A Microchip Electrophoresis Device Integrated with the Top-bottom Antiparallel Electrodes of Indium Tin Oxide to Detect Inorganic Ions by Contact Conductivity

Sheng-Yao Chang,* Ming-Yuan Lee,* and Ching-Chou Wu*†

*Department of Bio-industrial Mechatronics Engineering, National Chung Hsing University, Taichung City 402, Taiwan, ROC
**Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, Taichung City 402, Taiwan, ROC

A microchip electrophoresis (ME) device with off-channel contact conductivity detection (C4D) was constructed using top-bottom antiparallel indium tin oxide (ITO) electrodes and a cross-type microchannel. The 500-m wide top-bottom antiparallel decouplers were found to effectively decrease the interference of the electrophoretic current. The cross-type microchannel was formed by bonding the patterned negative photoresist microstructures and the two top-bottom opposed ITO-deposited glass substrates. Five seconds of 150 V/cm injection field and the 100 V/cm separation field equipped with the C4D of AC 200 mV excitation voltage provided adequate ME operational parameters to obtain the K⁺ and Na⁺ peaks separation. The ME devices obtained good coefficients in a range of 11000 μm for the K⁺ and Na⁺ detection. The calculated limit of detection was 1 μM. This design for off-channel and top-bottom antiparallel electrodes shows that C4D ME devices have great potential for the measurement of inorganic ions.

Keywords Contact conductivity detection, microchip electrophoresis, off-channel, top-bottom antiparallel electrode

(Received March 13, 2018; Accepted June 13, 2018; Published November 10, 2018)

Introduction

Microchip electrophoresis (ME) has been used in different micro-analytical applications because of its rapid separation, ease of mass production, high portability, and high compatibility with various detection techniques. ME has been widely applied to the biomedical, food production and environmental engineering fields for the determination of proteins, peptides, DNA, neurotransmitters, inorganic and organic ions, vitamins and reactive nitrogen species. In particular, electrochemical electrodes, including amperometry and conductometry, are among the most widely used ME detection strategies due to their ease of operation and flexible integration to form a µTAS. Compared to amperometric electrodes, conductometric electrodes with ME could have great promise for the detection of nonelectroactive species, including inorganic and organic ions by measuring the conductivity difference between the analytes and the BGE.

Conductometric electrodes equipped with ME use one of two strategies: contact detection and contactless detection. Capacitively coupled contactless conductivity detection (C4D) requires a more complex electrode configuration to decrease the unavoidable stray capacitance and higher AC detecting voltages (tens of Volts) than the contact conductivity detection (C4D). Moreover, the sensitivity of C4D is determined by the permittivity and thickness of the dielectric layer coated on the C4D electrodes. In contrast, C4D is easily constructed by microfabrication techniques and is simply measured with a conductometer. However, in general, C4D has a lower LOD (< μM) than C4D for inorganic ions because C4D electrodes can sustain a larger separation field due to the lack of the Faradaic effect on the electrodes. If the Faradaic effect induced by the separation field on the electrodes can be minimized, the contact-type detection also promises ME with great potential for the measurement of different analytes. The in-channel electrode is easily affected by the electrophoretic separation field, so a lower separation field (< 100 V/cm) was used. However, the end-channel ME detection presented the high LOD at the scale of tens of micromolars and a low resolution between K⁺ and Na⁺. Therefore, the ME electrode configuration is an important issue for developing more sensitive detection. Our previous study found that the decoupler design can decrease the Faradaic effect of the separation field on off-channel amperometric electrodes. However, to the best of our knowledge, no study has reported designs of off-channel ME contact conductivity electrodes.

In principle, the sensing properties of conductometric electrodes are related to the electrode’s cell constant (d/A; d, distance between two electrodes; A, the cross-sectional area of electrode). The greater the d/A is, the larger the impedimetric response is. Grass et al. proved that left-right antiparallel contact conductivity electrodes perpendicular to the ME channel easily presented a larger d/A and a better resistive response than front-rear parallel electrodes measured in an isotachophoresis PMMA-microchip, implying that it is easier for the left-right
antiparallel electrode configuration to obtain a larger $d/A$. In this study, top-bottom antiparallel indium tin oxide (ITO) electrodes were integrated with glass microchannels using the hot-bonding technique without expensive instruments to form the off-channel ME device. The antiparallel electrode design could decrease the stray capacitance. Moreover, the decoupler of off-channel ME could minimize the effect of the separation field on the contact conductivity electrodes. The area-varied ITO electrodes were used to form different $d/A$ values and to evaluate the effect of $d/A$ on the sensing properties of ME. $K^+$ and $Na^+$ ions were used as indexes to demonstrate the ME separation efficiency.

**Experimental**

**Reagents**

Histidine (His), MES, potassium chloride and sodium chloride were purchased from Sigma-Aldrich. A mixture of MES and His was used for BGE. Positive photoresist of S1818 and negative photoresist of SU8-3010 were respectively obtained from Shipley (Tokyo, Japan) and MicroChem (Newton, MA). Hydrochloric acid and nitric acid bought from Union Chemical Works (Hsinchu, Taiwan) were mixed as the ITO etchant. ITO-coated glass plates (ITO thickness: 260 ± 20 nm; resistivity ≤ 7 Ω cm) were bought from Anatech (Taipei, Taiwan). All chemicals were of reagent grade and were used without further purification. All solutions were prepared with water purified through a Milli-Q system.

**Fabrication of electrode and ME device**

The ITO-coated glass plates were used as the ME substrate and simultaneously fabricated as the decoupler and the working electrodes using microfabrication techniques. Prior to spin-coating of S1818 photoresist, the ITO plates were ultrasonically cleaned by isopropanol and acetone. After exposure and development, the patterned S1818 photoresist layer with about 2 μm in thickness was used as a masking material on the cleaned ITO plates to protect the decoupler and electrode regions. To increase the resistance to the ITO etchant, the S1818 photoresist was sequentially baked at 90 °C for 3 min, 120 °C for 3 min, and 150 °C for 10 min. Next, the S1818 photoresist-containing ITO plates were dipped in a mixture of HCl, HNO₃ and pure water (0.20:0.05:0.75, v/v) at 25 °C for 2 h. They were then dipped in acetone to remove the patterned S1818 photoresist to expose the ITO decouplers and electrodes. Four sets of the decoupler/electrode pairs were laid out on an ITO plate to increase the chip life span (as shown in Fig. 1(a)), and each decoupler was placed in front of one electrode. The gap between the decoupler and the electrode in each set was 250 μm. The distance between the electrode of the previous set and the decoupler of the following set was 100 μm. The width of each decoupler was 500 μm. Electrodes were fabricated in three different widths (50, 75 and 100 μm) for use in different microdevices to explore the effect of cell constant values on the C 2D. As shown in Fig. 1(a), a 10-μm thick SU8-3010 photoresist layer was formed on one of the patterned ITO electrode-containing plates as the wall of the ME microchannel and reservoir after exposure and development. Moreover, four holes were drilled in the ITO electrode-containing plates without the SU8-3010 coating corresponding to the location of the SU8-3010 photoresist reservoirs. In this study a hot-bonding technique was used to bond the patterned SU8-3010 photoresist layer (called the bottom plate of the ME device) and the other ITO electrode-containing plates (called the upper plate of the ME device). In order to lower the hot-bonding temperature, the SU8-3010 photoresist exposure only reached the 70% default dose (365 nm, 155 mJ/cm²). After alignment with a microscope, the aligned upper/bottom plates were placed on a home-made hot bonding machine with a normal pressure of 1.3 - 1.5 kgf/cm² and sequentially heated at 40 °C for 1 min, 65 °C for 3 min, and 80 °C for 5 min to form the SU8-3010 glass bond. Finally, the bonded ME device was overexposed at a 600 mJ/cm² dose (365 nm) to complete the curing of the SU8-3010 photoresist. Figure 1(a) shows the bonded ME device with the cross-type microchannel and the ITO electrodes. The ME channel was 10 μm in height and 75 μm in width. The distance from the intercross point of the ME channels to the sample reservoir, the sample waste reservoir and the buffer reservoir was 0.5 cm, and the effective separation length at the first-pair decoupler/electrode was 3.93 cm.

**ME procedure and measurement**

The ME high voltage was applied by using two digital high-voltage power supplies (MP-2AP; Major Science, New Taipei, Taiwan). The two power supplies can synchronize the voltage output. The EOF mobility of ME was estimated by the current-monitoring method, which uses 5 mM MES/His (pH 5.9) to replace the 10 mM MES/His (pH 5.9) filling the ME channel and applies a 100 V/cm field between the buffer reservoir and the buffer waste reservoir. The $K^+$ and $Na^+$ were prepared in pure water to enhance the stacking effect and used as an index to demonstrate the ME separation efficiency. During the injection mode, a 150 V/cm field was applied between the sample reservoir and sample waste reservoir, and the buffer reservoir and the buffer waste reservoir were floating. The impedance of the separation zone
was continuously measured using an LCR meter (4284A, Agilent, Santa Clara, CA) connected with the top electrode (vi of Fig. 1) and the bottom electrode (viii of Fig. 1) in a two-electrode mode. The LCR meter supplied the 5 – 200 mV peak-to-peak AC voltage (V_{pp}) and 100 kHz to determine the effect of V_{pp} excitation voltage on the sensing properties. Moreover, the measured impedance signal was recorded by a self-designed program with the 2 Hz acquisition rate through the LabVIEW GPIB (Austin, TX) connection.

**Results and Discussion**

**Chip fabrication**

Figure 1(b) shows optical images of the intercross region of the two microchannels and one set of off-channel electrodes. The average width of the microchannel built by the SU8-3010 walls was 75 ± 2.3 μm. The image of the ITO electrodes shows that the top and bottom antiparallel decouplers and electrodes are well aligned. The widths of the decoupler and conductometric electrode, shown in Fig. 1(b), were respectively 500 ± 1.6 μm and about 49 ± 1.2 μm, indicating the ITO etching process has good consistency with the mask design and good reproducibility.

**EOF and decoupler performance**

The separating efficiency and reproducibility of the ME device are determined by the strength and stability of EOF. The EOF mobility of the ME device was 3.7 ± 10^{-4} cm²/Vs, after activating the microchannels using 1 M NaOH, and could be maintained for 1500 s with only about a 3% decrease, indicating good stability within the period of ME operation.

The electrode configuration of off-chip ME uses a decoupler to prevent the separation field from imposing the faradaic effect on the later electrode. The faradaic effect could cause electrode deterioration and increased background noise. A good decoupler can absorb hydrogen to prevent the formation of hydrogen bubbles resulting from water electrolysis.

The decoupling efficiency was estimated by the background current passing from the decoupler as the separation field was raised from 40 to 180 V/cm in 5 mM MES/His BGE, as shown in Fig. S1 (see Supporting Information). The result shows that the background current measured at the 500 μm wide top-bottom antiparallel ITO decouplers was slightly smaller than that measured at a Pt wire, implying the 500 μm wide ITO decoupler had a decoupling efficiency similar to that of the Pt wire in the range of 40180 V/cm. Furthermore, although the top-bottom antiparallel ITO decouplers with a surface area of 300 × 75 μm and 500 × 75 μm were larger than the cross sectional area of the microchannel.

---

**Fig. 2** Electropherograms of 1 mM K⁺ and Na⁺ (a) injected for 5 s (i) and 10 s (ii) and measured at 5 mV_{pp}, (b) measured at 5 (i), 50 (ii), 100 (iii) and 200 mV_{pp} (iv), (c) in the MES/His (pH 5.9) BGE of 20 (i), 10 (ii) and 5 mM (iii), and (d) separated with separation fields of 130 (i) and 100 V/cm (ii) in 5 mM MES/His BGE. All experiments were performed using 100 μm wide top-bottom antiparallel electrodes at 200 mV_{pp} in 10 mM MES/His BGE under a 150 V/cm injection field for 5 s and the 130 V/cm separation field, except as otherwise noted.
250 mVpp, which is attributed to the Faradaic reaction of the data not shown when the excitation voltage was performed at S

significant increase to background noise. However, the larger mobility of the K+ ion. Although the K+ peak height was larger than the Na+ peak height due to the ratio of K+ and Na+, the off-channel ME device. The C2D electrode as the stronger separation field is applied to Na+ in ME. Repeatability was investigated using the following ME parameters of the 150 V/cm injection field: 5 s, 200 mV excitation voltage and 100 mV/cm separation field for 0.5 mM K+ and Na+ in 5 mM MES/His BGE, Fig. S2 (see Supporting Information). The migration time of K+ and Na+ was 42.8 ± 0.6 s (n = 3) with a RSD of 1.3%, and 54.7 ± 0.6 s (n = 3) with a RSD of 1.1%. The peak heights of K+ and Na+ were, respectively, 14.9 ± 0.3 kΩ (1.9% RSD) and 9.0 ± 0.4 kΩ (4.6% RSD). The small RSD implies high ME separation efficiency with good repeatability.

Effect of cell constant

Top-bottom antiparallel ITO electrodes with three different electrode widths were constructed to produce different cell constants. The cell constants calibrated by the three kinds of standard KCl solutions, 0.4 mM (resistivity: 17.3 kΩ cm), 1 mM (7.0 kΩ cm) and 4 mM (1.8 kΩ cm), the conductivity of which was determined by a conductimeter (Con510, Eutech Instruments), were respectively 24.2, 17.2 and 13.3/cm for the conductometric signal for the same concentrations of 1 mM K+ and Na+ measured at electrodes with a cell constant of 24.2/cm. Other conditions are the same as in Fig. 3. (b) The corresponding calibration curve of K+ (■) and Na+ (○).

Operational conditions of ME device

The ME operational conditions were investigated to determine the optimal separation efficiency. According to the mobility of K+ (7.6 × 10−4 cm2/Vs) and Na+ (5.2 × 10−4 cm2/Vs), the time required for Na+ to move from the sample reservoir to the intercross of the microchannels was calculated as 3.75 s with an EOF mobility of 3.7 × 10−4 cm2/Vs under a separation field of 150 V/cm. Figure 2(a) shows the electropherograms of 1 mM K+ and Na+ with injection times of 5 and 10 s, proving that the glass-based ME device can completely separate K+ and Na+. The K+ peak height was larger than the Na+ peak height due to the larger mobility of the K+ ion. Although the K+ peak height of the 10 s injection was 1.56 times larger than that of the 5 s injection, the resolution (2.9) of the K+/Na+ peak pair of the 10 s injection was lower than that (3.6) of the 5 s injection. Moreover, the peak shape of the 10 s injection presented an asymmetrical fronting peak, but the peak shape of the 5 s injection was more symmetric. Therefore, the 5 s injection is a better parameter for the sample injection.

Figure 2(b) shows the effect of excitation voltage on the conductometric signal for the same concentrations of 1 mM K+ and Na+. The Na+ peak height increased proportionally with the excitation voltage in the range of 5 – 200 mVpp with a good coefficient of determination (R2 = 0.992) and without a significant increase to background noise. However, the background noise increased obviously to reduce the S/N ratio (data not shown) when the excitation voltage was performed at 250 mVpp, which is attributed to the Faradaic reaction of the ITO electrode. Therefore, 200 mVpp was used in the following experiments to improve the S/N ratio.

The detection signal of the sample zone originates from the conductance difference between the sample zone and the BGE. The conductance of the sample zone is calculated from the BGE conductivity, the analyte conductivity and the transfer ratio of the analyte. The transfer ratio of the analyte is determined by its electrophoretic mobility, the BGE co-ion and the BGE counter-ion. Figure 2(c) shows the effect of BGE conductivity of 5, 10, and 20 mM MES/His (pH 5.9) on the sample zone detection signal. The peak heights of K+ and Na+ measured in the 5 mM MES/His BGE (117 S/cm) were respectively 2.85 times and 4.36 times greater than that measured in the 20 mM MES/His BGE (438 S/cm). The result indicates that the peak height increases as the BGE concentration decreases. Because the mobility of the BGE co-ion (His) is smaller than that of K+ and Na+, an asymmetric fronting peak is observed. To improve the S/N ratio, the 5 mM MES/His BGE was used to estimate the sensing properties of the ME devices.

Figure 2(d) shows the effect of the separation field (100 and 130 V/cm) on the detection signal. The 130 V/cm separation field induced a larger peak height and greater background noise than the 100 V/cm. The increase in background noise is attributed to the interference of the electrophoretic current on the C2D electrode as the stronger separation field is applied to the off-channel ME device. The S/N ratio of K+ and Na+ obtained with the 130 V/cm field was respectively 11.1 and 7.4, and 83.0 and 53.5 with the 100 V/cm. The results indicate that the 100 mV/cm field provides better separation and detection of Na+ and K+ in ME. Repeatability was investigated using the following ME parameters of the 150 V/cm injection field: 5 s, 200 mV excitation voltage and 100 mV/cm separation field for 0.5 mM K+ and Na+ in 5 mM MES/His BGE, Fig. S2 (see Supporting Information). The migration time of K+ and Na+ was 42.8 ± 0.6 s (n = 3) with a RSD of 1.3%, and 54.7 ± 0.6 s (n = 3) with a RSD of 1.1%. The peak heights of K+ and Na+ were, respectively, 14.9 ± 0.3 kΩ (1.9% RSD) and 9.0 ± 0.4 kΩ (4.6% RSD). The small RSD implies high ME separation efficiency with good repeatability.

Effect of cell constant

Top-bottom antiparallel ITO electrodes with three different electrode widths were constructed to produce different cell constants. The cell constants calibrated by the three kinds of standard KCl solutions, 0.4 mM (resistivity: 17.3 kΩ cm), 1 mM (7.0 kΩ cm) and 4 mM (1.8 kΩ cm), the conductivity of which was determined by a conductimeter (Con510, Eutech Instruments), were respectively 24.2, 17.2 and 13.3/cm for the
50, 75 and 100 μm wide electrodes. Figure S3 (Supporting Information) shows the electropherograms of 1 mM K⁺ and Na⁺ measured by the three kinds of top-bottom antiparallel ITO electrodes. The results show that the peak height clearly increased with the cell constant. The S/N ratio of the K⁺ and Na⁺ peaks measured by the 24.2/cm electrode was respectively 1.3 × 10³ and 1.0 × 10², which was 15.9 and 19.4 times the S/N ratio measured by the 13.3/cm electrode. The results indicate that the larger cell constant electrodes can obtain improved detection signals.

**Calibration curve of ME device**

The ME devices integrated with the off-channel top-bottom antiparallel electrodes with a cell constant of 24.2/cm were used to obtain the calibration curve. Figure 3 shows the electropherograms of 1 - 1000 μM K⁺ and Na⁺ and their corresponding calibration curves. The calculated plate number of all concentrations of K⁺ and Na⁺ exceeds 27.6 × 10⁴/m, and the largest plate numbers of K⁺ and Na⁺ were respectively 170.8 × 10⁴/m and 105.3 × 10⁴/m, which are greater than that of in-channel ³⁰⁻³². The ME devices equipped with conductivity detection. Furthermore, the resolution corresponding to 1, 50, 100, 500 and 1000 μM was respectively 4.1, 2.8, 2.2, 1.8 and 1.6. The good resolution results imply that the ME devices have great promise to separate other metal ions.

The calibration curves of K⁺ and Na⁺ can be represented by the power functions with regression equations of y (kΩ) = 5.72[K⁺]⁻²⁸ (μM) (R² = 0.994) and y (kΩ) = 4.77[Na⁺]⁻²⁵ (μM) (R² = 0.985) in the detecting range of 1 - 1000 μM. It is worth noting that the slope in the range of lower K⁺ and Na⁺ concentrations is larger than that in the range of higher K⁺ and Na⁺ concentrations. This is attributed to the influence of field-amplified sample stacking (FASS).³⁹ In the ME experiments, the K⁺ and Na⁺ ions were prepared in distilled water. The sample plug of lower K⁺ and Na⁺ concentrations has lower conductivity than the 5 mM MES/His BGE (117 S/cm), implying that the field strength across the sample plug is larger than that across the BGE zone. The FASS can drive the K⁺ and Na⁺ to accumulate in the interface between the sample plug and the BGE, causing a larger peak height. In contrast, the higher K⁺ and Na⁺ concentrations decrease the application of FASS. The resistance signals of 1 μM K⁺ and 1 μM Na⁺ separation peaks were respectively 5.9 and 5.0 kΩ, which are greater than three times the background noise (0.56 Ω) calculated within a 15 s interval prior to the K⁺ separation peak. The LOD for K⁺ and Na⁺ ion can be defined as 1 M. The electrode configuration and sensing properties of the ME device were compared with the results of previous studies as listed in Table S1 (Supporting Information). The combination of the FASS effect and the ME devices integrated with the top-bottom antiparallel electrodes and the off-channel C'D presented detection properties superior to the present in-channel and end-channel C'D ME devices. The findings suggest the off-channel C'D ME devices have great potential for use in the measurement of inorganic and organic analytes.

**Acknowledgements**

This work was financially supported by the Ministry of Science and Technology of Taiwan under the grants MOST104-2313-B-005-036-MY3 and MOST104-2622-B-005-007-CC2, and the “Innovation and Development Center of Sustainable Agriculture” from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) of Taiwan.

**Supporting Information**

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

**References**

1. X. Lin, Q. Chen, W. Liu, L. Yi, H. Li, Z. Wang, and J. M. Lin, *Biosens. Bioelectron.*, 2015, 63, 105.
2. A. Staňková, J. Marák, M. Rezeli, C. Páger, F. Kilár, and D. J. Kaniansky, *J. Chromatogr. A*, 2011, 1218, 8701.
3. P. Zhang, H. Nan, M. J. Lee, and S. H. Kang, *Talanta*, 2013, 106, 388.
4. I. Álvarez-Martos, M. T. Fernández-Abdul, A. Anillo, J. L. G. Fierro, F. J. García Alonso, and A. Costa-García, *Anal. Chim. Acta*, 2012, 724, 136.
5. P. Kubán and P. C. Hauser, *Electrophoresis*, 2015, 36, 195.
6. V. Solinová and V. Kašická, *J. Sep. Sci.*, 2006, 29, 1743.
7. D. B. Gunasekara, J. M. Siegel, G. Caruso, M. K. Hulvey, and S. M. Lunte, * Analyst*, 2014, 139, 3265.
8. D. Gunasekara, M. Hulvey, S. Lunte, and J. da Silva, *Anal. Bioanal. Chem.*, 2012, 403, 2377.
9. P. Troška, R. Chudoba, L. Danč, R. Bodor, M. Horčička, E. Tesárová, and M. Masár, *J. Chromatogr. B*, 2013, 930, 41.
10. J. Wang, M. Pumera, G. Collins, and I. Jelínek, *Analyst*, 2002, 127, 719.
11. P. Kubán and P. C. Hauser, *Lab Chip*, 2005, 5, 407.
12. C. B. Freitas, R. C. Moreira, and M. G. de Oliveira Tavares, *Talanta*, 2016, 147, 335.
13. H. Zhai, J. Li, Z. Chen, Z. Su, Z. Liu, and X. Yu, *Microchem. J.*, 2014, 114, 223.
14. J. Liu, F. Xu, S. Wang, Z. Chen, J. Pan, X. Ma, X. Jia, Z. Xu, C. Liu, and L. Wang, *Electrochem. Commun.*, 2012, 25, 147.
15. H. Shadpour, M. L. Hupert, D. Patterson, C. Liu, M. Galloway, W. Strijewski, J. Goettert, and S. A. Soper, *Anal. Chem.*, 2007, 79, 870.
16. W. K. T. Coltro, R. S. Lima, T. P. Segato, E. Carrilho, D. P. de Jesus, C. L. do Lago, and J. A. F. da Silva, *Anal. Methods*, 2012, 4, 25.
17. A. J. Zemann, *Electrophoresis*, 2003, 24, 2125.
18. R. M. Guijt, E. Baltussen, G. van der Steen, R. Schasfoort, S. Schlautmann, H. A. Billiet, J. Frank, G. W. van Dedem, and A. van den Berg, *Electrophoresis*, 2001, 22, 235.
19. M. Galloway, W. Strzyzewski, A. Henry, S. M. Ford, S. Llopis, R. L. McCarley, and S. A. Soper, *Anal. Chem.*, 2002, 74, 2407.
20. E. X. Vrouwe, R. Luttge, W. Olthuis, and A. van den Berg, *J. Chromatogr. A*, 2006, 1102, 287.
21. C. C. Wu, R. G. Wu, J. G. Huang, Y. C. Lin, and H. C. Chang, *Anal. Chem.*, 2003, 75, 947.
22. B. Grass, D. Siepe, A. Neyer, and R. Hergenröder, *Fresenius’ J. Anal. Chem.*, 2001, 371, 228.
23. J. Wang and M. Pumera, *Anal. Chem.*, 2002, 74, 5919.
24. S. G. Serra, A. Schneider, K. Malecki, S. E. Huq, and W. Brenner, in Proceedings of 3rd International Conference on Multi-Material Micro Manufacture, 2007, Borovets, Bulgaria, 43.
25. L. C. Chen, C. C. Wu, R. G. Wu, and H. C. Chang, *Langmuir*, 2012, 28, 11281.
26. M. C. Breadmore and P. R. Haddad, *Electrophoresis*, 2001, 22, 2464.
27. A. J. Bard and L. R. Faulkner, “Electrochemical Methods Fundamentals and Applications”, 2nd ed., 2001, John Wiley & Sons Inc., New York.
28. J. L. Beckers and P. Boček, *Electrophoresis*, 2003, 24, 518.
29. R. Bharadwaj and J. G. Santiago, *J. Fluid Mech.*, 2005, 543, 57.
30. M. Pumera, J. Wang, F. Opekai, I. Jelínek, J. Feldman, H. Löwe, and S. Hardt, *Anal. Chem.*, 2002, 74, 6378.