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Field-amplified polarity-switching sample injection in high-performance capillary electrophoresis

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ABSTRACT

In conventional electro-injection in high-performance capillary electrophoresis, although one is able to inject both positive and negative ions into the column, the number of negative ions injected is rather limited because of their movement against the electric field, assuming the column wall is negatively charged. If one simply reverses the polarity of the field, the electroosmotic flow will deter all positive and most of negative ions from injecting into the column. In the case of field-amplified sample injection, where samples are prepared in a low-conductivity buffer and injected electrically into the column, the number of positive ions injected is porportional to the field enhancement factor at the injection point. The negative ions will not be enhanced, but will be pushed away from the column by this high field strength. However, since the electroosmotic velocity of the bulk solution is much slower than the electrophoretic velocity of sample ions under the enhanced field, one can inject and concentrate both positive and negative ions into the column by switching the polarity of the electrodes at the proper time. Furthermore, one can also achieve selected charge discrimination.

INTRODUCTION

High-performance capillary electrophoresis (HPCE) has become a major analytical technique for separating charged compounds [1–3]. In HPCE, a high voltage is applied across a fused-silica capillary column filled with an electrolytic buffer. Charged species introduced at one end of the column migrate under the influence of the electric field to the other end of the column. The migration velocity of a particular ion species is a combination of the electrophoretic velocity of that species and the bulk electroosmotic velocity of the buffer. In many cases, depending on the column properties and the buffer conditions, the electrophoretic velocity is much less than the electroosmotic velocity. The net movement of the ions is in the same direction as the electroosmotic flow; generally positive ions migrate ahead of the neutral compounds followed by the negative ions.

One of the most interesting properties of HPCE is the electroomotic flow. The electroomotic force in a capillary column is produced by an electric field and transmitted by the drag of ions acting in a thin sheath of charged fluid adjacent to the silica wall of the column. The origin of the charge in the sheath is an unbalance between

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positive and negative ions in the bulk solution which balances a fixed charge on the silica wall thus generating a zeta potential ζ.

While the electrophoretic velocity of ionic species is simply the product of their electrophoretic mobilities and the electric field strength acting on them, the analysis of the electroosmotic velocity of the bulk solution is rather complicated. In columns where the electric field strength E and the zeta potential are constant throughout the whole column, the bulk electroosmotic velocity, v_{eo} can be expressed as $\mu_{oe}E$, where $\mu_{oe} = \varepsilon\zeta/4\pi\eta$ is called the electroosmotic mobility and ε is the dielectric constant and η is the viscosity of the buffer. However, in the case where there is a non-constant distribution in either field strength or zeta potential, the bulk electroosmotic velocity has to be averaged over all different local electroosmotic velocities weighted by the distribution factor [4–6]. This special property of electroosmotic flow in the averaging process turns out to be very useful in sample introduction, especially in field-amplified sample injection.

For conventional electro-injection in HPCE, samples are prepared in a buffer solution which has the same concentration as that used in separation. The number of ions, both positive and negative, injected into the column under this condition is rather limited. To increase the number of ions introduced into the column, the sample can be prepared in a highly diluted buffer or water, and then injected by electro-injection into the column. Because of the high resistivity of the sample solution, an enhancement in the electric field strength is set up at the injection point [7,8]. This field-amplified sample injection, first used in zone electrophoresis in a glass powder column by Haglund and Tiselius [9], can yield several hundred fold enhancement in the number of ions injected in HPCE [10–12]. In addition, the difference in the field strength distributions will casue injected sample ions stack into a narrow band once they migrate into the region of high-concentration buffer.

In the absence of electroosmotic flow, one can achieve sample enhancement for either positive or negatieve ions by simply choosing the proper polarity of electrodes. However, field-amplified sample injection only works for one type of ions if there is an electroosmotic flow. Under normal polarity, where the electroosmotic flow is toward the outlet of the column, the high field strength at the injection point will push away ions which have a negative mobility with respect to the electroosmotic flow. With reversed polarity, although the high field strength will pull the negative ions into the column, they will be carried out immediately from the injection end by the electroosmotic flow during sample injection.

Nevertheless, the electroosmotic velocity of the solution is a bulk property and has to be averaged over the whole column. As a result, it changes insignificantly from the pure buffer system during sample injection and is much slower than the electrophoretic velocity of sample ions under the enhanced field [6]. Therefore, by introducing a short plug of water prior to sample injection and selecting the proper polarity of the electrodes, one can inject and concentrate either positive or negative ions into the capillary column using field-amplified sample injection. Furthermore, if one switches polarities *during* the injection procedure at the proper time, an enhanced sample injection of both negative and positive ions under amplified conditions can be accomplished. This report will describe and present the experimental results of both charge discrimination sample enhancement injection and field-amplified polarity-switching injection.

EXPERIMENTAL

The experimental apparatus is very similar to the one described by Jorgenson and Lukacs [3]. Electrophoresis was carried out in a 100 cm × 365 μm O.D. × 75 μm I.D. fused-silica capillary column (Polymicro Technologies, Phoenix, AZ, USA). We used two power supplies in the system: one for injection, ±5 kV (Tennelec, Oak Ridge, TN, USA), the other for separation, −30 kV voltage (Glassman, Whitehouse Station, NJ, USA). The high-voltage end of the injection power supply, which can be switched between ±5 kV, was connected to a platinum wire dipped into the reservoir filled with low-concentration electrolyte or the reservoir filled with the sample solution. This power supply was connected to the system only during sample injection.

After sample injection, the ground end of the power supply for separation was connected to a platinum wire dipped into the reservoir filled with high conductivity at the inlet end of the column. The high-voltage end of the power supply, which was set at −30 kV, was connected to a platinum wire dipped into the reservoir at the outlet end of the column.

Chemical and electrolytes

To minimize Joule heating effects, a buffer of 2-N-(morpholine)ethanesulfonic acid (MES) and histidine (HIS) at pH 6.2 was chosen for our study. A stock solution of 100 m*M* with respect to both MES and HIS was prepared. Four amino acids, two positive ions: phenylthiohydantion (PTH)-arginine, PTH-histidine, and two negative ions: PTH-aspartic acid, PTH-glutamic acid, were chosen to illustrate our technique. A stock solution contained 2.1 mg PTH-arginine, 2.0 mg PTH-histidine, 2.8 mg PTH-aspartic acid and 3.2 mg PTH-glutamic acid in 10 ml of water was made. The sample was then diluted down to about $5 \cdot 10^{-5}$ *M* in both water and 100 m*M* MES–HIS. All reagents were purchased from Sigma (St. Louis, MO, USA).

RESULTS AND DISCUSSION

Charge discrimination enhanced injection

It is well known in electrophoresis that one can achieve sample enhancement by preparing the sample in a highly dilute buffer or water. Fig. 1 is a schematic diagram showing our experimental procedures for positive ion injection. A short plug of water is introduced into the capillary column before sample injection to establish enhanced electric field. The sample, which is dissolved in water, is then injected electro-kinetically into the column under this enhanced field. Once the positive ions reach the concentration boundary, they slow down and stack into a narrow band. Two to three orders of signal enchancement have been achieved using field amplified sample injection [12]. Fig. 2a and b compare the electropherograms of our sample solution using conventional electro-injection and field amplified sample injection. The absorbance unit in Fig. 2b is 100 times the unit in Fig. 2a. Peaks A and B in Fig. 2 are the two positive ions, PTH-arginine and PTH-histidine, respectively. Peak C is the neutral and water signal. Peaks D and E in Fig. 2a are the two negative ions, PTH-aspartic acid and PTH-glutamic acid, respectively.

In the conventional electro-injection, although there is a large bias against negative ions, they still can migrate into the column as long as the absolute value of their

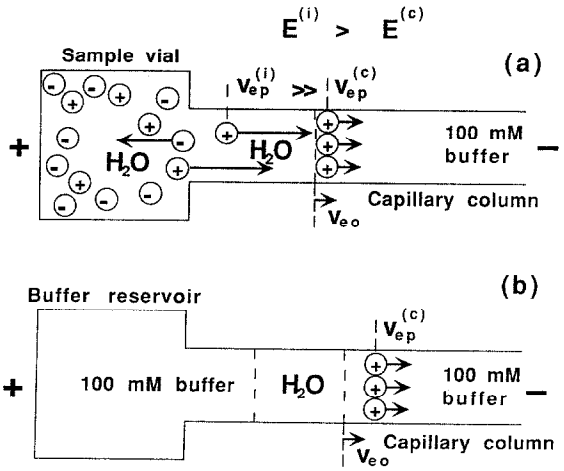

Fig. 1. A schematic diagram showing field-amplified sample injection for positive ions only: A short plug of water is introduced into the column before sample injection to set up the high field strength. (a) The sample prepared in water is then injected into the column using a positive voltage with respect to the other end of the column. (b) After sample injection, the inlet end of the column is switched into the high concentration buffer to start the separation process. $E^{(i)}$ is the electric field strength and $v_{ep}^{(i)}$ is the electrophoretic velocity of the ions at the injection end. $E^{(c)}$ and $v_{ep}^{(c)}$ are the field strength and electrophoretic velocity in the rest of column. v_{eo} is the averaged electroosmotic velocity of the bulk solution.

mobility is smaller than the electroosmotic mobility [10]. Fig. 2a shows that a small number of negative ions are detected using conventional electro-injection. When the sample is prepared in water, there is an enhanced electric field strength at the injection point. The negative ions in the sample solution are now pushed away from the column by this high field strength. Fig. 2b shows no detection of negative ions in field-amplified sample injection. For comparison, a electropherogram without sample bias using gravity injection is shown in Fig. 3.

To inject only negative ions into the uncoated column under the enchanced field strength, a plug of water is introduced into the column first by raising the water reservoir up to 15 cm for 30 s. After the water plug is injected, the inlet end of the column is then switched from the water reservoir to the fourth reservoir containing the sample dissolved in the water. A -5 kV with respect to the outlet end of the column is applied for 10 s at the inlet causing negative ions to electro-migrate into the column. A subtle difference in injecting the negative ions is that the water (or low concentration buffer) plug is now moving out of the column from the injection end. Consequenlty, the length of this water plug should be long enough such that some of it remains inside the column at the end of the sample injection time. A schematic diagram showing the negative ion injection is displayed in Fig. 4. The positive ions will be pushed away from the column during injection under the reversed polarity. A very effective charge discrimination signal-enhanced injection is achieved.

After sample injection, the column is then switched back to high-concentration buffer and the separation voltage with the normal polarity is applied to the column. The bulk solution and the sample plug now migrate toward the detector and the negative ions migrate back toward the injection end as shown in Fig. 4b. Once the

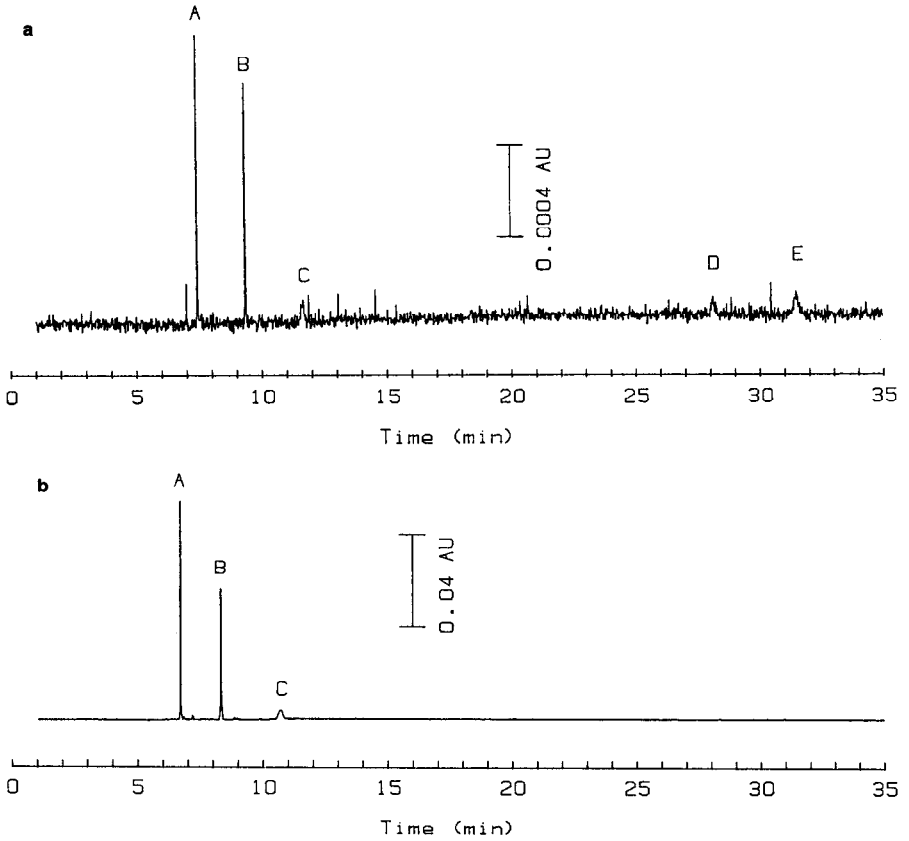

Fig. 2. (a) Electropherogram using conventional electro-injection: column was filled with 100 m*M* MES–HIS buffer, sample was also prepared in 100 m*M* MES–HIS buffer, −5 kV injection voltage for 10 s, −30 kV separation voltage; (b) electropherogram using field-amplified sample injection: experimental conditions are the same as in (a) except the sample was prepared in water. Peaks A, B, C, D and E correspond to PTH-arginine, PTH-histidine, neutral marker, PTH-aspartic acid and PTH-glutamic acid, respectively.

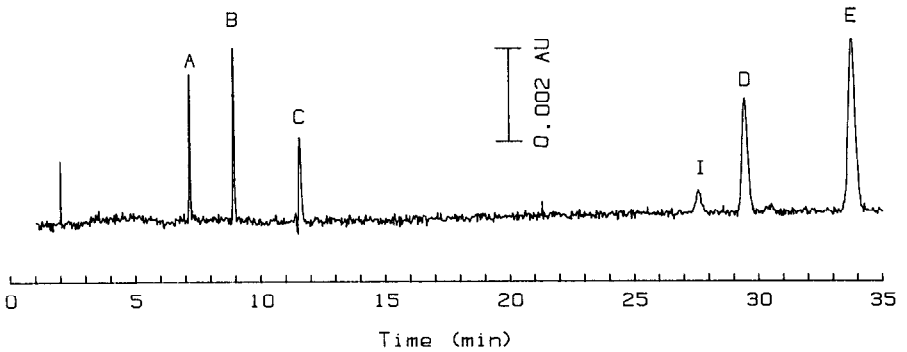

Fig. 3. Electropherogram using gravity injection. The experimental conditions are the same as described in Fig. 1. Peak I is the impurity.

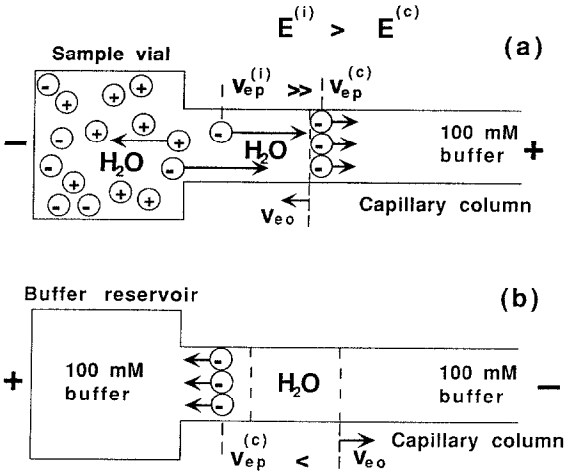

Fig. 4. A schematic diagram showing field-amplified sample injection for negative ions only. (a) A short plug of water is introduced into the column before sample injection, the sample prepared in water is then injected into the column using a negative voltage with respect to the other end of the column. (b) After sample injection, the inlet end of the column is transferred into the high concentration buffer and the polarity of the electrodes is switched back to normal setting to start the separation process.

negative ions reach the high-concentration region, they will experience the normal field strength and slow down into a narrow band. If the absolute value of their electrophoretic mobility is smaller than the value of the bulk electroosmotic mobility, the negative ions in the low electric field region will change direction again and be pushed passed the detector at the outlet end of the column.

Fig. 5 shows the electropherograms resulting from the charge discrimination injection of our sample solution. It shows large enhanced negative ion signals, peaks D and E, with no detection of positive ions. Compared with conventional electro-injection shown in Fig. 2a, a factor of 500 in signal enhancement for negative ions is observed. Also note that the impurities, peaks I in Fig. 5, are concentrated so they are now detectable whereas in conventional electro-injection, Fig. 2a, these compounds are not seen.

Field-amplified polarity-switching injection

Field-amplified sample injection described above only works for one type of ion, either positive or negative ions depend on the electrode configuration. By switching the polarity of the electrodes at proper times during injection, one can manage to inject and concentrate both positive and negative ions into the column. This process is shown schematically in Fig. 6.

In field-amplified polarity-switching injection, samples of both positive and negative ions are prepared in the low-conductivity buffer. We first inject, either by gravity or by electro-injection, a plug of low-conductivity buffer of water into the column before sample introduction. Then, a large number of positive ions can be injected into the column under a positive high voltage with respect to the outlet end of the column for a time period t_1 as described earlier. We then switch the voltage to the

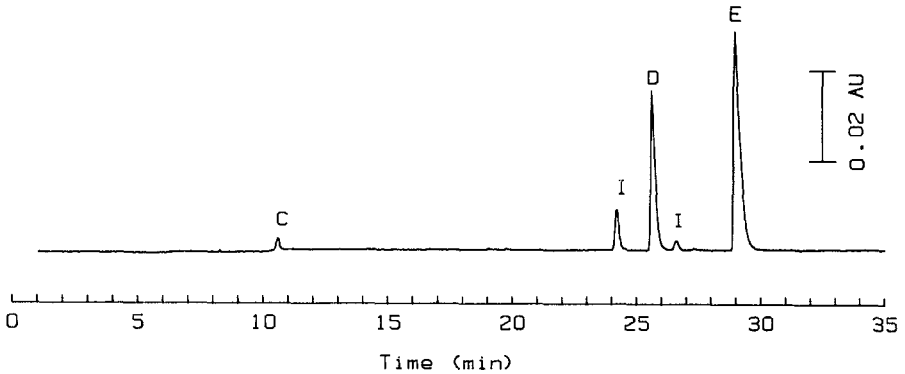

Fig. 5. Electropherogram using field-amplified sample injection with reversed polarity. The experimental conditions are the same as described in Fig. 1. A small amount of neutral molecules (C) and large negative ion peaks (D en E) were detected, no detection of positive ions. Two small peaks (I) are the impurities inside the sample solution which are barely detectable in gravity injection.

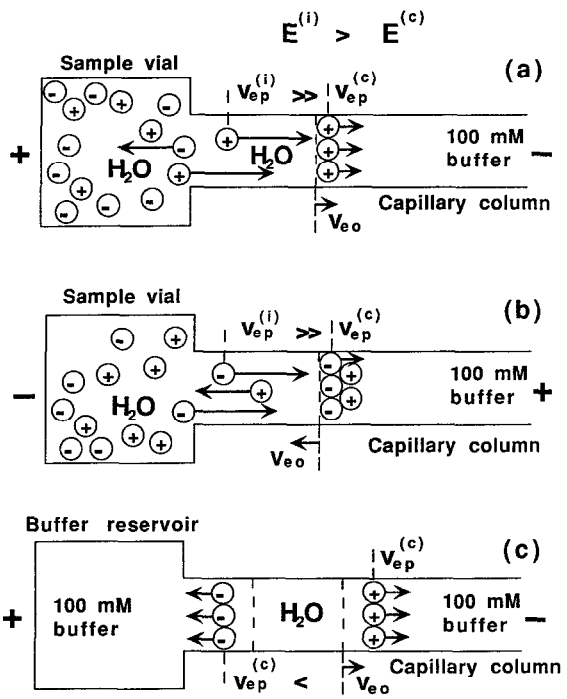

Fig. 6. A schematic diagram showing field-amplified polarity-switching injection. (a) A short plug of water is introduced into the column before sample injection. The positive ions are injected into the column first using a positive voltage with respect to the other end of the column. (b) The negative ions are then injected using reversed polarity. (c) After sample injection, the inlet end of the column is transferred into the high concentration buffer and the polarity of the electrodes is switched back to normal setting to start the separation process.

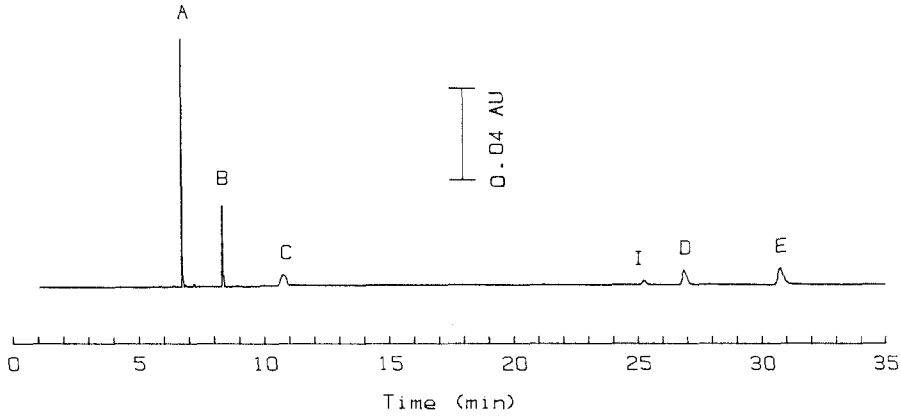

Fig. 7. Electropherogram using field-amplified polarity-switching injection. Both positive and negative ions were detected.

opposite polarity and cause the electroosmotic flow to migrate in the opposite direction and cause some of the positive ions to flow out of the column. However, since the electric field at the injection end of the column is very high, the negative ions which now have a very high electrophoretic velocity will overcome the electroosmotic flow and migrate into the column as we decribed earlier in charge discrimination injection. This reversed polarity can last for a shorter period of time t_2 such that part of the positive ions will remain inside the column.

After injection of both positive and negative ions, the inlet end of the column is switched back into the high conductivity buffer reservoir. The polarity of the high voltage is also switched back to the normal setting and the separation process begins. Both positive and negative sample ions will now migrate rapidly into the high-concentration regions, at opposite ends of the water plug and stack into narrow bands.

TABLE I

COMPARISON OF PEAK HEIGHTS FOR BOTH POSITIVE AND NEGATIVE IONS USING VARIOUS INJECTION METHODS

All peaks are normalized with respect to the gravity injection.

	PTH-Arginine	PTH-Histidine	PTH-Aspartic acid	PTH-Glumatic acid
Gravity injection	1	1	1	1
Conventional electro-injection sample in 100 m*M* MES–HIS	0.311	0.225	0.025	0.022
Field-amplified sample injection: positive ions only	28.04	13.44	0	0
Field-amplified sample injection: negative ions only	0	0	13.66	12.58
Field-amplified polarity-switching injection: both positive and negative ions	32.03	9.28	2.58	1.96

Different time programming schemes could be used to inject both positive and negative ions. One could also reverse the procedure, inject negative ions first then positive ions later.

Fig. 7 shows an electropherogram of the $5 \cdot 10^{-5}$ *M* sample mixture using 20 s −5 kV injection followed by 10 s +5 kV injection. A short water plug is also introduced into the column by raising the water reservoir up to 15 cm for 30 s prior to sample injection. All four ion peaks, two positive and two negative, are observed. Although one can also obtain signals of both positive and the negative ions using conventional electro-injection, the number of ions injected, especially negative ions, is greatly enhanced in field-amplified polarity-switching injection. Table I shows the comparison between peak heights normalized to the gravity injection for various electro-injection methods.

In conclusion, we have shown a rather simple electro-injection technique to concentrate *both* positive and negative ions into an uncoated column while retaining the high resolution in HPCE. Several orders of magnitude in signal enhancement was confirmed. In addition, selected charge discrimination was demonstrated, which might be very useful in real-life samples.

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