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# Capillary Electrophoretic Separation of Proteins under the Control of Radial Electric Field

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**Abstract:** Separation of basic proteins was performed using a homemade field-modulated capillary electrophoresis system. The resolution, elution and even wall adsorption can be regulated by adjusting the radial rather than axial voltage applied. Selection of running buffer and pH was found to be critical.

**Keywords:** Capillary electrophoresis, protein separation, radial electric field control.

In capillary electrophoresis (CE) of proteins, wall adsorption may lead to serious efficiency loss or even no peak detected. Although such adsorption can be suppressed by performing the separation at an extreme pH ($\leqslant$2 or >11), deactivating the capillary wall, or using special buffer additives[1-3], they are not suitable for dynamic control. Since 1994, we have started to investigate some physical approaches such as magnetic field modulation, electric field regulation and ultrasonic vibration. As a consequence, the electric field regulation was found to be promise and explored[4]. In this paper, we will discuss an interesting application, that is, field-modulated separations of basic proteins. Unexpectedly, the wall adsorption can be reduced and even regulated by adjusting the radial rather than axial voltage applied. The regulation will become easy at pH<3, as is in the case of electroosmosis control.

## Experimental

A homemade CE system discussed elsewhere[5] was used. In addition to axial voltage, an extra radial voltage was applied to a CE capillary through a sheath buffer created by casing the capillary with a wide-bore tube. They are both flexible fused silica columns from Hebei Yongnian Optical Fibre Factory (Hebei, China), with size of 75 μmID × 28.5 / 46.0 cm (separation length / total length) and 400 μmID × 32.0 cm, respectively. Proteins of cytochrome C, lysozyme, α–chymotrypsinogen A and myoglobin from Sigma (St. Louis, MO) were dissolved in water at a concentration of 0.5, 0.5, 1 and 1 mg/ml, respectively. Sodium phosphate, citric acid, dimethyl sulfoxide, hydrochloric acid

and sodium hydroxide were all analytical-reagent grade from Beijing Chemical Plant. The water used was doubly distilled.

**Results and Discussion**

*Buffer Selection* is critical to control the separation of proteins. The buffers most suitable for electroosmosis control are also suitable for protein separation. As shown in **Figure 1A** and **B**, citrate is better than phosphate since it generates more sensitive response to the radial voltage. Also, *lower pH* is better than higher (compare B and C). Possibly, citrate may generate a looser electric double layer than phosphate since citric acid is a weaker electrolyte than phosphoric acid. At low pH, wall charge will be reduced, which also leads to forming a loose electric double layer. These imply that the conditions of tending to form loose electric double layer are preferred.

**Figure 1.** Response of electroosmosis to radial voltage using different buffers at 0.01mol/l. An anode-to-cathode flow was set as positive.

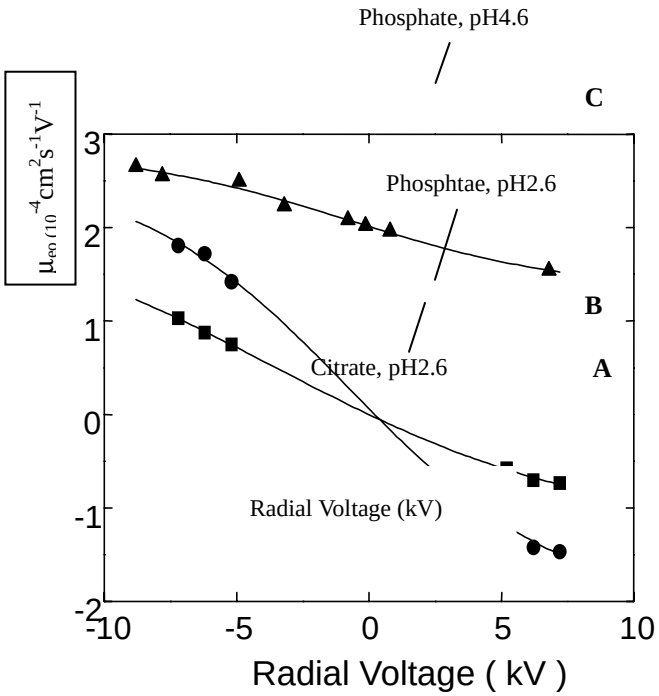
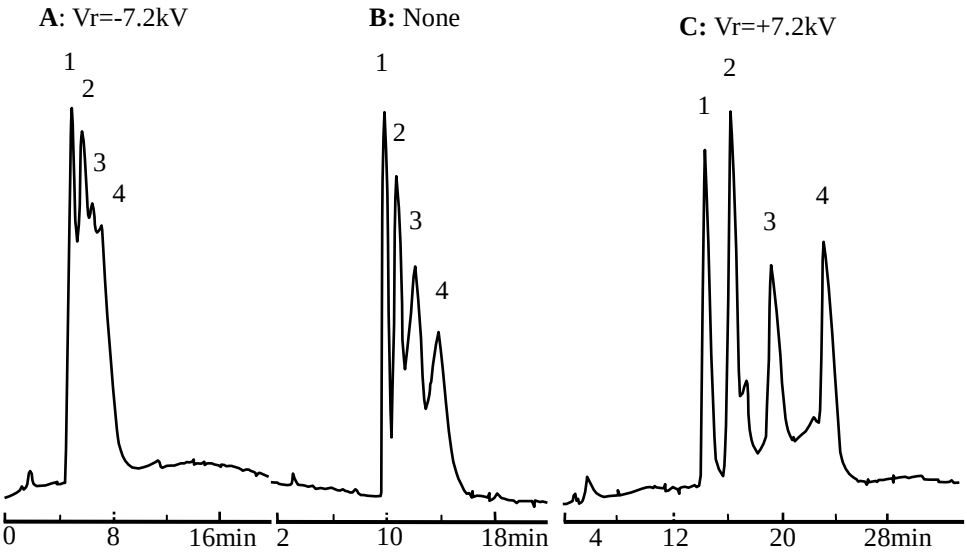


**Figure 2.** Separation of basic proteins at 6.9kV axial voltage and different radial voltages.




Buffer: 0.01 mol/l citric acid, pH2.6; UV-detection: 206nm; Sample injection: 7kV for 15s; Peak: 1=Lysozyme, 2=Cytochrome C, 3=α–Chymotrypsinogen A, 4=Myoglobin.

As expected, the elution time and the resolution of proteins can easily be regulated by changing the radial voltage. **Figure 2** and **Table 1** show that the resolution between cytochrome C and lyzosyme increases from 0.65 to 1.82 as $V_r$ (=potential outer minus inner) rises from –7.2kV to 7.2kV. Seemingly, this could be resulted from the prolonged separation time which will unavoidably result in peak broadening due to diffusion, and especially wall adsorption. But surprisingly, here the peak variances decrease as the

**Table 1.** Peak data detected using 0.01mol/L citric acid at pH2.6

| $V_r$ (kV) | Resolution (Rs*) | $t_R$ (Elution time, min) | | $\sigma^2$ (Total spatial variances, $cm^2$)* | |
|---|---|---|---|---|---|
| | | Lysozyme | Cytochrome C | Lysozyme | Cytochrome C |
| – 7.2 | 0.65 | 6.0 | 6.4 | 0.53 | 0.68 |
| – 6.2 | 0.68 | 6.4 | 6.8 | 0.37 | 0.61 |
| – 5.2 | 0.72 | 6.8 | 7.3 | 0.25 | 0.54 |
| None | 1.24 | 8.0 | 8.8 | 0.24 | 0.44 |
| 5.2 | 1.50 | 10.9 | 12.0 | 0.20 | 0.20 |
| 6.2 | 1.57 | 12.1 | 13.2 | 0.16 | 0.19 |
| 7.2 | 1.82 | 12.7 | 14.1 | 0.15 | 0.17 |

*. $R_s=[t_{R(2)}-t_{R(1)}]/[w_{1/2(2)}-w_{1/2(1)}]$, $\sigma^2=(Lw_{1/2})^2/(t^2_R 8\ln 2)$, where L=separation length, $w_{1/2}$=half height peak width, subscript (1) and (2) denote adjacent peaks.

separation time or radial voltage increases (**Table 1**). The only possibility is the wall adsorption controlling the peak broadening. In this case, peak width is proportional to adsorption degree. This also reveals that the wall adsorption can be regulated through the variation of radial voltage. Regulating the wall adsorption degree is useful not only for protein separation but also for removing undesired components from samples.

**Acknowledgment**

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