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Performance of Pyrolyzed Photoresist Carbon Films in a Microchip Capillary Electrophoresis Device with Sinusoidal Voltammetric Detection

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Pyrolyzed photoresist films (PPF) are introduced as planar carbon electrodes in a PDMS−quartz hybrid microchip device. The utility of PPF in electroanalytical applications is demonstrated by the separation and detection of various neurotransmitters. PPF is found to form a stable, low-capacitance, durable layer on quartz, which can then be used in conjunction with a microchip capillary electrophoretic device. Sinusoidal voltammetric detection at PPF electrodes is shown to be very sensitive, with a detection limit (S/N = 3) of 100 nM for dopamine, corresponding to a mass detection limit (S/N = 3) of 2 amol. The selectivity of analysis in the frequency domain is demonstrated by isolating each individual signal in a pair of analytes that are chromatographically unresolved. Effectively decoupling the electrophoresis and electrochemical systems allows the electrodes to be placed just inside the separation channel, which results in efficient separations (80 000−100 000 plates/m).

The use of carbon as an electrode material is widespread, due to its low cost, large potential window, and low background.[1] The latter is of particular importance in electroanalytical applications, where sensitivity is a crucial parameter. For these reasons, a majority of the recent reports of capillary electrophoresis with electrochemical detection (CE-EC) performed on microchips have utilized carbon electrodes, whether they are carbon fibers or paste immobilized in a poly(dimethyl siloxane) (PDMS) channel,[2−4] screen-printed thick-film electrodes,[5−11] or the more conventional carbon fiber microdisk electrodes simply aligned at the outlet of the channel.[12−14] A disadvantage of using carbon in this fashion for electrochemical detection in microchips is that it cannot be fabricated photolithographically and, therefore, cannot take advantage of the mass production processes utilized to manufacture other components of the chip. In this report, we demonstrate the use of pyrolyzed photoresist films (PPF) for electrochemical detection in a PDMS−quartz hybrid microchip.

The PPF surface is exceptionally smooth (<0.5 nm rms) when compared to other carbon electrode surfaces, such as polished glassy carbon (GC) and vacuum heat-treated GC disks, whose root-mean-square surface roughness was found by STM to be 4.1 and 4.5 nm, respectively.[15,16] Additionally, curing in a reducing atmosphere minimizes carbon oxidation, leading to a low oxygen/carbon atomic (O/C) ratio that is relatively stable toward air oxidation. The smoothness and low O/C ratio result in a surface with a low capacitance, contributing to the low background levels observed.[15] The ability to create sensitive carbon electrodes through lithographically patterning photoresist opens up many useful possibilities for chip design. For example, PPF electrodes can be fabricated in a planar arrangement that allows for placement in the separation channel. Although this detection geometry is not optimal,[14] the rigidity and stability of alignment are necessary to create a truly integrated device. Additionally, positioning the electrode at the channel outlet decreases peak asymmetry and increases efficiency, as well as increasing sensitivity when the electrophoresis and electrochemical circuits are decoupled. In this work, PPF electrodes on quartz are used in hybrid devices formed by molding channel structures into the elastomer PDMS. The use of polymers as substrates for microchip devices is advantageous due to their low cost and ease of fabrication.[17]

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Although much of the work done to date with chip-based separations has involved the use of laser-induced fluorescence detection,[18−22] electrochemical detection has received much interest recently as an alternative detection scheme for on-chip analyses.[2−6,8−10,12,14,20,23−25] EC detection is more amenable to miniaturization, is less expensive than optical detection, and has the necessary sensitivity to detect the small volumes encountered in microfluidics.

Sinusoidal voltammetry (SV) is an electrochemical detection technique that is very similar to fast-scan cyclic voltammetry, differing only in the use of a large-amplitude sine wave as the excitation waveform and analysis performed in the frequency domain. In SV, the raw time domain is collected from the electrochemical cell and converted into the frequency domain in order to better decouple the faradaic signal from the background components, as well as to generate a unique "fingerprint" frequency spectrum to aid in identification and isolation of chemical species. We have demonstrated the utility of the frequency spectrum to selectively isolate oligonucleotides tagged with four unique ferrocene derivatives,[26] as well as to null out each signal in a mixture of two carbohydrates with a chromatographic resolution of less than 0.8.[23] Selectivity can also be improved through manipulation of the applied potential window; [27] herein both techniques will be demonstrated for a mixture of neurotransmitters.

In addition to its selective capabilities, SV has been shown to be a very sensitive detection technique for the analysis of native amino acids,[28] carbohydrates,[29] neurotransmitters,[27] nucleotides,[30] and DNA[31] by flow injection analysis, with detection limits for dopamine at the picomolar level.[27] SV has been coupled to capillary gel electrophoresis for the detection of redox-labeled oligonucleotides,[26] achieving single-base resolution.[32] Recently, SV has been applied to on-chip detection of native carbohydrates at copper electrodes.[23] In this work, the analysis of neurotransmitters with SV is used to investigate the applicability and performance of PPF in a microchip CE electroanalytical system. The measurement sensitivity, durability, and reproducibility of this surface are explored. Additionally, the selectivity and sensitivity of SV are optimized. The selectivity of SV is illustrated by isolating a pair of unresolved analytes through the use of the frequency domain. The use of lithographically fabricated carbon electrodes in microchip-based separations allows for the design of a rugged, PDMS−quartz hybrid device with integrated electrochemical detection, upon which selective, efficient (80 000−100 000 plates/m), and sensitive (amol mass detection limit) analyses are performed.

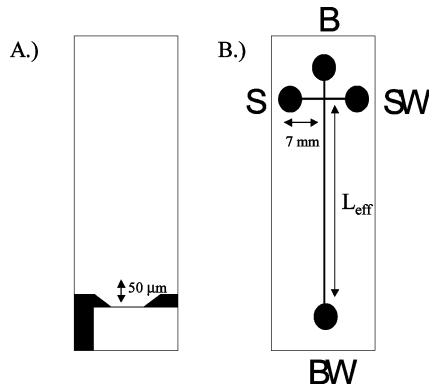

Figure 1. Electrode geometry and microchip layout. (A) Illustration of PPF electrode geometry. The electrode is 50 μm wide at the center working area. (B) Channel dimensions: 20 μm wide, 7 μm deep, 7 mm from each sample (S), sample waste (SW), and buffer (B) wells to the injection T, effective separation length (L_{eff}) varied (see Results and Discussion section).

EXPERIMENTAL SECTION

Reagents. 3-Hydroxytyramine hydrochloride (dopamine) (98%, Aldrich Chemical Co., Milwaukee, WI), isoproterenol hydrochloride (Sigma Chemical Co., St. Louis, MO), epinephrine (Sigma Chemical Co.), L-ascorbic acid (ACS grade, Fisher Scientific, Fair Lawn, NJ), L-3,4-dihydroxyphenylalanine (L-dopa) (Sigma Chemical Co.), 2-(*N*-morpholino)ethanesulfonic acid (MES) (99.5%, Sigma Chemical Co.), sodium phosphate monobasic (ACS grade, Fisher Scientific), and sodium phosphate dibasic (ACS grade, Fisher Scientific) were used as received. All solutions were made with water first deionized and then passed through a Milli-Q water purification system (Millipore Corp., Bedford, MA). Stock solutions (10 mM) of dopamine, isoproterenol, epinephrine, and L-dopa were made in 0.1 M hydrochloric acid and were diluted in the electrophoresis buffer prior to use. A stock solution of ascorbic acid was made in the electrophoresis buffer. All solutions were prepared daily, filtered, and degassed.

Fabrication of Pyrolyzed Photoresist Films. AZ4330 positive photoresist (Clariant Corp., Somerville, NJ) was spin-coated onto a fused quartz plate (7.62 cm by 1.9 cm) at 6000 rpm for 30 s using a Bidtec SP100 spin coater. Three coatings were used to obtain the desired thickness, which is on the order of 4−6 μm. The resist is then soft-baked for 60 s at 110 °C. A positive transparency mask containing a 50-μm-wide feature was used to define the electrode dimensions (Figure 1A). The photoresist was exposed through this mask to a UV flood source at 10 mW/cm^2 for 20 s using an OAI light source system (OAI, Milpitas, CA). The resist was then developed in a 4:1 (v/v) dilution of AZ 400K developer (Clariant) in water. The remaining resist was hard baked on a hot plate for 10 min at 90 °C. The pyrolysis procedure has been described previously.[15,33] Briefly, pyrolysis occurred in

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a tube furnace (Lindberg) fitted with a quartz tube flushed by forming gas (95% N_2 + 5% H_2, flowing at 100 sccm/min) for 20 min at room temperature. Under continuous gas flow, the temperature was increased at the rate of 10 °C/min to 1000 °C, held at 1000 °C for 60 min, and then cooled to room temperature. The PPF electrodes were in some cases treated with piranha solution (7:1 H_2SO_4/H_2O_2), which oxidized the surface (see Results and Discussion section). (***Caution!*** *Piranha solution is a powerful oxidizing agent that reacts violently with organic compounds; it should be handled with extreme care.*) In all other cases, the electrodes were sonicated in isopropyl alcohol (IPA) containing activated carbon for 5 min, in IPA alone for 5 min, and then in water for 5 min.

Fabrication of PDMS Channel. The PDMS replica was formed through the use of a silicon master, which was donated by Applied Biosystems, Inc. (Foster City, CA). This process has been described previously.[17,34] The resulting feature was found by profilometry to be 20 μm wide and 7 μm high. This feature was used to mold the channel into PDMS. The monomer and curing agent (Sylgard 184 silicone elastomer kit, Dow Corning, Midland, MI) were mixed in a 12.5:1 ratio, degassed, and poured into the silicon master. This was then cured in an oven at 60 °C for 45 min.

Assembly of PDMS Chip. The PDMS replica was removed from the master, and 3.5-mm wells were created with a hole punch. In each device constructed, the sample (S), sample waste (SW), and buffer (B) wells were positioned 0.7 cm from the T intersection, while the buffer waste (BW) (detection reservoir) was positioned at various distances (1, 4.5, or 4.8 cm) from this intersection, as detailed in the Results and Discussion section (Figure 1B). The PDMS replica was then sealed to the electrode plate. During sealing, the electrode was positioned with the leading edge inside the channel outlet through the use of a microscope. The two were reversibly sealed in an oven at 60 °C for 15 min. With the exception of the device used in the rapid analysis of dopamine and ascorbic acid, the buffer waste well was filled with electrophoresis buffer and pressure was applied to fill the untreated channels. The channels of the rapid analysis device (1-cm effective separation length) were pretreated with 0.1 M NaOH for 48 h prior to use, rinsed with water, and filled with electrophoresis buffer. The Ag/AgCl reference electrode was attached to a stereotaxic micropositioner and positioned as close to the PPF working electrode as possible with the aid of a microscope. Although a salt bridge was not used, the reference electrode was regenerated daily to ensure potential stability. The fluid level of the wells was monitored throughout the experiments to maintain proper function and to eliminate any hydrodynamic pressure-induced flow.[35]

Electrophoresis Procedures. A four-channel power supply built in-house was used for voltage control of the four buffer wells. The high-voltage supply was powered by a 12-V battery, and utilized three C30 HV modules (Emco High Voltage Corp., Sutter Creek, CA) to supply up to +3000 V to three of the buffer wells. The remaining channel, to be used with the buffer waste well in proximity to the working electrode, simply involves a high-voltage relay (SO2DNA253, Kilovac, Santa Barbara, CA), which allows it to switch between floating and ground states. Each channel incorporates such a digitally controlled relay. Relays were protected from excessive voltage, current, or temperature by a quad high side driver (LMD18400, National Semiconductor, Santa Clara, CA). Digital control of the high side driver and relays, as well as analog control of the HV modules, was accomplished through the use of a laptop. The software was written in-house (Labview, National Instruments), and potential was supplied to the power supply by a PCMCIA card (DA8P-12U, Quatech, Akron, OH). The electrophoretic current was monitored as the voltage drop across a 10 kΩ resistor in series with the buffer waste well channel. The entire power supply was battery-operated and therefore electrically isolated from the electrochemical detection system.

Injection Conditions. All injections were accomplished by applying a positive voltage to the sample well and grounding the sample waste well, with the buffer and detection wells floating. During separation, a positive voltage is applied to the buffer well with the detection well grounded, and less positive pull-back voltages were applied to the sample and sample waste wells.

dc Amperometric Detection. Amperometric detection was accomplished using a two-electrode setup at a constant detection potential (700−800 mV vs Ag/AgCl). Electropherograms were collected with a time resolution of 0.2 s (5 Hz) and were filtered at 2 Hz. The final step consisted of application of a five-point boxcar averaging routine.

Sinusoidal Voltammetry. The excitation signal was a 3-Hz sine wave with a 500-mV amplitude, which was digitally generated by National Instruments hardware (PCI-4451, National Instruments, Austin, TX) and controlled by Labview software. The data acquisition card has 16-bit resolution with a maximum sampling rate of 200 Ksamples/s. The signal was filtered by a Cyberamp 380 (Axon Instruments, Foster City, CA) with the 3-dB point set at 3 times the excitation frequency (10 Hz) and then applied to the electrochemical cell by a potentiostat (Geneclamp 500, Axon Instruments Inc., Foster City, CA). The current response was amplified by the potentiostat and filtered by the Cyberamp, with the 3-dB point set to 4 times the frequency of the tenth harmonic (120 Hz). The data acquisition software was designed in-house and digitally samples the data at 128 times the excitation frequency, or 384 Hz.

All further processing was performed by programs written in-house using a combination of Labview (National Instruments) and Matlab (The Mathworks, Inc., Englewood Cliffs, NJ). Each cycle of 128 points was added onto itself 3 times to generate one scan of 512 points, consisting of four identical cycles. This allows the lower frequency elements to be accurately represented, without compromising the time resolution. Each scan was then fast Fourier transformed, generating the time course and frequency domain. Only the first 10 harmonics were saved to disk to minimize the size of the data set.[28] Generation of the frequency domain information has been described previously,[28] but briefly, the current response can be thought of as a series of instantaneous current vectors. Before introduction of the analyte, the current response was composed solely of the background current vector, which typically has a fixed phase angle. When the analyte was injected, the current response included contributions from both the signal and the background vectors. To isolate frequency

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domain information characteristic of the signal vector only, the background vector was subtracted from the entire data set. The time point where the signal magnitude was at its maximum was used to obtain the optimum phase angle and current magnitude for a particular analyte.

Digital Lock-In. The time course (current vs time (scan number)) at each harmonic was also obtained, through the use of a digital equivalent to a lock-in amplifier.[28] The current response was monitored at the optimum phase angle for the analyte, and only the component of the background at this phase angle remains in the displayed time course. The most effective background elimination occurred when the phase angle of the background was ±90° from that of the signal. The resultant time course data was then low-pass filtered by applying a five-point boxcar averaging routine.

The digital lock-in approach can also be used to supply an additional degree of selectivity to this analysis. Monitoring the time course at ±90° out of phase with the optimum phase angle of an analyte will null out its signal. This can be used to identify a species (by its absence)[26−28] or to fully isolate unresolved species (Figure 7).[23]

Selectivity can also be gained through manipulation of the applied potential window. When the formal potential of an analyte is centered in the potential window, the signal of that analyte is present almost exclusively in the odd harmonics. This is due to the symmetry of the formal potential in the applied potential window.[27] The absence of signal in the even harmonics can be utilized to isolate species that are unresolved chromatographically (Figure 6).

Cyclic Voltammetry. The software used to obtain cyclic voltammograms was written in-house and has been described previously.[27] A digitally generated triangle waveform was applied at either 1 or 4.2 V/s (3 Hz) from −200 to 500 mV (vs Ag/AgCl). The signal was amplified and filtered at 24 times the scan frequency by the potentiostat and collected by the National Instruments hardware (PCI-4451). Postprocessing was performed with software using a combination of Labview (National Instruments) and Matlab software (The Mathworks, Inc.). User-defined background scans are subtracted from the entire data set to generate the background-subtracted current versus potential trace. The background-subtracted time domain can be obtained through subtraction of a background potential region from the entire data set.[27]

RESULTS AND DISCUSSION

The fabrication of PPF electrodes has been demonstrated on silicon and glassy carbon substrates, neither of which is suitable for electrophoresis applications; they are too conductive. Although electrochemical experiments, where the maximum potential applied is in the 1−2-V range, have been performed using PPF electrodes fabricated on silicon with no conductivity contribution from the substrate itself,[15,33] the high voltages (kilovolts) applied in electrophoretic separations will short even through oxidized, undoped silicon (unpublished observation). The substrate used must be stable at the pyrolysis temperature of 1000 °C, which is not the case for the soda lime plates commonly used for the fabrication of planar electrodes.[20,23,25,36−38] This led to the use of fused quartz, upon which the PPF adheres well to form a stable, durable film (discussed later). Quartz was also found to reversibly seal to PDMS in the formation of these devices, and removal of the PDMS layer did not detach the PPF from the quartz, even after the electrode plates were used in multiple devices.

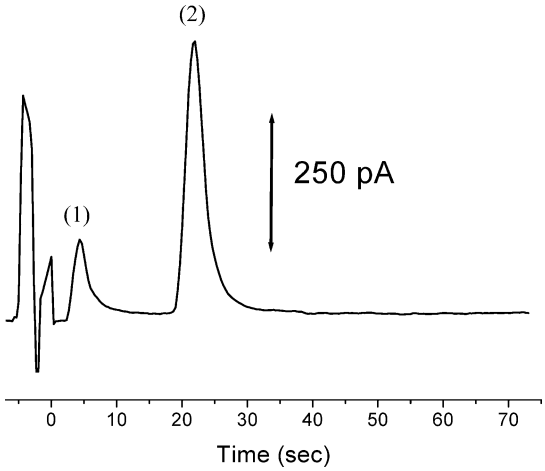

Figure 2. Rapid separation of 5 μM dopamine (1) and 250 μM ascorbic acid (2), first harmonic (3 Hz) time course. Electrophoresis buffer, 25 mM phosphate, 2 mM NaCl (pH 7.4); separation voltage, +750 V (440 V/cm) with +405 V pull-back voltage; injection, +1 kV for 3 s. Effective separation length of channel is 1 cm. SV detection was accomplished by applying a 3-Hz sine wave from −50 to 550 mV (vs Ag/AgCl).

Analytical Applications of the CE-EC Microchip Device. Figure 2 depicts the rapid separation of dopamine and ascorbic acid, performed in under 30 s on a chip with an effective separation length of 1 cm. Dopamine, which is a common target analyte for microdialysis applications, is eluted within 10 s with no compromise in sensitivity. Dopamine is generally found in the low-nanomolar range during in vivo microdialysis studies, which in many cases necessitates the collection of dialysate over a longer period of time to obtain a measurable quantity.[39] It is fully resolved from the response of a much larger concentration of ascorbic acid, which is typically found at the low-micromolar level.[40] The speed of analysis demonstrated, in addition to the fact that analyses in a microchip format have the ability to be multiplexed, could allow for the rapid analysis of a large number of samples.[22,41] The initial spike in the electropherogram is due to the injection process. It is interesting to note that the baseline reequilibrates rapidly after injection and that the baseline is very stable considering the high field applied (440 V/cm). This illustrates how effectively the electrophoresis and electrochemical systems are decoupled and the stability of the PPF background current.

A separation of four neuroactive molecules, dopamine, epinephrine, isoproterenol, and L-dopa, on a microchip with an effective separation length of 4.8 cm is shown in Figure 3. The resolutions among dopamine, epinephrine, and isoproterenol are 1.05 and 1.07, respectively. The efficiencies of each peak are

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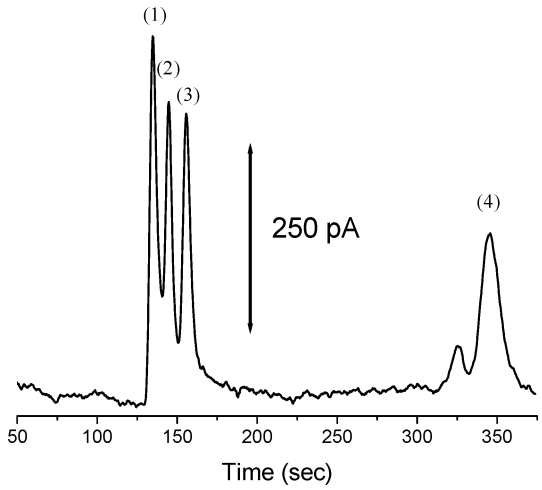

Figure 3. Separation of 10 μM dopamine (1), 25 μM epinephrine (2), 25 μM isoproterenol (3), and 100 μM L-dopa (4) at the first harmonic (3 Hz). Electrophoresis buffer, 50 mM MES, 20 mM phosphate, 2 mM KCl (pH 5.3); separation voltage, +1 kV (180 V/cm) with +470 V pull-back voltage; injection, +2 kV for 2 s. Effective separation length of channel is 4.8 cm. SV detection was accomplished by applying a 3-Hz sine wave from −100 to 400 mV (vs Ag/AgCl).

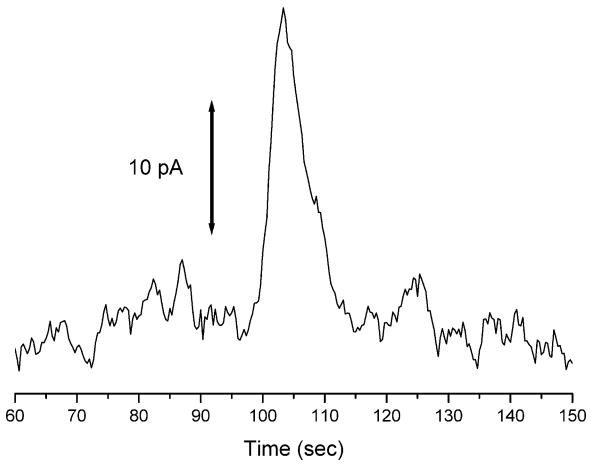

Figure 4. First harmonic time course elution of dopamine at a concentration (300 nM) near its detection limit. Electrophoresis buffer, 50 mM MES, 20 mM phosphate, 2 mM KCl (pH 5.3); separation voltage, +1 kV (190 V/cm) with +470 V pull-back voltage; injection, +2 kV for 3 s. Effective separation length of the channel is 4.5 cm. SV detection was accomplished by applying a 3-Hz sine wave from −56 to 444 mV (vs Ag/AgCl).

between 80 000 and 100 000 plates/m, which is similar to reported values for CE-EC on chip.[3,14,42] These data illustrate the ease of manipulation of experimental parameters, in this case effective separation length, when PDMS is used. Each CE-EC device used in this report was fabricated from the same mold and was tailored to fit the requirements of the application.

Sensitivity of SV and Comparison to Other Electrochemical Detection Techniques. The detection limit for dopamine at a piranha-oxidized electrode was determined by analyzing the response of an injection close to the detection limit (Figure 4). A 300 nM sample of dopamine was injected, and the extrapolated detection limit (S/N = 3) was found to be 100 nM. This corresponds to a calculated mass detection limit of 2 amol, when taking the geometric (20 × 20 × 7 μm) volume of the T intersection and then adding the volume of the separation channel section that could be occupied by the sample due to linear diffusion during the injection time. This value is slightly lower than the detection limits found at an unoxidized PPF electrode, such as in Figure 3, where the calculated detection limits for dopamine, epinephrine, isoproterenol, and L-dopa were found to be 160 nM, 500 nM, 515 nM, and 3.6 μM, respectively. The effect of this surface treatment is discussed in a later paragraph.

The sensitivity of SV was compared to that of dc amperometry and cyclic voltammetry (CV) by performing identical injections of dopamine on the same chip. The detection limit (S/N = 3) of dc amperometry ranged from 300 to 800 nM, depending on the detection potential applied (700−800 mV vs Ag/AgCl) (data not shown). CV was performed by applying −200 to 500 mV versus Ag/AgCl at 3 Hz (4.2 V/s), and its detection limit (S/N = 3) was determined to be 8 μM (data not shown). The sensitivity of SV and frequency domain analysis was shown to surpass commonly employed electrochemical detection methods in this system anywhere from 3-fold to over 1 order of magnitude. The slight improvement in sensitivity of SV relative to dc detection is supplemented by the impressive selectivity of SV, which dc amperometry lacks.

The limits of detection found in this system when dc amperometry is used compare favorably with literature detection limits for neurotransmitters detected by dc amperometry at electrodes utilized in a planar arrangement. At planar platinum electrodes, detection limits for dopamine range from 500 nM to 4 μM[20,25,36] and that for catechol detected at a planar gold electrode was reported to be 4 μM.[37] Additionally, the analysis of catechol was performed in a system similar to this one, which utilized a carbon fiber electrode in a planar arrangement with respect to the channel outlet, with detection limits that ranged from 500 nM to 4 μM.[2,3] This suggests that the sensitivity displayed in this work is partially a result of the extremely low background levels displayed by the PPF electrodes themselves, which is further enhanced through the use of frequency domain electrochemical detection.

Linearity and Stability of PPF-Based CE-EC Devices. The current response to injections of dopamine was linear from 300 nM to 50 μM (R^2 = 0.9996) with a sensitivity of 100 pA/μM. It was found that the devices were stable for up to 4 days if the channels remained hydrated during storage. After 4 days, the electroosmotic flow increased significantly, which made chip operation difficult. This effect has been reported elsewhere[34,37] and is a factor of the changing PDMS surface and not a result of electrode failure. The peak current and migration time were reproducible over 4 days, with RSDs (n = 8) of 9 and 5%, respectively. The PPF electrodes themselves were extremely stable and could be faithfully reused with new PDMS channel-containing substrates. The only cause for failure of the PPF electrodes was removal of the film by unintentional mechanical abrasion.

Surface Treatment of PPF. Background-subtracted cyclic voltammograms of dopamine and isoproterenol are shown in Figure 5. The PPF electrode used in this study was oxidized in piranha solution to increase the surface O/C ratio. Electrochemical oxidation of PPF has been shown to improve dopamine kinetics.[15]

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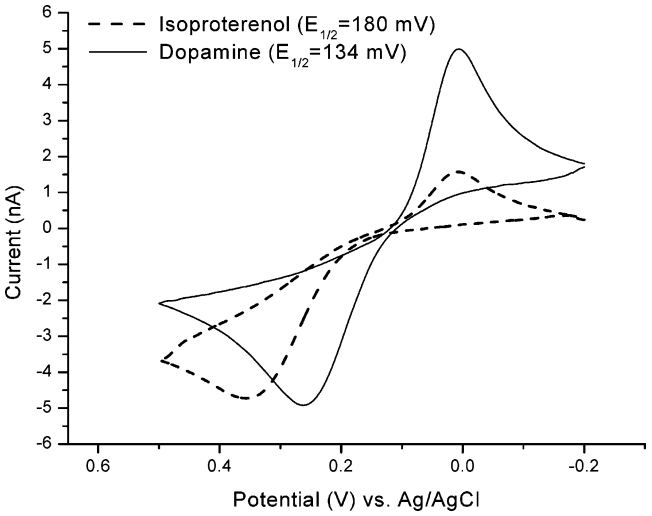

Figure 5. Background-subtracted cyclic voltammograms of dopamine and isoproterenol. Background subtraction was accomplished by injecting 100 μM solutions of each analyte for 5 s at +2 kV. Electrophoresis buffer and separation voltages identical to those used in Figure 4. Cyclic voltammetry was performed by applying −200 to 500 mV (vs Ag/AgCl) at 1 V/s.

This treatment was also applied to the electrodes used in the concentration study and undoubtedly aided in achieving the low detection limits for this molecule. The formal potentials of dopamine and isoproterenol were found to be 134 and 180 mV (vs Ag/AgCl), respectively. The peak splitting for dopamine was 240 mV in this work, which is slightly larger than the value reported previously for oxidized PPF (111 mV).[14] This is most likely due to the different techniques used to oxidize the surface; electrochemical oxidation of the PPF surface was not attempted in this study.

Selective Isolation of Signal through Manipulation of the Applied Potential Window. It has been shown that the response of an analyte will be distributed almost exclusively into the odd harmonics if its formal potential is centered in the applied potential window.[27] We can use this effect to achieve selectivity between two analytes that are not separated chromatographically, as illustrated in Figure 6. The applied potential window is centered at 180 mV versus Ag/AgCl (the formal potential of isoproterenol) in sections 1A and 1B of Figure 6. The first harmonic of an injection of dopamine and isoproterenol is shown in Figure 6.1A, and a peak for both species is apparent, with a chromatographic resolution of 0.9. Figure 6.1B is the fourth harmonic of the same data set, and it is apparent that there is no contribution to the signal from isoproterenol. Similarly, the applied potential window is centered at 134 mV versus Ag/AgCl (the formal potential of dopamine) in Figure 6-2C and 2D, and there is no signal for dopamine in the even harmonic (Figure 6.2D, second harmonic). This technique is a simple way in which to selectively detect two analytes (with known formal potentials) through manipulation of the applied excitation potential window.

Selective Nulling of Signal through the Digital Lock-In Technique. Utilizing the digital lock-in technique is an additional means for enhancing selectivity in SV, by eliminating an analyte's signal through manipulation of the monitored phase angle (see

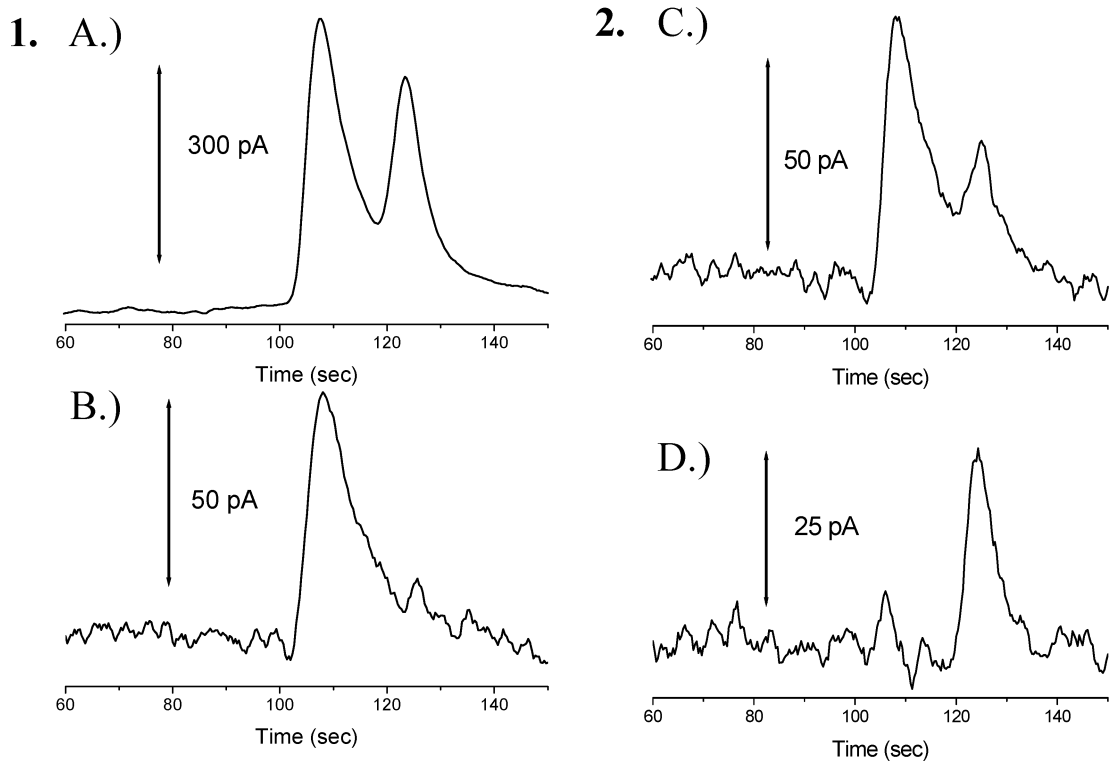

Figure 6. Manipulation of the applied potential window to enhance selectivity. Separation of 5 μM dopamine and 5 μM isoproterenol. All electrophoresis conditions were identical to those in Figure 4. SV detection was accomplished by applying a 3-Hz sine wave with a 500-mV amplitude with a variable offset. 1. Excitation offset centered on the half-wave potential of isoproterenol (180 mV (vs Ag/AgCl)). First harmonic (3 Hz) time course (A) and fourth (12 Hz) harmonic time course (B). 2. Excitation offset centered on the half-wave potential of dopamine (134 mV (vs Ag/AgCl)). Third harmonic (9 Hz) time course (C) and second harmonic (6 Hz) time course (D). The resolution between dopamine and isoproterenol is 0.9.

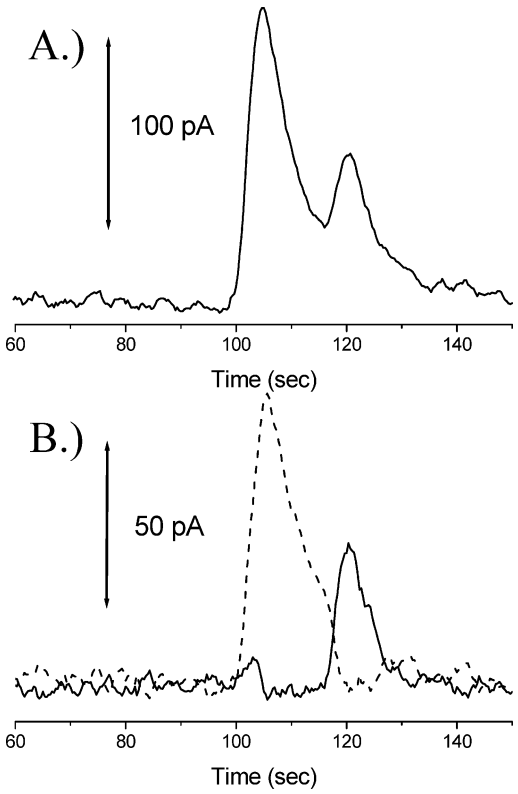

Figure 7. Utilization of the digital lock-in technique to enhance selectivity. Separation of 10 μM dopamine and 10 μM isoproterenol shown at the fifth harmonic (15 Hz). The electrophoresis conditions are identical to those in Figure 4. SV detection was accomplished by applying a 3-Hz sine wave from −116 to 384 mV (vs Ag/AgCl). (A) Phase common signal: time course monitored at the phase common angle of 137 °. (B) Phase-nulled signals: time course monitored at −90° out of phase (60°) with the dopamine optimum phase angle (150°) (solid trace), and time course monitored at +90° out of phase (213°) with the isoproterenol optimum phase angle (123°) (dashed trace). The resolution between dopamine and isoproterenol is 0.9.

Experimental Section). Figure 7 displays an injection of dopamine and isoproterenol similar to that discussed above, also with a resolution of 0.9. The fifth harmonic is shown, where the difference between the optimum phase angles of dopamine and isoproterenol is 27°. The signal at the phase common angle (137°), or the average of the two optimum phase angles, is shown in Figure 7A. There is a contribution from both species at this phase angle. The dashed plot in Figure 7B is the same data monitored at 90° out of phase (213°) with the optimum phase angle of isoproterenol (123°), which effectively nulls out the signal of isoproterenol. Dopamine is nulled out in the solid trace of Figure 7B by monitoring at 90° out of phase (60°) with its optimum phase angle (150°). Frequency domain analysis can be employed in either of the two above-mentioned modes to enhance the selectivity of the electrochemical measurement.

CONCLUSION

The applicability of PPF electrodes in microchip-based devices has been investigated. PPF was found to form a stable and durable layer upon quartz, which can be used apparently indefinitely to create numerous PDMS−quartz hybrid devices. The devices displayed a linear response over 3 orders of magnitude and could be used in reproducible analyses over days when stored appropriately. The ability to lithographically fabricate carbon electrodes will allow for the design of truly integrated CE-EC microchips that can be reproducibly created using IC technology, require no alignment once constructed, are rugged, and have few to no moving parts. Effective decoupling of the electrochemical and electrophoresis fields permits the planar electrodes to be placed at least partially in the separation channel and still retain a stable background level, even when high fields are applied, allowing for efficient separations (80 000−100 000 plates/m). Coupled to SV for the detection of neurotransmitters, PPF is shown to be very sensitive, with a mass detection limit of 2 amol. The multifaceted selectivity of SV was demonstrated in the analysis of a pair of analytes that are not baseline-separated chromatographically. CE on-chip with SV detection at PPF electrodes can be envisioned as a tool to explore a wide variety of analytical problems due to its ease of fabrication, the ability to change experimental parameters easily when PDMS is used as the channel substrate, and the ruggedness of the devices due to truly integrated detection, as well as selectivity that will allow for the analysis of complicated systems, and the sensitivity clearly demonstrated by these devices.

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