Journal of Chromatography A, xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Electropreconcentration, gate injection, and capillary electrophoresis separation on a microchip

Honggu Chun

Department of Biomedical Engineering, Korea University, 466 Hana Science Hall, 145 Anamro, Seongbukgu, 02841, Seoul, South Korea

ARTICLE INFO

Article history:
Received 9 June 2018
Received in revised form 17 August 2018
Accepted 25 August 2018
Available online xxx

Keywords:
Electropreconcentration
Ion concentration polarization
Capillary electrophoresis
Gate injection

ABSTRACT

The nanochannel-based electropreconcentration is not compatible with successive capillary zone electrophoresis (CZE). In this study, the incompatibility is theoretically discussed and experimentally proven, and then, the development of a monolithic glass microfluidic chip for performing integrated electropreconcentration and CZE separation is described. The sample is electropreconcentrated at the interface of a micro- and nanochannel where electric double layer overlap conditions exist. Because an ion-depletion region develops at the leading front of the preconcentrated plug, a field-enhanced sample stacking effect occurs which limits the separation efficiency unless compensated for. The ion-depletion region was confirmed by monitoring the solution conductivity at discrete points in the microchannel during the preconcentration step. The solution conductivity decreased >20-fold during the preconcentration step. To overcome the effects of this region, a cross-intersection was used to shunt the ion-depleted buffer away from the analysis channel while reintroducing the running buffer. When the preconcentrated sample plug arrives at the cross-intersection, it is gate injected into the analysis channel so that fresh running buffer surrounds the plug. Under these conditions, three-peptide mixture was preconcentrated ~200-fold in 60 s and the preconcentrated plug was successfully resolved with better than 1% relative standard deviations in migration times.

1. Introduction

Microfluidic chips are advantageous in terms of ease of automation, lower costs and reagent consumption compared to LC or HPLC. However, the small channel dimension limiting the detection pathlength makes detection in microchips challenging. To overcome this problem, sensitive detection techniques have been developed including laser induced fluorescence (LIF) (limit of detection ~10^{-13} M) [1], radioisotope (~10^{-10} M) [2], electrochemical (~10^{-8} M) [3], and photothermal absorbance detection (~10^{-9} M) [4]. The limit of detection can also be enhanced by on-line sample preconcentration techniques, such as field-enhanced sample stacking [5,6], isotachophoresis [7], sweeping [8,9], filtering [10,11], solid-phase extraction [12], focusing [13], and nanochannel-based electropreconcentration [14–21]. Among these, electropreconcentration has received considerable attention due to its high efficiency. Since Pu et al. demonstrated ion-enrichment and depletion effect using a linear micro/nano/microchannel structure [22], many groups reported similar approaches using nanochannels, nanopores and nanoporous polymers [16,23–26]. Then, Wang et. al. introduced a T-intersection structure with improved experimental results [14]. The nanochannel-based sample preconcentration is called electropreconcentration [27,28] or ion concentration polarization (ICP) [18,29] and has been analyzed with various approaches [14,27,28,30–34]. Integration of the electropreconcentration with other analytical methods or instruments would enable unparalleled sensitive sample analysis [35,36].

In this study, the development of the first successful integration of a T-intersection charge-selective electropreconcentrator with capillary zone electrophoresis (CZE) separation in a monolithic glass microchip is reported. To understand why the electropreconcentration cannot be directly coupled with CZE, the electropreconcentration mechanism is reviewed based on previous analytical work [27]. Briefly, a glass channel surface is negatively charged in a neutral pH buffer. Fig. 1a shows the intersection of the microchannel and two side channels labeled W containing nanochannels (gray areas). The microchannel situated to the left of the nanochannels contains the sample (S) while the area to the right is the analysis (A) channel. When the electric double layer (EDL) thickness is comparable to the nanochannel depth, the nanochannels become cation-permselective. For instance, when applied potentials at the sample, waste, and analysis channels are 200 V, floating, and 100 V, respectively, an electroosmotic flow (EOF) is generated along the sample and analysis channels. The nanochannel flow-resistance inhibits sample leakage towards the

E-mail address: chunhonggu@korea.ac.kr

https://doi.org/10.1016/j.chroma.2018.08.053
0021-9673/

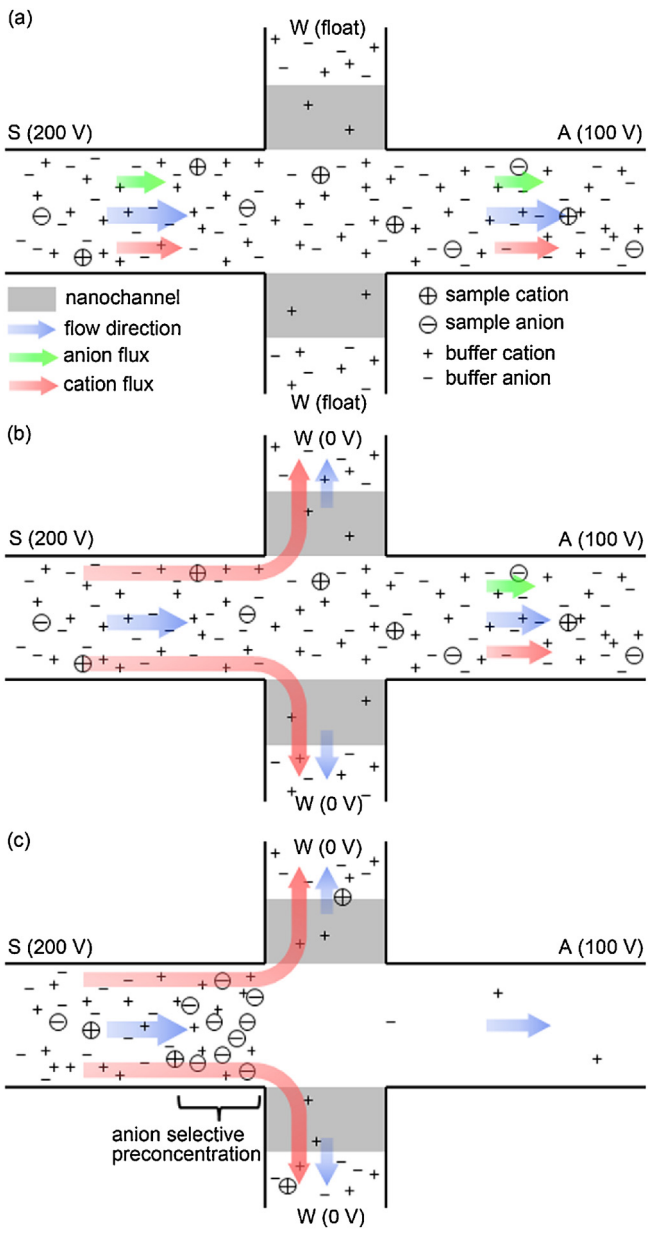

Fig. 1. The electropreconcentration process. Glass substrate micro- and nanochannel surfaces are negatively charged at neutral pH buffer, making the nanochannel cation-permselective. (a) Potentials at S and A create EOF in the microchannel that drives sample solution towards the analysis reservoir when W is floating. (b) When 0 V is applied to W, buffer and sample cations are extracted through the cation-permselective nanochannel, resulting in nanochannel EOF. The nanochannel volumetric flow is less than the sample channel volumetric flow, therefore, the excess sample channel flow generates a pressure driven flow towards the analysis channel. By applying an appropriate potential to the analysis channel, the sample channel net anion flux can be suppressed. (c) By maintaining the condition, the buffer and sample cations are continuously extracted through the cation-permselective nanochannels while the buffer and sample anions are redistributed based on their electrophoretic mobilities near the micro/nanochannel junction, resulting the anion-selective preconcentration. Because cations are continuously extracted through the nanochannels and net anion flux is suppressed, the resulting flow towards the analysis channel is ion-depleted.

waste channels under these conditions (Fig. 1a) and the EOF in the sample and analysis channels will be identical. When applied potentials at the sample, waste, and analysis channels are 200 V, 0 V, and floating, respectively, buffer and sample cations (if the sample cation size is smaller than the nanochannel depth) will be extracted through the cation-permselective nanochannel, generating a nanochannel EOF.

However, the nanochannel electroosmotic (EO) mobility is lower than the microchannel EO mobility when the nanochannel is in EDL overlap condition, and therefore, the volumetric nanochannel flow is less than the sample channel volumetric flow despite the higher electric field in the nanochannel [27,37]. The excess sample channel flow will generate a pressure driven flow towards the analysis channel, resulting in anion leakage down the analysis channel and no sample preconcentration [27,38–40].

This sample channel anion flow can be controlled by the analysis channel potential to counter the pressure driven flow. The anion leakage towards the analysis channel can be suppressed by applying 100 V to the analysis channel while holding the sample and waste potentials at 200 and 0 V, respectively (Fig. 1b). The 100 V was chosen to be higher than the potential at anodic side of the nanochannel [27]. Under these conditions, the buffer and sample cations are continuously extracted through the cation-permselective nanochannels while the net anion flow is blocked and the buffer and sample anions are redistributed based on their electrophoretic mobilities near the micro/nanochannel junction (Fig. 1c). In most cases, the sample anion electrophoretic mobility will be smaller than the buffer anion, resulting in the sample anion stacking at the low voltage side of the sample channel near the micro/nanochannel junction during the redistribution. This phenomenon is interpreted as anion-selective preconcentration. Cations or neutral samples are not preconcentrated under these conditions as they are continuously extracted through the nanochannels. The anion- and cation-selective electropreconcentration with a negatively- and positively charged channels, respectively, have been theoretically and experimentally proven [19,27,36,41]. Additionally, because cations are continuously extracted through the nanochannels and net anion flux is suppressed, the resulting flow towards the analysis channel will become ion-depleted (Fig. 1c). This ion-depletion reduces the buffer conductivity, creating an electric field enhancement [18]. When the preconcentrated anion sample plug is injected to the analysis channel by simply floating the W channels, anions in the field-enhanced region migrate rapidly toward the higher potential side. Once the anions pass the interface between the field-enhanced region and the running buffer, where the electric field is not enhanced, their migration speed is reduced and anions are stacked at the interface, known as the field-enhanced sample stacking [6,9,42]. As a result, the injected preconcentrated anion sample plug migrates stacked at the interface between the ion-depletion region and the running buffer and is not CE separated.

For electrophoretic separation of analytes preconcentrated by the field-enhanced sample stacking, micelle or spacer needs to be added [43–45]. On the other hand, the preconcentrated analytes needs to be injected into a homogeneous buffer or gel for the electrophoretic separation. Cong et. al. demonstrated a CE separation of a preconcentrated sample plug by introducing the plug to an EOF-suppressed separation channel using a pneumatic microvalve on a polydimethysiloxane chip [46]. The separation capacity and detection sensitivity would be improved if a monolithic glass or quartz substrate chip is developed. Foote et al. preconcentrated protein analytes using a non-EDL overlapped porous silica membrane as a size-selective filter between EOF-suppressed microchannels and showed capillary gel electrophoresis [24]. The nonlinear preconcentration profile as well as shifted separation peaks caused by electrophoretic sample collection/migration in the EOF-suppressed channel would be improved by applying the charge-selective electropreconcentration based on the T-intersection structure. Remarkably, Wang et al. reported CZE separation of model analytes in their original paper of electropreconcentration based on the T-intersection structure by simply floating the waste channel [14], which cannot be CZE separated as discussed above. Considering the preconcentration mechanism of anion redistribution based on

Please cite this article in press as: H. Chun, Electropreconcentration, gate injection, and capillary electrophoresis separation on a microchip, J. Chromatogr. A (2018), https://doi.org/10.1016/j.chroma.2018.08.053