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# Mobility-based selective on-line preconcentration of proteins in capillary electrophoresis by controlling electroosmotic flow

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**Abstract**

A simple method to perform selective on-line preconcentration of protein samples in capillary electrophoresis (CE) is described. The selectivity, based on protein electrophoretic mobility, was achieved by controlling electroosmotic flow (EOF). A short section of dialysis hollow fiber, serving as a porous joint, was connected between two lengths of fused silica capillary. High voltage was applied separately to each capillary, and the EOF in the system was controlled independently of the local electric field intensity by controlling the total voltage drop. An equation relating the EOF with the total voltage drop was derived and evaluated experimentally. On-line preconcentration of both positively charged and negatively charged model proteins was demonstrated without using discontinuous background electrolytes, and protein analytes were concentrated by approximately 60–200-fold under various conditions. For positively charged proteins, positive voltages of the same magnitude were applied at the free ends of the connected capillaries while the porous joint was grounded. This provided a zero EOF in the system and a non-zero local electric field in each capillary to drive the positively charged analytes to the porous joint. CE separation was then initiated by switching the polarity of the high voltage over the second capillary. For negatively charged proteins, the procedure was the same except negative voltages were applied at the free ends of the capillaries. Mobility-based selective on-line preconcentration was also demonstrated with two negatively charged proteins, i.e. β-lactoglobulin B and myoglobin. In this case, negative voltages of different values were applied at the free ends of the capillaries with different values, which provided a non-zero EOF in the system. The direction of EOF was the same as that of the electrophoretic migration velocities of the protein analytes in the first capillary and opposite in the second capillary. By controlling the EOF, β-lactoglobulin B, which has a higher mobility, could be concentrated over 150-fold with a 15 min injection while myoglobin, which has a lower mobility, was eliminated from the system.


*Keywords:* Electroosmotic flow; Preconcentration; Proteins

## 1. Introduction

Capillary electrophoresis (CE) has become one of the most powerful techniques in bioanalytical separations, primarily due to its high separation efficiency. However, CE suffers from poor concentration detection limits with UV absorption detection due to the very small sample volume (1–5 nl) and greatly reduced optical pathlength (25–150 μm) as compared to conventional liquid chromatography. Many efforts have been made to improve these concentration detection limits. In addition to new detection cell designs, on-line preconcentration methods have been extensively investigated [1–27]. These on-line preconcentration methods are designed to compress a large sample volume into a narrow plug so that the high efficiency of CE will not be lost. In general, these methods can be classified into two categories. First, charged analytes are concentrated at the boundaries of different background matrices by manipulating their electrophoretic migration velocities. Methods in this group include field-amplified sample stacking [3–9], large-volume sample stacking [10,11], pH-mediated stacking [12,13] and isotachophoresis [14–18]. Second, analytes (not necessarily charged) in a low strength solvent are concentrated by absorption into (or adsorption onto) a stationary or pseudostationary phase. Methods in this group include solid-phase extraction [19–21], membrane preconcentration [22,23], affinity chromatographic preconcentration [24,25] and sweeping techniques in micellar electrokinetic chromatography [26,27].

There are also on-line preconcentration methods especially designed for proteins. Hjertén et al. [28,29] described several approaches for protein samples by moving the protein analytes toward a non-buffering pH gradient, a small pore polyacrylamide gel, a piece of dialysis tubing, a gra-

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dient in conductivity, or by a combination of displacement electrophoresis and a counter flow. Wu et al. [30] described a method for on-line protein preconcentration by connecting a short length of semipermeable dialysis hollow fiber to the inlet of a fused silica capillary. An injection voltage was applied only across the fiber. Protein analytes electromigrated into the fiber and became concentrated. The problem with this method is that the experimental design was quite complicated, and the experimental set-up was difficult to build. Wei and Yeung [31] described a similar but much simpler approach by etching a short section of fused silica capillary with hydrofluoric acid instead of using a dialysis hollow fiber. Protein analytes could be concentrated by 25–100 times by performing various concentration approaches such as on-line stacking and isotachophoresis. Unfortunately no direct evidence was presented to specify the pore size and distribution in the etched capillary wall. The authors mentioned that large buffer ions could also be concentrated using the etched capillary, which could cause destacking in certain situations. Another problem of both of these methods is that preconcentration was only performed under acidic conditions where electroosmotic flow (EOF) on the capillary wall was minimized; otherwise a strong EOF would carry the protein analytes out of the concentration area and destroy the concentration effect.

One issue that has seldomly been addressed by preconcentration methods for CE is selectivity. High selectivity could help eliminate interfering components and simplify the subsequent CE separation step. The selectivities of methods in the first category are based on charge; that is, analytes with the same charge are concentrated while those with opposite charge are removed. Methods in the second category usually have higher selectivities, ranging from those based on hydrophobicity in solid-phase extraction to those based on high specific interaction in affinity chromatography. Hori et al. [32] described an off-line concentration method called "countercurrent electroconcentration." In this method, the electrophoretic velocities of the charged analytes were countered by a hydrodynamic flow. By controlling the hydrodynamic flow velocity, selective concentration of three naphthalene sulfonic acids based on electrophoretic mobility was demonstrated. Polson et al. [33] described an on-line approach based on the same mechanism using latex spheres as model analytes. Such mobility-based selectivity is attractive for on-line preconcentration because it can be easily controlled by adjusting experimental conditions such as pressure and voltage without changing any hardware. Recently, mobility-based selectivities have also been demonstrated for differential sample transport in microfluidic devices. Culbertson et al. [34] described an electroosmotically induced hydraulic pump for microfluidic devices by using a tee structure with one inlet channel and two outlet channels. A pressure-induced flow was created in both outlet channels by using polymer coatings to selectively reduce EOF in the ground channel. The pump could differentially transport ions with different mobilities to the two outlet channels. A similar technique, called "selective ion extraction" was described by Kerby et al. [35]. By combining hydrodynamic and electrokinetic flow control in microfluidic devices, mixtures of compounds sent to a T-junction on a chip could be completely separated into different channels on the basis of their electrophoretic mobilities.

In this paper, a simple approach for mobility-based selective on-line preconcentration of proteins in CE is described and demonstrated. While more work is required to fully develop this method, the preliminary results presented here show the potential of this approach.

## 2. Theory

The EOF velocity in a fused silica capillary, $v_{eo}$, can be expressed as:

$$v_{eo} = \mu_{eo} E = \frac{\varepsilon \zeta}{\eta} E \quad (1)$$

where $\mu_{eo}$ is the electroosmotic mobility, $E$ the electric field intensity, $\zeta$ the zeta potential of the capillary inner surface, and $\varepsilon$ and $\eta$ are the dielectric constant and viscosity of the background electrolyte, respectively. Several methods have been investigated to control the direction and magnitude of EOF in a fused silica capillary, including chemically modifying the capillary surface [36], using a buffer additive [37], applying a radial electric field [38,39] and connecting capillaries with different $\zeta$ potentials [40,41]. All of these methods are based on modification of the $\zeta$ potential. Eq. (1) also shows that the EOF velocity is proportional to the applied electric field intensity. However, because the electrophoretic migration velocity of the analyte, $v_{ep}$, is also proportional to the local electric field intensity:

$$v_{ep} = \mu_{ep} E \quad (2)$$

where $\mu_{ep}$ is the electrophoretic mobility of the analyte, $v_{eo}$ cannot be controlled independently of $v_{ep}$. In order to decouple the EOF from the local electric field intensity, either a non-uniform $\zeta$ potential or a non-uniform electric field intensity must be used. An easy method to form a non-uniform electric field intensity in CE is to use two high voltage sources as described by Dasgupta and Liu [42]. Such a design was used in this paper as shown schematically in Fig. 1. Two sections of fused silica capillary with the same inner diameter were connected through a porous joint which can be either a short section of dialysis fiber or an etched capillary. The free end of each capillary and the porous joint were immersed in three buffer reservoirs and filled with the same background electrolyte. High voltage was applied at the free end of each capillary while the porous joint was grounded. The porous joint served three purposes, that is, to allow electrical conductivity, to prevent passage of protein analytes, and to minimize cross-wall bulk flow. The positive

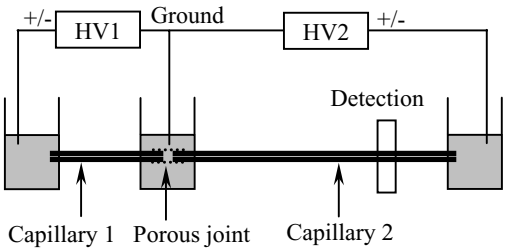



Fig. 1. Schematic diagram of the on-line preconcentration system with EOF control.

direction of the system was defined as from the free end of capillary 1 to the porous joint, or from the porous joint to the free end of capillary 2, as shown in Fig. 1.

The EOF velocity in each capillary, $v_{eo,i}$, can be expressed as [41]:

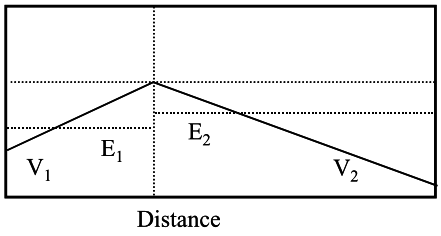
$$v_{eo,i} = \mu_{eo} E_i + \frac{\Delta P_i {r_i}^2}{8\eta L_i} \tag{3}$$

where $E_i$ is the local electric field intensity, $L_i$ and $r_i$ are the length and radius of each capillary, respectively, $\Delta P_i$ is the pressure drop induced by the unmatched EOF flow on each capillary, and $i$ can be 1 or 2. For a noncompressible liquid, the continuity principle implies that the volumetric flow rates in both capillaries are equal. For capillaries with the same radius and, thus, the same cross-sectional area, the EOF velocities in both capillaries are the same. Assuming steady, fully developed flow in both capillaries, the pressure drops in both capillaries should satisfy $\Delta P_1 = -\Delta P_2$. Substituting these conditions into Eq. (3), the EOF velocity in the system is obtained as:

$$v_{eo} = \frac{\mu_{eo} E_1 L_1}{L} + \frac{\mu_{eo} E_2 L_2}{L} = \frac{\mu_{eo}(V_1 + V_2)}{L} \tag{4}$$

where $V_1$ is the voltage drop from the free end of capillary 1 to the porous joint and $V_2$ is the voltage drop from the porous joint to the free end of capillary 2. The total length ($L$) of both capillaries is equal to $L_1 + L_2$. It can be seen from Eq. (4) that the EOF velocity is proportional to the total voltage drop divided by the total length of both capillaries (this is not an electric field intensity, although it has the same units) and is independent of the local electric field. Meanwhile, the electrophoretic migration velocity of the analyte is still only proportional to the local electric field intensity. In this way, the EOF velocity can be controlled independently.

The principle of mobility-based selective preconcentration of proteins is shown schematically in Fig. 2. Here we only consider negatively charged proteins (a treatment for positively charged proteins can be easily derived in a similar manner). Consider two negatively charged proteins with mobilities $\mu_{ep1}$ and $\mu_{ep2}$, where $|\mu_{ep1}| < |\mu_{ep2}|$. Setting voltages $V_1 < 0$, $V_2 > 0$, and $V_1 + V_2 > 0$, then according to Eq. (4), an EOF will exist in the system with direction from the free end of capillary 1 to the free end of capillary 2. In capillary 1, the electrophoretic migration of the protein analytes is in the same direction as the EOF, while in capillary 2, the directions are opposite to each other. Let $\bar{\mu}_{ep} = (\mu_{ep1} + \mu_{ep2})/2$ and set voltages $V_1$ and $V_2$ such that:



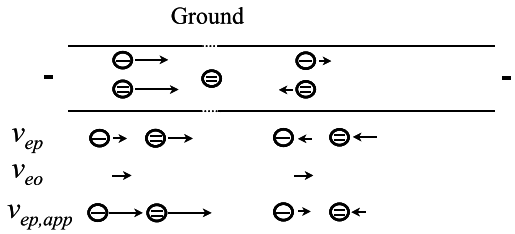




Fig. 2. Schematic diagram of the principle of mobility-based selective on-line preconcentration.

$$\frac{\mu_{eo}(V_1 + V_2)}{L} + \frac{\bar{\mu}_{ep} V_2}{L_2} = 0 \tag{5}$$

Then for the protein with mobility $\mu_{ep1}$ in capillary 1, the apparent electrophoretic migration velocity is positive, which means that the protein will migrate from the free end of capillary 1 to the porous joint; in capillary 2, the apparent electrophoretic migration velocity is also positive, which means that the protein will migrate from the porous joint to the free end of capillary 2. Therefore, it will be pushed out of the system and not concentrated. On the other hand, for the protein with mobility $\mu_{ep2}$, the apparent electrophoretic migration velocity is positive, which means that the protein will migrate from the free end of capillary 1 to the porous joint; in capillary 2, the apparent electrophoretic migration velocity will become negative, which means that the protein will migrate back to the porous joint once it enters capillary 2. Therefore, it will be concentrated in the porous joint and mobility-based selective concentration will be achieved. Solving Eq. (5), we obtain:

$$V_2 = \frac{-V_1}{1 + (\bar{\mu}_{ep} L / \mu_{eo} L_2)} \tag{6}$$

Since voltage $V_1$ will determine the speed of concentration, it should be set to a value as high as possible without causing too much Joule heating. Once voltage $V_1$ is set, voltage $V_2$ can be obtained from Eq. (6).

## 3. Experimental

### 3.1. Chemicals and materials

The dialysis hollow fiber was a modified cellulose fiber from Membrana (Wuppertal, Germany) with an internal diameter of 200 μm and a dry wall thickness of 8 μm. The molecular mass cut-off (MWCO) of this fiber was 10 000. Untreated fused silica capillary tubing (50 μm i.d. × 186 μm o.d.) was purchased from Polymicro Technologies (Phoenix, AZ, USA). Model proteins, β-lactoglobulin B from bovine milk ($M_r$ = 18 400), myoglobin from horse heart ($M_r$ = 16 890), cytochrome *c* from horse heart ($M_r$ = 13 370), lysozyme from chicken egg white ($M_r$ = 13 930) and ribonuclease A from bovine pancreas ($M_r$ = 13 700), were obtained from Sigma (St. Louis, MO, USA). DMSO (dimethyl sulfoxide), Tris[tri(hydroxymethyl)aminomethane], phosphoric acid, boric acid and sodium hydroxide were also obtained from Sigma. The buffer solutions were prepared with deionized water from a Millipore water purifier, filtered through a 0.22 μm filter, and degassed with an ultrasonic vibrator before use.

### 3.2. Instrumentation

A schematic diagram of the experiment setup is shown in Fig. 1. Two lengths of fused silica capillary were inserted into a short section of dialysis hollow fiber and glued with epoxy. The distance between the ends of the capillaries inside the fiber was 1 mm. One section of capillary with a length of 10 cm was used for protein concentration (capillary 1), and the other section of capillary with a length of 45 cm was used for CE separation (capillary 2). This fiber-capillary assembly was then fixed to a plastic buffer vial by epoxy. Two high voltage sources with switchable polarity (Model CZE 1000R, 30 kV, 300 μA, Spellman, Hauppauge, NY, USA) were used simultaneously in the experiments. A 2 mm long detection window was made by burning off the polyimide coating on capillary 2 at 30 cm from the end connected to the fiber, and on-line detection at 214 nm was achieved by using a UV-Vis absorption detector with fiber optics detection accessory from ThermoQuest (Model UV3000, Riviera Beach, FL, USA). Since high voltage was used in these experiments, care should be taken to avoid electrical shock.

## 4. Results and discussion

### 4.1. Porous joint

The key component of the selective on-line preconcentration system is the porous joint. Two different types of porous joint could have been used: polymeric dialysis hollow fiber or etched capillary. In our experiments, a short section of dialysis hollow fiber was used because the fiber, which has been used for hemodialysis, has a well-defined pore structure and demonstrates low adsorption of proteins. The electrical current remained stable during the preconcentration step which indicated a high dialysis rate of background electrolytes through the fiber wall. One major concern was the hydraulic permeability of the dialysis hollow fiber, which is the flow rate across the fiber wall under pressure. For a significantly high flow rate, the conditions for Eq. (4) would become invalid. The hydraulic permeability of the fiber was 5.4 ml $h^{-1}$ $m^{-2}$ $mmHg^{-1}$ as specified by the manufacturer. From simple calculations, the flow rate was found to be less than 0.5% of that in a 10 cm × 50 μm i.d. capillary induced by the same pressure drop. Therefore the flow across the fiber wall was considered to be negligible.

### 4.2. EOF control

Eq. (4) was evaluated experimentally. DMSO was used as EOF marker, and the running buffer was 100 mM boric acid, adjusted to pH 8.2 with Tris. The voltage drop applied to capillary 1 was set at −5 kV, while the voltage drop applied to capillary 2 was varied from 0 to 10 kV and, thus, the total voltage drop was varied from −5 to 5 kV. A 0.2% DMSO solution in the running buffer was hydrodynamically injected for 2 s by applying a nitrogen pressure of 1 psi. When the total voltage drop was negative, DMSO eluted from the end of capillary 1, and when the total voltage drop was positive, DMSO eluted from the end of capillary 2. The results are shown in Fig. 3. The EOF velocity increased linearly with the total voltage drop, and changed direction when the polarity of the total voltage drop was changed. Very good linearity was found between the EOF velocity and the total voltage drop ($R^2$ = 0.9999), as expected from Eq. (4). The reproducibility of the EOF velocity was also determined, and the R.S.D. of the EOF velocities shown in Fig. 3 were all less than 1.4% ($n$ = 6). Since the voltage drop over capillary 1 was kept constant, the local electric field intensity in capillary 1 was also constant; however, the EOF velocity changed

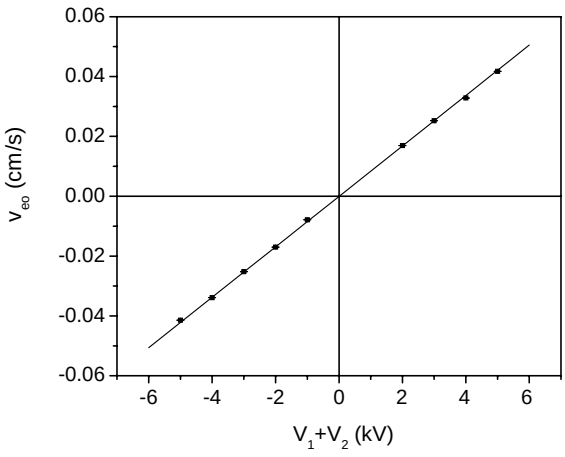



Fig. 3. Dependence of EOF velocity on total voltage.

as the total voltage drop over the system was changed. Therefore, the EOF could be controlled independently of the local electric field. Such ability to control EOF gives the current system more flexibility to perform on-line preconcentration in CE compared with the methods described by Wu et al. [30] or Wei and Yeung [31]. Furthermore, this method works well under basic conditions since the EOF can be controlled by the total voltage drop. As we demonstrate below, it is also possible to perform mobility-based selective on-line preconcentration in CE with this system.

### 4.3. On-line preconcentration of proteins

According to Eq. (4), if we set $V_1 + V_2 = 0$, then the EOF velocity in the system is zero. However, the electrophoretic migration velocity is not zero, because it is only proportional to the local electric field intensity. For positively charged proteins, if we set $V_1 > 0$, $V_2 < 0$, and $V_1 + V_2 = 0$, then wherever the protein is, it will be forced to the porous joint by the local electric field and become concentrated. Fig. 4A shows a typical electropherogram of three positively charged model proteins after on-line preconcentration. The sample was a protein mixture containing 1 μg ml$^{-1}$ cytochrome *c*, lysozyme and ribonuclease A in the running buffer (50 mM phosphoric acid, adjusted to pH 3 with Tris) which was electrokinetically injected from the free end of capillary 1 after both capillaries and the other two reservoirs were filled with running buffer. The voltage drops for injection were set at $V_1 = 5$ kV and $V_2 = -5$ kV. The injection time was 60 min. The electrical currents over capillaries 1 and 2 were 21 and 5 μA, respectively, and remained stable despite the long injection time. During this step, as can be seen from Fig. 1, cations in the background electrolytes such as hydrogen and Tris ions migrated across the fiber wall into the outside buffer reservoir, while anions such as phosphate ions migrated across the fiber wall into the porous joint. The stable current during the long injection step indicated that there was no ion accumulation in the porous joint because of the high permeability of buffer ions across the fiber wall. This was important for preconcentration because locally accumulated buffer ions would cause problems such as Joule heating or de-stacking.

The long 60 min injection of a very low concentration sample (1 μg ml$^{-1}$) was selected to demonstrate the advantage of concentrating a sample from a dilute solution. However, 15, 30, and 45 min injections of 10 μg ml$^{-1}$ protein sample were also tested and gave similar results. The linearity of peak area versus injection time ($R^2$) was approximately 0.9.

After injection, the concentrated sample was hydrodynamically transferred (because the EOF was very low under acidic conditions and the electric field in the porous joint was low since it had a larger internal diameter than the capillary) into capillary 2 for CE separation by applying a nitrogen pressure of 1 psi at the end of capillary 1 for 20 s. The voltage drops for separation were set at $V_1 = 2.2$ kV and $V_2 = 10$ kV, which were chosen to provide a uniform electric field intensity across the whole system and to avoid disturbance of the EOF. For comparison, the same protein sample was also analyzed using CE without preconcentration. In this case, only one capillary of the same dimensions

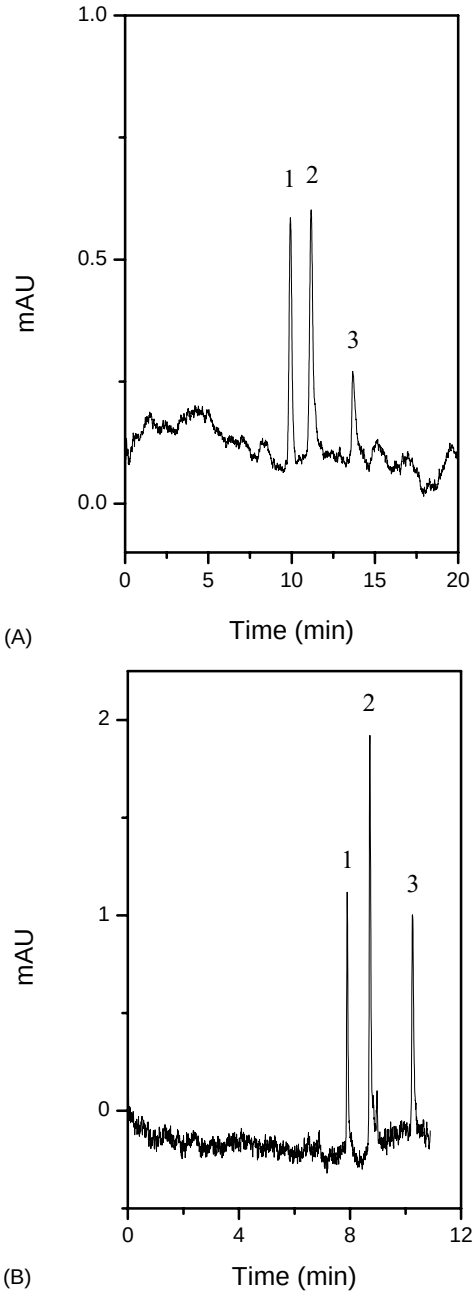



Fig. 4. Electropherogram of positively charged model proteins (A) with on-line preconcentration and (B) without on-line preconcentration. Experimental conditions: (A) 50 mM phosphoric acid, pH 3, run buffer; protein mixture containing 1 μg ml$^{-1}$ cytochrome *c*, lysozyme and ribonuclease A in the run buffer; injection voltages $V_1 = 5$ kV and $V_2 = -5$ kV, 60 min injection time; separation voltages $V_1 = 2.2$ kV and $V_2 = 10$ kV; 214 nm UV detection. (B) Protein mixture containing 100 μg ml$^{-1}$ cytochrome *c*, lysozyme and ribonuclease A in the run buffer; 1 psi injection for 2 s; 10 kV separation voltage. Peak identifications: (1) cytochrome *c*, (2) lysozyme, (3) ribonuclease A.

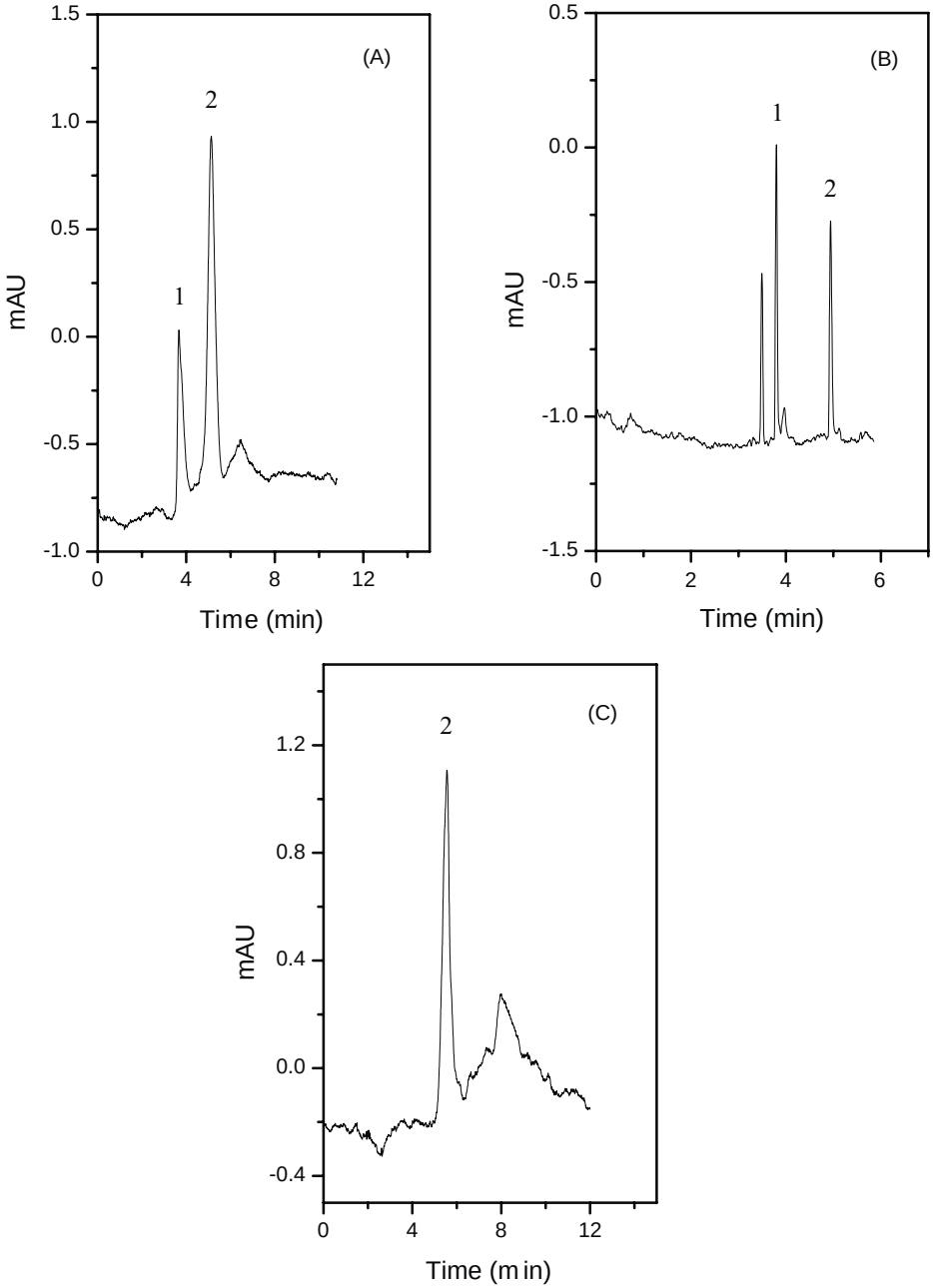



Fig. 5. Electropherograms of negatively charged model proteins (A) after on-line preconcentration, (B) without on-line preconcentration, and (C) after mobility-based selective on-line preconcentration. Experimental conditions: (A) 100 mM boric acid, pH 8.2, run buffer; protein mixture of 5 μg ml$^{-1}$ myoglobin and β-lactoglobulin B in the run buffer; injection voltages $V_1 = -5$ kV and $V_2 = 5$ kV; 15 min injection time; separation voltages $V_1 = 2.2$ kV and $V_2 = 10$ kV. (B) Protein mixture of 100 μg ml$^{-1}$ myoglobin and β-lactoglobulin B in the run buffer, 1 psi injection for 2 s; 10 kV separation voltage. (C) Injection voltages $V_1 = -5$ kV and $V_2 = 6.3$ kV; other conditions were the same as in (A). Peak identifications: (1) myoglobin, (2) β-lactoglobulin B, the peak before myoglobin in (B) was DMSO.

as capillary 2 was used, and no peak was detected in the electropherogram (data not shown).

A highly concentrated sample, a protein mixture containing 100 μg ml$^{-1}$ cytochrome *c*, lysozyme and ribonuclease A in the running buffer, was then analyzed using CE without preconcentration. The sample was injected by applying a nitrogen pressure of 1 psi for 2 s, and the result is shown in Fig. 4B. Pressure injection was used because there is no injection bias in pressure injection and, therefore, it represents the actual concentrations of proteins in the sample. Higher efficiency was achieved with direct CE analysis, which is probably due to the difference in sample volume. From a simple calculation, the sample volume in CE after preconcentration, which is the volume of the porous joint, was approximately 34 nl, while that in direct CE was only 4.7 nl. Some migration time shifts are also observed by comparing

Fig. 4A and B, which is probably due to inaccurate control of voltages and EOF modification during sample injection. By comparing the peak areas and concentration differences in the two electropherograms, concentration factors for cytochrome *c*, lysozyme and ribonuclease A were estimated to be 229, 184 and 128, respectively. The difference in concentration factors for different proteins is due to electrokinetic injection bias [3]. To further increase the concentration factor, a porous joint with the same inner diameter as the capillaries should be used, which is the present focus of our efforts in this area.

For negatively charged proteins, if we set $V_1 < 0$, $V_2 > 0$, and $V_1 + V_2 = 0$, then the local electric field would force the proteins to concentrate in the porous joint. Fig. 5A shows a typical electropherogram of two negatively charged model proteins after on-line preconcentration. The sample contained 5 μg ml$^{-1}$ myoglobin and β-lactoglobulin B in the running buffer (100 mM boric acid, adjusted to pH 8.2 with sodium hydroxide). The injection voltages were set at $V_1 = -5$ kV and $V_2 = 5$ kV, and the injection time was 15 min. After injection, the voltages were switched to $V_1 = 2.2$ kV and $V_2 = 10$ kV, and the concentrated sample was electrokinetically transferred into capillary 2 by EOF for CE separation. Electrokinetic injection was used instead of hydrodynamic injection since the EOF flow was strong enough in this case to transfer the concentrated sample band quickly. The two proteins were well separated after preconcentration. For comparison, the same sample with higher concentration, a protein mixture containing 100 μg ml$^{-1}$ each of myoglobin and β-lactoglobulin B with 0.2% DMSO in the running buffer, was then analyzed using CE without preconcentration. The sample was injected by applying a nitrogen pressure of 1 psi for 2 s, and the result is shown in Fig. 5B. By comparing the peak areas and concentration differences in the two electropherograms, concentration factors for myoglobin and β-lactoglobulin B were estimated to be 60 and 167, respectively. An unknown broad peak eluted after β-lactoglobulin B, which was not observed when concentrating positively charged proteins in acidic conditions as shown. The pH value of the buffer used here was 8.2 and, under these conditions, the electroosmotic mobility, $\mu_{eo}$, was large ($6.68 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ as determined experimentally). Capillary inner surface modification, such as protein adsorption during the injection step, could slightly change $\mu_{eo}$ in capillary 1, which would lead to unmatched EOF in the system. Such unmatched EOF could then disturb the concentrated protein band and push part of the band back to capillary 1, which would lead to additional peak broadening in the electropherogram. On the other hand, $\mu_{eo}$ was small under acidic conditions and, therefore, no such ghost peak appeared.

Mobility-based selective on-line preconcentration was also demonstrated for two negatively charged proteins, i.e. myoglobin and β-lactoglobulin B. In the running buffer (100 mM boric acid adjusted to pH 8.2 with sodium hydroxide), β-lactoglobulin B had a more negative electrophoretic mobility than myoglobin. Therefore, β-lactoglobulin B could be selectively concentrated from a mixture of myoglobin and β-lactoglobulin B by setting the EOF to a specific value. The electrophoretic mobilities of myoglobin and β-lactoglobulin B in the running buffer, and the EOF mobility were determined experimentally as −0.47, −1.70 and $6.68 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$, respectively. The required voltage drops for selective on-line preconcentration of β-lactoglobulin B were found to be $V_1 = -5$ kV and $V_2 = 6.3$ kV according to Eq. (6). The preconcentration procedure was the same as that used for negatively charged proteins, except the sample reservoir was replaced by a buffer reservoir and the same voltages were applied for another period of time. This step was used to ensure that myoglobin was purged completely from capillary 2 before CE separation was started. Different periods of time were tested and the results indicated that 10 min was long enough. Fig. 5C shows a typical electropherogram of β-lactoglobulin B after selective on-line preconcentration. Compared with Fig. 5A, the peak for myoglobin disappeared which indicates that selective preconcentration was achieved. For an injection time of 15 min, a concentration factor of 152 was achieved for β-lactoglobulin B. Slightly lower concentration factor was achieved compared with Fig. 5A. The unknown broad peak also became larger, which supports the explanation given above for this broad peak.

## 5. Conclusions

Mobility-based selective on-line preconcentration of protein samples in CE was demonstrated by controlling EOF using two high voltages, each over a capillary connected to each other through a short section of dialysis hollow fiber. The EOF velocity was found to be proportional to the total voltage drop over the two capillaries and independent of the local electric field. Mobility-based selectivity was achieved by setting the EOF velocity to a specific value; proteins with high mobilities were concentrated while those with low mobilities eluted. The advantage of the current system was that the selectivity could be easily changed by changing the operating parameters (the voltages) without any change in system hardware. Compared with conventional CE, broad peaks were typically observed when using the current on-line preconcentration system, which was mainly due to the large-volume of the porous joint. To further improve the system performance, a porous joint with the same inner diameter as the separation capillary and smaller volume is needed.

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