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Dual-Channel Method for Interference-Free In-Channel Amperometric Detection in Microchip Capillary Electrophoresis

Chuanpin Chen[†] and Jong Hoon Hahn^{*,†,‡}

School of Environmental Science and Engineering and Department of Chemistry, BK School of Molecular Science, Pohang University of Science and Technology, San 31 Hyoja-Dong, Pohang 790-784, South Korea

A novel in-channel amperometric detection method for microchip capillary electrophoresis (CE) has been developed to avoid the interference from applied potential used in the CE separation. Instead of a single separation channel as in conventional CE microchips, we use a dual-channel configuration consisting of two different parallel separation and reference channels. A working electrode (WE) and a reference electrode (RE) are placed equally at a distance 200 μm from its outlet on each channel. Running buffer flows through the reference channel. Our dual-channel CE microchips consist of a poly(dimethylsiloxane) (PDMS) upper plate and a glass lower plate to form a PDMS/glass hybrid chip. Amperometric signals are measured without any potential shift and interference from the applied CE potential, and CE separation maintains its high resolution because this in-channel configuration does not allow additional band broadening that is notorious in end-channel and off-channel configurations. The high performance of this new in-channel electrochemical detection methodology for CE has been demonstrated by analyzing a mixture of electrochemically active biomolecules: dopamine (DA), norepinephrine, and catechol. We have achieved a 0.1 pA detectability from the analysis of DA, which corresponds to a 1.8 nM concentration.

Capillary electrophoresis (CE) has been the separation method of choice for microchip-based analytical systems due to easy injection and separation by applying high voltages to microchannel networks without mechanical complications.¹ Various detection methods have been employed for microchip CE,² but electrochemical detection (ECD) techniques are most favorable because electrodes can be readily integrated onto microchips through conventional lithographic processes.^{3–6} The most widely used ECD technique for microchip CE is amperometry^{6–9} that has the

advantage of high sensitivity over conductometry^{10–12} and potentiometry.^{12–14} It has been found, however, that the electric potential for microchip CE separation would interfere with the amperometric detection system and harm the grounded potentiostat.^{15,16} In order to overcome the obstacle, many different ways to isolate the amperometric detector from the separation voltage interference have been developed, which can be grouped into end-channel,^{17–23} off-channel,^{24–33} and in-channel^{15,34–36} detection methods according to electrode configuration.

* To whom correspondence should be addressed. Phone: +82-54-279-2118. Fax: +82-54-279-5805. E-mail: hahn@postech.ac.kr.

[†] School of Environmental Science and Engineering.

[‡] Department of Chemistry.

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End-channel detection is most widely applied in both conventional and microchip CE,^{17–23} where the working electrode (WE) is placed at tens of micrometers from the end of a capillary column or a separation microchannel and the counter electrode is grounded and placed behind the WE. In this configuration, the WE feels nearly ground potential because resistance drops precipitously at the interface between the narrow-bore separation channel and the huge buffer waste reservoir, which effectively reduces the interference from the applied CE potential with amperometric detection.⁶ However, serious band broadening in the region between the outlet of the separation channel and the WE causes poor separation efficiency and low detection sensitivity in the end-channel mode.⁶ To restrict this band broadening, a sheath-flow supported system was proposed by Ertl et al.²⁰ In addition to the problem of band broadening, end-channel detection is not completely free from the interference caused by the CE potential, which induces fluctuations in the background and thus degrades the signal-to-noise ratio.⁶ Nyholm and his colleagues showed that the use of microband array electrodes could reduce the interference.^{21–23} In the end-channel mode, the optimum potentiostatic voltage applied between the WE and the reference electrode (RE) is sensitively varied by the positions of both electrodes, which are hard to keep fixed from one chip to another in fabrication and thus causes poor chip-to-chip reproducibility in ECD.¹¹

To avoid such problems of end-channel detection, various off-channel techniques have been developed,^{24–33} where a grounded decoupler is built in front of the WE mounted within the separation channel so that the amperometric detection is operated in a field-free region. Many different materials have been employed in creating the decouplers: for example, porous materials,^{25,26} cracked and etched capillaries,²⁷ membranes,^{28–30} and metals.^{31–33} However, it has been found that an increase in band broadening develops in the region between the decoupler and the WE,^{6,17,29,33} which is mainly due to the change in the flow profile across the decoupler: from the electroosmotic flat profile to the hydrodynamic parabolic one. A decrease in detection sensitivity, due to the leakage of analytes through porous decouplers, and the short lifetime of decouplers have limited the wide use of off-channel detection techniques.³¹

The intrinsic limitations of the end-channel and off-channel configurations have encouraged development of in-channel detectors, where the WE is placed under the CE potential within the separation channel, equipped with means that can minimize the interference from the electrical potential for CE.¹⁵ For example, an electrically isolated potentiostat has been developed to make it possible to place the WE in the separation channel directly.¹⁵ This in-channel system decreases the plate height by 4.6-fold compared with the end-channel ECD. Nevertheless, the separation voltage causes a potential shift between the WE and RE, which deteriorates the detection signal significantly and thus demands prior determination of appropriate potential for the amperometric detection of each analyte using hydrodynamic voltammetry (HDV). In order to improve the performance of the microchip detection with amperometric signal stability, in this report, we developed a novel dual-channel microchip configuration to dem-

onstrate the complete prevention of interference from the CE separation potential in the in-channel configuration. In this configuration, two different but identical microchannels are replica molded onto the PDMS surface and integrated onto a glass plate to form PDMS/glass hybrid chips. The gold WE and RE are positioned equally at 200 μm on each channel from the outlet. This dual-channel method creates interference-free amperometric detection in microchip CE, demonstrated for the separation of neurotransmitters in the present study. This is supported by the observation of high-resolution CE separation peaks without band broadening that occurs seriously in end-channel and off-channel configurations. Here, we presented a systematic characterization of the dual-channel system developed.

EXPERIMENTAL SECTION

Chemicals and Materials. SU-8 50 negative photoresist (Micro Chem. Corp., Newton, MA), Sylgard 184 silicone elastomer and curing agent (Dow Corning, Midland, MI), catechol (CA) (Sigma Chemical Co., St. Louis, MO), norepinephrine (NE) (Sigma Chemical Co., St. Louis, MO), 3-hydroxytyramine hydrochloride (dopamine, DA) (98%, Aldrich Chemical Co., Milwaukee, WI), 99.9% platinum (1 mm diameter) (Aldrich), 99.9% gold (1 mm diameter) (Aldrich), 100 mm silicon wafers (Silicon Inc., South Korea), and 25 \times 75 \times 1 mm microslides (VWR Scientific Inc., West Chester, PA) were all purchased. The running buffer of 20 mM concentration and pH 9.5 was prepared using boric acid (Sigma Chemical Co. St. Louis, MO) and NaOH (Sigma Chemical Co. St. Louis, MO).

Apparatus. A cutting plotter (CE 2000–120, Graphtec Corp., Japan), a spin coater (Delta 80, Suss MicroTec, Germany), and a UV aligner (CA-40TS, Shinu System, South Korea) were used to make SU-8 masters for replica molding of PDMS. For patterning electrodes, gold was deposited onto glass slides in a thermal evaporator (Vacuum Science, South Korea). A molded PDMS plate was irreversibly bonded with an electrode-patterned glass slide by corona discharge treatment³⁷ with a Tesla coil (BD-10A, Electro Technic Products Inc., Chicago, IL). Microfluidics for CE separation was controlled by a computerized high-voltage supplying system including a high-voltage power supply (MP10P24, Spellmen, Huntsville, NY), a high-voltage relay (K81C245, Kilovac, CA), and a homemade voltage dividing system. Voltages were applied to solutions in reservoirs of the microchip via platinum electrodes. A LabView (National Instruments, Austin, TX) program written in-house and a multifunction I/O board (PCI-6014, National Instruments, Austin, TX) were used for instrument control and data acquisition. Images were obtained using a Nikon TE300 microscope equipped with a CCD camera (TE/CCD, Princeton Instruments, Trenton, NJ) that was controlled by an on-air TV system (Sasem, South Korea). A FAS2 Femtostat potentiostat (Gamry Instruments Inc., PA) was employed for ECD.

Microchip Fabrication. Dual-channel CE microchips were fabricated by a photolithography process^{4,5} and an irreversible bonding method.³ Microchannel networks and microelectrode patterns were designed with AutoCAD (Autodesk Inc., CA). A master for replica molding of the PDMS upper plate was made by forming a 20 μm -thick SU-8 microchannel pattern on a silicon

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wafer, and then the master was silanized.³⁶ A 10:1 mixture of PDMS oligomer and crosslinking agent was poured onto the master and then degassed under vacuum. After at least 2 h of curing at 65 °C, a PDMS replica with the negative relief of a channel network was peeled from the master. The reservoirs for sample, buffer, etc. were shaped by punching holes at the dead ends of the channels. On the lower glass plate, a pair of working and reference Au microelectrodes were patterned. Briefly, the glass slide was cleaned with piranha solution (7:3 H₂SO₄/H₂O₂) followed by 20 nm Cr and 200 nm Au layers being deposited by sequential thermal evaporation. Two Au electrodes of 50 μ m width were patterned on the glass slide by the photolithography process involving photoresist exposure using a mask, development, and wet chemical etching. The plate thus processed was rinsed with piranha solution.

The PDMS and glass plates thus prepared were bonded irreversibly for the complete fabrication of a microchip. The PDMS surface facing the lower glass plate was treated with a corona discharge generated from a Tesla coil. Immediately after the treatment, the two plates were brought into conformal contact and kept in a 65 °C oven for 2 h, which secured the irreversible seal. The channels thus formed were leak-free and withstood pressure up to 40 psi under which the microchannels could be easily filled with a buffer solution without bubble formation.

Microchip CE–ECD. The CE microchip shown in Figure 1 has a microchannel network that combines two identical, conventional T-shaped CE microchannels sharing one common sample waste channel. The width and depth of all channels are the same. However, the width of the sample waste channel is made to be double to accommodate flows from the twin systems without building up resistance. A sample is injected and detected in the separation channel, while the reference channel allows only the blank buffer at the same flow rate. The in-channel configuration of the WE and RE is done by positioning them closer to the outlets of the separation and reference channels, respectively. A Pt wire (1 mm diameter) in the buffer waste reservoir served as the counter electrode in ECD as well as the grounded electrode for the CE separation. All the electrodes are placed closer to the channel outlets to minimize the iR drop. The Pt electrode is placed close to the channel outlets: the gap is about 100 μ m. Because the resistance of the buffer in the channel is about 1.5 k Ω / μ m, the distance from the electrode to the outlet of the channel is 200 μ m and the background current in ECD is lower than 10 nA normally, the iR drop between the WE and the outlet of the channel is lower than 3 mV. It is negligible comparing with the potential applied for ECD.

For CE separation, positive potentials of 1000, 700, and 300 V are applied to both of the buffer reservoirs, the sample and the blank reservoirs, and the sample waste reservoir, respectively. The gated injection method^{38,39} is employed, and the sample and blank buffer plugs are injected into the separation and reference channels, respectively, by electrically floating the potentials at the buffer reservoirs for 1 s using the high voltage relay. All ECDs are performed with a potentiostat in a faradic cage.

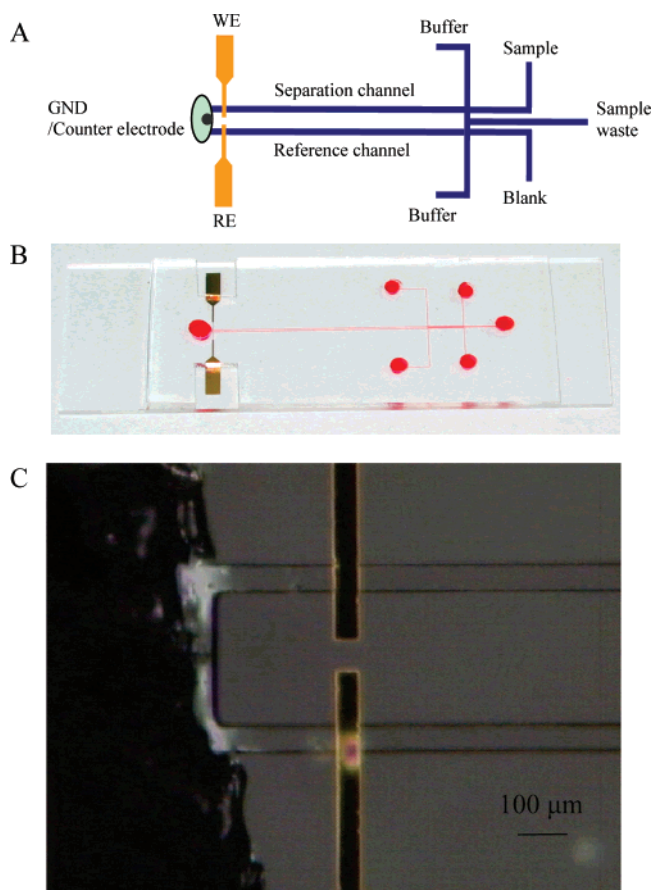


Figure 1. (A) Schematic of the microchannel network in the dual-channel CE microchip with an in-channel electrochemical detector. The separation and reference channels have the same length: 3 cm. Lengths of all other channels are shown in proportion to those of these two channels. All channels have the same cross section of 50 μ m width and 20 μ m depth, except the sample waste channel that is double in width. (B) Photograph of the microchip. Channels are filled with red ink for visualization. (C) CCD image of Au electrodes mounted in the exit of dual-channel.

Safety Considerations. The high-voltage power supply, the high-voltage relay/dividing system, and their associated electrical connectors should be placed in containers and/or properly grounded to not get harmed by an electrical shock.

RESULTS AND DISCUSSION

Interference from the CE Potential. In our dual-channel CE microchips, both the WE and RE are positioned at the same distance from the outlets of two identical channels, respectively, and the same separation potential (250 V/cm) from a single high-voltage power supply is applied to each channel. It is, therefore, expected that this configuration allows both the WE and RE to experience the same electric potential and its fluctuations. However, the fluctuations in the separation voltage would not interfere with the amperometric detection because those on both electrodes are perfectly correlated in time and magnitude and thus are cancelled out in measuring the current between the two electrodes. This would be quite a contrast to a conventional in-channel ECD configuration with a single channel, where the fluctuations interfere seriously the ECD.

To check the interference, we have modified the dual-channel chip so that it could perform in-channel ECD by placing a

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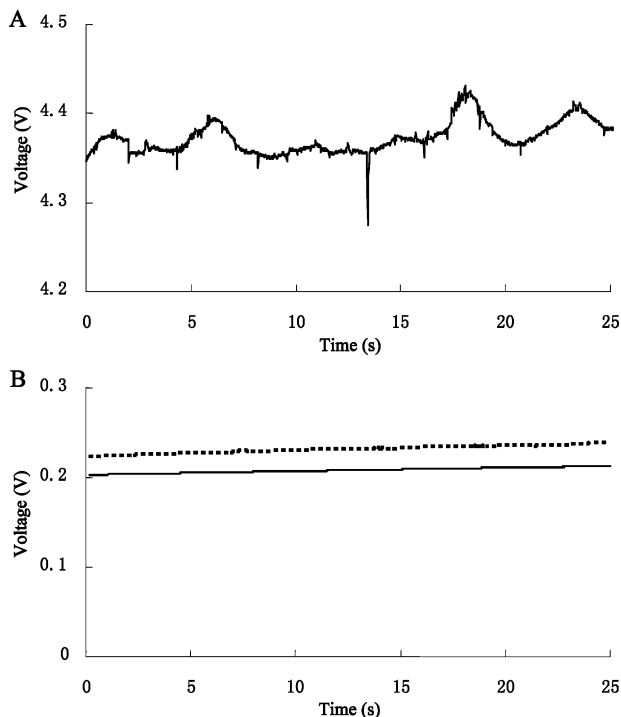


Figure 2. The time profiles of the open-circuit voltage between the WE and RE (A) in a conventional in-channel ECD configuration and (B) in the dual-channel configuration: with (—) and without (---) the CE high voltage. Running buffer, 20 mM boric acid (pH 9.5); CE potential, 250 V/cm.

homemade Ag/AgCl RE in the buffer waste reservoir and then measured the open-circuit voltage between the WE and RE while applying the separation potential. Figure 2A shows the time profile of the voltage. It is noted that the signal contains a serious drift and also short-term fluctuations. In contrast, the dual-channel configuration gives a very stable open-circuit signal in the presence of the separation high voltage, which is as stable as the signal in the absence of the separation potential (Figure 2B). Similar measurements were made on different dual-channel microchips ($n = 5$) fabricated under similar conditions. We observed almost a constant voltage–time profile, however, some oscillations at ± 10 mV, for all the dual-channel microchips studied, indicating very precise control of the microchannel fabrication by replica molding.

The open-circuit voltage measurements in Figure 2 demonstrate that our dual-channel configuration is very effective in avoiding the interference from the CE potential. Figure 3 compares the electropherograms of dopamine (DA) obtained using two different channel configurations in ECD: single in-channel (part A) and dual in-channel (part B) configurations. Again, serious drift and high-frequency fluctuations are inevitable in the electropherogram of the single-channel configuration but not with the dual-channel chip that was essentially free of noise. Note that the baseline of Figure 3B is almost noise free even at the low nA region (see the magnified trace).

Dual-channel microchips with different positions of the RE with respect to the WE are fabricated to investigate its effect on the electropherogram of DA detection. For example, when the RE is positioned at $100\ \mu\text{m}$ upstream from the WE (WE is at the same position as in the microchip of Figure 3), the optimum applied voltage (WE vs RE) obtained by HDV is $-1.5\ \text{V}$, which is shifted

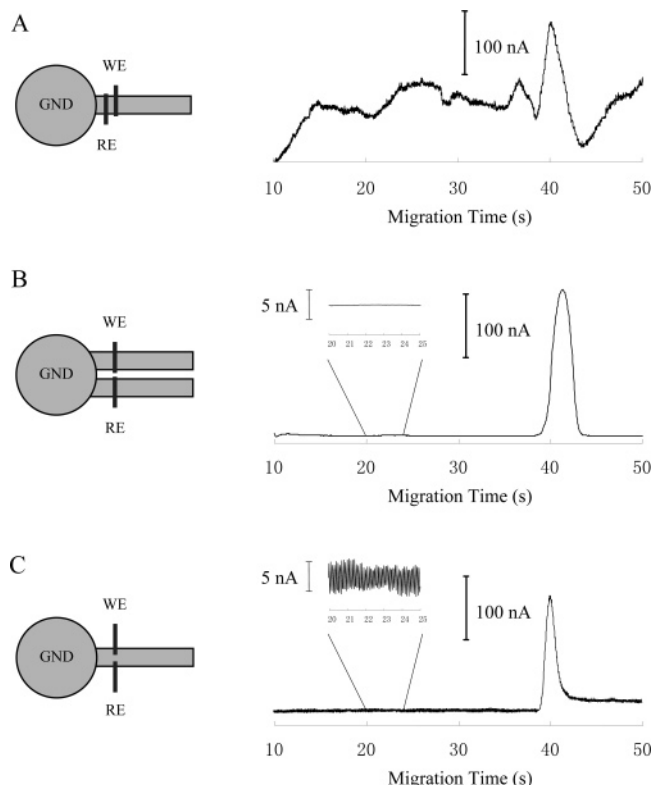


Figure 3. Electropherograms of DA observed with different WE–RE configurations. (A) Single in-channel configuration. The distance of the upstream side of the WE from the buffer waste reservoir is $200\ \mu\text{m}$, that of the downstream side of the RE is $100\ \mu\text{m}$, and the gap between the two electrodes is $50\ \mu\text{m}$. The applied voltage between the two electrodes (E) is $3.0\ \text{V}$. (B) Dual in-channel configuration. The distances of the forward sides of the WE and RE from the buffer waste reservoir are the same, $200\ \mu\text{m}$; $E = 0.7\ \text{V}$. A concentration of DA of $5\ \text{mM}$ is injected for $1\ \text{s}$ and run with $20\ \text{mM}$ boric acid buffer (pH 9.5). The electric potential of $250\ \text{V/cm}$ is applied to each separation channel. A part of the baseline of the electropherogram is magnified down to the low nA scale. (C) Single in-channel, head-to-head WE–RE configuration. The distance of the upstream sides of the WE and RE from the buffer waste reservoir is $200\ \mu\text{m}$, and the gap between the WE and RE is $20\ \mu\text{m}$; $E = 0.7\ \text{V}$. A part of the baseline of the electropherogram is magnified down to the low nA scale as in part B.

negatively by $2.2\ \text{V}$ to the applied voltage on the microchip of Figure 3B. On the other hand, the optimum voltage is shifted positively when the RE is positioned at $100\ \mu\text{m}$ downstream from the WE. Although a shift in applied voltage for amperometry is involved in misaligning the RE from the WE, its electropherogram is free of noise, similar to Figure 3B. Therefore, if the potential shift is compensated for ECD, a perfect alignment between the WE and the RE may not be necessary. This is an advantage to facilitate chip fabrication particularly when precise lithographic techniques are not available or applicable.

It might be suggested quite possibly that the same effect of noise reduction could be achieved if the two electrodes are positioned head-to-head in the same channel. We have fabricated such a microchip with a $20\ \mu\text{m}$ gap between the heads of the WE and the RE and checked the noise generated during CE separations. Figure 3C shows the electropherogram of $5\ \text{mM}$ DA, which was obtained using the head-to-head configuration. The serious drift in the electropherogram of the single in-channel, sequential

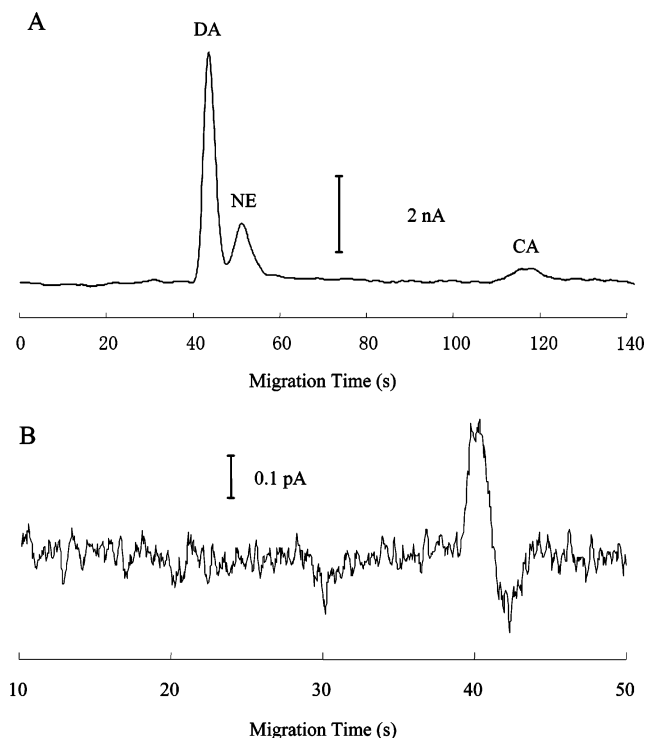


Figure 4. Electropherograms of (A) an equimolar mixture of DA, NE, and CA (100 μ M each) and (B) 5 nM DA. $E = 0.7$ V. Running buffer, 20 mM boric acid (pH 9.5); CE potential, 250 V/cm.

configuration (Figure 3A) is now removed almost completely because the WE and RE suffer the same effect from the CE potential. The high-frequency fluctuations still remain noticeable due to an overlapped potential in the detection zone. The closeness of both the WE and RE and the presence of the higher potential field on the WE, generated by the additional potential for amperometric detection, may help with the overlap of the diffusional field lines and disturb the electroosmotic flow in the detection zone and, hence, the noisy background signal in the electropherogram of the head-to-head configuration. This possibility is absent in the dual-channel configuration due to the different channels for the WE and RE. Thus, it is concluded that our dual-channel configuration provides the best performance among different in-channel configurations.

Separation Performance and Detection Limit. A mixture of neurotransmitters, DA, NE, and CA (100 μ M each) in boric acid buffer (pH 9.5) has been employed to evaluate the CE separation/ECD performance of our dual-channel microchips. Figure 4A demonstrates its high performance. For example, the plate number of the DA band is 1900 and its sensitivity is 59 pA/ μ M, which is much better than an end-channel system under similar separation conditions.⁴⁰ The electropherogram is nearly constant for 50 runs. This indicates higher stability and performance of the sensor in the dual-channel configuration than in the

off-channel systems, where the performance of the decoupler degrades rapidly with continuous separation runs.³¹ Figure 4B shows the DA detection limit at 1.8 nM with a signal-to-noise ratio of 3:1. The detection limit is much lower than that of the off-channel microchip CE–ECD system, which showed a detection limit of 25 nM.³⁰

The chip-to-chip reproducibility of the dual-channel ECD for microchip CE ($n = 5$) is studied and showed high stability of the sensor signal with the relative standard deviation for peak heights equal to 1.7%. Generally, it is difficult to align the WEs at the exact position manually under the CCD camera when different chips are fabricated. Hence, the smaller changes in the WE position may lead to poor chip-to-chip ECD reproducibility in the end-channel mode due to the misalignment of the WEs.⁴¹ This problem is insignificant in our dual-channel microchip for the fact that the variation in the distance from the WE (or RE) to the outlet of the microchannel does not change the optimum applied voltage between the WE and RE. The equal distances (from electrode to the channel outlet) in both microchannels can make an exact offset between the potential shifts (due to the CE potential) on both electrodes.

CONCLUSIONS

Our dual-channel method to avoid interference from the CE potential has proved to have high performance in detection and separation through this work. By removal of both long-term drift and short-term fluctuations from the baselines of the electropherograms, femtoampere-level ECD has been achieved, where analytes of low nanomolar concentrations can be detected. This detectability of the dual-channel technique is very promising in making ECD the more frequent detection method of choice for microchip CE. For trace on-chip analysis, laser-induced fluorescence detection techniques have usually been employed.³⁷ Sources and detectors in those techniques are hardly fabricated onto microchips, although some advancements have been made to integrate optical components into microfluidic chips.³ ECD is, however, well fit for microchip systems because electrodes and/or signal control/acquisition electronics can be readily integrated by lithography. Now that the dual-channel technique with excellent detectability has been developed, it is expected that the technique will be a standard format of ECD in microchip CE and greatly expand the practicability of microchip CE. Future directions include the application of this dual-channel microchips for separating clinically, environmentally, and pharmaceutically relevant compounds and the development and integration of the hand-held units for point-of-care analysis.

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