Cation-Selective Electropreconcentration

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S1. Preconcentration charge selectivity

The concentration polarization phenomenon is based on ion-permselectivity and interplay between electroosmotic and electrophoretic mobilities. The resulting preconcentration is selective to co-ion (cation in this study).

The following figure repeats the figure 2(a) and shows channel cross-section (not in scale). The white and red arrows show the flow and electric field directions, respectively.

![Diagram](image)

The micro- and nanochannel EOF directions were reversed with polyE-323 coating and therefore same as the counter-ion (fluorescein in this study) electrophoresis direction. As a result, when we apply potentials to reservoirs, we can observe the fluorescein from the sample channel continuously pass through the nanochannel into the waste channels. However, we can also observe the partial fluorescence enhancement at the nanochannel and waste channel junction (red circles in the cross sections). This phenomenon can be explained with the waste channel shape. We designed the waste channel in U-shape for convenient solution filling. This U-shape results in symmetric electric field pattern along the A-B cross-sectional line, and therefore, the electric field is cancelled at the nanochannel and waste channel junction. When the fluorescein molecule reaches the nanochannel and waste channel junction, the electrophoretic migration suddenly decreases due to the reduced electric field while the flow rate is maintained due to the incompressibility of water, and therefore, the fluorescent intensity is partially enhanced. However, the enhancement factor is limited to only 2-3 times.

S2. Preconcentration process

In figure 3, the Rhodamine 6G preconcentration data at high initial concentration was collected in shorter period than at lower initial concentration. During the electropreconcentration, the fluorescent intensity increased linearly at the beginning, and then, the concentrating effect leveled off, showing saturation. This saturation level is linearly correlated with the buffer ionic
strength. However, the sample plug length continued to increase at this point, maintaining the rate at which molecules were being trapped. For this reason, the molecular amount concentrated (figure 3(b)) was calculated based on integrated fluorescent intensities over the entire preconcentrated plugs, showing a linear increase.

In this study, we showed preconcentration data with initial Rhodamine 6G concentration of 100 nM, 1 μM, and 10 μM. In the 10 μM experiment, the saturation level was reached in short time compared to other lower initial concentration experiments and the plug size increased faster. Therefore, a 4x objective lens was used for image acquisition in the 10 μM experiment while a 10x objective lens was used for the 100 nM and 1 μM experiments. The following image compares the 100 nM and 10 μM data (Figure S-1).

**Figure S-1.** Preconcentration process with different initial sample concentrations. Images are contrast adjusted for better visualization.

Although we used the widest field-of-view objective lens available for the 10 μM experiment, the plug size increased faster and filled the field-of-view only in 3 min. After 3 min, we verified that the plug size increased stably by changing the imaging location, but it is not possible to quantify the molecular amount with the partial image of the plug. Therefore, we showed shorter period for the 10 μM data where quantification is possible in figure 3.

The figure 4(a)-(b) and (c)-(d) compares the cation-selective preconcentration efficiency for the three systems at pH 3.4 and 7.4, respectively. The ionic strength of the 0.005% formic acid + 5% IPA buffer was similar to that of the 1 mM phosphate buffer. The maximum concentration of the preconcentrated plug at pH 7.4 was comparable to that at pH 3.4 for the polyE-323- and the TMSVE-coated-nanochannel systems (Figure 4(c)). However, the rates of sample collection were reduced (Figure 4(d)) because the surface charge density, hence the EOF rate, of polyE-323 or TMSVE decreased in the weakly alkaline phosphate buffer. In particular, the flow rate of the DADMAC system (green circle) was highly reduced. Therefore, the preconcentrated plug shows
high intensity at the beginning but diffused with time as shown in the figure S-2. This pH dependent flow rate of the DADMAC system can be found in other literature.²

Figure S-2. Preconcentration process of the DADMAC membrane system at pH 7.4.

S3. TRITC-tagged albumin preconcentration

The preconcentration experiment was repeated with 1 and 15 µM of TRITC-tagged albumin in a 0.01% formic acid + 5% IPA buffer at pH 3.3 (Figure S-3) in the polyE-323-coated micro- and nanochannels. The potentials applied at the S, W, and D reservoirs were 0, 50, and 20 V, respectively. The albumin is positively charged (~+12) at the pH of the buffer.³ The preconcentration process was stable, and the total number of preconcentrated TRITC-tagged albumin molecules increased linearly for both initial concentrations.

Figure S-3. Progress of the cation-selective preconcentration of TRITC-tagged albumin at initial concentrations of 1 µM (○) and 15 µM (●), respectively, in a 0.01% formic acid + 5% IPA buffer at pH 3.3 with polyE-323-coated micro- and nanochannels.

S4. pH dependency of TMSVE-coated microchannel EO mobility

In figure 4, the maximum concentration of the preconcentrated plug at pH 7.4 was comparable to that at pH 3.4 for the polyE-323- and the TMSVE-coated-nanochannel systems (Figure 4(c)).
However, the rates of sample collection were reduced (Figure 4(d)) because the surface charge density, hence the EOF rate, of polyE-323 or TMSVE decreased in the weakly alkaline phosphate buffer. Figure S-4 shows the electroosmotic (EO) mobility in the TMSVE-coated microchannels measured by a gated-injection of Rhodamine B (10 µM). All buffers were 10 mM ammonium acetate, pH-adjusted by adding HCl or NaOH. The EO mobility in the TMSVE-coated microchannel was more sensitive to pH compared to that in the polyE-323-coated microchannel, resulting in a slower rate of sample collection (Figure 4(d)).

![Figure S-4](image)

**Figure S-4.** EO flow at different pH values for a TMSVE-coated, 12-µm-deep microchannel.

### S5. Sample adsorption

During cation-selective preconcentration of biological samples, the adsorption of the sample onto the glass surface could result in surface charge modification, flow instability, and loss of a potentially irreplaceable sample. To compare the adsorption of a biological sample to different channel coatings, a cationic peptide, TRITC-tagged angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe), was preconcentrated in the polyE-323- and TMSVE-coated-nanochannel systems. The Arg and His ensure the sample to be cationic in the buffer (0.005% formic acid + 5% IPA, pH 3.4). After preconcentration for two hours, the sample channel was washed with buffer, and then a fluorescence image was acquired (Figure S-5). The adsorption of the peptide in the TMSVE-coated-nanochannel system was strong, whereas in the polyE-323-coated-nanochannel system, it was weak, presumably due to the high electrostatic repulsive force of polyE-323.
Figure S-5. Peptides/proteins sample adsorption comparison between a polyE-323 and TMSVE coated channel. The white dashed lines show the nanochannel locations. The cation-selective electrophoreconcentration was performed with a cationic peptide sample, angiotensin III, for 2 h. The sample channel was washed with buffer and then fluorescent image was taken. (a) The polyE-323 coated channel showed little sample adsorption while (b) TMSVE coated channel showed strong sample adsorption.

References