Published in final edited form as:

J Electroanal Chem (Lausanne Switz). 2013 March 15; 693: 28–33. doi:10.1016/j.jelechem.2013.01.022.

Microfabricated Microelectrode Sensor for Measuring Background and Slowly Changing Dopamine Concentrations

Adam K. Dengler and Gregory S. McCarty*

Department of Biomedical Engineering, North Carolina State University, Raleigh, NC, USA

Abstract

The electrochemical detection of neurotransmitters *in vivo* has centered on fast scan cyclic voltammetry (FSCV) due to its temporal resolution, sensitivity and chemical selectivity. FSCV is a differential technique that records phasic (second-to-second) changes in the concentration of electroactive neurotransmitters such as dopamine (DA). To isolate the currents due to fluctuations in analyte concentration, in other words to make these phasic measurements, requires the subtraction of a large background current. The subtraction of this background and its volatility renders FSCV unable to determine background or slowly varying concentrations of electroactive analytes. However, there is still a need to readily determine the background and slowly changing concentrations of electroactive analytes in tissue. For example, the background concentrations of DA vary throughout the brain and can affect the dynamics of dopaminergic systems. So, this report presents a microfabricated electrochemical sensor for measuring background and slowly changing concentrations of DA *in vitro* with the selectivity and sensitivity of FSCV. The sensor is comprised of two microfabricated microelectrodes which are spaced 8 μm apart. Varying the applied potential of the outer electrode manipulates the local concentration of electroactive species including concentration at the inner electrode. These changes are measured at the inner electrode using FSCV. The resulting signal with calibration can determine the background and slowly changing concentration of DA with the selectivity and sensitivity of FSCV. In this study the background of DA is determined *in vitro* using this sensor. The DA signal is shown to be the result of adsorption/desorption at the outer electrode. Interference from ascorbate on the DA signal is shown to be minimal for this approach.

INTRODUCTION

Measuring the extracellular concentration of neurotransmitters in the brain has attracted significant attention. Information about extracellular neurotransmitter concentrations has been useful in improving understanding about a range of pathologies and in understanding fundamental relationships between behavior and neurochemistry.^[1] The extracellular concentration of neurotransmitters may be classified as either phasic, occurring on short (~1 s) time-scales, or tonic, slowly changing baseline concentrations that vary over minutes to hours.^[2] Indeed, dopamine (DA) neurons have both tonic and phasic firing patterns^[2] which contribute to the extracellular concentration of DA in the brain.^[3] Measurements with positron emission tomography (PET) have estimated baseline extracellular concentration of DA in the 120 nM range.^[4] PET is a noninvasive measurement which uses radio-labeled DA

© 2013 Elsevier B.V. All rights reserved.

*To whom correspondence should be addressed. gsmccart@ncsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

antagonists to quantify endogenous DA based on known values of DA agonist binding. Baseline concentrations or basal levels of DA have been measured in the low nM range with microdialysis across brain regions^[5], but this method has several drawbacks, including low temporal resolution and tissue damage.^[6] On the other hand, electrochemical methods utilizing carbon microelectrodes offer minimal damage^[7] and excellent spatial and temporal resolution^[1], but hitherto have been restricted to measuring rapid fluctuations in concentration that ride on top of an unknown basal concentration. Background-subtracted fast scan cyclic voltammetry (FSCV) has been used extensively to study phasic DA release^[1], including in awake and behaving animals treated with cocaine.^[8] In FSCV the potential of a microelectrode is rapidly cycled (>100V/s) using a triangle waveform. This rapidly changing potential generates a large background current, due to charging of the electrical double layer and electrode surface processes. In addition to these background processes, Faradaic currents associated with redox reactions of the electroactive molecule(s) in the solution are generated. Traditional *in vivo* FSCV measurements utilizes background subtraction, in other words the current measured at a chosen time is defined as the background current and subtracted from all measurements. The resulting CVs can be displayed to determine differential changes in the concentration of the electroactive species. This background subtraction removes contribution from background levels of electroactive species. Moreover, the background signal varies over the period of minutes^[9], making it difficult to measure phasic changes over periods greater than this. Carbon fiber microelectrodes are especially sensitive to catecholamines when using FSCV due to the adsorption of the catecholamine to the electrode surface at negative holding potentials.^[10] Additionally, cycling to a higher potential range (1.3 V vs. Ag/AgCl) increases adsorption by overoxidation of carbon surface.^[11] This FSCV procedure has created a useful technique for studying *rapidly fluctuating* catecholamine in tissue.

Here, we present a microfabricated sensor coupled with a voltammetry protocol capable of measuring baseline concentrations of an electroactive species using the selectivity and sensitivity of FSCV. The electrodes are made of pyrolyzed photoresist (PPF), which have a similar chemical composition to glassy carbon.^[12] Furthermore, PPF electrodes are comparable to carbon fiber microelectrodes for FSCV detection of DA.^[13] The sensor is designed for integration onto our existing microfabricated platform. This platform has already been demonstrated for *in vivo* measurements of phasic DA signaling and is small enough to cause limited tissue damage.^[14] This study is a proof of concept for the microfabricated sensor design and procedure. The sensor is comprised of parallel carbon band microelectrodes in close proximity of one another. The 'outer' microelectrode forms two bands which enclose and run parallel to either side of the 'inner' band electrode. A square wave potential applied to the outer electrodes influences local concentrations of electroactive species and the inner electrode monitors the local concentration of electroactive species with FSCV. In this study the origin of the large fluctuations in concentration observed in the FSCV signal at the inner electrode is shown to be DA adsorption/desorption from the outer electrode. Interference to the DA signal from ascorbate is shown to be negligible.

EXPERIMENTAL

Reagents and Solutions

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and used as delivered unless specified otherwise. Electrode calibrations and experiments were done in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM NaH₂PO₄, 2 mM K₂H₂PO₄, pH = 7.4). Stock solutions of DA and ascorbic acid were prepared in 0.1 N HClO₄ and diluted with PBS to the target concentrations directly before use.

Microfabrication of PPF Microelectrodes

Microelectrodes were made from PPF^[12-13] using procedures similar to our previous works for generating microelectrode arrays and are detailed elsewhere.^[14] The PPF microelectrodes are fabricated on a silicon wafer insulated with silicon nitride. The PPF electrodes are patterned and the non-active portions of the sensors are insulated with a second silicon nitride layer. The geometry for the electroactive part of the device is illustrated in Figure 1 and consists of an inner carbon band 10 μm wide $\times$ 100 μm long electrode flanked by two interconnected band electrodes of identical dimensions spaced 8 μm apart to either side.

Instrumentation and Software

Fast Scan Cyclic Voltammetry data was collected using a “Dual waveform UEI” potentiostat (UNC Chemistry Electronics Shop, Chapel Hill, NC) in two electrode mode and TH-1 software coded in LabVIEW (National Instruments, Austin, TX, U.S.A.). In all cases a triangular waveform from -0.4 V to 1.3 V at 400 V s^{-1} was applied to the inner working electrode at a repetition frequency of 3 Hz. The inner electrode was held at -0.4 V between cycles. A square potential waveform of $t_{\text{acc}} = 15\text{ s}$ at $E_{\text{acc}} = 0\text{ V}$ then $t_{\text{stp}} = 5\text{ s}$ at $E_{\text{stp}} = 1\text{ V}$ was applied to the outer working electrodes unless otherwise stated. The waveform was both generated and recorded with an ADC/DAC card PCI-6251 (National Instruments). The FSCV waveform was filtered through a low pass 2 kHz filter to remove digitization effects. Data analysis and plotting was done in Microsoft Excel and GraphPad Prism (GraphPad Software, San Diego, CA, U.S.A.) respectively. Data are reported as mean $\pm$ standard deviation.

Voltammetric Experiments

Connections between the electrochemical preamplifier and bonding pad were made using silver epoxy, silver wire, and soldered gold pins. A custom flow-bath setup accommodated the perfusion of solutions over the electrodes. Electrodes were surrounded with Polydimethylsiloxane (PDMS) wells which were bonded to the wafer with epoxy. Continuous flow of solutions was controlled by a multistaltic pump (Buchler Instruments, Lenexa, KS) which bi-directionally connected inlet vials and an outlet waste vial to the PDMS wells via polymer tubing and 22-gauge Luer-lock syringe tips (B-D., NJ, U.S.A.).

RESULTS & DISCUSSION

Operation of the sensor

The sensor operates in a manner similar to adsorptive stripping voltammetry^{[14][15]}, from which nomenclature is borrowed, except in our case the inner and outer electrodes each have specialized functions. Figure 2 details the FSCV signals collected from the inner electrode during operation of the sensor. This figure also helps outline the operation of the sensor and the data analysis procedures. Operating conditions were as described in the experimental section. The sensors are submerged in a solution typically containing 1 μM of DA in PBS. As a control, before and after each DA experiment, the experimental conditions are run in a solution of PBS buffer without DA to detect possible chemical and electronic artifacts contributing to the signal. Figure 2a shows a representative electrochemical color plot of the signal collected at the inner (FSCV) microelectrode in a solution containing 1 μM of DA in PBS. Figure 2c shows a representative electrochemical colorplot of the signal collected at the inner electrode in a PBS solution. Comparing Figure 2a to 2c, features are obvious when the potential of the outer electrode is increased from 0 V to 1.0V in a DA containing solution. (The outer electrode potential is shown above the color plots.) Throughout this work a background voltammogram is taken at a time corresponding to a minimal current at the DA oxidation peak potential and subtracted from the rest of the data. This aids in

visualization of the variations; however, the quantity measured is a difference (vide infra) and thus unaffected by this choice. Note that plotting the data this way results in features that look very similar to traditional bolus injections of DA, but are not. In fact these features correspond to changes in local DA and DOQ concentrations due to the potential applied to the outer electrode. This assignment was confirmed by the CV, shown in Figure 2e. The CVs in Figure 2e were collected at the positions highlighted with the dotted line in Figure 2a and c. Note the CV for PBS without DA is almost featureless. Utilizing the described procedures a sensor can be used for tens of hours before it fails.

The color plot for the DA containing solution illustrates a periodic signal with the current magnitude increasing when the outer electrode is increased to the stripping potential. Figure 2b illustrates the peak DA oxidation current collected at the inner microelectrode as a function of time (the solid line in Figure 2a). In addition, the potential of the outer electrode is superimposed on to aid understanding (dotted line Figure 2b). The current versus time plot for the PBS solution is included, Figure 2d, for completeness. In Figure 2b, Roman numerals highlight the positions used to interpret the data during the accumulation and stripping portions of the outer electrode cycles. Highlighted in Figure 2b with Roman numerals and black circles are: (I to II) a pulsatile increase in the local concentration of DOQ and DA due the increased potential on the outer electrode, (II to III) diffusion and oxidation at the outer electrode rapidly decreasing the local concentration of DA, (III to I) after the stripping step, the outer electrode is returned to an accumulation potential which temporarily decreases the local DA concentration as it re-adsorbs to the outer electrode and then a slow and constant recovery in the local DA concentration. The difference between the maximal current at the E_p following the stripping, (51.8 nA at II) and the current measured immediately before the stripping step (2.2 nA at I), is denoted ΔI (in this case $\Delta I = 49.6$ nA) and with calibration can be used as a measure of background DA concentration. In practice each value of ΔI reported in this work is an average over at least three consecutive cycles of the outer electrode waveform.

Figure 2f illustrates the proposed mechanism of the sensor for monitoring the background concentration of DA. Figure 2f (top) illustrates the accumulation step where the potential of the outer electrode is held at a low potential, E_{acc} , for a specified period of time, t_{acc} . This accumulation step enables DA adsorption to carbon surfaces.^{[15][16]} Subsequently, the potential is stepped to a high potential, E_{stp} , to strip off the DA from the outer electrodes. E_{stp} oxidizes the adsorbed DA to dopamine-ortho-quinone (DOQ). The DOQ then desorbs and diffuses away from the outer electrode increasing the local concentration DOQ. The inner microelectrode continuously measures the local concentration of DOQ and DA using background subtracted FSCV. The DOQ is reduced by and adsorbs to the FSCV electrode during the holding time in between scans when the holding potential is -0.4 V vs. Ag/AgCl. The resulting FSCV signal is such that a pulsatile increase in DOQ appears similar to a DA bolus. The change in signal at the inner electrode due to the application of E_{stp} to the outer electrode can then be converted into a concentration for DA, using a calibration. Our data suggests that the similarity of the DOQ signal to a DA bolus is because of adsorption/oxidation kinetics of DA and DOQ at the electrode surface and is in accordance with previous research on DA/electrode kinetics.^[10]

Effect of DA concentration

In order to calibrate the sensors, the dependence of the ΔI on DA concentration was investigated. Figure 3a shows the concentration dependence of four sensors. Points are averages between at least three consecutive ΔI and bars are standard deviations. All sensors show very similar responses with ΔI increasing in a non-linear fashion with the concentration. There is some small variation in the magnitude of the response between

devices, which is likely due to variability in the production process. Comparison between devices is facilitated through normalization of ΔI . Figure 3b shows $\Delta I/\Delta I_{5\mu M}$ for the four sensors displayed in 3a, where the ΔI at various concentrations is normalized to the ΔI at 5 μM . The points represent the mean ΔI of all four sensors and the error bars the standard deviation. The nonlinear dependence of ΔI with concentration can be explained by desorption/adsorption of DA to the outer electrodes as they are cycled from E_{acc} up to E_{stp} and E_{stp} down to E_{acc} respectively. At low concentrations, adsorption sites are more readily available and so the slope of the curve is relatively larger. At higher concentrations, most of the adsorption sites on the surface are occupied by DA and the curve levels off in accordance with site saturation. The concentration range of 200 nM to 5 μM represents the middle to lower range of sensitivity for the device. Although 200 nM is larger than the expected low nM levels of tonic DA, it is expected that the sensitivity of this proof of concept device can be improved if needed, for example by decreasing the distance between outer and inner electrodes (currently 8 μm).

The magnitude of the ΔI signals ranges between approximately 10 nA for 200 nM to 55 nA for 5 μM (Figure 3b). The noise level for the current system resides within ± 0.5 nA and so the signal to noise ratio for I_p is not a concern at these concentrations. The heterogeneity between sensor pairs is not especially significant ($\sim 20\%$) and so for clarity only normalized and then averaged data will be presented.

Device response as a function of holding time and potential

In an effort to improve understanding of the underlying physics governing operation of the sensors and as a possible avenue to improving sensitivity we investigated how ΔI varied as a function of E_{acc} , E_{stp} and t_{acc} , and the results are presented in Figure 4. The response for each data set was normalized to the value of ΔI for the standard waveform parameters described in the experimental section and a DA concentration of 1 μM , which is denoted as ΔI^* in Figure 4. The point used for normalizing each data set is highlighted by a circle in Figure 4a, b, and c. Figure 4a shows normalized average $\Delta I/\Delta I^*$ responses from four different electrodes as E_{acc} was varied from -0.6 V to 0.6 V in 0.2 V intervals. The stripping potential was $E_{stp} = 1.0$ V, time held at the accumulation potential was $t_{acc} = 15$ s, time at the stripping potential was $t_{stp} = 5$ s. Data shown are the mean and error bars are standard deviation of the four devices. $\Delta I/\Delta I^*$ decreased with more positive holding potentials with the greatest decrease recorded between 0 V to 0.2 V. This trend is explained by the decrease in the adsorption of DA to the outer electrode during accumulation. The adsorption of DA to carbon microelectrodes is known to be a function of potential.^[11] DA is positively charged at the PBS buffer pH of 7.4 by protonation of the amino group.^[10b] As the outer electrodes are held at increasing positive potentials (>0 V) during the accumulation step, electrostatic repulsion decreases the amount of DA adsorbed and thus lowers the magnitude of $\Delta I/\Delta I^*$. The electrostatic interaction between holding potential and side chain charge affects the adsorption of catechols to a carbon electrode.^[10a, 13a] Surprisingly, there is no significant change in the magnitude of $\Delta I/\Delta I^*$ for $E_{acc} < 0$ V. Investigation into the adsorption of DA at carbon fiber microelectrodes has shown increasing adsorption in this range^[11, 16], suggesting that mass transfer limited DA adsorption in this system is causing this trend.

Figure 4b shows the change in $\Delta I/\Delta I^*$ as E_{stp} was varied from 0.2 V to 1.2 V in 0.2 V steps. The accumulation potential, accumulation time and stripping time were held constant, $E_{acc} = 0$ V, $t_{acc} = 15$ s, and $t_{stp} = 5$ s. $\Delta I/\Delta I^*$ increases from 0.2 to 0.6 V, levels off between 0.6 to 1.0 V, and increases once again when increasing the peak potential from 1.0 to 1.2 V. The increase in $\Delta I/\Delta I^*$ from $E_{stp} = 0.2$ to 0.6 V can be explained by the increased desorption of DA from the outer electrodes and some DA oxidation as the electrode potential approaches the peak oxidation potential of DA. At the potentials greater than 0.6 V vs Ag/AgCl, the peak oxidation potential of DA, it is expected that most of the DA oxidizes to DOQ and

desorbs from the outer electrode. Potential ranges beyond 1.2 V were not investigated as they lead to surface chemistry instabilities^[13a] which could convolute the signal.

To further evaluate the effect of DA adsorption on the magnitude of the current signal, the holding time, t_{acc} , was varied. Accumulation potential, stripping potential and stripping time were held constant, $E_{acc} = 0$ V, $E_{stp} = 1$ V, and $t_{stp} = 5$ s. $\Delta I/\Delta F^*$ increased non-linearly with increasing holding time over in the tested time range (Figure 4c). This dependence can be explained by the DA adsorption to the outer electrode carbon surface. Time spent at the holding potential allows for DA in bulk solution to diffuse to and adsorb at sites on the outer electrode. At the longer holding times the DA desorption/adsorption is approaching equilibrium for the given solution concentration, resulting in $\Delta I/\Delta F^*$ magnitude leveling off with increasing time. Reducing the holding time reduces the time in which DA accumulates by mass transfer and adsorption, resulting in a smaller $\Delta I/\Delta F^*$. Note that since the same conditions were used to normalize the data in Figure 4a, 4b and 4c the magnitude of $\Delta I/\Delta F^*$ can be directly compared. For accumulation times greater than fifteen seconds $\Delta I/\Delta F^*$ continues to increase. This result indicates that saturation of the adsorption sites on the electrode was not the cause of the observed plateau in Figure 4a. If site saturation were the cause of this plateau, then signals greater than the reference point should not be possible, but are clearly present in 4b ($V_{stp} = 1.2$ V) and 4c ($t_{acc} = 25, 55$ seconds). This result supports the conclusion that mass transfer limited DA adsorption to the outer electrode for holding potentials less than zero volts.

Interference of Ascorbic Acid

Ascorbic acid (AA) is a readily oxidizable species commonly found in biological tissue. AA is typically found in relatively large concentration (hundred micromolar) in tissue and is a common interferent during electrochemical studies in tissue. Electrochemical signal from AA could interfere with the DA signals recorded with our sensor. In order to test the effects of AA on the tonic sensor, the response of the sensor was tested with **200 nM of DA** with and without **200 μ M of AA**. Figure 5 shows the average cyclic voltammograms collected during the experiment for DA with and without AA. The dotted lines in Figure 5 correspond to the standard deviations of the collected signal for four sensors. As expected this CV confirms the presence of DA. While some differences in the CV are apparent at upper oxidative potentials, the CV is unaffected in the range of the oxidation potential for DA (~ 0.6 V vs. Ag/AgCl), and so ΔI should be relatively unaffected. Indeed, four devices were tested and no significant difference ($p = 0.271$) was observed between the ΔI response with and without AA, $\Delta I = 9.98 \pm 0.29$ nA and $\Delta I = 10.5 \pm 0.38$ nA, respectively. This result suggests that AA will not interfere with our measurements. As with traditional FSCV measurements, multidimensional statistical methods such as principle component analysis can be utilized to further improve selectivity^[17].

CONCLUSIONS

These proof of concept studies show the operation of a sensor that enables slowly varying concentrations of an electroactive species to be quantified using selective and responsive FSCV. These studies demonstrate that an electroactive species, DA, can be adsorbed to an electrode and then rapidly released by varying the potential of that electrode. The desorbed species, in this case DOQ, causes a change in the local concentration that can be measured by an independent microelectrode in close proximity using FSCV. Interference from ascorbic acid is shown to be negligible for these sensors, an important consideration for future *in vivo* measurements. These studies represent a first step to creating an electrochemical sensor for measuring the basal tone or tonic concentrations of an electroactive biochemical in tissue in real time. Future research will focus on improving

sensitivity of the device and optimizing potential waveforms for *in vivo* measurements of tonic DA.

Acknowledgments

The authors acknowledge financial support from the National Institutes of Health grant MH092786. The authors would like to thank Drs. R.M. Wightman and Martin Edwards for helpful discussions.

References

1. Robinson DL, Hermans A, Seipel AT, Wightman RM. Chem Rev. 2008; 108:2554–2584. [PubMed: 18576692]
2. Schultz W, Apicella P, Ljungberg T. Journal of Neuroscience. 1993; 13:900–913. [PubMed: 8441015]
3. Owesson-White CA, Roitman MF, Sombers LA, Belle AM, Keithley RB, Peele JL, Carelli RM, Wightman RM. J Neurochem. 2012; 121:252–262. [PubMed: 22296263]
4. Delforge J, Bottlaender M, Pappata S, Loc'h C, Syrota A. Journal of Cerebral Blood Flow and Metabolism. 2001; 21:613–630. [PubMed: 11333372]
5. Shou M, Ferrario CR, Schultz KN, Robinson TE, Kennedy RT. Analytical Chemistry. 2006; 78:6717–6725. [PubMed: 17007489]
6. Watson CJ, Venton BJ, Kennedy RT. Analytical Chemistry. 2006; 78:1391–1399. [PubMed: 16570388]
7. Jaquins-Gerstl A, Michael AC. Journal of Neuroscience Methods. 2009; 183:127–135. [PubMed: 19559724]
8. Heien MLAV, Khan AS, Ariansen JL, Cheer JF, Phillips PEM, Wassum KM, Wightman RM. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:10023–10028. [PubMed: 16006505]
9. Hermans A, Keithley RB, Kita JM, Sombers LA, Wightman RM. Analytical Chemistry. 2008; 80:4040–4048. [PubMed: 18433146]
10. a) Bath BD, Martin HB, Wightman RM, Anderson MR. Langmuir. 2001; 17:7032–7039. b) Bath BD, Michael DJ, Trafton BJ, Joseph JD, Runnels PL, Wightman RM. Analytical Chemistry. 2000; 72:5994–6002. [PubMed: 11140768]
11. Heien M, Phillips PEM, Stuber GD, Seipel AT, Wightman RM. Analyst. 2003; 128:1413–1419. [PubMed: 14737224]
12. Ranganathan S, McCreery R, Majji SM, Madou M. Journal of the Electrochemical Society. 2000; 147:277–282.
13. a) Takmakov P, Zachek MK, Keithley RB, Walsh PL, Donley C, McCarty GS, Wightman RM. Analytical Chemistry. 2010; 82:2020–2028. [PubMed: 20146453] b) Zachek MK, Takmakov P, Moody B, Wightman RM, McCarty GS. Analytical Chemistry. 2009; 81:6258–6265. [PubMed: 19552423]
14. Zachek MK, Park J, Takmakov P, Wightman RM, McCarty GS. Analyst. 2010; 135:1556–1563. [PubMed: 20464031]
15. Kopanica R, K M. Pure & Applied Chemistry. 1989; 61:97–112.
16. Heien MLAV, Phillips PEM, Stuber GD, Seipel AT, Wightman RM. Analyst. 2003; 128:1413–1419. [PubMed: 14737224]
17. a) Heien MLAV, Johnson MA, Wightman RM. Analytical Chemistry. 2004; 76:5697–5704. [PubMed: 15456288] b) Keithley RB, Heien ML, Wightman RM. Trac-Trends in Analytical Chemistry. 2009; 28:1127–1136.

Highlights

- Designed and built a sensor to measure slowly changing or background levels of dopamine
- Demonstrated the sensor's operation and performance
- Determine the mechanism that governs the signal generated by the sensor
- Demonstrated that ascorbate does not interfere with sensor's operation

Figure 1.

A schematic depicting the geometry and operation of a microfabricated microelectrode sensor. (a) Top-down and cross-sectional view of the sensor. The working electrodes are shown in striped grey and comprise of three parallel bands ($100 \times 10 \times 0.4 \mu\text{m}$ each) separated by 8 μm gaps. The outer electrodes flank the inner electrode and are controlled by a single lead. (b) Bright field microscopy image of the sensor, scale bar (white) is 100 μm .

Figure 2.

FSCV signal collected during operation of the sensor in PBS solutions. (a) Background-subtracted FSCV color plot over several outer electrode stripping cycles in 1 μM DA solution. Top shows the waveform applied at the outer electrode. (b) Current vs. time trace of the color plot (black solid line, left axis) overlaid with potential vs. time at the outer electrode (dotted line, right axis). Stepping the outer electrode to a positive potential (I to II) induces a spike in oxidation current while stepping back to a lower potential (III) decreases current followed by slow recovery. The difference in current from I to II is defined as ΔI . (c) Background-subtracted FSCV color plot over several outer electrode stripping cycles in PBS solution sans DA. (d) Same as 2b in PBS solution sans DA. (e) Background-subtracted cyclic voltammogram of an individual pulse at location II in 2b for 1 μM DA solution (dashed line) and PBS solution sans DA (dotted line). All electrode potentials reported versus Ag/AgCl. (f) Schematic representation of sensor operation. FSCV is continuously applied at the center electrode to monitor changes in DA. The outer electrode influences local DA concentration by alternating between a low accumulation potential and a high stripping potential. The resultant difference in the FSCV signal between the two stages, ΔI , is concentration dependent.

Figure 3.

ΔI as a function of DA concentration. (a) Non-normalized ΔI magnitudes for four electrode pairs. Points are averages between several consecutive ΔI for a given electrode and bars are standard deviation. (b) ΔI over various DA concentrations normalized to ΔI at $5\ \mu\text{M}$. Points are averaged between four electrode pairs and error bars are standard deviation.

Figure 4.

Normalized signal, $\Delta I / \Delta I^*$, as a function of outer electrode stripping waveforms in PBS solution with 1 μ M DA. ΔI^* is the circled data point in each graph and refers to ΔI recorded with a standard outer electrode waveform ($E_{acc} = 0$ V, $t_{acc} = 15$ s, $E_{stp} = 1$ V, $t_{stp} = 5$ s) for comparison between variables. Points are averaged between four electrode pairs and error bars are standard deviation. Insets illustrate the parameters varied. (a) ΔI vs. accumulation potential, E_{acc} , at the outer electrode. (b) ΔI vs. stripping potential, E_{stp} , at the outer electrode. (c) ΔI vs. accumulation time at the outer electrode, t_{acc} . All electrode potentials reported versus Ag/AgCl.

Figure 5. Average cyclic voltammograms at two conditions: (1) 200 nM DA and (2) 200 nM DA with 200 μM ascorbic acid. Dotted lines represent standard deviation ($n=4$). Electrode potentials reported versus Ag/AgCl.