

## Specific Oxygen-Containing Functional Groups on the Carbon Surface Underlie an Enhanced Sensitivity to Dopamine at Electrochemically Pretreated Carbon Fiber Microelectrodes

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The in vivo use of carbon-fiber microelectrodes for neurochemical investigation has proven to be selective and sensitive when coupled with background-subtracted fast-scan cyclic voltammetry (FSCV). Various electrochemical pretreatments have been established to enhance the sensitivity of these sensors; however, the fundamental chemical mechanisms underlying these enhancement strategies remain poorly understood. We have investigated an electrochemical pretreatment in which an extended triangular waveform from  $-0.5$  to  $1.8$  V is applied to the electrode prior to the voltammetric detection of dopamine using a more standard waveform ranging from  $-0.4$  to  $1.3$  V. This pretreatment enhances the electron-transfer kinetics and significantly improves sensitivity. To gain insight into the chemical mechanism, the electrodes were studied using common analytical techniques. Contact atomic force microscopy (AFM) was used to demonstrate that the surface roughness was not altered on the nanoscale by electrochemical pretreatment. Raman spectroscopy was utilized to investigate oxide functionalities on the carbon surface and confirmed that carbonyl and hydroxyl functional groups were increased by electrochemical conditioning. Spectra collected after the selective chemical modification of these groups implicate the hydroxyl functionality, rather than the carbonyl, as the major contributor to the enhanced electrochemical signal. Finally, we have demonstrated that this electrochemical pretreatment can be used to create carbon microdisc electrodes with sensitivities comparable to those associated with larger, conventionally treated cylindrical carbon fiber microelectrodes.

### 1. Introduction

Carbon fiber microelectrodes are commonly employed to quantify rapid dopamine fluctuations both in vitro<sup>1–3</sup> and in vivo.<sup>4,5</sup> These electrodes have proven to be particularly useful in biological applications because of their biocompatibility, high tensile strength, low cost, wide potential window, and inert nature.<sup>6–8</sup> Although carbon materials have been extensively investigated for their distinct electronic and chemical properties, carbon fibers are significantly less well characterized than macro carbon electrode materials including highly ordered pyrolytic graphite and glassy carbon electrodes.<sup>8</sup>

The manufacturing of carbon fibers generally results in graphitic sheets that radiate out from the center of the fiber (radial type), align in concentric circles (onion type), or distribute randomly (random type) throughout the fiber.<sup>8</sup> The basal plane

forms the backbone of the graphitic lattice, and the edge plane contains a significant population of oxygen-containing functional groups.<sup>8</sup> Importantly, the final orientation of the graphitic structure largely determines the electrochemical performance. Because the edge plane of the graphitic sheet is more reactive than the basal plane, the desired shape of an electrode dictates the appropriate carbon fiber for a given application.

Many types of signal-improvement schemes have previously been used to enhance the electrochemical properties of carbon, including laser activation,<sup>9–11</sup> surface cleaning,<sup>12,13</sup> flame etching,<sup>14</sup> and electrochemical conditioning.<sup>15–19</sup> Of these,

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electrochemical methods are particularly common because of the facile and practical nature of this type of enhancement strategy. The sensitivity and selectivity of carbon fiber microelectrodes conditioned with extended wavelimits have been demonstrated in several published studies, both in vitro<sup>17,19</sup> and in vivo,<sup>19–21</sup> in which dopamine was detected in the presence of multiple interferents. A variety of spectroscopic techniques including X-ray photoelectron spectroscopy (XPS),<sup>22</sup> thermal desorption mass spectrometry,<sup>23</sup> enzyme-immobilized fluorescence microscopy,<sup>24–26</sup> and optical spectroscopy<sup>27</sup> have provided a general understanding of carbon surface functionality after electrochemical pretreatment of macro carbon electrode materials. Overall, the data suggest that electrochemical conditioning improves the response of the electrode by increasing the population of oxygen-containing functional groups on the carbon surface. However, the complex and dynamic nature of carbon surfaces precludes generalization across carbon materials, and the specific chemical mechanisms underlying electrochemical enhancement strategies remain poorly understood.

In this work, we describe a specific chemical mechanism underlying the enhanced adsorbance and sensitivity of carbon fiber microelectrodes conditioned with extended voltage scans prior to the detection of endogenous catecholamines using FSCV. AFM and Raman spectroscopy were used to demonstrate that electrochemical conditioning with a commonly used waveform increases carbonyl and hydroxyl group surface coverage without increasing nanoscale surface roughness. Upon conditioning with extended wavelimits to optimize sensitivity, the hydroxyl groups on the carbon surface were more extensively developed. The conditioning treatment described herein can be used to generate microdisc electrodes with a sensitivity approaching that of much larger, cylindrical microelectrodes. This treatment provides a viable means to achieve improved spatial resolution for measurements in discrete microenvironments such as in brain tissue, with an electrode geometry that can be more easily multiplexed into compact carbon arrays for simultaneous measurements in multiple channels.

## 2. Experimental Section

**2.1. Chemicals.** All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO) unless otherwise specified and were used without additional processing. All electrochemical experiments were carried out in physiological pH 7.4 buffer solution (15 mM Tris, 3.25 mM KCl, 1.20 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 2 mM Na<sub>2</sub>SO<sub>4</sub>, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, and 145 mM NaCl). All aqueous solutions were made using doubly distilled deionized water (Barnstead EasyPure II, Dubuque, IA).

**2.2. Electrode Fabrication.** Elliptical disk microelectrodes with a polished angle of 15° were fabricated using P-55s pitch-based carbon fibers, and cylindrical microelectrodes were fabricated using T-650 carbon fibers (Cytec Industries, Inc., Woodland Park, NJ) as previously described.<sup>13</sup> A Ag/AgCl reference electrode was employed (World Precision Instruments, Inc., Sarasota, FL).

**2.3. Flow Injection.** A syringe pump (New Era Pump Systems, Inc., Wantagh, NY) supplied a continuous buffer flow of 1 mL/min across the working and reference electrodes. Single 2 s bolus injections were accomplished with a six-port HPLC valve and air actuator controlled by a digital valve interface (Valco Instruments Co., Inc., Houston, TX). The working electrode was positioned in a custom electrochemical cell using a micromanipulator (World Precision Instruments, Inc., Sarasota, FL). The flow-injection apparatus was housed within a custom Faraday cage.

**2.4. Electrochemical Data Acquisition.** The triangular detection waveform ranged from −0.4 to +1.3 V,<sup>28–31</sup> and the electrochemical pretreatment waveform ranged from −0.5 to +1.8 V. These were output using a custom-built instrument for potential application to the electrochemical cell and current transduction (University of North Carolina at Chapel Hill, Department of Chemistry, Electronics Facility). TH-1 software (ESA, Chelmsford, MA) was used for waveform output with a DAC/ADC card (NI 6251 M). A second card (NI 6711) was used to trigger the DACs and ADCs and for synchronization of the electrochemical experiment with flow injection. Signal processing (background subtraction, signal averaging, and digital filtering (4-pole Bessel Filter, 2 kHz) was software-controlled.

Simulations of cyclic voltammograms were generated using DigiSim version 3 (Bioanalytical Systems, Inc., West Lafayette, IN).

**2.5. Surface Analysis.** AFM of the carbon surface was carried out with a Veeco D3000 microscope (Plainview, NY) using tips from Nanosensors (PPP-HCH, Neuchatel, Switzerland) with a nominal resonance of 280 kHz. Raman experiments were performed with a previously described custom-built spectrometer.<sup>29</sup> Spectra with a collection time of 120 s were obtained with a dry 100× objective while the electrode surface was submerged in a physiological pH 7.4 Tris buffer solution.

**2.6. Chemical Derivatization.** Surface derivatization of electrochemically pretreated electrodes with 2,4-dinitrophenylhydrazine (DNPH) (Alfa Aesar, Ward Hill, MA) was accomplished following the procedure of Fryling et al.<sup>27,32</sup> An equimolar ratio of concentrated HCl and KOH (Thermo Fisher Scientific, Inc., Pittsburgh, PA) was used as a Lucas test reagent.<sup>33</sup> Electrochemically pretreated electrodes were saturated for 1 min in the Lucas test solution. Structures were drawn using ChemDraw Ultra v11.0.

**2.7. Statistics.** Data are presented as the mean ± standard error of the mean. One-tailed Student's *t* tests were used to determine significance of means. Analysis of covariance (ANCOVA) was used for the comparison of linear regression

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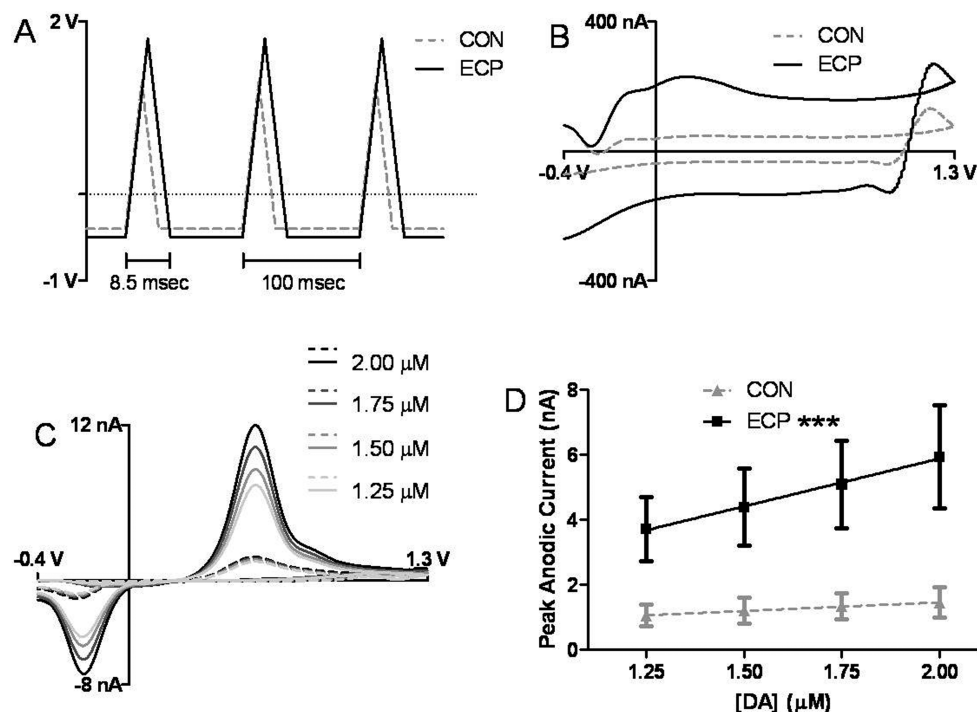
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**Figure 1.** Electrochemical pretreatment enhances electrode sensitivity. (A) Comparison of the electrochemical waveforms, applied at 400 V/s every 100 ms. The electrochemical pretreatment waveform ranged from  $-0.5$  to  $+1.8$  V. The control waveform used for electrochemical detection ranged from  $-0.4$  to  $+1.3$  V. (B) Representative background cyclic voltammograms collected at a single electrode, demonstrating the change in nonfaradaic charging current that results in response to electrochemical pretreatment. (C) Representative background-subtracted cyclic voltammograms collected at one electrode in response to varying concentrations of DA before (---) and after (—) electrochemical pretreatment. (D) Calibration curve that demonstrates increased sensitivity after pretreatment ( $n = 6$ ,  $p < 0.001$ , ANCOVA).

results. In Figure 6, data were analyzed using one-way ANOVA with Tukey's post hoc test for repeated measurements. In all cases, the statistical significance is designated as  $p < 0.05$ . Statistical and graphical analyses were carried out using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA).

### 3. Results and Discussion

**3.1. Electrochemical Pretreatment Enhances Electrode Sensitivity.** A freshly polished electrode was used to detect various concentrations of dopamine before and after the application of facile electrochemical pretreatment with FSCV. This experimental design allowed for the comparison of data at a single electrode, thus eliminating inconsistencies resulting from electrode-to-electrode variability.

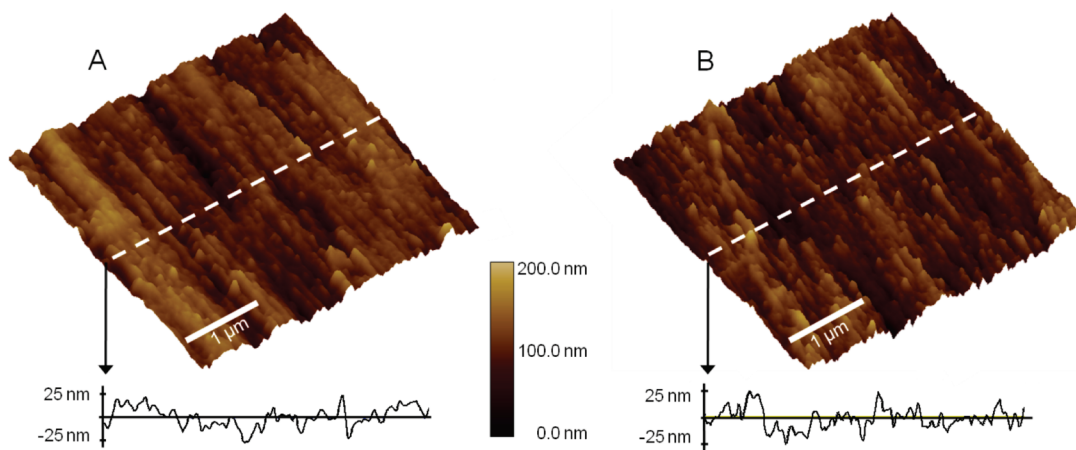
Figure 1A illustrates the waveforms used for detection and pretreatment, applied at a scan rate of 400 V/s and a frequency of 10 Hz. A waveform that is regularly used *in vivo*, ranging from  $-0.4$  to  $+1.3$  V, was applied for approximately 10 min until the electrode stabilized. Herein, this waveform will be termed the control waveform (CON), and it was used for the initial detection of dopamine. A pretreatment waveform (ECP) ranging from  $-0.5$  to  $+1.8$  V was subsequently applied for 5 min as a treatment that was intended to build surface oxides for improved electrochemical performance. Finally, the control waveform was again applied for at least 10 min to reestablish a stable background current prior to the final detection of dopamine. Figure 1B reveals the nonfaradaic background current generated in response to the application of each of these waveforms, showing a 3.6-fold increase in the capacitive charge upon the pretreatment process. Figure 1C demonstrates representative CVs for the detection of various dopamine concentrations at a single electrode before (---) and after (—) electrochemical pretreatment. Obvious increases in

the peak anodic and cathodic currents are evident after pretreatment. In Figure 1D, peak anodic currents were plotted against a range of physiological dopamine concentrations, and the 5.6-fold increase in the slope demonstrates that the electrochemical pretreatment significantly ( $p < 0.001$ , ANCOVA) increased the sensitivity for the detection of dopamine ( $n = 6$ ). The normalized sensitivities (accounting for electrode area) were  $0.17 \pm 0.01 \text{ mA } \mu\text{M}^{-1} \text{ cm}^{-2}$  prior to pretreatment and  $0.97 \pm 0.04 \text{ mA } \mu\text{M}^{-1} \text{ cm}^{-2}$  after conditioning. The sensitivity for the more commonly used cylindrical carbon fiber microelectrode was  $0.621 \text{ mA } \mu\text{M}^{-1} \text{ cm}^{-2}$  ( $n = 4$ ), a value not significantly different from that of the pretreated disk electrode ( $p > 0.05$ , ANCOVA).

To rule out the possibility that the increased signal inherent to an ECP-conditioned electrode was due solely to increased electrochemical cycling time (rather than to the extended wavelimits of the ECP waveform), a control experiment was conducted with a separate set of electrodes ( $n = 4$ ). For this group, the ECP conditioning period was substituted with a period of conditioning using the control waveform; however, the wavelimits were not extended. An average signal of  $0.9 \pm 0.1 \text{ nA}$  was measured for  $1 \mu\text{M}$  dopamine both before and after this conditioning, demonstrating that this additional cycling time did not improve the sensitivity to this analyte ( $p > 0.05$ , one-tailed paired  $t$  test). Thus, it was concluded that signal enhancement after ECP conditioning resulted from the extended wavelimits of the applied ECP waveform.

**3.2. Atomic Force Microscopy.** Because the electrochemical background current scales with electrode area and the application of the electrochemical pretreatment waveform increased the capacitive background current (Figure 1B), the enhanced electrochemical sensitivity may have resulted from an increase in electrode surface area. However, no obvious differences in the





**Figure 2.** Electrochemical conditioning does not alter surface roughness. Representative AFM images collected at (A) an unconditioned carbon microdisk electrode and (B) an electrode conditioned with the ECP waveform, with associated line plots. Surface roughness values were not significantly different between the two groups ( $n = 4$ ,  $p > 0.05$ , Student's  $t$  test).

electrode surface area or fiber diameter were detected with optical or scanning electron microscopy (SEM) when control electrodes were compared to those that were pretreated with the ECP waveform (data not shown). Thus, contact AFM of the carbon electrode surface was carried out to determine if the nanoscale surface roughness was altered by electrochemical pretreatment. The first sample set ( $n = 4$ ) established a baseline roughness for polished electrodes prior to the application of any waveform. The second sample set ( $n = 4$ ) was composed of polished electrodes that were cycled with the ECP waveform for 5 min.

Figure 2A,B shows representative AFM images and line plots from these sample sets. Quantitative analysis of these data indicated that the rms roughness values for the two surface treatments were  $13.8 \pm 0.6$  nm for a polished electrode and  $13.9 \pm 0.2$  nm for an ECP-cycled electrode. These values were not significantly different ( $p > 0.05$ , one-tailed  $t$  test). Thus, the electrode surface topography at this size scale did not play a significant role in enhancing the electrochemical signal.

**3.3. Adsorption Isotherm.** It has been previously reported that during the voltammetric detection of dopamine at carbon fiber microelectrodes, dopamine adsorbs to oxide groups on the carbon surface.<sup>12,19</sup> Thus, the increase in sensitivity resulting from electrochemical pretreatment may have been due to an increase in the number of adsorption sites on the carbon surface for dopamine. Therefore, the amount of adsorbed analyte on the carbon surface was determined before and after the electrochemical pretreatment. The equation for peak current due to an adsorbing species

$$i_p = (9.36 \times 10^5) n^2 \nu A \Gamma_{DA} \quad (1)$$

defines how the peak anodic current ( $i_p$ ) is proportional to both the surface coverage of the analyte ( $\Gamma_{DA}$ ) and the surface area of the electrode ( $A = 3 \times 10^{-6}$  cm<sup>2</sup>).<sup>34</sup> Given that the surface area of the electrode was not significantly altered by the pretreatment process, the number of electrons transferred ( $n = 2$ ), and the unchanged scan rate ( $\nu = 400$  V/s), it follows that the adsorbing qualities increased because of the electrochemical pretreatment. To investigate this further, a Langmuir isotherm was constructed to quantify the surface coverage of adsorbed dopamine. An adsorption isotherm is a method of relating the concentration

of analyte in the bulk solution around an electrode, the coverage of the analyte adsorbed to the electrode surface, and the electrical state of the system at a given temperature.<sup>34</sup> Langmuir isotherms are governed by the equation

$$\frac{\Gamma_{DA}}{\Gamma_s - \Gamma_{DA}} = \beta[DA] \quad (2)$$

where  $\beta$  is a constant,  $[DA]$  is the concentration of dopamine in the bulk solution, and  $\Gamma_s$  is the saturation coverage of dopamine on the electrode surface. This equation reduces the number of variables by assuming no interactions between adsorbed species, a homogeneous electrode surface, and at high concentration the ability to form a monolayer.<sup>34</sup> It has been shown that at low concentration the equation for the Langmuir isotherm can be further reduced to a simplified form, where  $b = \beta\Gamma_s$ , by assuming that  $\Gamma_{DA}$  is small in comparison to  $\Gamma_s$ .<sup>35,36</sup>

$$\Gamma_{DA} = b[DA] \quad (3)$$

The peak anodic currents for varying concentrations of dopamine were measured before and after electrochemical pretreatment. These experimentally measured currents are attributed to the sum of the diffusive and adsorptive currents at the electrode surface.<sup>12</sup> Thus, a purely diffusion-controlled environment was digitally simulated, and the current due to diffusion was subtracted from the experimental current to resolve the contribution from adsorption alone. Using eq 1, the surface coverage of adsorbed dopamine was extracted from the diffusion-corrected experimental data and plotted versus concentration (Figure 3).

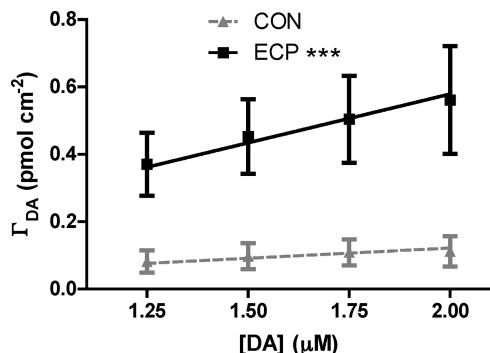
The 8-fold increase in the slope after the application of ECP indicates that increased adsorption of dopamine to the electrode surface significantly ( $p < 0.001$ , ANCOVA) contributes to the enhanced electrochemical signal ( $n = 6$ ). These data are consistent with previous reports demonstrating that increased adsorption at the electrode surface increases sensitivity.<sup>35–37</sup>

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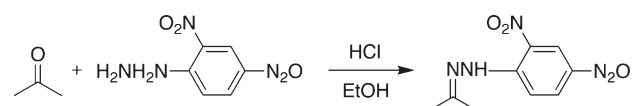
**Figure 3.** Electrochemical pretreatment increases the adsorption of dopamine to the carbon microelectrode surface. The Langmuir isotherms show increased surface coverage of DA at electrochemically pretreated electrodes, as compared to those conditioned with the control waveform ( $n = 6$ ,  $p < 0.001$ , ANCOVA).

**3.4. Raman Spectroscopy of a Carbon Fiber Microelectrode Surface.** The population of oxide-containing functional groups on the electrode surface may have been altered by the application of the electrochemical waveform. Indeed, carbon surfaces are prone to reactions with  $O_2$  and  $H_2O$  to form various oxygen-containing functional groups,<sup>8</sup> and the application of an extended electrochemical waveform to a bundle of carbon fibers has been shown to increase the oxygen-to-carbon ratio, as observed with XPS.<sup>38</sup> Here, Raman spectroscopy was used to determine the specific surface species responsible for enhanced dopamine adsorption to the carbon fiber surface. This method, which relies on the inelastic scattering of monochromatic light to provide information on molecular structure, was chosen for the exquisitely sensitive and nondestructive nature of the visible laser along with the ability to make measurements in situ through physiologically buffered solution.<sup>39</sup>

McCreery et al. have done extensive work using Raman spectroscopy to investigate graphitic and glassy carbon electrode surface structure, contributing greatly to the fundamental characterization of these carbon surfaces.<sup>8</sup> However, carbon subtypes are highly variable in bulk and surface chemical properties and cannot be generalized. In this work, spectra were collected to investigate the surface functional groups that develop on the surface of P-55 carbon fiber microelectrodes in response to electrochemical pretreatment. All measurements were performed in buffer solution. Consistent with previously published spectra for highly ordered pyrolytic graphite electrodes, three prominent Raman peaks were noted at 1340, 1610, and 1655  $cm^{-1}$  (Figure 4A).<sup>40</sup> The peak at 1340  $cm^{-1}$  was used in data normalization because this band is reliably stable and inherent to the graphitic lattice.<sup>40</sup> Figure 4A shows the averaged spectra for freshly polished electrodes ( $n = 3$ , gray) and for electrodes conditioned with the control CON ( $n = 3$ , black) and ECP ( $n = 3$ , red) electrochemical waveforms. Figure 4B is an enlargement of the 1500–1750  $cm^{-1}$  range. Application of the control waveform increased the intensity of the peaks at 1610 and 1655  $cm^{-1}$  as compared to an electrode that had no waveform applied. Electrodes conditioned with the ECP waveform exhibited a modest increase in Raman intensity at 1610  $cm^{-1}$  as compared to an

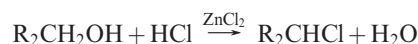
electrode that had no conditioning; however, the intensity did not reach the value measured at electrodes conditioned with the control waveform. In contrast, the intensity of the 1655  $cm^{-1}$  peak exceeded that collected at a control electrode conditioned with the control waveform, and this peak shifted to a slightly higher wavenumber. This distinct shift is likely due to the increased intercalation of small anions, cations, and neutrals between graphite layers as the surface oxides are increased.<sup>41</sup> A similar band has previously been observed for intercalation compounds and oxidized  $sp^2$  carbon as the result of an altered electronic environment and increased asymmetry at the carbon surface.<sup>42–48</sup>

Because XPS studies have shown that carbonyl and hydroxyl groups are the predominate surface oxide functionalities on carbon,<sup>49,50</sup> Raman spectroscopy was used to investigate electrochemically pretreated electrodes after selective derivatization of the surface. DNPH was used to target carbonyl groups selectively, forming a hydrazone derivative on the carbon surface.<sup>33</sup> The following condensation reaction illustrates the product of the addition of DNPH.



The resulting spectra showed a marked reduction in the 1610  $cm^{-1}$  peak, but the 1655  $cm^{-1}$  peak remained relatively unchanged (Figure 4C, blue,  $n = 6$ ) as compared to that of nonderivatized ECP-conditioned electrodes (red). This suggests that the 1610  $cm^{-1}$  peak is largely due to the presence of surface carbonyl groups, and the selective removal of these groups allows the shoulder at 1655  $cm^{-1}$  to become more apparent.

A classic condensation reaction was used to target hydroxyl functionalities selectively on the carbon surface. Organic chemistry dictates that an acid anhydride forms a covalent ester bond with alcohols but not with carbonyl groups.<sup>33</sup> Thus, the final sample set consisted of ECP-conditioned electrodes that had subsequently undergone the Lucas test for hydroxyl groups according to the following condensation reaction.



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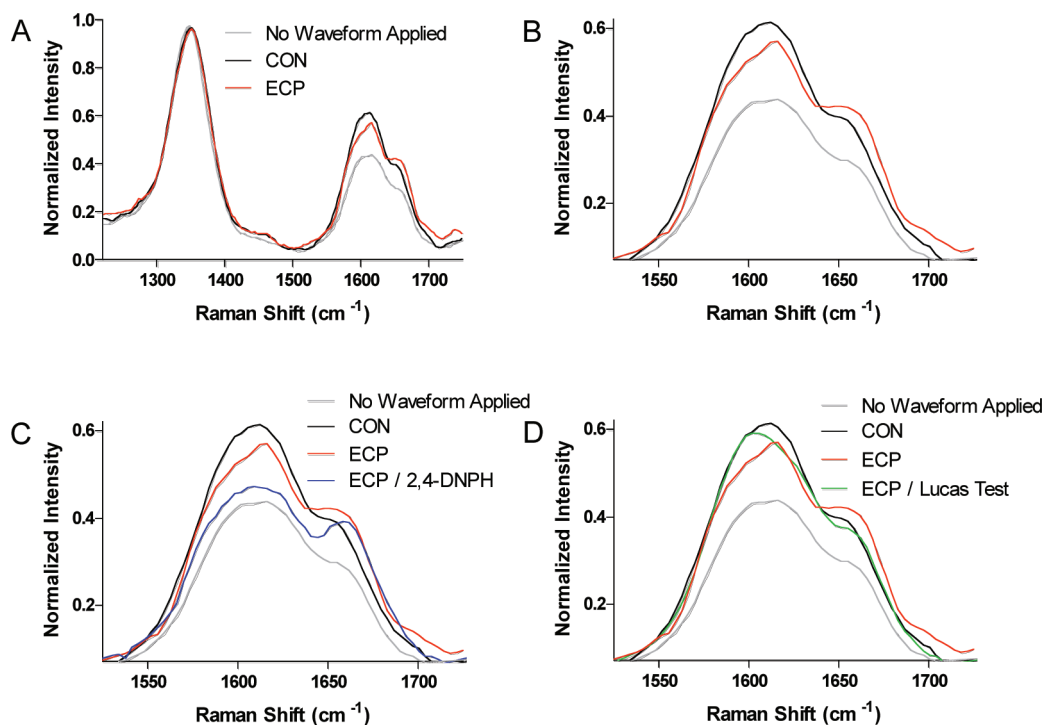
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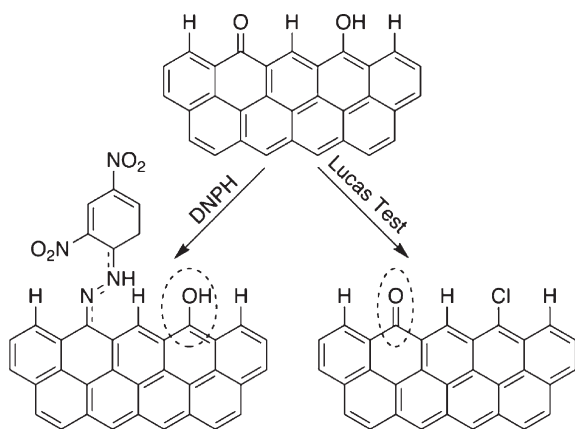
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**Figure 4.** Electrochemical conditioning increases surface carbonyl and hydroxyl groups. Averaged Raman spectra collected at the surface of carbon fiber microelectrodes, normalized to the  $1340\text{ cm}^{-1}$  peak. (A) Three prominent peaks of interest were noted in spectra collected from all sample sets: electrodes that had not been conditioned, electrodes conditioned with the control waveform (CON), and those conditioned with the electrochemical pretreatment (ECP) waveform. (B) Expanded view of a portion of plot A demonstrating the variation in the  $1610$  and  $1655\text{ cm}^{-1}$  peaks that results upon electrochemical conditioning. (C and D) Spectra collected after the specific derivatization of surface oxygen-containing functional groups. ( $n = 6$  for all spectra.)



**Figure 5.** Chemical derivatization of oxidized, edge-plane graphitic carbon at a microelectrode surface. Derivatization using DNPH specifically targets carbonyl groups (left). Condensation of the surface hydroxyl group using the Lucas test (right). Dashed ovals highlight the oxygen functionalities remaining after chemical modification.

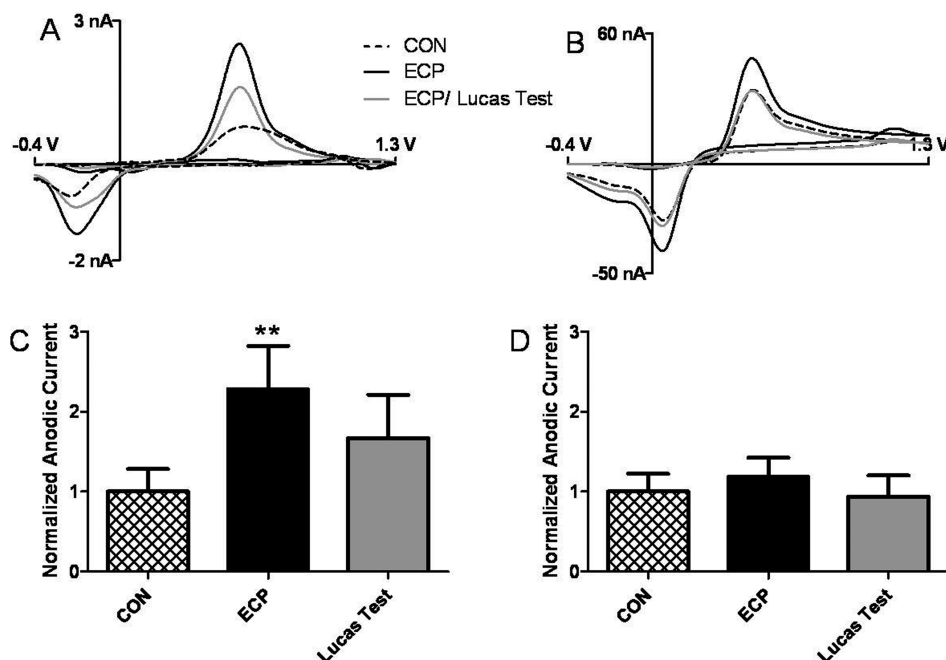
This modification was designed to remove the surface hydroxyl groups from an electrochemically pretreated electrode (Figure 5).

The Raman spectra for this sample set (Figure 4D, green,  $n = 6$ ) showed a marked reduction in the  $1655\text{ cm}^{-1}$  peak when compared to that of nonderivatized ECP-conditioned electrodes. This resulted in a spectrum that was strikingly similar to that acquired for the set of electrodes conditioned with the control waveform alone. Thus, the  $1610$  and  $1655\text{ cm}^{-1}$  peak intensities were largely attributed to the surface carbonyl and hydroxyl groups, respectively. The data are consistent with previously reported XPS spectra collected at a bundle of carbon fibers demonstrating that

the carbonyl surface functionality dominated upon application of lower potentials; however, the hydroxyl group population developed as the applied potential increased to approximately  $3.0\text{ V}$ .<sup>38</sup> Overall, these data indicate that the application of the ECP waveform selectively increases surface hydroxyl group coverage, and the formation of these hydroxyl functionalities significantly contributes to the enhanced electrochemical sensitivity to dopamine.

**3.5. Electrochemical Characterization of Chemically Modified Electrodes.** To verify this hypothesis electrochemically, electrodes were initially conditioned using the control waveform. Next, dopamine and ferrocenecarboxylic acid (FCA) were detected using background-subtracted FSCV before and after electrochemical pretreatment and again after subsequent derivatization of surface hydroxyl groups. FCA was chosen because it undergoes well-characterized, highly reversible oxidation. The detection of the more adsorbing species, dopamine, should be enhanced to a greater extent by oxide functionalities on the carbon surface.

Figure 6 illustrates the results of the detection of  $1\text{ }\mu\text{M}$  dopamine (Figure 6A,C) and  $1\text{ mM}$  FCA (Figure 6B,D). As expected, the electrochemical pretreatment significantly ( $p < 0.001$ ) increased the electrochemical signal for dopamine ( $n = 7$ ). Subsequent derivatization of the hydroxyl functionalities on the electrode surface significantly attenuated the signal enhancement; however, enhancement was not fully abolished. This is most likely due to the regeneration of surface hydroxyl groups upon renewed electrochemical cycling using the control waveform.<sup>36</sup> The electrochemical pretreatment process did not significantly improve the signal in response to FCA ( $p > 0.05$ ,  $n = 7$ ), a species whose electrochemical detection is largely diffusion-controlled (Figure 6C,D). The heterogeneous electron-transfer



**Figure 6.** Hydroxyl groups on the carbon fiber surface gained by electrochemical pretreatment with extended wavelimits underlie signal enhancement in the detection of DA. (A, B) Representative cyclic voltammograms for 1  $\mu$ M DA and 1 mM FCA, respectively, before and after the removal of hydroxyl groups using the Lucas test. (C, D) Normalized peak anodic currents for 1  $\mu$ M DA and 1 mM FCA, respectively, before and after the Lucas test. ( $n = 7$ ,  $p < 0.001$ , ANOVA.)

rate constants ( $k^0$ ) for the electrochemical detection of dopamine and FCA at pH 7.4, before and after pretreatment, were determined by digital simulation. For dopamine, there was a 2-fold increase in  $k^0$  for the electrochemical pretreatment process. For FCA, there was no significant change in the rate constant. This finding is in agreement with the results of previous studies that demonstrate enhanced electron transfer at electrochemically treated versus freshly cleaved carbon fiber microdisk electrodes, particularly in the oxidation of cationic catechols.<sup>18,51</sup>

#### 4. Conclusions

The results show that electrochemical pretreatment of a pitch-based P-55s carbon fiber microelectrode can be used to increase electrode sensitivity, creating an improved tool for electrochemical measurements in small microdomains. The electrochemical pretreatment described herein results in an increased population of surface oxides consisting largely of carbonyl and,

particularly at higher pretreatment potentials, hydroxyl functional groups that underlie an improved adsorptivity of dopamine on the carbon surface. Boasting a 5-fold increase in peak anodic current, nearly a 6-fold increase in sensitivity, and faster electron-transfer kinetics for the detection of dopamine, a pretreated carbon microdisk electrode offers a competitive alternative to a larger, cylindrical carbon fiber microelectrode for single-channel measurements and an optimized geometry that permits multiplexing into closely spaced multiple electrode arrays.

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