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Use of a Carbon-ink Microelectrode Array for Signal Enhancement in Microchip Electrophoresis with Electrochemical Detection

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Abstract

In this communication, we demonstrate that a carbon ink microelectrode array, where the electrodes are held at the same potential, affords significant signal enhancement in microchip electrophoresis with amperometric detection. The ability to fabricate an array of carbon ink microelectrodes with a palladium decoupler was demonstrated and the resulting electrodes were integrated with a valving microchip design. The use of an 8 electrode array led to a significant improvement in the limits of detection at the expense of separation resolution due to the increased detection zone size. It is also shown that microdialysis sampling can be integrated with the microchip device and a multi-analyte separation achieved.

Keywords

microchip electrophoresis; carbon-ink microelectrode; electrode array; palladium decoupler

Amperometric detection has become a popular detection mode for microchip electrophoresis (ME), primarily due to the facts that microelectrodes can be patterned with the same techniques used to fabricate the fluidic network and, as opposed to fluorescence detection, electrochemistry can be used to detect many analytes without the need for derivatization. Most of the papers in this area have used a single working electrode to evaluate issues such as electrode materials, electrode alignment, detection modes, and methods to isolate the detection electrodes from the electrophoresis separation voltage [1–9]. There have been reports of using multiple electrodes with ME and early work focused on operating dual electrodes at different potentials to monitor reversible redox couples [10–13]. While there have been reports of integrating electrode arrays with ME [14, 15], Henry's lab was the first to describe an array of electrodes that were individually addressed and operated simultaneously [16]. Improved detector selectivity and resolution were achieved by applying different potentials at each gold microelectrode.

Amatore's group recently described the use of serially configured thin-layer dual electrodes in a microchannel under laminar flow conditions [17, 18]. It was shown that operating the dual electrodes at the same potential (generator-generator mode) with optimized electrode widths, electrode spacings, and flow regimes can lead to an increased signal-to-noise ratio, as compared to a single electrode of the same overall surface area. When the dual electrodes are operated at the same oxidative potential, the increased current densities result from the ability of fresh, un-oxidized analyte to diffuse to the surface of the second electrode, as opposed to a larger single electrode where analyte may become depleted at the electrode surface. While it was shown that the optimally configured dual electrodes can lead to

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increased analytical performance compared to a single electrode, their study was limited to two platinum or gold electrodes and no separation was reported, as the analyte was continuously infused through a PDMS microchannel. The purpose of this paper is to investigate whether multiple working electrodes (up to 8) operating at the same potential can significantly improve the limits of detection for ME and amperometric detection.

The first aim of this work was to investigate whether this principle of signal enhancement holds true in a ME device with two working electrodes. As opposed to the work from Amatore where a continuous flow of analyte was used [17], in this study a mixture of analytes is injected into a separation channel, separated by electrophoresis, and detected at electrodes that are also integrated with a palladium decoupler so that the electrodes are in a field free region but remain within the fluidic network. The initial studies used palladium working electrodes that were fabricated on the same plate as the palladium decoupler. We chose to use typical electrophoresis channel dimensions as well as standard electrophoresis buffers and separation voltages; therefore, the main variable to investigate in these studies was the electrode array configuration. We have shown that a carbon ink microelectrode can be integrated with a palladium decoupler and is preferable for measuring catecholamines [13]; however, the size of these carbon microelectrodes ($\sim 10\text{ }\mu\text{m}$ in width and $\sim 1\text{ }\mu\text{m}$ in height) cannot be readily varied, as it is not possible to fabricate smaller electrodes with the micromolding procedure and PDMS will not fully seal over larger electrodes. Therefore, palladium electrodes were initially used since it is possible to easily vary their size/spacings and PDMS microchannels can reversibly seal over the thin-layer electrodes ($\sim 4000\text{ }\text{\AA}$) no matter the electrode dimensions. Using a valving microchip design (Figure 1A), a comparison was made between the use of a single $100\text{ }\mu\text{m}$ palladium electrode (in length) and two $50\text{ }\mu\text{m}$ serially configured palladium working electrodes that were separated by $100\text{ }\mu\text{m}$ (Figure 1B). In all studies, the width of the electrode is defined by the channel dimensions ($40\text{ }\mu\text{m}$). A $50\text{ }\mu\text{M}$ norepinephrine sample was injected (895 pL plug) into the electrophoresis channel and $+900\text{ mV}$ was applied to the working electrode(s) (vs. a Pt. quasi-reference). The average peak height ($n = 4$) for the $100\text{ }\mu\text{m}$ electrode was $61.9 \pm 4.7\text{ pA}$ while the average peak height for the two $50\text{ }\mu\text{m}$ electrodes with the $100\text{ }\mu\text{m}$ spacing was $169.1 \pm 12.1\text{ pA}$. Clearly, the multiple electrodes of equal surface area led to a signal enhancement, with the signal from the two $50\text{ }\mu\text{m}$ electrodes being ~ 2.7 times greater than the single electrode. A decrease in this signal enhancement was seen when the electrode spacing was increased to distances greater than $100\text{ }\mu\text{m}$, as the detection zone size (spacing from leading edge of the first electrode to trailing edge of the second electrode) increases when the electrode spacing is increased. Thus, in a microchip-based separation device, there is a balance between the signal enhancement due to the increased time for fresh analyte to diffuse to the second electrode and band broadening from the increased detection zone. This is especially evident when using a palladium decoupler, as the parabolic flow that results after the decoupler can lead to band broadening [3, 13]. From these data and experimental conditions, it was determined that $100\text{ }\mu\text{m}$ was the optimal spacing for further studies. Smaller spacings were not investigated since a previous study with carbon ink microelectrodes, which are typically $\sim 10\text{ }\mu\text{m}$ in width and $\sim 1\text{ }\mu\text{m}$ in height, showed that a spacing of less than $100\text{ }\mu\text{m}$ leads to an incomplete seal of PDMS over the electrodes [13].

Since carbon electrodes have minimal issues with fouling [5, 11] and our group has shown that carbon ink electrodes can be integrated with a palladium decoupler for monitoring ME experiments [2, 13, 19], the effect of using a carbon ink microelectrode array was next explored. Initially, a negative chrome mask containing 1, 4 or 8 lines separated by $100\text{ }\mu\text{m}$ was used to fabricate raised structures from which PDMS-based micromolding channels were made. As previously reported [13], a SU8-2 resist was initially used and the resulting $12\text{ }\mu\text{m} \times 1.2\text{ }\mu\text{m}$ microelectrodes were rectangular in shape (Figure 2A). When buffer was introduced into a PDMS-based valving microchip (Figure 1A) that was reversibly sealed

over an array of these rectangular microelectrodes, fluid leakage was evident around the electrode. This was due to an incomplete seal between the PDMS and the glass plate around the carbon electrode. While this may be partially due to the fact that the 3.5 mm thick valving chips do not conformally seal as well as thinner PDMS chips that can be used with other injection methods, it was found that rounding the carbon ink microelectrodes eliminated this leakage. These microelectrodes were made by creating rounded micromolding channels from the same negative mask, positive photoresist, and a double PDMS casting procedure [20]. Structures made with positive photoresist (AZ 4620) can be rounded by a heating step [21]. By first casting and curing PDMS onto the master and then casting a second PDMS layer onto the cured PDMS layer, rounded micromolding channels that replicate the negative mask are created. This rounding was confirmed by confocal microscopy of the resulting carbon microelectrode (Figure 2A). This technique led to the reproducible fabrication of carbon microelectrode arrays consisting of 1, 4 or 8 electrodes that could be reversibly sealed with a PDMS microchannel (Figure 2B). To our knowledge, this is the first description of a carbon ink electrode array that is integrated within a microchannel network.

To determine the amount of signal enhancement that is possible with an array of carbon ink microelectrodes, a 50 μM norepinephrine solution was injected (950 pL plug) into the separation channel, and, following an electrophoresis separation, detection was achieved at either 1, 4 or 8 electrodes (as depicted in Figure 2B), all operating at +900 V. The improvement in the signal-to-noise ratio is shown in Figure 3A. As is clearly seen, the ability to use electrode arrays for amperometric detection with ME leads to a significant signal enhancement. A limit of detection (LOD) comparison between the use of a single electrode versus an 8 electrode array was performed and it was found that the LOD for norepinephrine at a single electrode was 2.8 μM and use of 8 electrodes improved the LOD for norepinephrine to 170 nM. As is noted above, one point of concern in a separation system is the effect of the increased detection zone on band broadening. To further investigate the signal enhancement and to also determine if any decrease in separation resolution results from using an electrode array, a separation between 50 μM dopamine and 50 μM norepinephrine (which differ in structure by a hydroxyl group) was performed at both a single electrode and an 8 electrode array. The resulting signal enhancement and decreased resolution is evident in Figure 3B. When using a decoupler to integrate a separation with electrochemical detection, the area after the decoupler is a field-free region where parabolic flow dominates [3]. Furthermore, by increasing the detection window to 796 μm by using an 8 electrode array (compared to 12 μm for a single electrode) even more band-broadening can occur, as the detector observes the analyte band over a much increased area. To quantitate the amount of band broadening seen in this separation, the separation resolution between 2 peaks (R_s) was calculated by the formula

$$R_s = \frac{2(t_{r2} - t_{r1})}{w_1 + w_2} \quad (1)$$

where t_r is the retention time and w is the width at base, with baseline resolution being defined as a R_s value of 1.5 [22]. The R_s between dopamine and norepinephrine using a single electrode detection was calculated to be 2.0 while the R_s value between the 2 analytes when using an 8 electrode array was calculated to be 1.4, which is nearly baseline resolved and sufficient for quantitation purposes (Figure 3B). For these studies the use of more than 8 electrodes was not investigated. This data shows that the number of electrodes that can be used in this type of separation system is a balance between the desired LOD values and separation resolution.

To further demonstrate the advantages of using the electrode array for signal enhancement in ME with amperometric detection, microdialysis sampling (MD) was integrated with the microchip device. We have previously shown that the microchip design shown in Figure 1A can be coupled with MD sampling by simply placing the tubing coming from either a brain or linear MD probe into the syringe pump inlet [19]. The probe is perfused with a dialysate and, once this continuous stream of sample has entered the microchip, the PDMS valves are used to discretely inject a plug of dialysate into the separation channel for analysis with ME and amperometric detection. In this work, a linear probe was used to sample from solution containing dopamine, norepinephrine, and catechol (100 μ M each). The data from 3 consecutive injections (760 pL injection plug) of the dialysate is shown in Figure 4. As can be seen in the figure, norepinephrine and catechol are baseline resolved ($R_s = 2.5$). The average peak height for catechol was found to be 184.9 ± 14.2 pA, with the number of plates being 1490 (35,060 plates/m). The limit of detection ($S/N = 3$) for the MD/ME system was determined by sampling from a 500 nM catechol solution and found to be 260 nM. It is important to note that this includes recovery across the MD probe (which was found to be 77% with similar experimental conditions [19]) and Taylor dispersion as the analyte crosses the probe and is transferred to the chip [23]. This LOD is significantly improved over previous work from our lab using single carbon ink microelectrodes with MD sampling and ME, where the LOD for catecholamines was in the low micromolar range [19].

In summary, this work has shown that the principle of signal enhancement when using optimally spaced multiple electrodes in a generator-generator mode holds true in a ME device where the electrodes are integrated with a palladium decoupler. This was demonstrated using typical electrophoresis voltages and channel dimensions with both dual palladium working electrodes and an array of carbon ink microelectrodes. The use of an 8 electrode array led to a significant improvement in the LOD for norepinephrine (2.8 μ M for a single electrode versus 170 nM for 8 electrodes) at the expense of separation resolution due to the increased detection zone size. The applicability of using the electrode array for signal enhancement in ME with amperometric detection was demonstrated by integrating microdialysis sampling with the device. While this communication has demonstrated the advantages of using multiple electrodes held at the same potential for signal enhancement with ME and amperometric detection, there are areas for future study. This includes investigating the use of more electrodes in the array, the fabrication of smaller carbon microelectrodes so that a higher density of electrodes can be utilized, studying the effect of linear velocity on the signal enhancement, and improving the peak capacity of the separation component so that the increased detector zone size does not adversely affect the separation resolution.

Experimental

Fabrication and operation of PDMS-based bilayer valving microchips followed previously published methods [19, 21]. The separation channel was 40 μ m (in width) $\times$ 20 μ m (in depth) and the effective separation length was either 3.0 cm (for characterization studies) or 4.25 cm (for microdialysis separation). The electrophoresis buffers contained sodium dodecyl sulfate (SDS), as Culbertson's group has shown that the addition of SDS can eliminate analyte absorption as well as increase and stabilize the electroosmotic flow in PDMS devices [24]. The valving channels were filled with water and MAC valves (MAC Fluid Power Engineering, St. Louis, MO) were used to trigger injection valves #1 and #2 by means of a timer-based control unit [19, 21]. As shown in Figure 1A, sample (from a syringe or as sampled by a microdialysis probe) is continuously introduced into the microchip *via* a syringe pump (0.3 μ L/min). Injection into the separation portion of the device is accomplished by opening valve #2 (normally closed) and closing valve #1 (normally open) for a pre-determined time (typically less than 1 sec.) [19, 21]. Injection plug sizes were

determined by using fluorescein, a fluorescence microscope (IX71, Olympus America), and a Qicam Fast digital CCD camera (QImaging). Borosilicate glass rounds were sputtered with 200 Å titanium and 4000 Å palladium by Stanford's Nanofabrication Facility. The decoupler design and palladium connector were patterned onto the glass round using positive resist and wet etching [13]. The decoupler length was 2 mm, with the distance from the decoupler to the leading edge of the first working electrode being 250 µm, as was previously determined [3].

Two methods were used to fabricate PDMS carbon ink micromolding channels for patterning carbon ink electrodes. The first used a negative chrome mask (Advance Reproductions Corp.) and SU8-2 negative photoresist (MicroChem Corp.). A PDMS rectangular micromolding channel was made from the resulting master using a Sylgard 184 kit (Ellsworth Adhesives). A second method that results in rounded carbon ink microelectrodes used the same negative mask and a positive photoresist (AZ 4620 positive resist, AZ Resist) that was spun at 6000 rpm for 30 sec. onto a 4-in silicon wafer. Following a post-exposure bake temperature ramp that concluded at 120 °C for 5 mins to round the structure [21, 25], a double casting procedure of PDMS was used to replicate the negative mask [20]. This was accomplished by first casting PDMS (using a Sylgard 184 kit) onto the master and, once cured, this PDMS layer was removed and another aliquot of PDMS was cast against it. The second layer of PDMS was removed from the first PDMS layer and used to mold the carbon ink electrodes. Carbon ink microelectrodes were made by sealing the PDMS micromolding channels over the palladium connector, filling the channels with a carbon ink mixture, and, following several heating steps, removing the PDMS mold to leave the hardened carbon on the glass round [19, 26]. The rectangular and rounded carbon ink microelectrodes were imaged with a Keyence VK-9710 Violet Laser Scanning Confocal Microscope (Keyence Corp.) The accompanying VK Viewer software allowed collection of color 3-D image data over the entire electrode as well as a line scan to obtain a profile of each electrode.

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Fig. 1.

A) Schematic of the microchip device that was used in these studies, with the number of detection electrodes varying throughout (not to scale). Injections into the electrophoresis channel are made by opening valve #2 (normally closed) and closing valve #1 (normally open). Abbreviations B: buffer reservoir, BW: buffer waste reservoir, SW: sample waste reservoir, PB: pushback reservoir. B) Micrographs of 100 μm single palladium electrode relative to the palladium decoupler. C) Two 50 μm palladium electrodes that are separated by 100 μm . For B and C, the electrode width is fixed by the channel width (40 μm).

Fig. 2.

A) Height profiles extracted from confocal images of carbon microelectrodes made from either rectangular or rounded micromolding channels. For clarity, only one edge of each electrode is shown.

B) Micrographs of 1, 4 and 8 carbon ink electrodes sealed over a palladium connector electrode and inside the electrophoresis channel.

Fig. 3.

A) Signal to noise comparison for the injection and separation of norepinephrine, with amperometric detection at 1, 4 or 8 electrode(s) ($n=4$ for each point). B) Electropherogram of separation between dopamine (DA) and norepinephrine (NE) with a single electrode (bottom trace) and eight electrodes (top trace). Other variables: detection potential = +0.9 V, separation voltages: B = 700 V and PB = 200 V, buffer = 10 mM Boric acid with 25 mM SDS (pH 9.2).

Fig. 4.

Electropherogram demonstrating microdialysis sampling coupled with microchip-based electrophoresis and amperometric detection. A 5 mm linear probe was used to sample from a solution of dopamine (DA), norepinephrine (NE), and catechol (CAT), each at 100 μ M. Other variables: perfusate flow rate = 0.3 μ L/min, detection potential = +0.9 V, separation voltages: B = 1200 V and PB = 450 V, buffer = 10 mM Boric acid with 25 mM SDS (pH 9.2).