. Linear relationship between electroosmotic flow and current through the capillary tube with diamine phosphate as carrying electrolyte. Conditions are the same as those given on Figure 5.

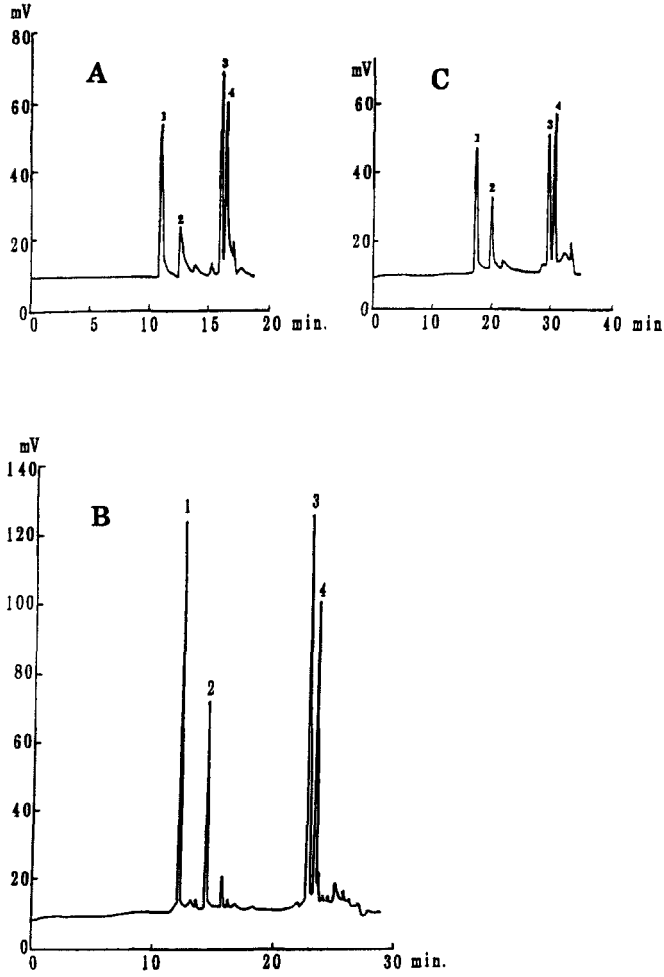

FIGURE 7. Improved separation of basic proteins with diamine phosphate as carrying electrolyte. (1) lysozyme (2) cytochrome C (3) trypsinogen (4) α-chymotrypsinogen A. (A) buffer, pH8. 5 60mM ethylenediamine; applied voltage, 15.5kV; current, 35uA; ambient temperature, 18℃; (B) buffer, pH8. 5 30mM 1, 3 -diaminopropane; applied voltage, 18kV; current, 65 - 68uA; ambient temperature, 20℃; (C) buffer, pH8. 5 30mM 1, 4 -diaminobutane; applied voltage, 18kV; current, 34.5uA; ambient temperature, 15℃.

TABLE 1

Separation Efficency of Every Peak in Figure 7.

Carrying electrolyte	[a]Thereotical plate number × 10^{-4}			
	peak 1	peak 2	peak 3	peak 4
ethylenediamine	1.4	0.5	7.7	2.8
1,3-diaminopropane	4.9	5.1	14.9	13.6
1,4-diaminobutane	1.2	1.1	3.3	4.9

[a]N=5.53(RT/WI), where RT is the retention time, WI is the peak width at half height.

TABLE 2

Retention Time Reproducibility of Every Peak in Figure 7.

Carrying electrolyte	RSD%(n=6)			
	peak 1	peak 2	peak 3	peak 4
ethylenediamine	1.22	0.95	1.53	1.51
1,3-diaminopropane	0.26	0.21	0.54	0.86
1,4-diaminobutane	0.81	0.73	0.61	0.57

electroosmotic mobility can also be arributed to excessive Jeale heat which caused temperature rise of the capillary tube and then viscosity reduction of the carrying solution although sometimes the applied voltage was not very high.

It should be noted that linear relationship between electroosmotic mobility and applied voltage was also obtained as

demonstrated in Figure 5, which meaned linear relationship between the reciprocal of viscocity and applied voltage.

Usefulness of ethylenediamine, 1, 3- diaminopropane and 1, 4 -diaminobutane as phophate as carrying electrolyte

In capillary zone electrophoresis (CZE) , electroosmotic mobility affects separation efficency and resolution. The use of ethylenediamine, 1, 3- diaminopropane and 1, 4- diaminobutane phosphate as carrying electrolyte gave slow electroosmotic mobility which can make low separption efficency but high resolution. In a limited range, the slowed electroosmotic mobility can be optimized to a approriate value by adjusting diamine concentration, pH and neutral salt concentration to make a desired separation. The dependence of electroosmotic mobility on these experimental parameters was well depicted in this study.

Besides the advantage above, the use of diamine phosphate as carrying electrolyte can also improve the separation of basic proteins, which is demonstrated in Figure 7. At operating pH8.5, the selected basic proteins possess positive charges, then they are absorbed by capillary inner wall through electric attraction in common carrying electrolyte solution, as indicated in reference (21). With diamine phosphate as carrying electrolyte, positive diamine ions competed for adsorption of basic proteins to capillary inner wall through electric attraction, then the separation of basic proteins was improved. The separation efficency and retention time reproducibility of every peak demonstrated in Figure 7 were shown in Table 1 and Table 2, respectively.

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