DEVELOPMENT OF ELECTROSPRAY IONIZATION INTERFACE-INTEGRATED MICROCHIP FOR MASS SPECTROMETRIC DETECTION IN ELECTROPHORESIS

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ABSTRACT

The development of an electrospray ionization (ESI) interface-integrated microchip for simple and highly sensitive analysis by microchip electrophoresis (MCE) directly coupled to ESI-mass spectrometry (MS) is reported. The developed device provided a good combination of MCE–ESI-MS with on-line sample concentration based on the concept of large-volume sample stacking with an electroosmotic flow pump (LVSEP). Fluorescence imaging showed the applicability of the developed device to both ESI-MS detection and on-line sample concentration by LVSEP.

KEYWORDS: Microchip, Electrophoresis, On-line sample concentration, LVSEP, ESI

INTRODUCTION

It is well-known that microchip electrophoresis (MCE) directly coupled with mass spectrometry (MS) is a high-performance and versatile analytical separation and detection technique because of its ability of high resolution, high identification and fast analysis as well as small consumption of samples [1], while it often confronts the low concentration sensitivity. In this study, large volume sample stacking with an electroosmotic flow pump (LVSEP) [2, 3], which is an on-line sample concentration technique for microscale electrophoresis, was applied to microchip zone electrophoresis (MCZE) directly coupled to a MS detector (LVSEP-MCZE–MS) for improving the detection sensitivity by using an electrospray ionization (ESI) interface-integrated microfluidic device with a V-shape channel configuration (Figure 1). Although the concept of the channel configuration for ESI was similar to previous papers [4, 5], the fabricated device differs from the previous reports in terms of following three points: (1) the separation channel is modified to suppress the cathodic electroosmotic flow (EOF) to apply LVSEP for the enhancement of the sensitivity; (2) LVSEP-MCZE eliminates both the complicated channel configuration and multi-step voltage operation for the sample injection; (3) LVSEP-MCZE provides the highly efficient preconcentration and separation since the effective separation length is almost kept as the original channel length.

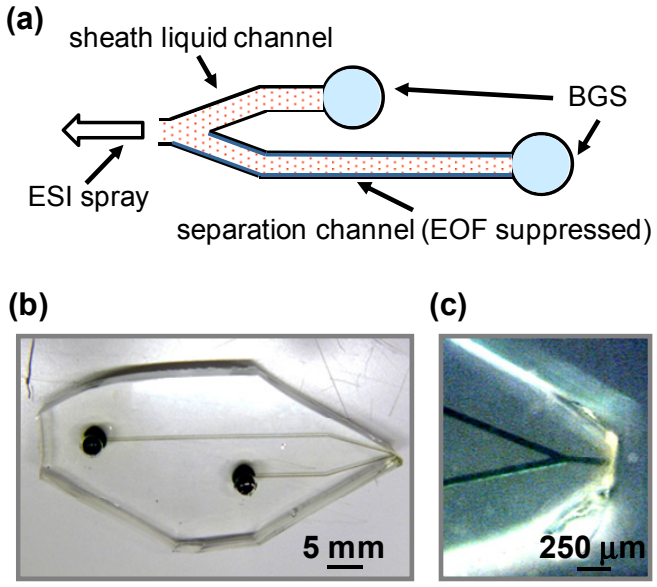

Figure 1: Schematic of the ESI interface-integrated microfluidic device with a V-shape channel configuration. (a) Channel design, (b) whole view of the fabricated device, and (c) magnified image of the ESI spray tip.

THEORY

The schematic of LVSEP-MCZE–MS using the developed device is shown in Figure 1: the inner surface of the sheath liquid channel was untreated, whereas the surface of the separation channel was modified by poly(vinyl alcohol) (PVA) to suppress the EOF. The operation procedures of LVSEP-MCZE–MS are schematically shown in Figure 2. In At the initial stage, the whole channel is filled with an anionic sample solution (S) with the low ionic strength (I), whereas the two buffer reservoirs are filled with a background solution (BGS) with the high I (Figure 1a). After applying the voltage, the BGS is introduced into the channels by the temporarily enhanced EOF due to the low I of the S (Figure 2a), and a Taylor cone is generated. The anionic analytes are concentrated by the field-amplified sample stacking due to the difference in the conductivity between the S and BGS (Figure 2b). Although the concentrated samples approach the cathodic reservoir by the fast EOF, the EOF in the separation channel is gradually suppressed by the removal of the S with low-I from the channel. However, in the sheath liquid channel, the fast EOF is continuously

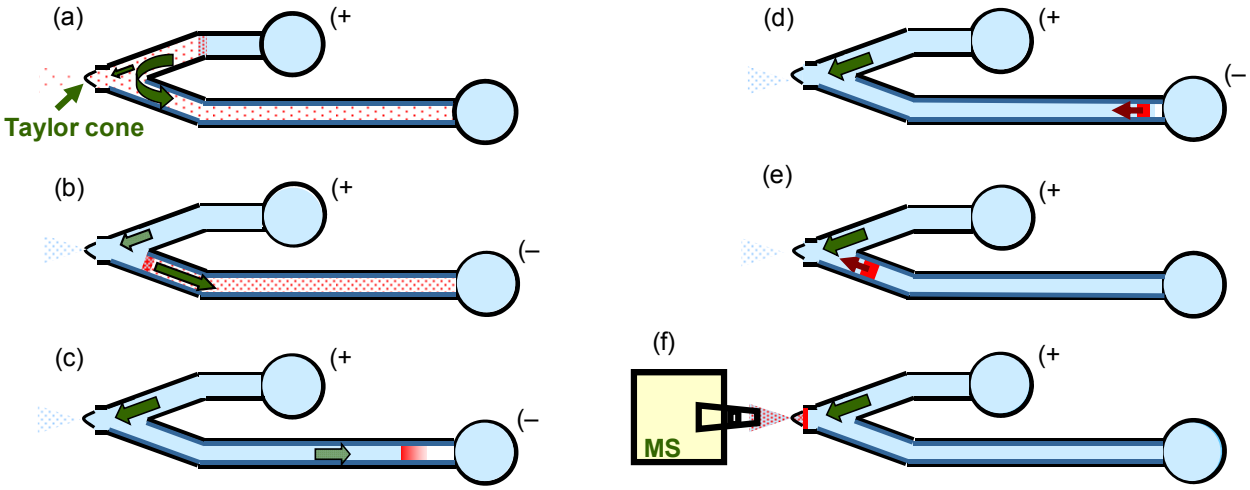

Figure 2: Schematic of LVSEP-MCZE–ESI-MS. (a) The initial stage, (b) preconcentration by field-amplified stacking of anionic samples, (c) decreasing the migration velocity of each analyte, (d) inversion of the migration direction of the analytes, (e) separation of the analytes by MCZE, (f) MS detection via ESI.

generated (Figure 2c). The anionic analytes can move toward the anode by their own electrophoretic mobilities (Figure 2d). The analytes are separated by the MCZE mechanism, and led to the sprayer via the cross section of the separation and sheath channels by the EOF in the sheath channel (Figure 2e). Finally, the ionized analytes are introduced into the MS detector (Figure 2f).

EXPERIMENTAL

The ESI sprayer integrated MCE microchip was fabricated by conventional soft lithography method using poly(dimethylsiloxane) (PDMS) as a chip material with an SU-8 mold. The inner surface of the separation channel was modified with PVA, while the sheath liquid channel was untreated. The MS detector used was a Shimadzu LCMS-2010 modified for MCE–MS.

RESULTS AND DISCUSSION

The generation of the Taylor cone and electrospray could be observed at the ESI sprayer during the MCE analysis (Figure 3), where sulforhodamine B was used as a test sample. Under an infusion analysis condition, a stable MS signal of caffeine was obtained by the positive mode for at least 4 min (Figure 4).

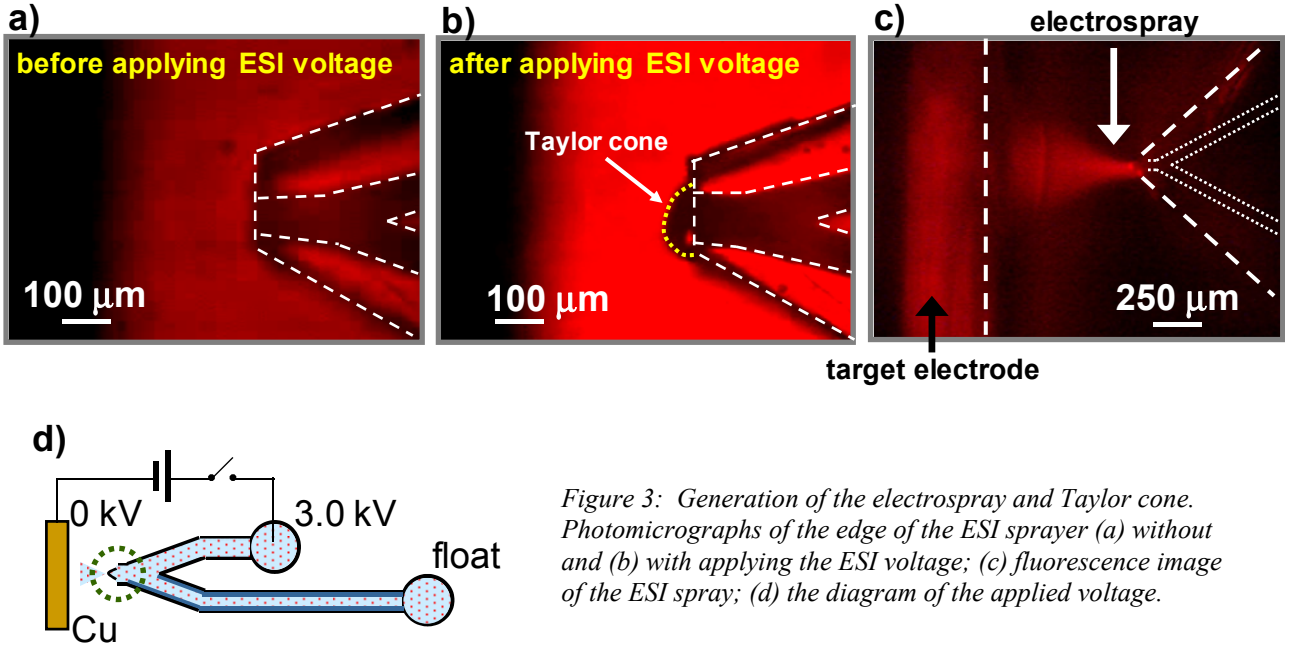

Figure 3: Generation of the electrospray and Taylor cone. Photomicrographs of the edge of the ESI sprayer (a) without and (b) with applying the ESI voltage; (c) fluorescence image of the ESI spray; (d) the diagram of the applied voltage.

Under the LVSEP-MCZE condition, both the stacking of anionic compounds and inversion of the moving direction nearby the anodic BGS reservoir were confirmed in the developed device (Figure 5).

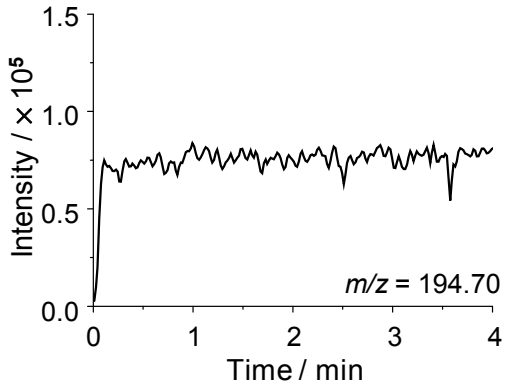

Figure 4: Mass electropherogram of 50 µM caffeine obtained by infusion ESI-MS. BGS, 1 mM ammonium formate (pH 6.7) containing 30% (v/v) acetonitrile.

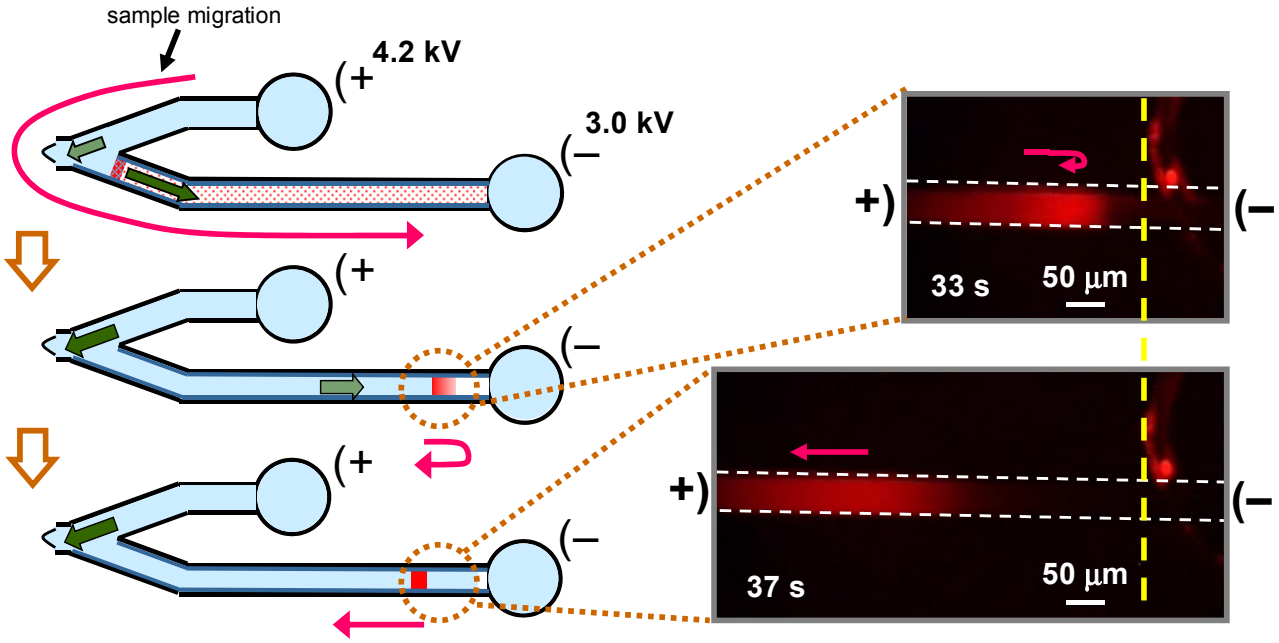

Figure 5: LVSEP-MCZE of sulforhodamine B on the fabricated microchip: (left) schematic of the sample migration and concentration, (right) fluorescence view of each sample migration; BGS, 25 mM ammonium carbonate (pH 8.4) containing 30% (v/v) methanol.

CONCLUSION

The applicability of the developed device to the LVSEP-MCZE and MCZE–ESI-MS analyses were confirmed. To achieve further sensitive detection by LVSEP-MCZE–ESI-MS, the fabrication of more sophisticated devices with downsizing the sprayer orifice is in progress for stabilizing the electrospray.

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