DEVELOPMENT OF A MICROFLUIDIC DEVICE FOR PERFORMING SAMPLE PRECONCENTRATION AND CAPILLARY ELECTROPHORESIS SEPARATION

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ABSTRACT

We report on a microfabricated device for sample preconcentration and capillary electrophoresis (CE) separation in a glass microchip. The device allows highly efficient anion-selective preconcentration to be performed with a micro-nano-microchannel structure. An integrated cross channel enables gate-injection of the preconcentrated sample into the analysis channel for CE separation. Sample preconcentration and corresponding enhanced CE separation signal have been demonstrated with TRITC-tagged peptides.

KEYWORDS: Preconcentration, Electrophoresis, Separation

INTRODUCTION

Protein or peptide analyses involve multiple separation steps due to the large number and dynamic concentration range. A common multidimensional separation method to resolve protein or peptide mixtures would be LC(liquid chromatography)-CE separation. For realizing the two-dimensional separation in one-dimensional microchannels, small portions of the first-dimension separated (LC, slow) sample need to be periodically injected into the second-dimension separation (CE, fast) channel. During the second-dimension separation, most majority (> 99%, i.e. analyte transfer efficiency is less than 1%) of the first-dimension separated samples are wasted to prevent signal overlapping, decreasing overall signal intensity. Therefore, it is desirable to preconcentrate (or collect) the first-dimension separated samples between each periodical injection to increase the analyte transfer efficiency (up to 100%) and corresponding detection efficiency.

THEORY

We have been exploring methods to concentrate samples prior to transfer to the CE separation channel, using an integrated preconcentration functional element on the microchip. The approach taken involves electrokinetic trapping mediated by nanochannels that are joined to a microscale separation channel. Differences in the surface-to-volume ratio of nano- versus micrometer deep channels contribute to modify ionic transport and result in a concentration or depletion of ions at the nanojunction, depending on the direction of an applied electric field. In effect the nanochannel becomes ion permselective provided the electric double layer of the nanochannel is near or at electrical double layer overlap conditions. This ion permselectivity is created by a concentration polarization at the micro-nano interface as counterions transfer across the nanochannel leaving an enrichment of coions. Over time, this depletion of counterions creates an energy barrier near the nanochannel that limits the permeability to ions opposite in charge to the nanochannel's surface [1-3].

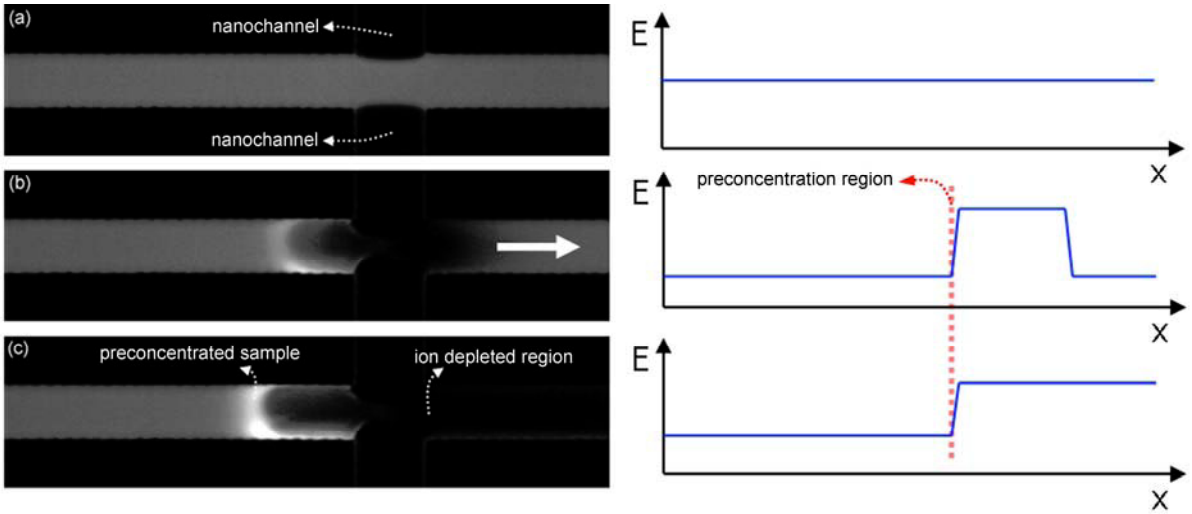

Figure 1: (Left) CCD images depicting the expansion of the ion-depletion region and the effects of a discontinuous electric field. The images were taken (a) 0, (b) 2, and (c) 4 s after applying the potentials for preconcentration. Potentials for S (sample, left channel), W (waste, across the nanochannels), and D (drain, right channel) were 60, 0, and 45 V, respectively. As the concentration proceeds, the ion-depletion region expands towards the drain channel. (Right) The ion-depletion decreases conductance and increases electric field strength, therefore, the electric fields are discontinuous as shown by the plots on the right. Each plot corresponds to the image directly to the left.

EXPERIMENTAL

The integration of the concentrator with CE required some design modifications because of the generation of discontinuous electric fields in the CE column (Figure 1). The ion-depletion region expands towards the drain (D) channel termini during the concentration. The conductivity of the ion-depleted region is lower than the sample solution conductivity, and therefore, the electric field is higher in the ion-depleted region. The discontinuous electric fields inhibit CE separations (Figure 2). Figure 2c shows a CE separation of TRITC-tagged peptides in a simple microchannel after an electrokinetic trapping concentration (Figure 2a). As shown, the sample is not separated because the peptides stacked at the interface and transport through the analysis channel at the same velocity as the electric field discontinuity (Figure 2c). This phenomenon is called field amplified sample stacking [4]. The electric-field discontinuity problem was solved by incorporating a cross channel (Figure 2b) for performing gated injections of the concentrated sample plug into the analysis channel (Figure 3). The ion-depleted solution flows into the drain channel while the buffer flows into the analysis channel during preconcentration (Figure 2b). The electric-field discontinuity in the analysis channel that inhibits the CE separation is thereby eliminated.

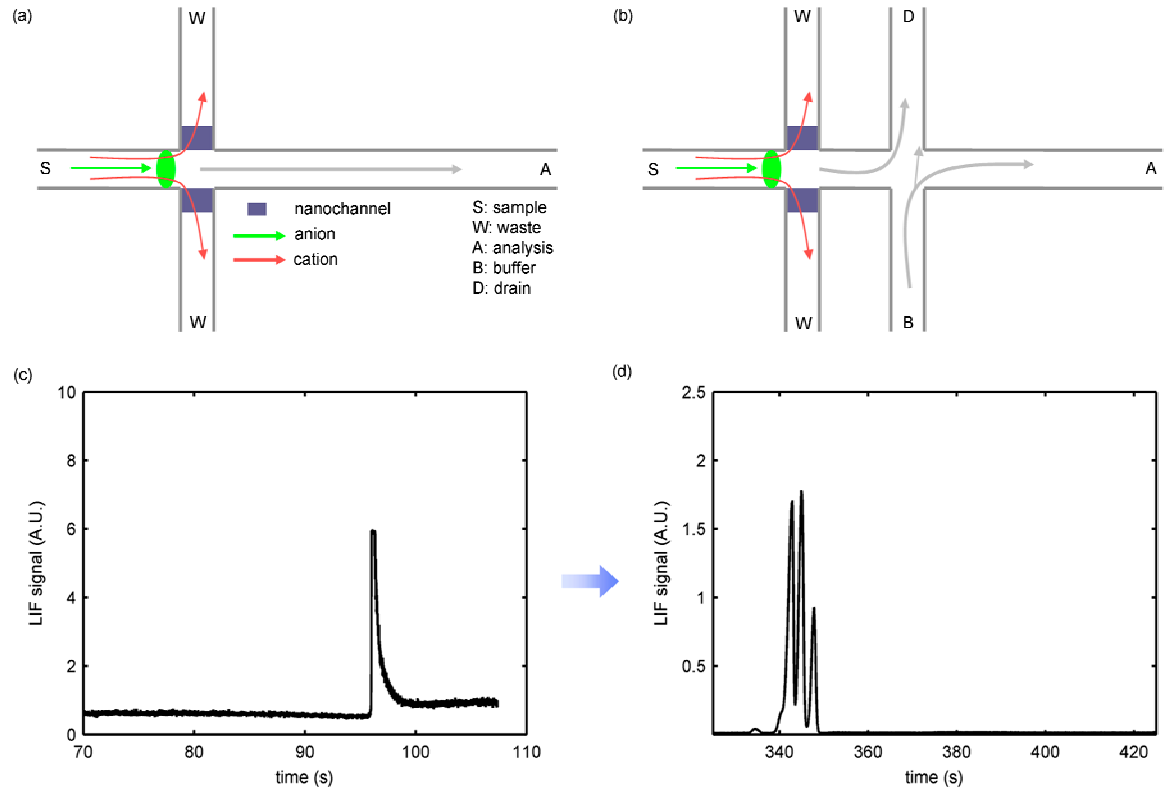

Figure 2: CE separation of preconcentrated sample. (a) shows a schematic for direct injecting the preconcentrated sample into a CE analysis channel. (b) shows a schematic for gated-injecting the preconcentrated sample into a CE analysis channel. (c) shows a direct injection result after preconcentrating four TRITC-tagged peptides (YPFVEPI, AGDV, WAGGDASGE, and DDDDD. Each peptide concentration was 2 μM in 1 mM phosphate buffer at pH 7.4). The conductivity mismatch due to an expanded ion-depleted solution in the analysis channel inhibits the CE separation and all samples are stacked at the interface. (d) shows a result with gated-injecting the preconcentrated sample. The analysis channel conductivity was matched with the preconcentrated sample plug by flowing the same buffer (B) as the sample solution. Therefore, the preconcentrated peptides can be successfully separated.

RESULTS AND DISCUSSION

Figure 2d shows an example of separation of preconcentrated sample after a gated injection. The stacked sample is clearly separated again. The separated peak intensities are lower than that of the stacked peak (Figure 2c), however, the sum of the separated peak areas is comparable to the stacked peak area, showing high analyte transfer efficiency.

CONCLUSION

In this study, an anion-selective preconcentrator is integrated with CE column in a glass microchip and showed enhanced CE separation signal, expanding application of the preconcentration to subsequent analysis technologies. This technology will be integrated between LC and CE columns to increase the analyte transfer efficiency up to 100%.

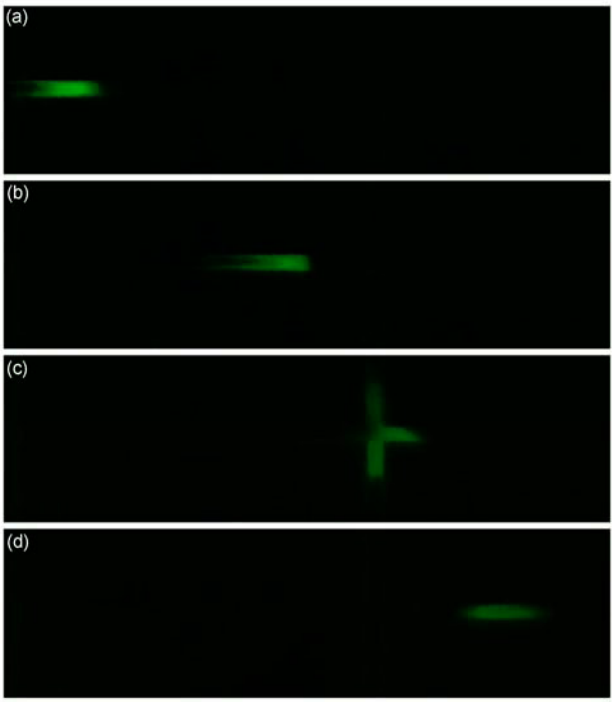

Figure 3: An example of gated injection of a preconcentrated sample. (a), (b), (c), and (d) are CCD images taken 0, 0.5, 1.2, and 2.6 s after the preconcentrated sample release. The preconcentrated sample plug is successfully gated injected into the analysis channel between (c) and (d).

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