Published in final edited form as:
Anal Chem. 2003 June 1; 75(11): 2710–2714. doi:10.1021/ac026354l.

On-Column Electrochemical Detection for Microchip Capillary Electrophoresis

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Abstract

The development of a cellulose acetate decoupler for on-column electrochemical detection in microchip capillary electrophoresis is presented. The capillary based laser-etched decoupler is translated to the planar format to isolate the detector circuit from the separation circuit. The decoupler is constructed by aligning a series of 20 30-μm holes through the coverplate of the microchip with the separation channel and casting a thin film of cellulose acetate within the holes. The decoupler shows excellent isolation of the detection circuit for separation currents up to 60 μA, with noise levels at or below 1 pA at a carbon fiber electrode. Detection limits of 25 nM were achieved for dopamine. This decoupler design combines excellent mechanical stability, effective shunting of high separation currents, and ease of manufacture.

Capillary electrophoresis (CE) is a powerful separation technique with excellent mass detection limits due to the small volume of sample typically injected. More recently, the application of microfabrication technology from the electronics industry has allowed chemists to further reduce the size of analytical instrumentation. The smaller integrated microchip systems provide many analytical benefits.[1] Among these benefits are higher field strength separations providing faster analysis with theoretically higher efficiencies (plates/cm), integration of the analytical scheme from sample preparation through analysis, and portability for on-site analysis. Unfortunately, the extreme miniaturization of the microchip format has made detection of analytes at low concentrations extremely difficult. This has resulted in the implementation of two primary detection schemes: laser-induced fluorescence (LIF) and electrochemical detection (EC). LIF is a highly effective detection scheme, resulting in the lowest concentration detection limits;[2] however, the implementation of LIF can be quite difficult. The number of laser wavelengths available is limited, often requiring derivitization of the sample. Additionally, the equipment is expensive, and alignment of the laser is complicated. Furthermore, the instrumental setup for typical LIF is often cumbersome, making the size of the detector module unrealistic when compared to the analytical microchip. On the other hand, EC is relatively simple, inexpensive, applicable to a wide range of analytes, and easily miniaturized.

There are two primary strategies for coupling EC to CE: end-column detection and on-column detection. In end-column detection, the electrode is positioned outside the capillary. When end-column detection is used, the effect of the separation field on the working electrode is minimized by use of extremely low separation currents. While the detector noise due to the separation current decreases as the working electrode is positioned further from the capillary outlet, detection sensitivity also decreases as a result of loss of analyte by diffusion in the detection cell. To prevent the loss of analyte through diffusion, on-column detection can be

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employed. In this arrangement, the separation potential must be grounded prior to the capillary outlet. Several methods for accomplishing this have been described for conventional CE.[3–7]

End-column electrochemical detection has been the predominate choice for the application of microchip CE–EC. Mathies and co-workers reported the first integrated microchip CE–EC system.[8] Wang et al. have published a number of papers describing the use of different electrode materials and alignment techniques.[9–11] Additionally, Lunte and co-workers have published dual electrode detection in the microchip format and the use of carbon fiber and carbon paste electrodes.[12–14] Gavin and Ewing have reported the use of a channel electrophoresis system for continuous sample injection and spatially resolved detection at an end-column array of 100 individually addressable electrodes.[15] Further reports of end-column electrochemical detection have been reviewed.[16,17] Although many papers have employed end-column CE–EC in the planar format, only two decouplers have been reported for on-column detection. First is the palladium field decoupler.[18] This decoupler uses a thin sheet of palladium in contact with the separation channel as the CE cathode. The separation is grounded at the electrode, and H_2 produced at the cathode is absorbed into the palladium. Noise levels at or below 15 pA were maintained with separation currents up to 11 μA, and a limit of detection (LOD) of 290 nM was achieved for dopamine. Second is the use of a microhole array, which connects the cathodic reservoir to the separation channel through the plastic coverplate of a microchip device.[19] In this report, the LOD of aminophenol was report to be 5 μM. A third approach for employing CE–EC in a microchip system, dubbed in-channel detection, uses a novel approach.[20] For this arrangement, an electrically isolated potentiostat is used with the working electrode placed just within the separation channel from the cathodic reservoir. Although an improvement in peak shape, as compared to end-column detection, was achieved, the detection limit for catechol was 4 μM. These results agree well with the reported microchip CE–EC LOD values, which are typically on the order of 10^{-6} or 10^{-7} M. Our goal was to translate what we have learned from the capillary-based laser-etched decoupler to the planar format.[3] Ideally, a decoupler should be capable of shunting high electrophoretic currents so that the separation is not limited to low ionic strength background electrolytes (BGE) or sample matrixes. This requires a decoupler with a high surface area, which is practical only if a highly mechanically stable decoupler can be manufactured. Furthermore, the polymer used as the decoupler should not act as an ion-exchange membrane, as is the case with Nafion. We have developed a microchip-based laser-etched decoupler that combines these characteristics with ease of manufacture. Separation currents as high as 60 μA have shown no increase in detector noise, and an LOD of 25 nM has been achieved for dopamine.

EXPERIMENTAL SECTION

Materials

Dopamine, hydroquinone, isoproterenol, graphite powder, and mineral oil were obtained from Sigma (St. Louis, MO). Cellulose acetate was obtained from Aldrich (Milwaukee, WI). All other chemicals were reagent grade or better and were used as received. All stock solutions were prepared at 10 mM in 0.1 M perchloric acid and diluted to the desired concentration in background electrolyte just prior to analysis. Cellulose acetate solutions were prepared as 6% (w/w) in acetone. BGE was prepared from the free acid by adjusting the pH with solid lithium hydroxide.

CE–EC Apparatus

Electrophoresis was driven by a high-voltage dc (0–30 kV) power supply (Spellman High Voltage, Hauppauge, NY). All potentials and injections were controlled manually. Experiments were performed at ambient temperature (~21 °C). A platinum auxiliary electrode and Ag/AgCl reference electrode were used. All potentials are reported versus the Ag/ AgCl

reference. The detector potentiostat was an LC-4CE from Bioanalytical Systems, Inc. (West Lafayette, IN). Data acquisition was through a PCI-MIO-16XE-50 A/D computer card, and programming was performed in-house using LabView software (National Instruments, Austin, TX). Carbon paste electrodes were made by first mixing graphite powder and mineral oil (70% w/w carbon) with a mortar and pestle. The carbon paste was then packed 5 mm into the end of a 2-cm-long capillary having a 150-μm i.d. and 365-μm o.d.. The end of a 70-μm tungsten wire was then dipped in silver paste (Ted Pella, Inc., Redding, CA) and threaded through the capillary from the other side. The wire was imbedded in the carbon paste to ensure good electrical contact and glued into place with super glue (3M, St. Paul, MN). Carbon fiber electrodes were made as previously described.[4] Briefly, a 33-μm carbon fiber (Avco Specialty Materials, Lowell, MA) was threaded through a 3 cm × 75 μm i.d. capillary and secured in place with silicone adhesive. The carbon fiber was trimmed so that 1 mm protruded from each end of the capillary. The capillary was placed 1 cm into a stainless steel needle and electrical contact between the carbon fiber and the needle was made with silver paste. All injections were performed by applying a negative voltage to the sample waste reservoir (Figure 1) while the buffer reservoir was left floating and the sample and decoupler reservoirs were grounded. After the double-T injector region was filled with sample, a separation potential was applied to the buffer reservoir, and pushback voltages were applied to the sample and sample waste reservoirs while the decoupler reservoir remained at ground.

Preparation of Microchips

Poly(dimethylsiloxane) (PDMS) was ordered from Fisher Scientific (Dow Corning, Midland, MI, SYLGARD elastomer kit 184) and mixed, 10 parts PDMS oligomer to 1 part cross-linking agent (w/w), as per the manufacturer's directions. Microchips were constructed as previously described.[21] Briefly, masters were constructed on silicon wafers with SU-8 10 photoresist (MicroChem Corp., Newton, MA). Negative masks were prepared in Freehand 8.0 (Macromedia Inc., San Francisco, CA) and produced with a resolution of 2400 dpi (Laser Graphics, Lawrence, KS). PDMS channel structures were made by casting the PDMS over the master and allowing it to cure at 75 °C for 1 h. The channels were 40 μm wide and 40 μm deep; all other dimensions are listed in the figures. All coverplates and channel structures were irreversibly sealed by placing the PDMS in an air plasma cleaner/sterilizer (Harrick, PDC-32G) for 45 s on medium setting. Glass coverslips were etched using a 35-W CO_2 laser (M25 class, Universal Laser, Denver, CO) set at 8% power and 3.1% speed, repeating the etching pattern 20 times to obtain cuts completely through the glass. Buffer and sample reservoirs were made by cutting a 1.7-mL screw top sample vial (Fisher Scientific, Pittsburgh, PA) in half and attaching the top to either glass or PDMS coverplates with silicone epoxy (GE Sealants & Adhesives, Huntersville, NC).

RESULTS AND DISCUSSION

Initial Decoupler Design

Initially, the simplest decoupler design was sought, and therefore, coverplates were constructed of PDMS. PDMS slabs, 5 mm thick, were cut to ~50 mm × 65 mm. Holes were cut through the PDMS coverplate to align with the four buffer reservoirs; another hole 1 mm in diameter, to serve as the decoupler; and additional holes 360 μm in diameter, for alignment of electrodes with the separation channel (Figure 1). The decoupler was created by casting a thin film of cellulose acetate (~100 μm) onto a glass plate. Small squares of cellulose acetate, measuring 2 mm on each side, were then cut out of the film and sandwiched between the channel structure and the coverplate so that the decoupler hole in the coverplate was centered on the cellulose acetate. This sandwich design was chosen since cellulose acetate cast within the decoupler hole would not adhere to the PDMS.

Evaluation of the Initial Decoupler Design

Although most of the separation current is shunted through the decoupler, a small portion always affects the detection circuit. Noise at the working electrode remains steady until the separation current has reached the limiting separation current (LSC).[3] At this point, the noise due to the separation current is larger than the background environmental noise of the electrochemical cell, and the detector noise is observed to increase dramatically with increasing separation current. The fraction of electrophoretic current grounding at the detector is controlled by the relative resistance through the decoupler to ground, as compared to the resistance through the remaining separation channel to the detector. Thus, it is observed that with higher surface area decouplers, less of the electrophoretic current affects the detector, and higher separation current can be employed before the LSC is reached. Evaluation of the decoupler requires determination of the detector noise as a function of current with the working electrode placed at different distances from the cathodic reservoir.

Figure 2 shows the detector noise as a function of separation current for electrodes placed at 0.5-cm increments from the decoupler. Although the noise values fluctuate, they remain close to 1 pA, regardless of how close the electrode is positioned to the decoupler. The noise values at the furthest point are actually higher than the noise levels at the closest position. This is most likely due to hydrodynamic effects, because it is possible for turbulent flow to arise from the channel flow passing the three previous electrode positioning holes. It is evident from these data that the decoupler effectively shunts the separation current up to at least 30 μA. Figure 3 shows an electropherogram obtained using a microchip with the sandwich decoupler. The peak shape was excellent, despite some tailing (most likely due to adsorption of analytes onto the PDMS), with the efficiency for the hydroquinone peak being 175 000. Furthermore, peak heights and noise levels indicated that detection limits between 10 nM and 100 nM could be expected. Unfortunately, the injection-to-injection reproducibility was extremely poor. The relative standard deviation for replicate injections of hydroquinone was >50%, and injections of catecholamines were significantly worse. Further investigation into this phenomenon indicated that leakage around the edge of the cellulose acetate was allowing the analytes to migrate into the cathodic reservoir. The cellulose acetate appeared to adhere to the PDMS during manufacture; however, once the decoupler was allowed to soak in solution, it no longer sealed against the PDMS of the cathodic reservoir. To prevent this leakage, polyurethane (The Polymer Technology Group Inc., Berkeley, CA), Nafion (Aldrich), and PDMS were each used individually or in combination to replace the cellulose acetate membrane. Neither polyurethane nor Nafion showed adherence to the PDMS once the decoupler was allowed to soak in buffer. Thin films of PDMS did adhere, but they were not useful as a decoupler because the resistance through the PDMS was too high.

Revised Decoupler Design

To provide a support that the cellulose acetate would adhere to, glass coverplates were selected to replace PDMS coverplates. Microscope coverslips measuring 48 mm × 65 mm and 250 μm thick were etched using a CO_2 laser. The laser was used to cut holes through the coverplate for the four buffer reservoirs and the decoupler (Figure 4). A series of 20 short laser cuts, spaced ~75 μm apart, were used to etch holes through the glass. The holes measured 75 μm wide and 100 μm long at the surface, with 30-μm circular openings at the bottom. The holes were then filled with polymer by moving a small drop of cellulose acetate over the surface of the cuts. The cellulose acetate was allowed to dry at room temperature for 1 h prior to being heated at 75 °C for 2 h to remove residual acetone. The cellulose acetate was found to adhere more reliably to the glass if it was allowed to dry slowly. Finally, the decoupler holes were aligned with the separation channel 1 cm prior to the channel outlet, and the channel structure and the coverplate were irreversible annealed. The cathodic reservoir was filled with 0.1 N NaOH, and the decoupler was allowed to soak for 1 h prior to use. Carbon fiber electrodes were inserted

300 μm into the outlet of the separation channel. Carbon fiber electrodes were used in this design instead of carbon paste electrodes, since the thin glass coverplates did not provide enough mechanical stability for a carbon paste electrode as in the initial design.

Noise values as a function of separation current for the revised decoupler design were also evaluated, and isolation of the detector cell from separation current up to 60 μA was achieved (Figure 5). This corresponds to a current density of 0.4 A/cm^2 for the microchip decoupler. This value compares well with the capillary-based laser-etched decoupler, when the relative resistances from the decoupler to the detector for both formats are taken into account. Two major benefits arise from the ability of the decoupler to maintain noise levels of 1 pA at the detector in the presence of such high separation currents. The first is the compatability of the separation device with high ionic strength BGE. This allows the development of methodology using a wide range of buffer types. The second advantage is the ability to operate at extremely high field strengths, thereby increasing resolution in the ultrafast separations now capable on microchip CE devices. In our studies, field strengths as high as 1700 V/cm were applied without affecting the detector noise, resulting in detection limits of 25 nM for dopamine (n = 3, RSD = 6.5 %, Figure 6). This is, to our knowledge, the lowest reported LOD for electrochemical detection with microchip CE and shows an order of magnitude improvement over the lowest previously reported values.[22] Zeng et al. attribute the low detection limits of their work to efficient alignment of the end-column working electrode. It is likely that our improvement in detection limits is the result of employing on-column detection, allowing more of the sample plug to be oxidized at the working electrode.

Effects of a Mixed Substrate Microchip

To facilitate the use of a cellulose acetate decoupler, it was necessary to employ a glass coverplate in the microchip system. As can be seen from comparison of Figures 3 and 6, a dramatic change in the separation time and peak shape has occurred. This is attributed in part to the significantly higher EOF on glass relative to PDMS. Although the al-PDMS system had a longer separation channel and lower field strength, experiments performed under the same conditions (data not shown) still showed a significantly higher EOF in the mixed substrate system. It has been observed in our laboratory that the EOF in PDMS drops off dramatically during the first week the chip is used. This could explain the dramatic differences between the EOF observed with PDMS versus glass. Because of the increased EOF in the glass/PDMS system, it was necessary to change the BGE from pH 7.0 to pH 2.75 to slow the transport of neutrals. For complex samples requiring more than a couple of peaks to be resolved in a few seconds, further suppression of the EOF may be necessary. Another drawback of the mixed substrate system is the peak broadening due to different EOFs occurring at different walls of the separation channel. Even though the peaks are only a few seconds wide at baseline, this dramatically reduces the number of analytes that can be resolved in the separation. Further work investigating the on-chip stacking of analytes would be valuable in lowering the detection limits and compressing sample bands, allowing higher efficiency separations. The unexpectedly low response for hydroquinone indicates a 5-fold higher LOD at 121 nM than that for dopamine. The peak for hydroquinone shows more band broadening than the dopamine peak, and when peak areas are taken into account, the hydroquinone shows a 2–3-fold lower response than expected.

CONCLUSION

The applicability of on-column electrochemical detection for microchip CE has been demonstrated. The planar format of the microchip device allows highly mechanically stable decouplers to be easily fabricated. With on-column detection for microchip CE–EC, further benefits of the microanalytical system can be realized. First, separations that take advantage

of extremely high field strengths can be developed. We have used field strengths as high as 1700 V/cm, which is 3–8 times higher than commonly employed. Even with such extreme field strength, the separation current is stable, and noise at the detector is maintained at 1 pA. Second, low limits of detection can be obtained in the presence of extreme separation conditions, rivaling the results achieved by LIF detection. Further work will be directed at sample handling of extremely low volume samples and on-chip sample band compression to optimize the efficiency of the separation.

Acknowledgements

This work was funded by a grant from the National Institutes of Health. We thank Stacy Arnett for many useful discussions and for providing all of the carbon fiber electrodes used in this work.

References

1. Bruin GJM. Electrophoresis 2000;21:3931–3951. [PubMed: 11192117]
2. Jiang G, Attiya S, Ocvirk G, Lee WE, Harrison D. J Biosens Bioelectron 2000;14:861–869.
3. Osbourn DM, Lunte CE. Anal Chem 2001;73:5961–5964. [PubMed: 11791566]
4. Park S, Lunte SM, Lunte CE. Anal Chem 1995;67:911–918.
5. Kok WT, Sahin Y. Anal Chem 1993;65:2497–2501.
6. O'Shea TJ, Greenhagen RD, Lunte SM, Lunte CE, Smyth MR, Radzik DM, Watanabe N. J Chromatogr 1992;593:305–312.
7. Wallingford RA, Ewing AG. Anal Chem 1987;59:1762–1766. [PubMed: 3631501]
8. Woolley AT, Lao K, Glazer AN, Mathies RA. Anal Chem 1998;70:684–688. [PubMed: 9491753]
9. Wang J, Tian B, Sahlin E. Anal Chem 1999;71:3901–3904.
10. Wang J, Tian B, Sahlin E. Anal Chem 1999;71:5436–5440.
11. Wang J, Chen G, Cheatrathi MP, Fujishima A, Tryk DA, Shin D. Anal Chem 2003;75:935–939. [PubMed: 12622387]
12. Martin RS, Gawron AJ, Lunte SM, Henry CS. Anal Chem 2000;72:3196–3202. [PubMed: 10939387]
13. Gawron AJ, Martin RS, Lunte SM. Electrophoresis 2001;22:242–248. [PubMed: 11288891]
14. Martin RS, Gawron AJ, Fogarty BA, Regan FB, Dempsey E, Lunte SM. Analyst 2001;126:277–280. [PubMed: 11284324]
15. Gavin PF, Ewing AG. J Am Chem Soc 1996;118:8932–8936.
16. Lacher NA, Garrison KE, Martin RS, Lunte SM. Electrophoresis 2001;22:2526–2536. [PubMed: 11519957]
17. Wang J. Talanta 2002;56:223–231.
18. Chen DC, Hsu FL, Zhan DZ, Chen CH. Anal Chem 2001;73:758–762. [PubMed: 11248889]
19. Rossier JS, Ferrigno R, Girault HH. J Electroanal Chem 2000;492:15–22.
20. Martin RS, Ratzlaff KL, Huynh BH, Lunte SM. Anal Chem 2002;74:1136–1143. [PubMed: 11924975]
21. Gawron AJ, Martin RS, Lunte SM. Electrophoresis 2001;22:242–248. [PubMed: 11288891]
22. Zeng Y, Chen H, Pang DW, Wang ZL, Cheng JK. Anal Chem 2002;74:2441–2445. [PubMed: 12038773]

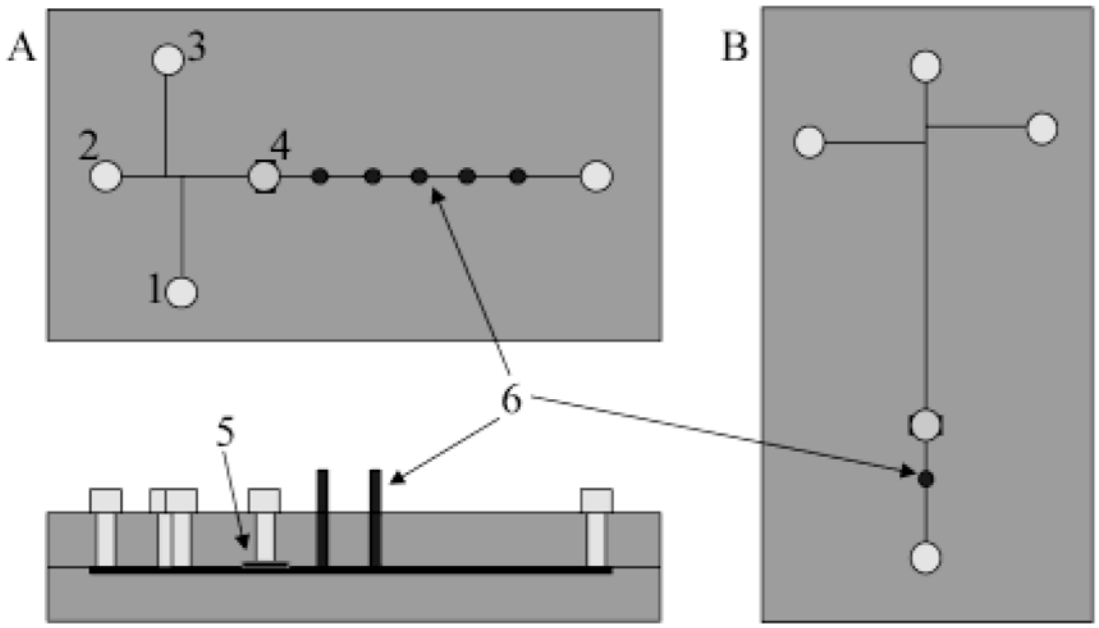

Figure 1.
Layout of the initial decoupler design: (A) Depiction of the chip design used to evaluate the performance of the decoupler. Five holes are drilled through the PDMS coverplate, in contact with the separation channel, each 0.5 cm further from the cathodic reservoir than the previous. A carbon paste electrode was placed in each of the first four holes, individually, with a platinum auxiliary placed in the hole directly behind it. (B) Depiction of the chip design used to obtain electropherograms of catecholamines and hydroquinone. The distance from the injection "T" to the decoupler is 3 cm. The distance from the decoupler to the working electrode is 1 cm. Labels: (1) sample reservoir, (2) buffer reservoir, (3) sample waste reservoir, (4) decoupler reservoir, (5) cellulose acetate film, and (6) electrodes. The injection "T", the injector sidearms, and the distance from the buffer reservoir to the injector are 500 μm, 13 mm, and 7.5 mm, respectively.

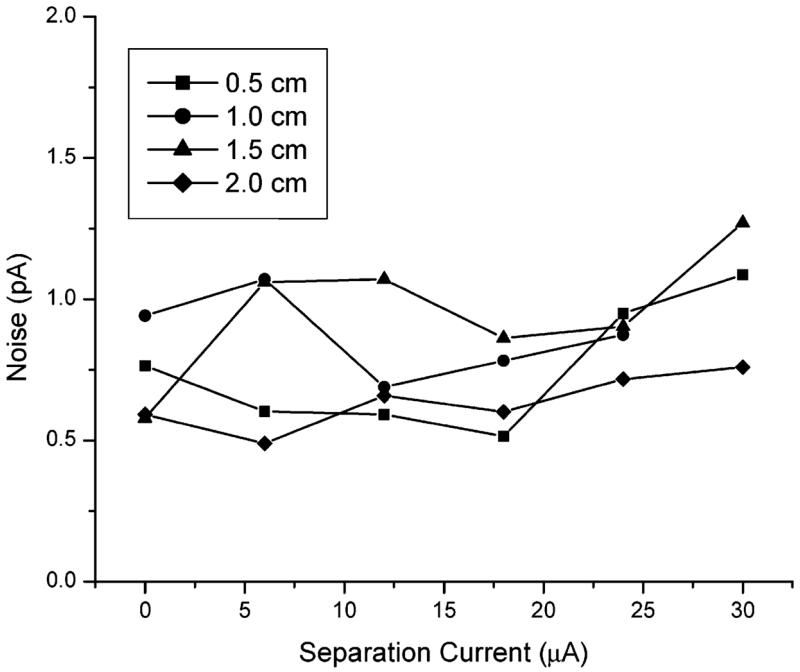

Figure 2.

Noise vs separation current for electrodes placed at increasing distances from the decoupler. Conditions: 10 mM H_3PO_4 adjusted to pH 7.0 with LiOH, carbon paste electrode at 800 mV.

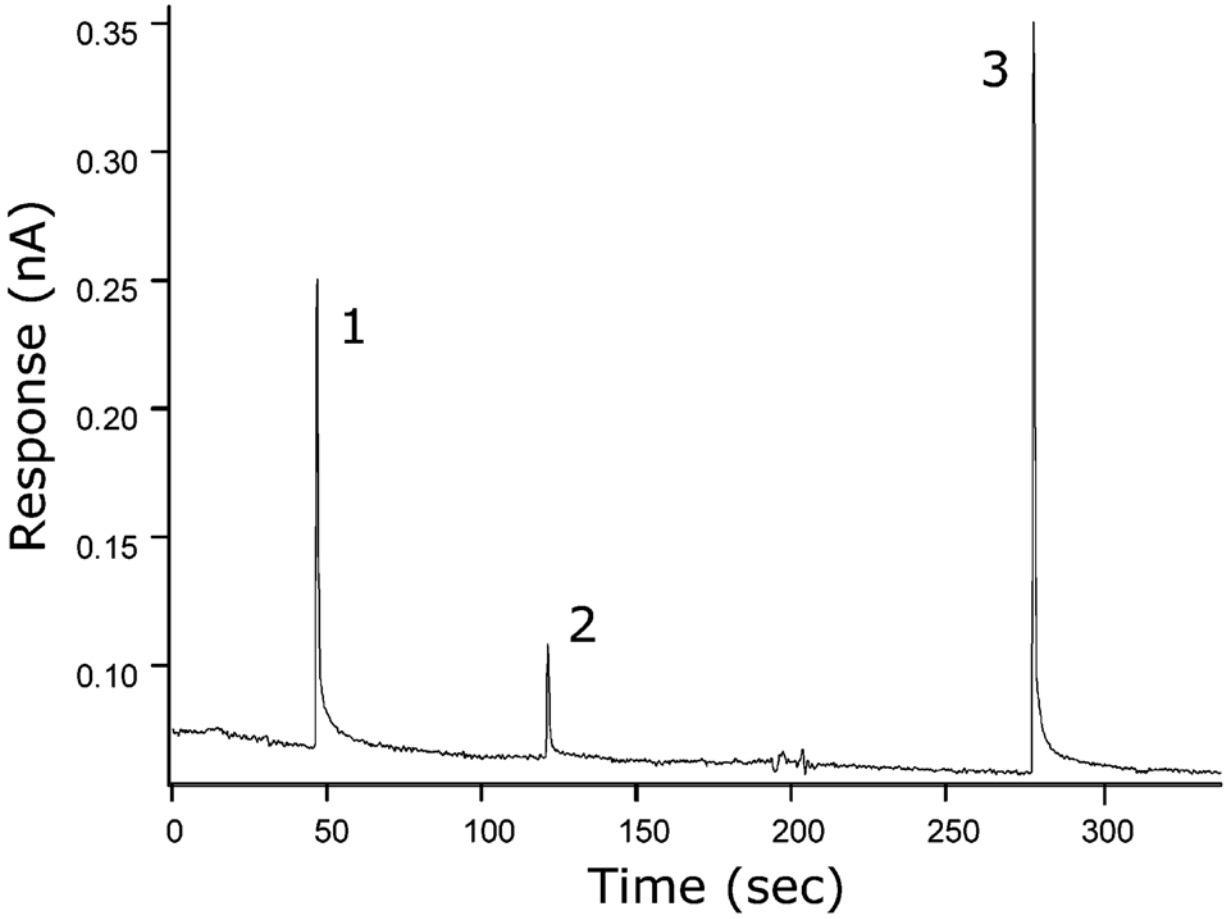

Figure 3.
Separation of catecholamines and hydroquinone. Labels: (1) 1 mM epinephrine, (2) 100 nM isoproterenol, and (3) 1 mM hydroquinone. Conditions: 10 mM H_3PO_4 adjusted to pH 7.0 with LiOH, carbon paste electrode at 800 mV, separation potential 3000 V, and injection potential 2600 V.

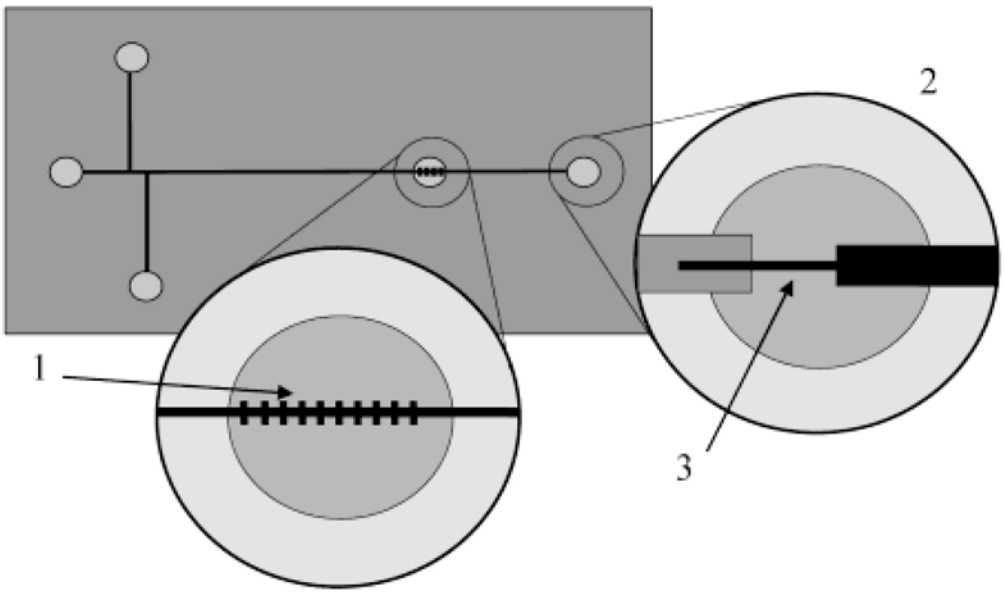

Figure 4.
Layout of the chip-based laser-etched decoupler. Labels: (1) decoupler holes, (2) detector reservoir, and (3) carbon fiber electrode. The distance from the buffer reservoir to the decoupler is 3 cm. The distance from the decoupler to the detector reservoir is 1 cm. The injection "T", the injector sidearms, and the distance from the buffer reservoir to the injector are 500 μm, 13 mm, and 7.5 mm, respectively.

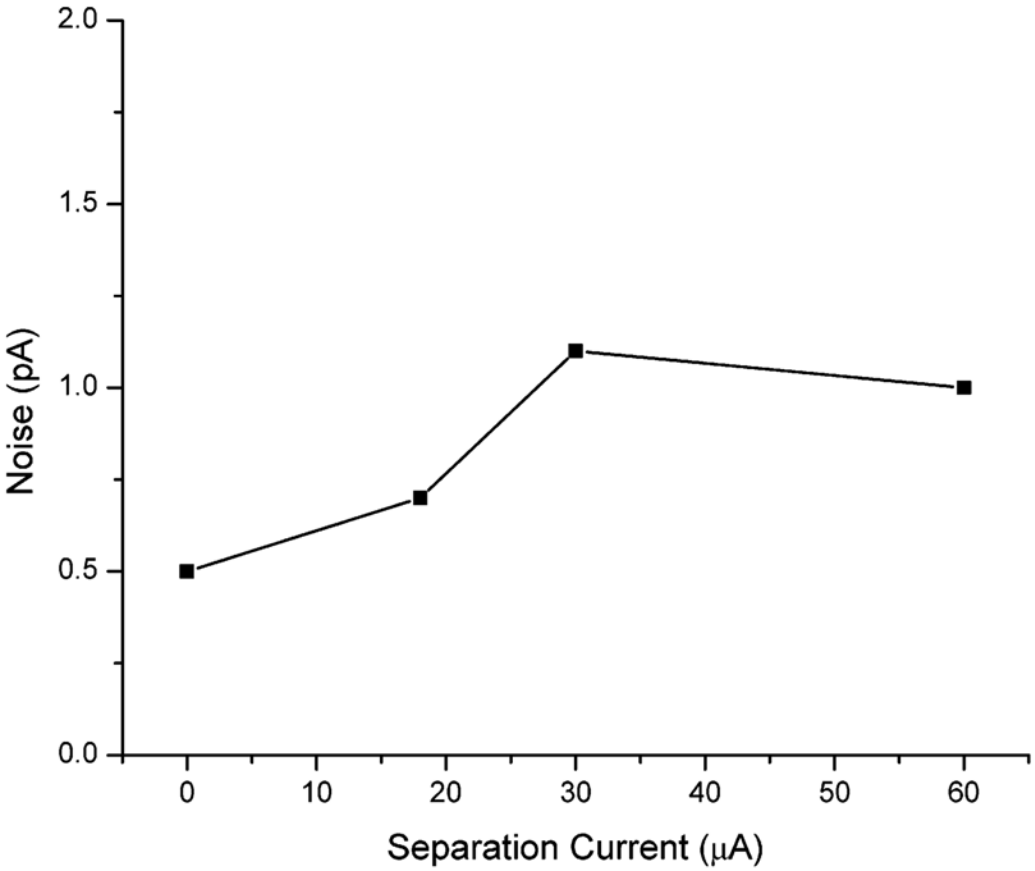

Figure 5.
Noise vs separation current for the laser-etched decoupler. Conditions: 10 mM H_3PO_4 adjusted to pH 7.0 with LiOH, carbon fiber electrode inserted 300 μm into channel at 800 mV.

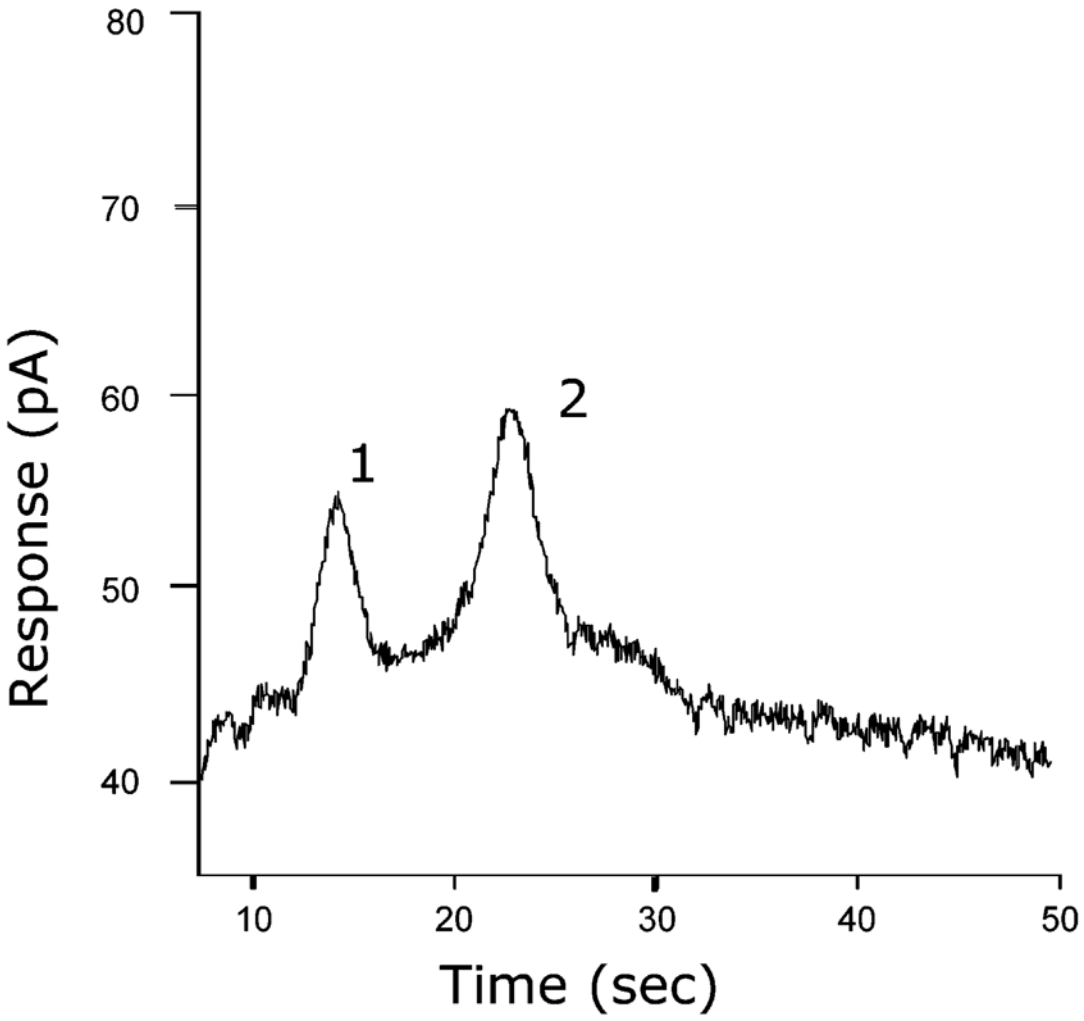

Figure 6.
Separation of dopamine and hydroquinone. Labels: (1) 82 nM dopamine and (2) 500 nM hydroquinone. Conditions: 10 mM H_3PO_4 adjusted to pH 2.75 with LiOH, carbon paste electrode inserted 300 μm into channel at 800 mV, separation potential = 5000 V, and injection potential = 4300 V.