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TECHNICAL INNOVATION

Cation-Selective Electropreconcentration

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2013,
Accepted 00th January 2013

DOI: 10.1039/x0xx00000x

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A cation-selective microfluidic sample preconcentration system is described. The cation sample was electropreconcentrated using a reversed-direction electroosmotic flow (EOF) and an anion-permselective filter, where an electric-double-layer (EDL) overlap condition existed. The anion-permselective filter between microchannels was fabricated by three different methods: 1) extending a positively charged, nanoporous, polymer membrane by photopolymerization of poly(diallyldimethylammonium chloride) (PDADMAC); 2) etching a nanochannel and then coating it with a positively-charged monomer, *N*-[3-(trimethoxysillyl)propyl]-*N′*-(4-vinylbenzyl)ethylenediamine hydrochloride (TMSVE); or, 3) etching a nanochannel and then coating it with a positively-charged, pre-formed polymer, polyE-323. The EOF direction in the microchannel was reversed by both TMSVE- and polyE-323 coatings. The cation-selective preconcentration was investigated using charged fluorescent dyes and TRITC-tagged peptide/proteins. Preconcentration in the three different systems was compared with respect to efficiency, dependence on buffer concentration and pH, tolerable flow rate, and sample adsorption. Both TMSVE- and polyE-323-coated nanochannels showed robust preconcentration at high flow rates, whereas the PDADMAC membrane maintained the anion-permselectivity at higher buffer concentrations. The TMSVE-coated nanochannels showed a more stable preconcentration process, whereas the polyE-323-coated nanochannels showed a lower peptide sample adsorption and robust efficiency under a wide range of buffer pH values. The system described here can potentially be used for the preconcentration of cationic peptides/proteins on microfluidic devices for subsequent analyses.

1. Introduction

Various sample preconcentration methods have been developed for improving detection sensitivity.[1-16] Among these, electropreconcentration has advantages of simple and straightforward implementation in a lab-on-a-chip setup, high efficiency for charged biomolecules, not requiring of spatial or temporal buffer changes, and good compatibility with subsequent analysis techniques, for instance capillary electrophoresis (CE).[1] Wang et al. demonstrated a million-fold preconcentration of peptide and protein samples using a nanochannel within a T-shaped microchannel; the preconcentration occurred on the anodic side of the nanochannel.[12]

Electropreconcentration is accomplished by applying an electric field across a nanochannel (or nanoporous membrane) that spans two microchannels when the electric-double-layer (EDL) approaches an overlap condition within the nanochannel.[17, 18] Under these conditions, the co-ion transport across the nanochannel is suppressed owing to the creation of a concentration polarization region at the micro/nanochannel interface. Consequently, the nanochannel becomes ion-permselective, allowing ions of the same charge as the nanochannel to be concentrated.[1, 19]

The majority of previous electropreconcentration studies were based on an anionic nanochannel or polymer, hence limited to anionic samples.[1, 7, 12-14, 20, 21] Many analytical methods, however, requires cationic samples. For example, electrospray ionization mass spectrometry (ESI-MS) coupled with reversed-phase liquid chromatography (LC) or capillary electrophoresis (CE) uses acidic buffer with a pH lower than sample peptide/protein pI, resulting in samples to be cationic.[22-25] A cation-selective preconcentration can significantly enhance the detection sensitivity for cationic analytes; this can be used to monitor the toxic cationic coagulants[26] after water purification. Furthermore, a combination of both an anion- and a cation-selective preconcentration system can actively select samples of specific pI range using the buffer pH to control the sample charge polarity.

Previous studies have predicted that cation-selective preconcentration may require surface coating.[1, 27] Recently, Sheridan et al. reported a cation-selective preconcentration experiment using a bipolar electrode focusing technology with limited concentrating rate and time.[28] In this study, we report a stable, high-yield cation-selective electropreconcentration on a microchip (Figure 1). The chip incorporates either an anion-permselective nanochannel, or a positively charged polymer membrane within the microchannel intersection. N-[3-(Trimethoxysilyl)propyl]-N′-(4-vinylbenzyl)ethylenediamine hydrochloride (TMSVE) or polyE-323[24, 29, 30] was used for the positively-charged surface coating of the anion-permselective nanochannel. On the other hand, a positively charged polymer, poly(diallyldimethylammonium chloride) (PDADMAC)[31] was prepared by photopolymerization in the microchannels, forming the anion-permselective polymer membrane. For the three

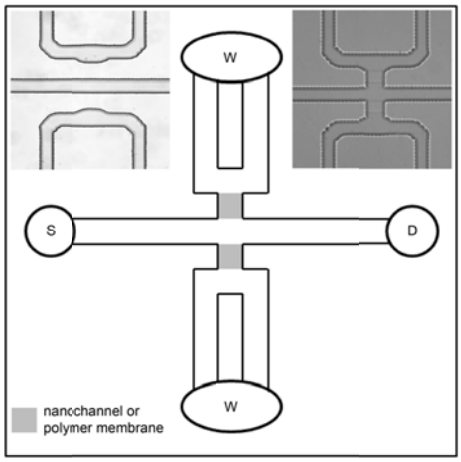

Fig. 1. A cation-selective electropreconcentration microchip. S, D, and W represent the sample, drain, and waste reservoir, respectively. The left and right insets show the actual images of the nanochannel and the polymer membrane, respectively.

systems (the TMSVE-coated nanochannels, the polyE-323-coated nanochannels, and the PDADMAC membrane), important characteristics of the preconcentration process, such as buffer concentration and pH dependency, efficiency, tolerable flow rate, and sample adsorption were compared.

2. Experimental Section

2.1 Reagents

The reagents were purchased from commercial sources as follows: Fluorescein disodium salt dihydrate from Acros Organics (Geel, Belgium); Rhodamine B, Rhodamine 6G, TRICT-tagged albumin, TMSVE, DADMAC, 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone and *N,N*′-methylene-bisacrylamide from Sigma (St. Louis, MO, USA); Angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe) from American Peptide (Vista, CA, USA); TRITC from Invitrogen (Carlsbad, CA, USA); 1,2-bis(3-aminopropylamino)ethane from LabKemi (Stockholm, Sweden); epichlorohydrine from Fluka Chemie (Buchs, Switzerland). The peptides were TRITC-tagged as previously described.[32] All solutions were prepared using deionized (DI) water filtered through a Barnstead Nanopure Filtration System (Boston, MA).

2.2 Microchip Fabrication

Glass micro- and nanochannel fabrication procedures were as previously described.[1] A chrome/photoresist-coated B270 glass slide with dimensions 4 × 4 inches and thickness 0.9 mm (Telic, Valencia, CA, USA) was used as the base substrate. The desired pattern was exposed on the substrate with a maskless SF-100 photoexposure system (Intelligent Patterning, St. Petersburg, FL, USA). The patterned substrate was developed using MF-319 (MicroChem Corp., Newton, MA, USA), and the chrome layer was etched using chrome etchant (Transene, Danvers, MA, USA). Next, channels were etched in the substrate using a 10:1 buffered oxide etchant (Transene). Each microchannel was 50-μm wide at full height, 12-μm deep, and 8-mm long from the nanochannel or the polymer membrane to the reservoir. The substrate was then diced into microchips

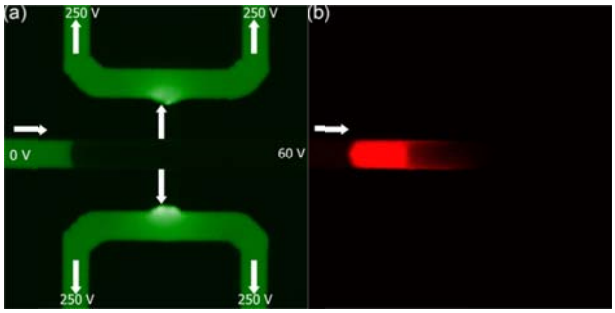

Fig. 2 Cation-selective electropreconcentration using polyE-323-coated micro- and nanochannels. (a) Fluorescein (green) leaks through the anion-permselective nanochannels into the waste channels. (b) Rhodamine 6G (red) is preconcentrated in front of the nanochannels.

(dimensions 1×1 inches) on a Basic Dicer II (Dicing Technology, San Jose, CA, USA). After removing the remaining chrome/photoresist layer, hexamethyldisilazane (HMDS; Acros Organics) and photoresist S1813 (MicroChem Corp) were spin-coated in succession on the substrate (30 s at 4,000 rpm each), and baked for 2 min at 95°C in a convection oven. The substrate was aligned under a mask to define the nanochannel pattern, and then exposed to UV light (365 nm) on a J200 UV Exposure System (OAI, Milpitas, CA, USA) at 4.8 mW cm^{-2} for 100 s. The nanochannel pattern was developed using MF-319 and etched to 40-nm nanochannel depth using a 10:1 buffered oxide etchant for ~3 s. Access holes (diameter 1 mm) were made on a MB-1000-1 powder blaster (Comco Inc., Burbank, CA, USA). The channel patterned substrate was bonded with a B270 blank glass substrate (dimensions 1×1-inch, thickness 0.9 mm). Cloning cylinders (diameter 6 mm) (Fisher Scientific, Fair Lawn, NJ, USA) were placed on top of the access holes and bonded using UV curable optical adhesive (NOA 63, Norland, Cranbury, NJ, USA) to provide fluid reservoirs.

2.3 Surface coating and polymer synthesis

PolyE-323 was synthesized and coated following the procedure of Hardenborg et al.[30] 1,2-Bis(3-aminopropylamino)ethane (17.65 g) dissolved in DI water (20 mL) was mixed with epichlorohydrine (9.3 g). The mixture was sealed and stirred at room temperature. After 48 h, DI water (100 mL) was added. The channel was coated with polyE-323 by flushing a polyE-323 solution (15%) adjusted to pH 7.0 with 1 M acetic acid for 1 h. The excess polymer was removed by flushing 50 mM ammonium acetate for 5 min.

For TMSVE coating, the channel was filled with TMSVE/acetic acid/DI water = 2:3:5 solution overnight, and then washed with isopropanol.

The PDADMAC membrane was fabricated as previously described.[31] Aqueous DADMAC solution (65%) in the presence of 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropio-phenone (2%) as the photoinitiator and *N,N*′-methylenebisacrylamide (2%) as the cross-linker was polymerized by exposing to UV light over the desired pattern mask to yield PDADMAC membrane.

2.4 Optical System and Flow Control

The progress of the sample preconcentration process was monitored on a TE300 inverted microscope (Nikon, Tokyo,

Japan) equipped with a 10× objective, a high pressure mercury lamp, and a NTE/CCD-512-EBFT CCD 16-bit resolution camera (Roper Scientific, Trenton, NJ, USA). The fluorescence image data was analysed using custom Matlab (The MathWorks, Natick, MA, USA) code. The high voltage was supplied to the chip reservoirs from an 8-channel, high-voltage power supply (E10128, EMCO, Sutter Creek, CA, USA) connected to individual relays and current sink resistors (10 MΩ), and controlled by a customized LabVIEW program using the analogue output of a DAQ card (PCI-6713, National Instruments).

3. Results and Discussion

3.1 Cation-selectivity

To verify that the preconcentration was cation-selective, a mixture of 10 µM Fluorescein (anionic) and Rhodamine 6G (cationic) in a 0.005% formic acid + 5% isopropyl alcohol (IPA) buffer at pH 3.4 was electropreconcentrated using the polyE-323-coated micro- and nanochannel system. The positive surface charge of polyE-323, which is coated on the surface of the glass channel, reverses the EOF direction towards the anodic side. The potentials applied at the S, W, and D reservoirs were 0, 250, and 60 V, respectively. Figure 2(a) shows that the anionic Fluorescein from the S reservoir was not preconcentrated but continuously passed through the anion-permselective nanochannel into the waste channels (upper and bottom U-shaped channels). While anions were extracted through the anion-permselective nanochannels, an ion-depletion region developed in front of the nanochannel pairs to maintain charge neutrality. This concentration polarization resulted in the cationic Rhodamine 6G being preconcentrated in front of the ion-depleted region (Figure 2(b)) (see Supplementary Information, Preconcentration charge selectivity).

The cation-selective preconcentration process in the TMSVE-coated micro- and nanochannel system was investigated at various initial Rhodamine 6G concentrations (100 nM, 1 µM, and 10 µM) in a 0.005% formic acid + 5% IPA buffer at pH 3.4. The potentials applied at the S, W, and D reservoirs were 0, 250, and 60 V, respectively. The degree of preconcentration was estimated by a comparison with the fluorescence intensities of Rhodamine 6G solutions of known concentrations (Figure 3(a)). For each sample preconcentration experiment, the fluorescence intensity increased linearly at first, but then saturated. However, the plug length of the preconcentrated sample continued to increase, maintaining a linear rate of preconcentration with respect to the total sample molecules (Figure (3b)) (see Supplementary Information, Preconcentration process).

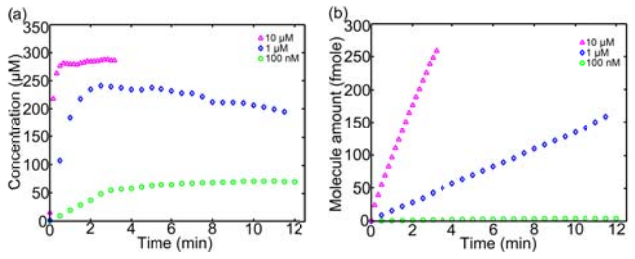

Fig. 3. Progress of the cation-selective Rhodamine 6G preconcentration in the TMSVE coated micro- and nanochannels. (a) Fluorescent intensity (µM), and (b) number of molecules (fmole) in the preconcentrated plug.

In addition, the preconcentration experiment with TRITC-tagged albumin in the polyE-323-coated micro- and nanochannels showed stable process (see Supplementary Information, TRITC-tagged albumin preconcentration).

3.2 Comparison of the three systems in cation-selective electropreconcentration

The efficiency of the three systems described in this work, i.e., 1) PDADMAC-membrane in TMSVE-coated microchannels; 2) TMSVE-coated micro- and nanochannels, and 3) polyE-323-coated micro- and nanochannels in the cation-selective preconcentration was compared. The sample was Rhodamine 6G (10 µM) in a 0.005% formic acid + 5% IPA buffer at pH 3.4 (Figures 4(a) and (b)), and in an 1 mM phosphate buffer at pH 7.4 (Figures 4(c) and (d)). The potentials applied at the S, W, and D reservoirs were 0, 250, and 100 V for the PDADMAC membrane system and 0, 250, and 60 V for the TMSVE- or polyE-323-coated nanochannel systems, respectively. All three systems showed stable cation-selective preconcentration at pH 3.4. The maximum concentration of the preconcentrated plug was higher in the polyE-323-coated-nanochannel system compared to the TMSVE-coated-nanochannel system (Figure 4(a)), but the plug size increased faster in the TMSVE-coated-nanochannel system, resulting in similar rates of sample collection (Figure 4(b)). 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 (see Supplementary Information, pH dependency of TMSVE-coated microchannel EO mobility). Preconcentration in the PDADMAC membrane system was less efficient in terms of the maximum concentration level and collection rate at low (~1 mM) buffer ionic strength (Figure 4). However, preconcentration in the PDADMAC-membrane system kept stable after an increase in the buffer ionic strength up to 10 mM, whereas there was no preconcentration in the polyE-323- or the TMSVE-coated nanochannel systems under these conditions. The pore size of

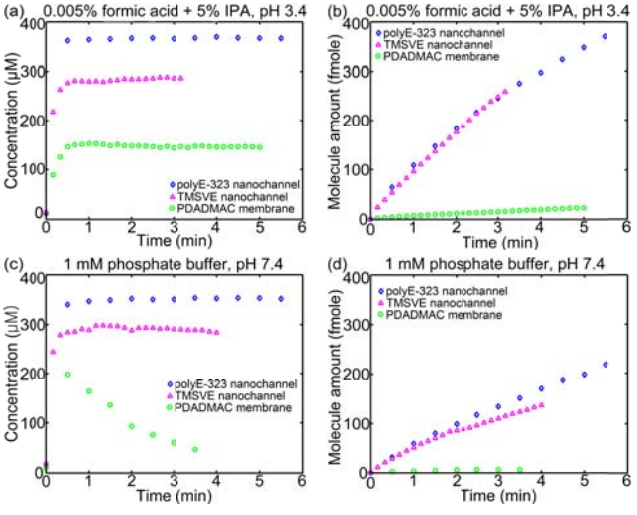

Fig. 4. Progress of the cation-selective sample preconcentration of Rhodamine 6G (10 µM) in (a), (b) a 0.005% formic acid + 5% IPA buffer at pH 3.4, and (c),(d) an 1 mM phosphate buffer at pH 7.4, respectively.

the PDADMAC membrane is believed to be smaller than the nanochannel depth (40 nm), maintaining the EDL overlap condition at a higher buffer ionic strength. The tolerable electric field, hence the flow rate, was higher in the polyE-323- or the TMSVE-coated-nanochannel system compared to that in PDADMAC-membrane system. Preconcentration in the polyE-323- and TMSVE-coated-nanochannel systems was stable when the difference of potentials applied at the S and W reservoirs was as high as 4 kV, whereas the PDADMAC membrane was broken at a potential difference of 500 V.
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. Experimental data showed less peptide adsorption in the polyE-323-coated-nanochannel system compared to the TMSVE-coated-nanochannel system (see Supplementary Information, Sample adsorption).

4. Conclusions

Herein we describe the cation-selective electropreconcentration using anion-permselective TMSVE- or polyE-323-coated nanochannels or a positively charged nanoporous polymer (PDADMAC) placed between the positively-surface-coated microchannels. Preconcentration was robust in both polyE-323- and TMSVE-coated-nanochannel systems in a buffer of low (~1 mM) ionic strength, whereas the same was true for the PDADMAC-membrane system at a high (~10 mM) ionic strength. The electric-field tolerance and the flow rate were higher in both polyE-323- and TMSVE-coated-nanochannel systems than those in the PDADMAC-membrane system. The polyE-323 coating showed a better performance over different pH values, and a weak sample adsorption compared to the TMSVE coating. Cation-selective preconcentration is essential for biological sample analyses in acidic buffer. This work can be extended to the serial coupling of cation- and anion-selective preconcentrations to selectively preconcentrate samples having a specific range of pI by changing the pH of the buffer.

Acknowledgements

This research was partially supported by Korea University (grant K1325081) and the National Research Foundation of Korea (NRF) (grants NRF-2013M3A6A4046061, NRF-2012-0009556, NRF-2011-0013976, and NRF-2011-0031866)

Notes and references

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