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Evaluation of in-channel amperometric detection using a dual-channel microchip electrophoresis device and a two-electrode potentiostat for reverse polarity separations

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Abstract

In-channel amperometric detection combined with dual-channel microchip electrophoresis is evaluated using a two-electrode isolated potentiostat for reverse polarity separations. The device consists of two separate channels with the working and reference electrodes placed at identical positions relative to the end of the channel, enabling noise subtraction. In previous reports of this configuration, normal polarity and a three-electrode detection system were used. In the two-electrode detection system described here, the electrode in the reference channel acts as both the counter and reference. The effect of electrode placement in the channels on noise and detector response was investigated using nitrite, tyrosine, and hydrogen peroxide as model compounds. The effects of electrode material and size and type of reference electrode on noise and the potential shift of hydrodynamic voltammograms for the model compounds were determined. In addition, the performance of two- and three-electrode configurations using Pt and Ag/AgCl reference electrodes was compared. Although the signal was attenuated with the Pt reference, the noise was also significantly reduced. It was found that lower LOD were obtained for all three compounds with the dual-channel configuration compared to single-channel, in-channel detection. The dual-channel method was then used for the detection of nitrite in a dermal microdialysis sample obtained from a sheep following nitroglycerin administration.

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Keywords

Dual-channel; Electrically isolated potentiostat; In-channel electrochemical detection; Microchip electrophoresis; Noise

1 Introduction

Since its introduction in the early 1990s [1–3], microchip electrophoresis (ME) has become a popular analytical method due to the ability to perform fast and efficient separations, minimal consumption of sample and reagents, parallel processing, and the capacity to perform on-site analysis [4, 5]. Electrochemical detection (EC) methods, in particular, amperometry and conductometry, are attractive approaches for microchip-based portable analysis systems because the detector and associated instrumentation can be miniaturized, and microelectrodes can be produced using standard microfabrication protocols. There are many examples of successful implementation of ME-EC, and these have been reviewed [5–9]. These devices have been used for monitoring various types of analytes in complex matrices such as cell lysates, foods, and beverages [7, 10]. Also, these devices have potential applications in lab-on-a-chip devices (e.g. lab on animal and lab on robot to monitor drugs, toxins, and behavior), environmental and homeland security applications (monitoring toxins and explosives), and pharmaceuticals analysis (monitoring drug degradation and counterfeit drugs) [7, 10–13].

With the exception of noncontact electrochemical techniques, such as capacitively coupled contactless conductivity detection (C^4D), electrochemical detection for microchip electrophoresis can be difficult to implement due to interference from the electric field used for the separation. Several strategies have been reported to alleviate this problem. The most common approach is to place the working electrode outside the microchannel, since the electric field strength drops rapidly in this region [14]. Unfortunately, this strategy can lead to a substantial loss of separation efficiency, due to the diffusion of the analyte plug into the relatively large outlet reservoir. Decouplers have also been used to isolate the separation voltage from the working electrode in ME-EC by grounding the separation voltage in the channel prior to the detector [15]. In particular, palladium (Pd) and platinum (Pt) decouplers that allow the separation voltage to be applied without interfering with the electrode potential have been described [15–18]. However, since Pd adsorbs hydrogen and not oxygen, it can be applied only for normal polarity separations [15–17]. Another useful alternative configuration for ME-EC is to position the working electrode slightly in-channel and use an isolated or floating potentiostat to protect the detector from the high separation voltage [14, 19]. This approach has been applied in the reverse polarity mode for the detection of nitrite and nitric oxide generated from diazeniumdiolates [20]. This configuration leads to higher separation efficiencies and better resolution compared to the end-channel configuration [14,20]. However, with in-channel detection, the potential of the working electrode is influenced by the separation voltage, and the apparent half-wave potentials for compounds are shifted positive under reverse polarity conditions [14]. In addition, baseline noise can be significantly increased compared to that with end-channel detection due to voltage fluctuations in the power supply.

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An approach for minimizing the effect of voltage fluctuations on electrochemical detector response in the in-channel configuration using a dual-channel microchip was reported by Chen and Hahn in 2007 [21]. In their design, two independent gold electrodes were placed within parallel microchannels at exactly the same position in relation to the end of the channel. The electrode in one of the channels was used as a reference where only BGE was injected, and the electrode in the other channel was used for detection of the analytes following electrophoretic separation. In this configuration, the noise induced by the separation voltage is cancelled out since both reference and working electrodes were placed at a point of isopotential inside their respective channels. This configuration maintains the high efficiency separation advantage of in-channel detection while also providing lower LOD. The Hahn group has employed this dual-channel design for the determination of catecholamines [21] and aminophenols [22, 23], and has reported LOD in the low nanomolar range using normal polarity separation conditions.

In this paper, the dual-channel approach is evaluated for reverse polarity separations of negatively charged model compounds with different half-wave potentials using the in-channel configuration and a two-electrode isolated potentiostat. The signal, noise, and LOD for these analytes were determined and compared to those obtained using in-channel ME-EC in single-channel devices. The method was then applied to the determination of nitrite in a microdialysis sample.

2 Materials and methods

2.1 Chemicals and materials

All reagents were of analytical grade and used as received. The following reagents and materials were used for these studies: SU-8 10 photoresist and SU-8 developer (MicroChem, Newton, MA, USA); AZ 1518 photoresist and 300 MIF developer (Mays Chemical, Indianapolis, IN, USA); photolithography film mask (50 000 dpi; Infinite Graphics, Minneapolis, MN, USA); 100 mm (4″) silicon (Si) wafers (Silicon, Boise, ID, USA); borosilicate float glass (4″ × 1.1 mm; Precision Glass and Optics, Santa Ana, CA, USA); Sylgard 184 Silicone Elastomer Kit: PDMS (Ellsworth Adhesives, Germantown, WI, USA); titanium etchant (TFTN; Transene, Danvers, MA, USA); epoxy paste and Cu wire (22 gauge; Westlake Hardware, Lawrence, KS, USA); conductive liquid silver paint (Ted Pella, Redding, CA, USA); sodium nitrite ($NaNO_2$), sodium iodide, ascorbic acid, sodium azide, boric acid, tetradecyltrimethylammonium bromide (TTAB), glutathione (GSH), and tyrosine (Tyr) (Sigma, St. Louis, MO, USA); acetone, 2-propanol (isopropyl alcohol, IPA), 30% H_2O_2, and NaOH (Fisher Scientific, Fair Lawn, NJ, USA).

2.2 Solutions and sample preparation

Stock solutions of nitrite, H_2O_2, GSH, ascorbic acid (AA), Cys, and Tyr (10 mM each) were prepared in ultrapure water (18.2 MΩ cm) (Millipore, Kansas City, MO, USA) and stored at 4°C. Standard solutions for analysis by ME-EC were prepared by dilution of the stock solutions in the BGE, which consisted of 10 mM boric acid with 2 mM TTAB adjusted to pH 11 with 1 mM or 10 mM NaOH.

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2.3 Instrumentation

A dual-channel high voltage power supply (HV Rack, Ultra-volt, Ronkonkoma, NY, USA) controlled by Labview software (National Instruments, Austin, TX, USA) was employed in these experiments. (*Caution! To avoid electrical shock, the high-voltage power supply should be used with extreme care.*) Gated injection [14, 24] and electrophoretic separation were accomplished through the application of negative potentials of −1400 or −2400 V to the BGE reservoirs (C and D), and −1200 or −2200 V to the sample and background reservoirs (A and B), while BGE waste and sample waste reservoirs were kept at ground (GND) (E and F) (Fig. 1A). Lower separation voltages were employed with microchips with a 3.5 cm separation channel, and higher separation voltages were used with devices with a 5 cm separation channel. The injection time was 1 s, and the separation lasted 50–60 s.

2.4 PDMS/glass microchip fabrication

The fabrication of PDMS-based microfluidic devices has been described previously by our group [14, 25]. Briefly, SU-8 10 negative photoresist (for channels patterning) was spin-coated on a 100 mm Si wafer to a thickness of 15 ± 1 μm using a Cee 100 spin coater (Brewer Science, Rolla, MO, USA). The wafer was then transferred to a programmable hotplate (Thermo Scientific, Asheville, NC, USA) for a soft bake at 65°C for 2 min and then at 95°C for 5 min. Microfluidic channel design was created using AutoCad LT 2004 (Autodesk, San Rafael, CA, USA) and printed onto a transparency film at a resolution of 50 000 dpi (Infinite Graphics, Minneapolis, MN, USA). The coated wafer was covered with the transparency film mask and exposed to 344 mJ/cm^2 using an in-line UV flood source (ABM, San Jose, CA, USA). Following the UV exposure, the wafer was post-baked at 65°C for 2 min and 95°C for 10 min. The wafer was then developed in SU-8 developer, rinsed with IPA, and dried under nitrogen. A final “hard bake” was performed at 175°C for 2 h. The thickness of the raised photoresist, which corresponds to the depth of the PDMS channels, was measured with a surface profiler (Alpha Step-200, Tencor Instruments, Mountain View, CA, USA). The PDMS microstructures were made by casting a 10:1 mixture of PDMS elastomer and curing agent, respectively, against the patterned Si master.

Dual-channel devices containing a separation channel and a reference channel with lengths of 3.5 or 5 cm (from the intersection to the end of the separation channel) were used for this study. The sidearm lengths were as follows: A-x2, B-x1, x1-y1, and x2-y2 were all 0.75 cm; C-y1, D-y2, and E-z were 1.5 cm; and y1-z and y2-z were 0.25 cm (Fig. 1A). The width and depth of the electrophoresis microchannels were 40 and 15 μm, respectively. The width of the sample waste channel (z-E) was 80 μm. Holes for the reservoirs were created using a 4 mm diameter biopsy punch (Harris Unicore, Ted Pella Redding, CA, USA) except in the case of the waste/GND reservoir, which was created using a 6-mm diameter biopsy punch. The PDMS substrate containing the dual-channel, configuration was reversibly sealed to a borosilicate glass plate containing a 15 μm Pt band working electrode (WE) and a 15 or 50 μm Pt reference electrode (RE). The separation channel, reference channel, and Pt electrodes were carefully aligned exactly at the end of the outlet of the channel (Fig. 1B).

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2.5 Electrode fabrication and electrochemical detection

All electrochemical measurements were performed using 15 μm Pt working electrodes. All Pt electrodes were fabricated in-house using a magnetron sputtering system (AXXIS DC magnetron sputtering system, Kurt J. Lesker Co., Jefferson Hills, PA, USA). The electrode fabrication protocol, which has been previously described [11], was used for preparation of these electrodes.

Electrochemical detection was accomplished using a wireless isolated potentiostat (model 8151P 2-channel and 9051 single-channel, Pinnacle Technology, Lawrence, KS, USA), operating at 10 Hz sampling rate (gain 500 0000 V/A, resolution 27 fA) and 13 Hz (gain = 500 0000 V/A, resolution = 47 fA) in a two-electrode configuration. Pinnacle Acquisition Laboratory software (PAL or Sirenia) was used for all data acquisition. A BAS LC-4C potentiostat was used for the study that employed a three-electrode system. In this case, end-channel configuration was used (the working electrode was placed 5 μm from the channel end toward the waste reservoir). The working electrode consisted of a 15 μm Pt band electrode. For the electrode in the reference channel either a 15 or 50 μm Pt band electrode was employed (Fig. 1ARE). A Ag/AgCl reference (Bioanalytical Systems, West Lafayette, IN, USA) was placed in the separation ground (waste “F”) reservoir for characterization of Pt electrodes (Fig. 2A and B). To facilitate perfect alignment of the electrodes in the two channels, the microchip was placed under an inverted microscope (Nikon Ti-U, Melville, NY, USA). The ground electrode was placed as close as possible to the channel end in the single-channel to effectively ground separation current (Fig. 2A). However, the separation ground electrode was placed after the Ag/AgCl electrode or away from the channel end for obtaining a symmetric separation field distribution for both channels (Fig. 2B).

2.6 Microchip electrophoresis

ME separations were carried out on a PDMS/glass hybrid device using gated injection [14, 24]. The BGE consisted of a solution of 10 mM boric acid with 2 mM TTAB, adjusted to pH 11 with NaOH. In this case, the TTAB is used to reverse the EOF and is present at a concentration below the critical micelle concentration [25]. After the complete filling of the channels with BGE using negative pressure, the desired standard or sample solution was placed in the HV sample reservoir (Fig. 1A).

2.7 Microdialysis samples

A BASi loop microdialysis probe (BAS) with 1 cm membrane length and 30 kDa molecular weight cut-off was used in these studies. A syringe pump (CMA, North Chelmsford, MA, USA) set at a flow rate of 1 μL/min was used for perfusion. The microdialysis probe was inserted into left dorsal side of a female domesticated sheep after performing anesthesia at the local site. Initially, 50 mM phosphate with 119 mM NaCl was perfused through the probe and background was collected at 10-min intervals for 1 h. Then the perfusate was switched to 4.8 mg/mL nitroglycerin in 50 mM phosphate and 119 mM NaCl solution and dialysate was collected every 10 min for 2 h. These dialysate samples were stored immediately in a −80°C freezer. In these studies, the 90-min 10 μL sample was thawed and diluted with 10 μL of BGE and injected into the chip. All animal procedures were performed at Purdue University under veterinarian supervision using approved protocols.

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3 Results and discussion

It has been demonstrated that higher separation efficiencies and better resolution are obtained for ME-EC when the electrode is placed inside the microchannel as opposed to end-channel configuration [14,19]. However, placing the electrode in the channel can lead to increased noise and shifts in the apparent redox potential for the analytes of interest [14, 19]. Chen and Hahn described a dual-channel design with unique sample and reference channels to minimize noise due to the high voltage used for separation [21–23]. They employed positive polarity and an isolated potentiostat in a three-electrode configuration. In this paper, we report the performance of this detection strategy under reverse polarity conditions using a custom-made isolated potentiostat that employs a two-electrode configuration. The effect of electrode composition and position on noise and potential shift in the dual in-channel configuration was investigated and compared to results obtained using the single channel configuration.

3.1 Two-electrode versus three-electrode systems for dual-channel dual-electrode amperometric detection

For successful implementation of amperometric detection in liquid chromatography or electrophoresis, it is important to minimize the IR drop between the working and reference electrodes. In the dual-channel configuration described here, resistance between the reference and working electrodes can be high due to the small dimensions of the microchannels, especially in cases where a low conductivity BGE is employed or the electrodes are placed deep in the separation channel. Therefore, using the two-electrode system, we found, not surprisingly, that placement of working and reference electrodes deep inside the two separate channels, without a counter electrode, led to baseline instabilities and irreproducible results.

The interaction of the separation voltage with a working electrode placed in an in-channel configuration leads to a negative half-wave potential shift under reverse polarity conditions using a single channel method [14,26,27]. In the case of the dual-channel configuration, where both the working and reference/counter electrodes are placed deep within a channel, there is additional positive potential (with respect to ground) on both electrodes due to the separation voltage [14, 27]. However, this does not affect the potential applied to the working electrode (with respect to the reference electrode) by the potentiostat, because that value is based on the potential difference between the working and reference electrodes. Since with the two-electrode configuration the electrode placed in the reference channel acts as both a reference and counter electrode, the separation voltage-induced potential can affect the detector performance because the negative shift in potential (positive-induced potential) will affect the rate of any reduction reactions occurring at the reference/counter electrode.

3.2 The effect of separation field on detector response

No system peaks were reported in the original studies using the dual-channel approach for normal polarity separations [21,22]. However, we have observed system peaks when the electrodes were placed deep inside the channel under reverse polarity conditions with both the single-channel and dual-channel configurations with the two-electrode potentiostat. The

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appearance of these system peaks is most likely related to the change in the composition of the BGE during the gated injection or different conductivity zones in the buffer that can modify the electrical double layer at the working electrode and induce a charging current.

To investigate this phenomenon further, the effect of the electrode position within the channel on the appearance of system peaks was investigated, and the results are depicted in Fig. 3. System peaks were generated when both working and reference electrodes were placed in the channel at a distance of either 200 or 50 μm from the channel outlet as can be seen in Fig. 3A and B, respectively. The system peaks could be eliminated only by placing the electrodes exactly at the end of the channel. A very low noise at the working electrode (1–4 pA) was observed using this new electrode position (Fig. 3C).

3.3 Effect of reference electrode size and placement and counter electrode

In the dual-channel configuration described above, a Pt electrode is placed in the reference channel; it acts as a pseudo-reference electrode as well as the counter electrode. In order to determine the effect of reference electrode placement and type on the potential shift at the working electrode in the in-channel configuration, a 15-μm Pt working electrode with a 15-μm Pt pseudo-reference was compared to a 15-μm Pt working electrode with a Ag/AgCl reference placed in the waste reservoir. (Once the Pt pseudo-reference electrode is replaced with a Ag/AgCl electrode, the electrode configuration in dual-channel microchip can be approximated to our single-channel in-channel system as shown in Fig. 2A). The relative placement of the reference and working electrodes and separation ground for the two different experimental setups is shown in Figure 2. Hydrodynamic voltammograms (HDV) obtained for nitrite, iodide, Tyr, and H_2O_2 with Pt reference (both Pt working and reference electrodes were placed at exact channel end) versus Ag/AgCl reference (Fig. 2B) are shown in Fig. 4A–D. A potential shift can be expected in both these scenarios due to reference electrode material and the position of the electrode relative to the WE. The HDV of Tyr and iodide, which are two easily oxidized species (Fig. 4B and D), exhibit clear negative shifts in half-wave potentials.

This potential shift is not as obvious in the HDV of nitrite or hydrogen peroxide, which are more difficult to oxidize (Fig. 4A and C). A negative shift can be seen in HDV of nitrite and H_2O_2 with a Pt reference in dual-channel configuration plotted up to +0.9 V and +1.0 V, respectively. Above +1.1 V, there was sudden increase in signal for both species. However, potentials greater than +1.1 V were not used in these studies because we have observed that Pt electrodes that have been deposited on glass often crack or peel off during the ME-EC experiments. This occurs with both the single- and dual-channel devices and is independent of the type of reference electrode that is used (Pt or Ag/AgCl). Therefore, a working electrode potential of +1.1 V versus both Pt and Ag/AgCl reference was employed for the remaining studies.

Significantly lower current responses were observed for nitrite, tyrosine, and H_2O_2 with the Pt pseudo-reference compared to the Ag/AgCl reference (Fig. 5A shows data for nitrite). However, the baseline noise was much lower with a Pt pseudo-reference compared to a Ag/AgCl reference due to cancellation of the separation voltage fluctuations (Fig. 6 and Table 1) [21]. The low peak currents with the Pt reference electrode are almost certainly due to the

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rate of the reduction reaction at the reference and IR drop due to the lack of a counter electrode.

The effect of the size of the Pt reference electrode on peak current was also investigated using a 50-μm Pt electrode in the reference channel and a 15-μm Pt working electrode in the parallel separation channel. The Pt pseudo-reference electrode size was increased such that the faradaic processes at the working electrode were not limited by the reactions at the reference/counter electrode [27]. Figure 5B shows peak height comparison using two harder-to-oxidize species, nitrite and azide, with a 50-μm Pt reference versus Ag/AgCl reference using a dual-channel setup. Under these conditions, both the peak current and baseline noise increased (~17 pA) (Table 1). This shows that a larger Pt electrode placed in the parallel channel is a better choice for the reference, but comes at the expense of an increase in noise.

For the reference electrode comparisons shown in Fig. 5A and B, a two-electrode configuration with a Pinnacle isolated potentiostat was employed. Lastly, an experiment similar to that shown in Fig. 5A was performed by placing a Pt wire counter electrode in waste “F” reservoir and using a three-electrode configuration with a conventional BAS potentiostat (Fig. 5C). In this case, Pt working and reference electrodes were placed 5 μm from the end of the channel. Figure 5C shows a comparison of the nitrite signal using a Pt reference with that obtained using a Ag/AgCl reference. A higher signal (approximately 2 times) was obtained with a 15-μm Pt reference as opposed to a Ag/AgCl reference. This could be due to a negative potential shift that is more apparent when a counter electrode is present (Fig. 4A). However, there are difficulties when an electrode is placed even 5 μm away from the channel outlet toward the waste reservoir using the conventional three-electrode potentiostat. In particular, the high voltage used for separation can damage both the electrode and potentiostat electronics.

Table 1 provides a summary of the baseline noise and background currents obtained using different reference electrodes in dual- and single-channel microchip electrophoresis systems with in-channel electrochemical detection. The dual-channel configuration with a 15-μm Pt reference electrode exhibited the lowest baseline noise and background currents compared to other designs.

3.4 Separation performance and detection limits

Our group has published protocols for the separation and detection of oxidative and nitrosative stress markers using ME with amperometric detection using single-channel simple-T microchips and an isolated wireless potentiostat [14]. The use of the isolated potentiostat afforded electrode placement at the exact end of the channel (referred to as in-channel configuration) without the need for a decoupler and minimized the interference of the high separation voltage [14]. Despite the higher resolution and sensitivity of in-channel detection in single-channel ME, the baseline noise is very high (~25 pA). The majority of this noise could be due to fluctuations in the separation voltage. The dual-channel strategy described in this paper significantly reduced the baseline noise (4 pA) and background current (to approximately 0.8 nA), which led to improved LOD compared to the single-channel approach (Fig. 6).

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It can be seen that the lower LOD obtained with the dual-channel configuration are mainly due to a reduction of the baseline noise. The baseline noise obtained for the dual- and single-channel configurations were 4 pA and 25 pA, respectively. The noise could be further reduced to 0.98 pA without significant reduction of peak height by applying a digital filter (moving average, 5 points window). LOD at a S/N of 3 for nitrite, H_2O_2, Tyr, GSH, and AA were 0.58, 0.75, 0.14, 0.82, and 0.21 μM, respectively. The LOD that were obtained in the single and dual in-channel configurations using nitrite and H_2O_2 as model compounds are shown in Table 2. Application of a digital filter to data obtained using the dual-channel configuration lowered the LOD of nitrite and H_2O_2 about threefold, while the decrease was approximately twofold with the single-channel configuration.

3.5 Detection of nitrite in microdialysis samples

Microdialysis samples collected from subcutaneous nitroglycerin perfusion studies in sheep were used to test the applicability of a dual-channel in-channel detection scheme for biological applications. ME-EC analysis of a microdialysis sample obtained 90 min after infusion of nitroglycerin showed a peak for nitrite, the primary metabolite of nitroglycerin in the skin. The nitrite peak was identified by spiking the sample with a nitrite standard (Fig. 7). To quantitate the amount of nitrite that was produced, a calibration curve was prepared over the concentration range of 3.1–50 μM (R^2 = 0.997). The estimated concentration of nitrite in the microdialysis sample obtained 90 min after infusion with nitroglycerin was determined to be 68 μM.

4 Concluding remarks

The in-channel electrode configuration using a dual-channel microchip described for normal polarity by Hahn's group was evaluated for reverse polarity separations using a two-electrode isolated potentiostat. We found that placement of the electrode deep inside the channel as originally reported by Chen and Hahn could not be employed due to the appearance of system peaks and the instability of the electrode under those conditions. However, placing the electrodes at the exact channel end (similar to our previous in-channel amperometric detection studies with simple-T microchips) obviated the system peaks and improved electrode lifetime. Baseline noise obtained using the dual-channel configuration (4 pA) was significantly lower than that observed with single-channel in-channel detection (25 pA).

A clear negative potential shift between Pt reference and Ag/AgCl reference was observed during HDV studies for I^- and Tyr, but the shift was not clear with nitrite and H_2O_2. The peak current was substantially decreased when a Pt electrode was employed as a reference compared to a Ag/AgCl reference. The most likely reasons for the decrease in signal are the lack of counter electrode in the two-electrode system, IR drop between the working and reference electrodes, the effect of separation field, and the instability of the 15-μm pseudo-Pt reference. The type of reference electrode also influenced the peak heights when using the dual-electrode configuration. Despite these issues, lower LOD were observed with the dual-channel configuration due to the reduction in baseline noise compared to the single-channel configuration. The dual-channel method was then used for nitrite detection in a sheep

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microdialysis sample, and nitrite concentration levels of 68 μM were found following 90 min of continuous perfusion of nitroglycerin.

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Abbreviations

AA	ascorbic acid
GSH	reduced glutathione
HDV	hydrodynamic voltammograms
IPA	2-propanol
ME-EC	microchip electrophoresis coupled to electrochemical detection
RE	reference electrode
Tyr	tyrosine
WE	working electrode

References

1. Manz A, Harrison DJ, Verpoorte EMJ, Fettinger JC, Paulus A, Luedi H, Widmer HM. J Chromatogr. 1992; 593:253–258.
2. Harrison DJ, Manz A, Fan Z, Luedi H, Widmer HM. Anal Chem. 1992; 64:1926–1932.
3. Jacobson SC, Hergenroder R, Koutny LB, Warmack RJ, Ramsey JM. Anal Chem. 1994; 66:1107–1113.
4. Arora A, Simone G, Salieb-Beugelaar GB, Kim JT, Manz A. Anal Chem. 2010; 82:4830–4847. [PubMed: 20462185]
5. West J, Becker M, Tombrink S, Manz A. Anal Chem. 2008; 80:4403–4419. [PubMed: 18498178]
6. Auroux PA, Iossifidis D, Reyes DR, Manz A. Anal Chem. 2002; 74:2637–2652. [PubMed: 12090654]
7. Mark JJP, Scholz R, Matysik FM. J Chromatogr A. 2012; 1267:45–64. [PubMed: 22824222]
8. Ghanim MH, Abdullah MZ. Talanta. 2011; 85:28–34. [PubMed: 21645665]
9. Coltro WKT, Lima RS, Segato TP, Carrilho E, Pereira de Jesus D, Lucio do Lago C, Fracassi da Silva JA. Anal Methods. 2012; 4:25–33.
10. Martín A, Vilela D, Escarpa A. Electrophoresis. 2012; 33:2212–2227. [PubMed: 22887146]
11. Scott DE, Grigsby RJ, Lunte SM. Chem Phys Chem. 2013; 14:2288–2294. [PubMed: 23794474]
12. Chen G, Lin Y, Wang J. Talanta. 2006; 68:497–503. [PubMed: 18970349]
13. Berg C, Valdez DC, Bergeron P, Mora MF, Garcia CD, Ayon A. Electrophoresis. 2008; 29:4914–4921. [PubMed: 19130571]
14. Gunasekara DB, Hulvey MK, Lunte SM. Electrophoresis. 2011; 32:832–837. [PubMed: 21437918]
15. Lacher NA, Lunte SM, Martin RS. Anal Chem. 2004; 76:2482–2491. [PubMed: 15117187]
16. Mecker LC, Filla LA, Martin RS. Electroanalysis. 2010; 22:2141–2146. [PubMed: 21572540]

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17. Kovarik ML, Li MW, Martin RS. Electrophoresis. 2005; 26:202–210. [PubMed: 15624172]
18. Wu CC, Wu RG, Huang JG, Lin YC, Chang HC. Anal Chem. 2003; 75:947–952. [PubMed: 12622389]
19. Martin RS, Ratzlaff KL, Huynh BH, Lunte SM. Anal Chem. 2002; 74:1136–1143. [PubMed: 11924975]
20. Gunasekara DB, Hulvey MK, Lunte SM, Fracassi da Silva JA. Anal Bioanal Chem. 2012; 403:2377–2384. [PubMed: 22415023]
21. Chen C, Hahn JH. Anal Chem. 2007; 79:7182–7186. [PubMed: 17708674]
22. Chen CP, Teng W, Hahn JH. Electrophoresis. 2011; 32:838–843. [PubMed: 21413030]
23. Chen C, Hahn J. Environ Chem Lett. 2011; 9:491–497.
24. Zhang G, Du W, Liu BF, Hisamoto H, Terabe S. Anal Chim Acta. 2007; 584:129–135. [PubMed: 17386595]
25. Hulvey MK, Frankenfeld CN, Lunte SM. Anal Chem. 2010; 82:1608–1611. [PubMed: 20143890]
26. Wallenborg SR, Dorholt SM, Faibushevich A, Lunte CE. Electroanalysis. 1999; 11:362–366.
27. Contento N, Bohn P. Microfluid Nanofluid. 2014; 17:1–10.

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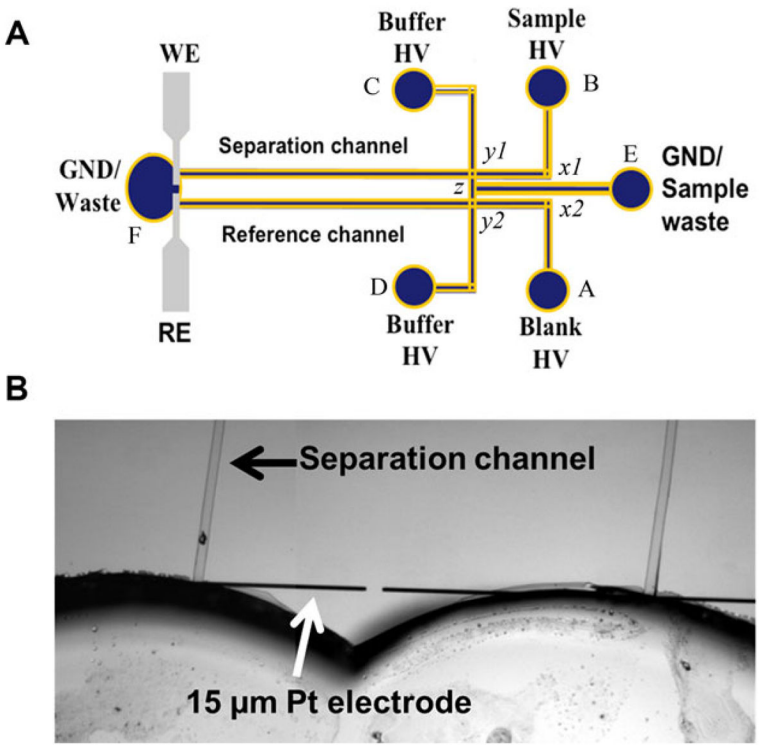

Figure 1.

(A) Schematics of the dual-channel microchip with in-channel electrode configuration. All reservoirs except the waste/GND reservoir have a diameter of 4 mm, which is equivalent to a volume of 20 µL. The waste/GND reservoir has a 50 µL capacity. (B) Image of placement of 15 µm Pt electrodes.

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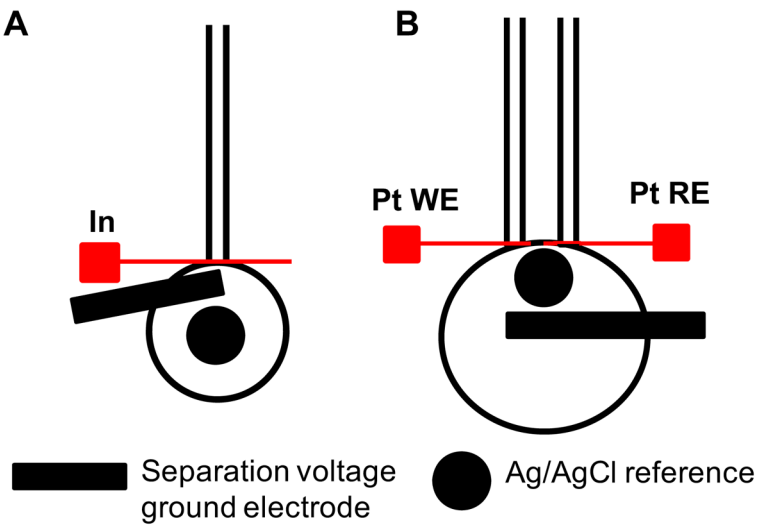

Figure 2.
The placement of Pt and Ag/AgCl reference electrodes (A) single-channel (B) dual-channel ME-EC.

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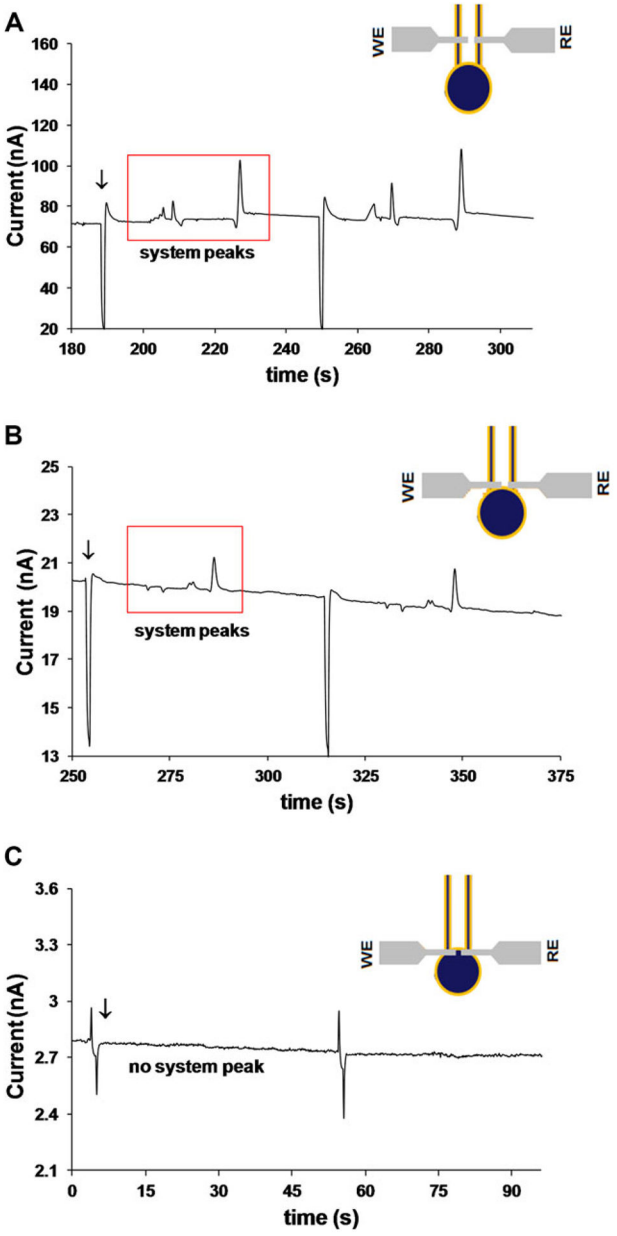

Figure 3.

Investigation of appearance of system peaks with electrode position. Electrodes were aligned at (A) 200, (B) 50, and (C) 4 μm from the channel outlet. Conditions: BGE: 10 mM boric acid with 2 mM TTAB at pH 11; gated injection (↓) of 1 s; detection potential: +1.1 V (against Pt).

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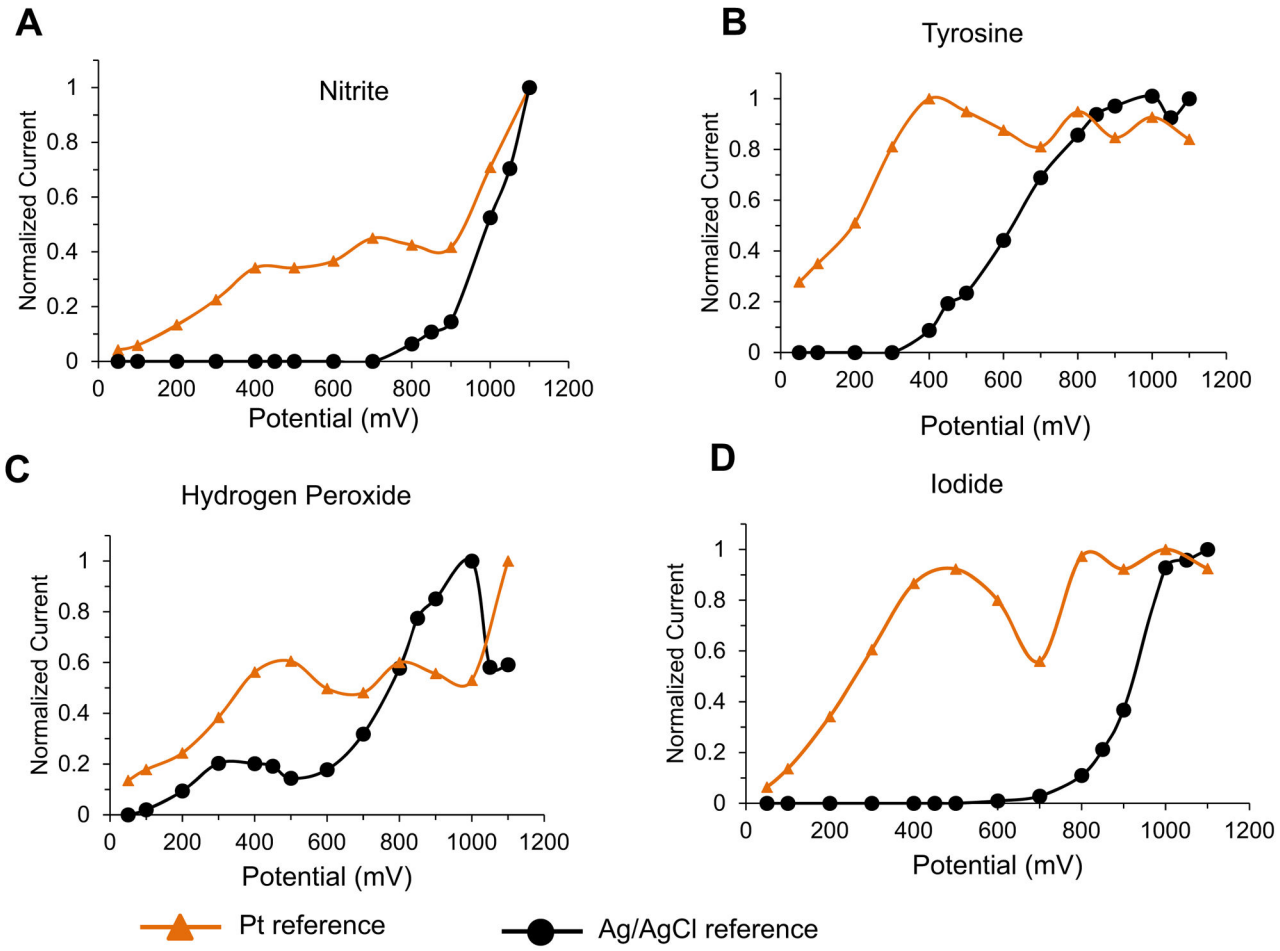

Figure 4.

HDV of (A) nitrite (B) Tyr (C) H_2O_2 (D) I^- using 15 μm Pt and Ag/AgCl reference electrodes. The working electrode is a 15 μm Pt electrode. A dual-channel microchip with reference electrode placement similar to that shown in Fig. 2B was used for these studies. Conditions: BGE: 10 mM boric acid with 2 mM TTAB at pH 11.

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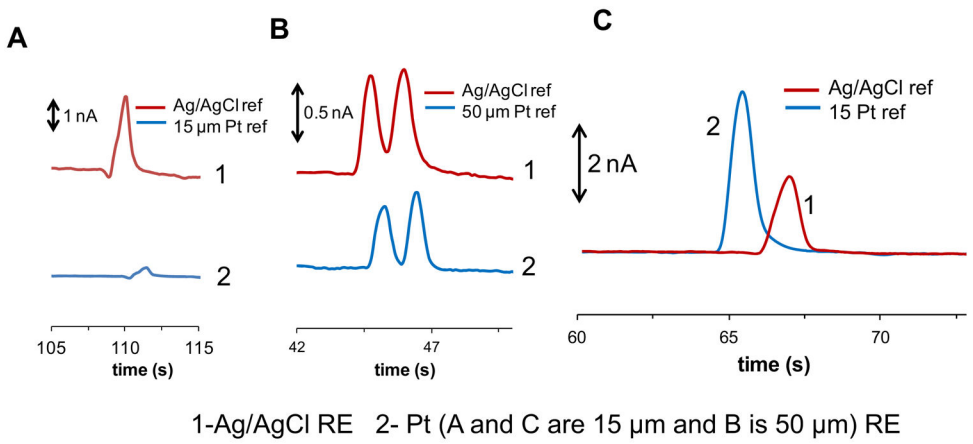

Figure 5.

A comparison of peak current of 100 µM nitrite with (A) 15 µm Pt reference and Ag/AgCl reference, (B) 50 µm Pt reference and Ag/AgCl reference, and (C) 15 µm Pt reference and Ag/AgCl using a three-electrode system. For A and B, a two-electrode system was used with a Pinnacle potentiostat; for C, a BAS potentiostat was used. For all studies, dual-channel microchips were used with reference electrode placement similar to that shown in Fig. 2B. Conditions: BGE: 10 mM boric acid with 2 mM TTAB at pH 11. The working electrode is a 15 µm Pt electrode.

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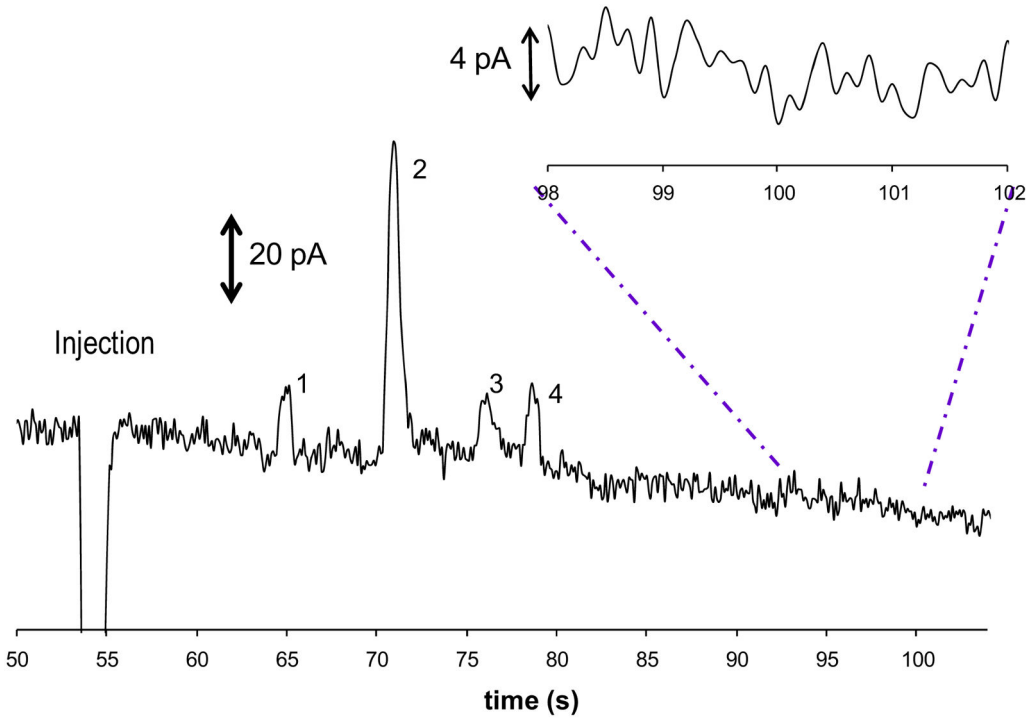

Figure 6.

Electropherogram of an equimolar (3.1 μM) mixture of (1) nitrite, (2) Tyr, (3) H_2O_2, and (4) unknown. Inset figure shows an enlarged portion of the baseline. Conditions are the same as given in Fig. 4.

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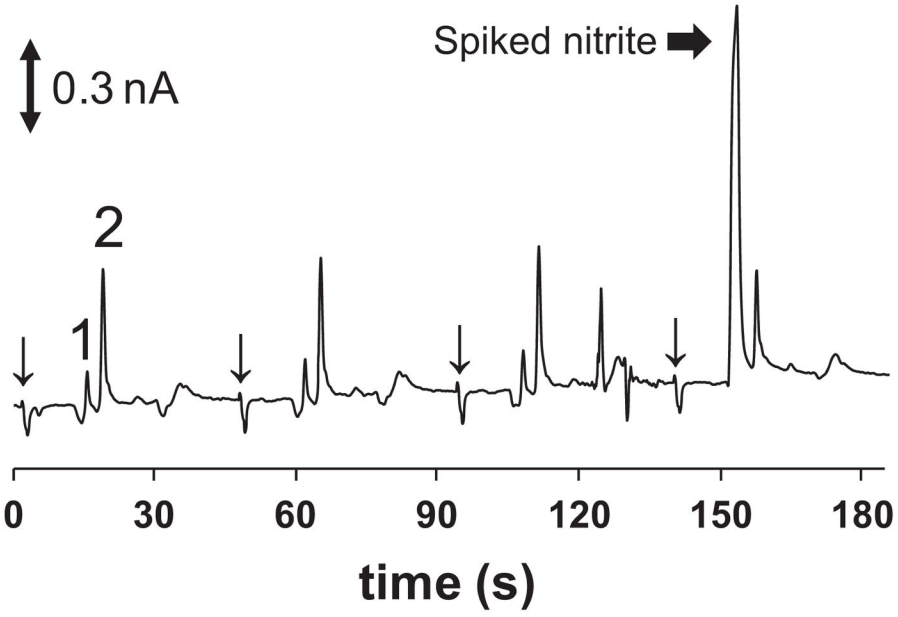

Figure 7.

Electropherograms for analysis of nitrite (peak 1) in sheep perfusate samples. Peak 2 is an unidentified analyte. Conditions as in Fig. 4. Two microliters of 10 mM nitrite standard solution was spiked during the analysis to match the migration time. Arrows indicate injections.

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Table 1

Comparison of approximate baseline noise and background currents when different reference electrodes were employed in dual- and single-channel microchips

Parameter	Dual-channel			Single-channel
	15 μm Pt reference	50 μm Pt reference	Ag/AgCl reference	Ag/AgCl reference
Noise (pA)	4	17	27	25
Background current (nA)	2.8	4.7	4.2	~4

A Pinnacle isolated potentiostat in the two-electrode configuration was used for these studies.

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Table 2

Comparison of LOD and baseline noise in single- and dual-channel ME with in-channel amperometry

Channel configuration	LOD (μM)			Noise (pA)
	Nitrite	H_2O_2	Tyrosine	
Dual-channel – 15 μm Pt WE and RE (before digital filter)	1.87	2.5	0.53	4
Dual-channel – 15 μm Pt WE and RE (after digital filter)[a)]	0.58	0.75	0.14	0.98
Single-channel – 15 μm Pt WE and Ag/AgCl RE [1]	2.06	13.88	–	25–27
Single-channel – 15 μm Pt WE and Ag/AgCl RE (after digital filter)[a)]	1.44	7.37	–	9.5–11.5

WE, working electrode; RE, reference electrode.

[a)]Moving average, 5 points window.