

Novel Modifications to Carbon-based Electrodes to Improve the Electrochemical Detection of Dopamine

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Abstract: In this work, we describe three simple modifications to carbon electrodes which were found to improve the detection of an *exemplar* neurotransmitter (dopamine) in the presence of physiological interferents (ascorbic acid and/or uric acid). First, the electro-oxidation of ascorbic acid, as a pretreatment, at boron-doped diamond electrode (BDE) interfaces is studied. This treatment did suppress the detection of ascorbic acid oxidation signal, but only in a manner suitable for single-use detection of high concentrations of dopamine (i.e. $> 1 \mu\text{M}$). Second, the hydrogenation of BDE by electrochemical cathodic treatment and plasma hydrogenation was investigated. Large cathodic, applied potentials (i.e. $> -5 \text{ V}$) and hydrogen plasma pre-treatment of BDE lead to the *partial* and *complete* oxidization of ascorbic acid before dopamine, respectively. The consequence at hydrogen-plasma treated BDE is the *complete* electrochemical separation of these two species without any typical catalytic reactions between the analytes. Third, the modification of glassy carbon electrodes with carbon black nanoparticles is explored. This modification enables the

simultaneous detection of ascorbic acid, dopamine and uric acid, significantly enhancing the sensitivity of dopamine. Dopamine was best detected using the unconventional route of detecting 5,6-dihydroxyindole, which is made possible by use of carbon-black nanoparticles. The potential of all three studied modifications to be of electro-analytical use is highlighted throughout this work.

1. INTRODUCTION

Living cell-solid state device interfacing is anticipated to be one of the key bio-electronic synergies of the 21st century, especially in the fields of smart sensing, biotechnology, communications and medicine.¹⁻³ One of the most fascinating topics in this area is *neuron interfacing* – the coupling of electronic devices to neurons.⁴⁻⁷ It offers the possibility for future non-invasive long-term neural signal recording from multiple neurons within artificial or natural neural networks. Neurotransmitters (e.g. dopamine, norepinephrine, serotonin, etc.) are extremely important to neuronal signaling. The relative amounts of neurotransmitters are known to be related to a variety of neurological conditions and diseases. *In-situ* and real-time quantification of neurotransmitters by a neuronal-solid-state device would revolutionise research in neurological diseases and their treatments.⁸⁻⁹

A neuronal solid-state device based on electrochemistry is a promising approach. The detection of various neurotransmitters, such as dopamine¹⁰⁻¹⁵, serotonin¹⁶, norepinephrine¹⁷⁻¹⁸, adenosine¹² is possible on electrochemical solid-state devices based on carbon-based electrodes^{10-11, 17-22}, metal electrodes²³⁻²⁷, silicon electrodes^{7, 28-29}, polymer-modified electrodes^{14, 30}, and porphyrin-modified electrodes.³¹⁻³² At present, the major hurdle in this area, aside from the questions of electrode stability and biocompatibility, is the detection of neurotransmitters in the presence of physiological interferents. Neurotransmitters and organic interferents oxidise at similar potentials, which impede the detection of the neurotransmitter *via* unwanted chemical effects (e.g. catalysis, complexation).¹⁵ For the detection of dopamine

(DA), a catecholamine neurotransmitter, interferents such as ascorbic acid (AA) and uric acid (UA) impede the electroanalysis of DA in physiological conditions due to two main issues. First, AA, UA, and DA oxidize at similar potentials. Second, oxidized DA is reduced by unoxidized AA and then re-oxidized at electrode surface.¹⁵ These two issues influence the accuracy and precision of the electrochemical response of DA.

Boron doped diamond electrode (BDE) is a strong candidate to address this problem with a number of advantages including biocompatibility, robustness, conductivity, chemical inertness, availability in a range of sizes from the macro to nano-scales, and relatively low-cost compared to novel metal-based materials.³³⁻³⁵ However, it is not a good choice in its unmodified form for the detection of DA due to such electrode being unable to electrochemically separate DA from organic interferents in solution.^{14, 21, 36} A further modification is usually requested. For instance, diamond interfaces have been variously modified to achieve this aim by polymer films^{14, 30, 37}, self-assembled monolayers³⁸⁻³⁹, metal catalysts⁴⁰, electrochemical treatment^{11, 41}, oxygen plasma⁴², hydrogen plasma⁴³⁻⁴⁴, carbene chemistry⁴⁵, wet chemical methods⁴⁶. However, extra introduced modifiers enhance the uncertainty of detection of neurotransmitters under physiological environment, such as lower biocompatibility and unknown reaction with other physiological species. Therefore it is of great interest to investigate a method to activate diamond interface without introducing modifiers to detect DA from the interferents of AA and UA.

Another novel idea is to detect DA outside the potential range that DA, AA and UA would overlap each other. It is reported that redox peaks of 5,6-dihydroxyindole (DHI), which is generated from the cyclisation, oxidation and rearrangement of dopamine-quinone, were observed at multi-walled carbon nanotubes (MWCNTs) -poly electrode, f-MWCNTs-poly films electrode and carbon nanotubes-ionic liquid gel modified electrode.⁴⁷⁻⁵⁰ Detecting DHI provides a possible approach to determine DA because the DHI concentration is

proportional to the DA concentration in the solution, and the low detection potential can avoid the interference from AA and UA. However, only a few papers are concerned about the redox reaction of DHI, and to date, no sensor has been reported to determine DA via DHI. The reason may be that the peak current of DHI decayed quickly in continuous voltammetric scans, and most in situ DA determinations have to take place in a neutral solution where the electrochemical response of DHI is weak. Carbon black (CB) stands out as a good candidate to solve these problems, in the light that CB is well-known for its strong adsorption of organic species therefore could enhance the electrochemical response. Together with other advantages like low cost and simple modification procedure, carbon black becomes a promising alternative to other modified sensors.

In this work, we investigated three possible and facile modifications to diamond electrodes to improve the detection of an *exemplar* neurotransmitter (dopamine) in the presence of interferents (ascorbic acid and/or uric acid). First, the electro-oxidation of ascorbic acid at BDE interfaces is studied here for the first time, inspired by previous studies showing pre-treatment in ‘organic media’⁵¹⁻⁵² (e.g. ethanol, quinizarin) to usefully modify diamond electrode surfaces. Second, the hydrogenation at BDE by electrochemical cathodic treatment and plasma hydrogenation was investigated.⁵³ Third, the modification of glassy carbon electrodes with carbon black nanoparticles is explored. To-date none of these modifications has been studied for the detection of neurotransmitters.

2. METHODS

Materials All chemicals were purchased from Sigma Aldrich, unless otherwise noted. Fresh solutions of *L*- ascorbic acid, dopamine hydrochloride, and uric acid were prepared daily in phosphate buffers (pH 7.4, 0.1 M) prepared from $K_2HPO_4 \bullet 3H_2O$ (Sigma Aldrich), KH_2PO_4 (Sigma Aldrich), and deionized, distilled water (dd- H_2O ; MilliQ, Q-Guard-1 Filter). Contamination was removed from electrodes by *Aqua Regia*, which was prepared by mixing

a 1:3 ratio (v/v) of nitric and hydrochloric acid (both from Sigma Aldrich). Piranha solution was made from a 3:1 (v/v) solution of concentrated H₂SO₄ (Fischer Scientific) and dilute H₂O₂ (30%, Alfa Aesar).

Electrochemical Methods Throughout this work, experiments were conducted using a three-electrode electrochemical cell, which had a working electrode, a platinum counter-electrode and an Ag/AgCl reference electrode. These were all connected to a computer-controlled potentiostat (PGSTAT128N, Metrohm Autolab UK) with the General Purpose Electrochemical System software (v. 4.9).

Polycrystalline, boron-doped diamond electrodes (Element 6, 10 mm x 10 mm x 0.6 mm, [B]>10²⁰ cm⁻³) were used as the working electrode. These electrodes were mounted in a PTFE holder, with exposed electrode area of 0.071 cm². Prior to use, the diamond electrodes were polished with alumina powder slurry (particle size: 1 μm, followed by 0.3 μm). Electrodes were then cleaned by ultrasound in dd-H₂O and acetone. Unless otherwise indicated, boron-doped diamond electrodes were electrochemically cleaned by cyclic voltammetry (− 1.7 V and 2.5 V in 0.1M HNO₃, 15 scans, scan rate: 0.1V s⁻¹). Electrodes prepared in this manner are referred to as unmodified BDE, notwithstanding atmospheric surface oxidation after BDE is cleaned. Electrochemical measurements were performed at ≈ 20°C. Cyclic voltammetry (CV) was performed by using a scan rate of 0.1 V s⁻¹. Square-wave voltammetry (SWV) was employed using the following parameters: 10 mV amplitude, 1 mV step potential, and frequency of 10 Hz.

The Monarch 430 carbon black (Cabot Corporation, CB M 430, diameter 27±10 nm) was obtained from James M Brown Ltd (Staffordshire, UK). Carbon black suspension was prepared by adding 1 mg powder into 1 mL milli-Q water, and ultrasonicing for 1 hour before use. The particle size in the suspension was determined to be about 32 nm. 10 μL carbon materials suspensions were drop-coated onto glassy carbon electrode with surface area of

0.0707 cm², and dried in the fume hood to form a carbon black-glassy carbon electrode (CB-GCE). The amount of the carbon materials on the surface is about 0.01 mg.

A homemade electrochemical cell was created using the methods of Hoffmann *et al.*⁵³ They showed that boron-doped diamond electrodes can be hydrogen terminated by applying negative potentials in acidic media. Some minor modifications were made by us: first, platinum deposition onto the working electrode was prevented by surrounding the Pt counter-electrode with a Vycor porous glass membrane (BAS Inc.) closed off at one end by Araldite glue; second, tweezers were used to hold the glass membrane and prevent cracking by thermal expansion; third, the cathodic pretreatment was applied using two power supplies, connected in series, capable of applying –35 V for 3 minutes to the working electrode; fourth, the surface area exposed to the treatment was 0.071 cm². No Pt deposition occurred when modifying BDE using this apparatus, as confirmed by the lack of hydrogen evolution peaks near 0.2 V for any cathodic BDE scanned in 0.1 M HNO₃.^{53,54}

X-ray Photoelectron Spectroscopy (XPS): XPS experiments were conducted using an home-built X-ray photoelectron spectrometer at 10^{–9} – 10^{–10} Torr, using an Al K α source (1486.6 eV) at 10 kV anode potential and 10 mA emission current. A Fixed Analyzer Transmission mode was used to obtain spectra, using a pass energy of 50 eV or 25 eV, for survey and core level XPS scans. All peak fitting was done using XPS Peak Fit (v. 4.1) software using Shirley background fits, with peak areas normalized to their atomic sensitivity factors.⁵⁵ XPS peaks were calibrated to the C1s peak at 285.0 eV. The reported peak area ratios have an error of 10 - 15%.⁵⁶

Contact Angle Measurements: Contact angle measurements were conducted using pendant droplets of distilled water delivered to the electrode surface via a gas-tight glass syringe operated by a dc motor drive. The sample and the surrounding air were held constant at 20 \pm 0.1°C in a thermostatically controlled box, using a circulating water bath. The internal

contact angle of six droplets at differing locations at the electrode was measured from digital photographs originating from a CCD camera with a telecentric lens aligned on an optical bench. The average contact angle and standard deviation is reported here.

3. RESULTS AND DISCUSSION

3.1 Modification of Boron-doped Diamond by Organic Media

Before considering the electrochemical effect of modifiers, the response of unmodified BDE towards DA and AA must be established. Cyclic voltammograms of 0.1 mM DA and 0.1 mM AA in phosphate buffer at unmodified BDE are shown in Fig. 1A (i and ii). The first oxidation potentials occur at 0.59 V for DA ($E_{pa, DA}$) and 1.1 V for AA ($E_{pa, AA}$), as expected. There is a second oxidation peak for DA at 1.2 V. Unmodified BDE has higher selectivity for DA oxidation ($2.89 \times 10^{-5} \text{ A cm}^{-2}$) than for AA oxidation ($9.72 \times 10^{-6} \text{ A cm}^{-2}$). These results are consistent with the known electrochemistry of DA and AA at unmodified BDE in physiological conditions.^{15,10,20,32} The second oxidation peak of DA is likely a further oxidation of dopamine-o-quinone (DA-o-quinone), dopamine semi-quinone (DAsemi-quinone), or some other oxidation products of DA. Mixed solutions of AA and DA in phosphate buffers mimicked physiological conditions, where AA:DA ratio is at least 10:1. Unmodified BDE exposed to a mixture of 0.1 mM DA and 1 mM AA detects both as a single oxidation wave at $\approx 0.5 \text{ V}$ ($E_{pa, AA/DA}$), with current larger than that for AA or DA alone (see Fig. 1A, iii). It demonstrates that unmodified BDE cannot detect DA and AA together. In the absence of AA, $i_{pa, DA}$ increases with increasing [DA], having a linear response of $20.5 \mu\text{A mM}^{-1}$ ($R^2 = 0.99$ and $N=4$, see Fig. 1B and its *inset*). Increasing [AA] with fixed [DA] enhances $i_{pa, AA/DA}$ by $15.6 \mu\text{A mM}^{-1}$ ($R^2 = 0.98$ and $N=5$, see *inset* to Fig. 1C); this particular current response is due either to the direct consequence of increasing [AA], to the regeneration of DA from its oxidation products *via* an EC' mechanism with unoxidized AA,

or both.¹⁵ The above results emphasize the known difficulty of detecting DA in the presence of AA at physiological conditions.

Electrochemical pre-treatment of BDE in an organic media is a simple strategy to modify its surface chemistry and electronic behavior.⁵¹⁻⁵² Here, BDE was pre-treated using anodic potential (+2V) for 20 seconds in 'organic media' consisting of 0.1 mM AA in a phosphate buffer of pH 7.4 (*method label*: +2VAA). As shown in Fig. 1D, this modification does not affect the cyclic voltammetric current of DA oxidation significantly, compared to the unmodified BDE. Meanwhile, the normal detection of AA oxidation at unmodified BDE occurs at 1.1 V, but this is not possible after +2VAA pre-treatment and is only regained after rinsing the modified electrode with water, as shown in Fig. 1E. Therefore, the +2VAA pre-treatment causes the electrode to become inactive towards AA oxidation. The above suggests that the +2VAA pre-treatment creates a weakly bound layer of oxidized AA which was easily rinsed off, that effectively block AA oxidation signal.

The stability of the oxidized AA layer can be appreciated by comparing Figures 1F and 1G. When a +2V AA pre-treated electrode is used to detect AA without any prior rinsing with water (Fig. 1F) one notices that a stable AA oxidation signal at 1.1 V is seen only after the first voltammetric scan. By contrast, when a +2VAA pre-treated electrode is rinsed prior to the measurement (Fig. 1G) AA is detected on the first scan, with voltammetric current decreasing upon subsequent scans towards a stable current. In both cases, the stable current is comparable to simple fouling of BDE by AA. This comparison highlights the weakly bound nature of the AA overlayer at the +2VAA pre-treated electrode.

Square wave voltammetry results in Fig. 1H show the detection of DA at BDE using the +2V AA method pretreatment, exposed to solutions containing a fixed [AA] of 1 mM with varied [DA] (from 0 to 10 μ M). At low [DA] the oxidation peak is at a potential expected for AA. Then, as [DA] increases the potential of this peak moves negatively, along with a

progressively smaller peak current. At higher [DA], the DA oxidation peak appears at the expected DA oxidation potential with a peak current that increases proportionally to [DA]. These changes happen despite constant [AA] in the electrolyte. These results suggest that the AA-modifier to BDE is not suitable for electroanalysis of DA in the presence of AA at low [DA]. However, it is suitable when [DA] is high, consistent with the idea that AA is blocked from oxidizing at the interface.

The results of Fig. 1H are similar to those reported at other BDE interfaces. Kondo *et al.*¹⁵ observed a similar effect at carboxyl-terminated diamond using differential pulse voltammetry. They explained this effect as being a result of AA acting as a sensitizer for DA detection, in which unoxidised AA undergoes EC' catalysis with a product of DA oxidation (DA semi-quinone) to produce additional detectable DA. The results of Roy *et al.*¹⁴ for poly (*N,N*-dimethylaniline) modified BDE are similar to those presented in Fig. 1H in that AA signal is observed to decrease in the presence of increased [DA]. They ascribed this to the polymer acting as a physical barrier and/or electrostatically repelling AA, with the result that AA has a lower diffusion rate than DA.

3.2 Hydrogenation of Diamond Electrodes: Cathodic and H-Plasma Treatments

3.2.1 Cathodic Pre-Treatments

BDEs can be oxidised by low, positive potentials to exhibit effects on the separation of AA and DA oxidation signals.^{11, 14, 42, 57} However, the detection of the true AA or DA current is not possible, as this pre-treatment does not eliminate the EC' mechanism between these two species in mixed solutions. Thus, the electroanalysis of DA at oxidized BDE is unreliable. Pre-treatment using negative potentials at BDE for AA and DA separation has been reported, but not widely adapted.⁵⁷⁻⁵⁸ This particular pre-treatment is simple and introduces little contamination to the diamond surface, making it an attractive modification

for neurotransmitters detection in physiological environments.

A comparison between BDE having electrochemical pre-treatments with positive or negative potentials was made. BDE were modified by applying several positive (anodic) or negative (cathodic) potentials for 3 minutes in HNO_3 or H_2SO_4 , respectively, as the latter is a better reducing agent than the former. Fig. 2A and 2B respectively show the results of this comparison for the individual detection of DA and AA. In both figures, the +2V pre-treatment did not cause a significant shift of $E_{\text{pa,AA}}$ or $E_{\text{pa,DA}}$, compared to unmodified BDE. In contrast, the -2, -5 and -10V pre-treatments lowered $E_{\text{pa,AA}}$ from 0.96V to 0.55 V, 0.46 V, and 0.27 V, respectively; $E_{\text{pa,DA}}$ was lowered from 0.66 V to 0.52 V, 0.34 V, and 0.38 V, respectively. In addition, ever increasing cathodic pre-treatment lowers $E_{\text{pa,AA}}$ to ever lower potentials, while $E_{\text{pa,DA}}$ remains at a relatively fixed potential. (This useful trend may realize the ideal situation of $E_{\text{pa,AA}} < E_{\text{pa,DA}}$.) Also, in Fig. 2A, one observes the peak-to-peak separation for the DA redox pair at cathodic BDE to be smaller than that observed at +2V pre-treated BDE. All the above results are consistent with DA and AA oxidation being more favorable at cathodic BDE than at unmodified or +2V modified BDE. None of the above results are affected by Pt deposition from the counter electrode during cathodic pre-treatment⁵³, as confirmed by the lack of hydrogen evolution peaks near 0.2 V for any cathodic BDE scanned in 0.1 M HNO_3 .⁵⁴

Thus far, the trends suggest that AA oxidation could be shifted by applying even higher cathodic potentials to BDE during pre-treatment. This can be done by using the method of Hoffmann *et al.*⁵³ Their methodology was replicated using a home-made electrochemical cell as described in the experimental section. This highly cathodic pre-treatment is capable of inducing dramatic changes in surface hydrophobicity. Diamond electrodes oxidized in aqua regia were conducted, with resultant contact angles $< 5^\circ$ in all cases. After electrodes were modified by applying -35 V for 3, 9, or 20 minutes leading to

increased contact angle (respectively, $53.8^{\circ} \pm 11.8^{\circ}$, $58.8^{\circ} \pm 4.4^{\circ}$, $63.8^{\circ} \pm 3.0^{\circ}$). The higher cathodic pre-treatments impart ever more uniform hydrophobicity to the diamond surface as duration increases. Lower hydrophobicity is seen here than that achieved by Hoffmann *et al.*⁵³, which may reflect a lesser degree of hydrogenation in this work. A 3 minute pre-treatment at -35V has shifted $E_{\text{pa, AA}}$ and $E_{\text{pa, DA}}$ to 0.45 V and 0.25 V respectively in the absence of the other species. As seen in Fig. 2C, this modified electrode detects both species in a mixed solution of 0.1 mM DA and 1 mM AA as a single broad oxidation peak at 0.25 V . This represents a further decrease of $E_{\text{pa, AA/DA}}$ compared to BDE modified using smaller cathodic potentials and this is a significant shift compared to the position of $E_{\text{pa, AA}}$. However, our results do not show conclusively that the complete electrochemical separation of AA and DA occurs at cathodic treated BDE.

Hydrogen-plasma (HP) modification of diamond is known increase sub-surface hydrogen content and produce defect free, clean and hydrophobic BDEs, which is thought to lead have electrochemical advantages, particularly for the detection of negatively charged organic molecules in physiological conditions^{53, 59-60} These properties and observations motivate the study of this straightforward modification to BDEs here. Diamond electrodes were first cleaned by CV in 0.1 M HNO_3 and exposed to high temperature, hydrogen plasma (H_2 gas, 45 Torr , 600°C , 1100W) for 10 minutes. After treatment, the electrodes were sonicated for 30 s in methanol to remove any residual sp^2 carbon after the HP-modification.⁶⁰ Electrodes were used once and re-hydrogenated before further use. As shown in Fig. 3, the surface chemistry of hydrogenated BDE is contaminant-free. The electrodes have low oxidation content ($\text{O1s/C1s} < 0.04$). This interface is highly hydrophobic with contact angles exceeding 90° . These results are expected for uniformly hydrogen-terminated diamond surfaces.

Fig. 2C shows the results of incubating a HP-BDE in a buffer solution containing 0.1

mM DA and 1 mM AA. Here two well-separated oxidation peaks separated by more than 200 mV are observed. This separation satisfies the minimum requirements to realize the full electrochemical separation of AA and DA.¹³ The identity of these peaks was determined by SWV, as shown in Fig. 2D. The individual oxidation signal for AA and DA occurred at 0.00 V and 0.15 V, respectively. Ideally, the DA oxidation peak should not be affected by [AA]. To test this, HP-BDE was exposed to solutions containing a fixed [DA], while [AA] was varied. Fig. 2D and 4 show the relationship between $i_{pa,DA}$ and [AA] to be weak ($0.1 \mu A mM^{-1}$) compared to unmodified BDE ($15.6 \mu A mM^{-1}$). If AA does not oxidize before DA, then one would expect unoxidized AA to catalyze the reduction of oxidized DA, thereby enhancing the DA signal artificially. This is the origin of the strong relationship between $i_{pa,DA}$ and [AA] at unmodified BDE. The fact that this relationship is very weak at HP-BDE suggests that AA is fully oxidized prior to DA oxidation; there is no unoxidised AA in solution to reduce oxidation products of DA. Therefore, the EC' catalytic cycle between unoxidised AA and oxidation products of DA has been eliminated at the interface of HP-BDE. To our knowledge, this is the first time such a result has been seen on diamond modified without the addition of non-diamond material.

Hydrogen-termination of diamond surfaces by plasma modification is a superior method to detect DA in the presence of AA. This performance is likely due to clean and contaminant-free diamond surfaces, increased sub-surface concentration of hydrogen, decreased negative surface charge at physiological conditions and increased surface hydrophobicity.^{53, 60} All of these properties are known to improve electron transfer of small, negatively charged molecules at interfaces.^{44, 60-61} The improved AA oxidation kinetics at hydrogenated diamond are probably facilitated by increased hydrophobic interactions and reduced electrostatic repulsion between AA and hydrogenated surfaces.⁵⁷ As a consequence,

the elimination of the EC' reaction between AA and DA is possible at well-hydrogenated diamond electrodes, as the oxidation kinetics of AA are faster than DA.

The complete separation of AA and DA occurs on hydrogen plasma modified diamond, but only *partial* separation occurs at BDE modified with large cathodic potentials. Faster AA oxidation kinetic is observed on the 'cathodic BDE', but without the accompanying electrochemical separation of the AA and DA oxidation signal seen at HP-BDE interfaces. This observation suggests that both modifications lead to BDE being similarly hydrogenated, just not to the same extent. The extent of surface hydrogenation is likely dependent on the magnitude of the applied cathodic potential. Larger cathodic pre-treatments involving application of more cathodic potential than -35V for a longer time frame may impart more of the characteristics of a clean, hydrophobic, and hydrogenated surface to BDE, in an extremely facile manner. This study is proof-of-principle that diamond electrodes modified with large cathodic potentials and hydrogen plasma are of electro-analytical use for neurotransmitter detection.

3.3 CB-GCE

The strong ability of CB to adsorb organic molecules makes it a potential candidate to effect the separation of organic molecules from interference. CB exhibits the best conductivity among all the carbon materials⁶², with a smaller particle size⁶² and a larger surface area than graphite.⁶³ In this work, the low-cost, properties, and facile use of CB is exploited towards the detection of an exemplar neurotransmitter in the presence of interferes. Here, DA is determined at CB-GCE by two methods: first, the direct oxidation of DA at CB-GCE at $+0.15\text{ V}$ and the simultaneous determination of AA, UA and DA; second, the detection of DA *via* the determination of DHI at -0.3 V (see section 3.3.1)). It is shown that both methods exhibit good sensitivity, selectivity and detection limit.

3.3.1 DA oxidation at CB-GCE

In the absence of other species, cyclic voltammograms of AA, DA, and UA at CB-GCE in 0.1 M phosphate buffer are shown in Fig. 5, with the responses at bare GCE shown for comparison. The larger background current at CB-GCE indicates a larger double layer capacity than a bare GCE. On the bare GCE, the oxidation of AA, UA, and DA is irreversible, occurring at +0.22 V, +0.29 V, and +0.16 V, respectively. By contrast, at CB-GCE, AA, UA, and DA exhibit reversible redox behavior with peak couples at $-0.07/-0.22$ V, $+0.26/+0.25$ V, $+0.14/+0.13$ V, respectively. Another pair of peaks is only observed at CB-GCE at $-0.30/-0.31$ V, which is attributed to the DHI redox couple. The DHI is generated from the cyclization, oxidation and rearrangement of dopaminequinone, as observed and demonstrated at Multiwall Carbon Nanotubes (MWCNTs)-poly (3,5-dihydroxy benzoic acid) electrode, *f*-MWCNTs-based polymer film electrode and carbon nanotube/ionic liquid gel-modified electrode.⁴⁷⁻⁵⁰ The presence of CB leads to negative shifting of the oxidation potential of all three species, effects enhanced current densities, promotes the reduction reaction, and enables DHI to be detected.

The simultaneous detection of AA, UA, and DA are shown in Fig. 5D. This figure shows the second redox voltammetric cyclic with 2 mM AA, 0.1 mM UA and 0.1 mM DA in 0.1 M phosphate buffer at a bare GCE and a CB-GCE. These conditions mimic physiological ones (e.g. blood plasma) where the concentration of UA and DA are 20 times smaller than AA.⁶⁴ At the bare GCE, Only one broad oxidation peak is observed between 0.1 and 0.3 V, implying severe overlap of the oxidation signal of AA, DA, and UA. However, at a CB-GCE, four pairs of peaks are well-resolved. Using the results of Fig. 5A to 5D the redox couples at $-0.29/-0.31$ V, $0.03/-0.1$ V and $+0.20/+0.15$ V can be assigned to DHI, AA and DA, respectively. The oxidation peak of UA is at +0.31 V, with its broad and ill-featured

reduction wave at +0.3 V. The above demonstrates that the electrochemical redox behaviour of AA, DA, and UA can be well separated at CB-GCE.

3.3.2 DHI at CB-GCE: *via* chemical generation of DA

Detecting DHI provides a possible approach to determine DA because the DHI concentration is proportional to the DA concentration in the electrolyte, and the low detection potential can highly avoid the inference from AA and UA. However, few reports study the redox reaction of DHI and to the best of our knowledge, no sensor has been reported to determine DA *via* DHI. The lack of study concerning this approach may be linked to the fact that the peak current density of DHI decays quickly in continuous voltammetric scans and that most of *in situ* DA determination is conducted at physiologically neutral solution where the electrochemical response of DHI is weak.

Notwithstanding the above limitations, the electrochemical response of DA and DHI at a CB-GCE was further investigated here. Fig. 6A suggests that the emergence of the DHI redox couple at -0.29 and -0.31 V was only observable in the second voltammetric scan, after the oxidation of DA. This is in agreement with the known mechanism of DHI generated from oxidized DA; this DHI is then available to undergo redox reactions at CB-GCE. To further investigate this relationship between DA and DHI, cyclic voltammetry was conducted in the same buffer with ever increasing [DA] at a potential range which does not oxidize DA. The result of this experiment is shown in Fig. 6B and no distinct DHI signal can be observed. Dopamine can be introduced to this system by applying $+0.4$ V for 10 seconds as a pre-condition prior to conducting the analogous experiments as those described by Fig. 6B. The resulting profiles in Fig. 6C show a pair of peaks when DA is present having current density proportional to [DA]. From this one confirms that oxidation of DA is necessary to generate DHI at CB-GCE.

3.3.3 DA, DHI determination at CB-GCE using SWV

As noted earlier, DA can be potentially determined by both directly oxidation or via detecting DHI reduction at CB-GCE. The direct determination of varying [DA] at pH 7.4 within a potential range where only DA is oxidized is shown in Fig. 7A. In the absence of DA, no peak is detected, with a well-resolved peak at +0.15V assigned to DA oxidation emerging as [DA] increases. The associated peak current density *versus* [DA] plots is shown as an insert, with a $0.13 \mu\text{A } \mu\text{M}^{-1}$ relationship and a detection limit of $0.06 \mu\text{M}$ (3S/N).

Varying [DA] can be determined at CB-GCE *via* the DHI reduction signal. First, DA was oxidized at a CB-GCE with an applied pre-condition of +0.6 V held for 10 s in 0.1 M phosphate buffer. Square wave voltammetry (SWV) was then performed within a potential range in which DA was not oxidised, with results shown in Fig. 7B pertaining to [DA] ranging from 0 to $8 \mu\text{M}$. Dopamine is required to observe any feature *ca.* -0.3 V . A plot of this peak current density *versus* [DA] yields a linear relationship of $0.61 \mu\text{A } \mu\text{M}^{-1}$ and a calculated detection limit of $0.013 \mu\text{M}$ (3S/N) within a range of [DA] from 0.1 to $40 \mu\text{M}$. These results are superior to the method of direct DA detection discussed above.

Interference of AA and UA

The detection of DA in mixed solutions is made difficult by the presence of AA and UA at 20-1000 times higher concentration, depending on the sample type (e.g. human body, environmental).^{7, 48} Here, physiological conditions are mimicked at CB-GCE interfaces in 0.1 M phosphate buffer solutions containing 2 mM AA, 0.1 mM UA, and increasing [DA] (see Fig. 10C). As [DA] increases, the peak current density of AA and UA does not change and decreases slightly, respectively; for DA the peak current density increases and does not overlap that of AA or UA. Using the standard addition method, 0.05 mM DA was detected 3

times in solutions containing 0.05 mM UA, 1mM AA, and 10 mM AA; the results of these experiments are shown in Table 1. The recovery is 96.3% in UA and 108.6 – 113.5% in AA, thereby indicating that detection of DA at CB-GCE by this method is relatively free of interference from UA and 20 times AA. However, in large excess of AA the interference remains significant.

An analogous set of experiments was conducted to observe the influence of AA toward the redox reaction of DHI. First, a precondition of +0.6V for 10 s was applied at CB-GCE in 0.1 M phosphate buffer containing 0.05 mM DA at 0 to 10 mM AA. Cyclic voltammetry was then conducted between –0.4 to 0V (i.e. no DA oxidation). The DHI redox was observed at *ca.* –0.3 V and was found to be hardly affected by a large [AA] (see Fig. 10D). The recovery using this particular method is closer to 100% (see Table 1). This result reflects both the indirect methods improved the accuracy for the detection of DA and that it is subject to less AA interference.

As noted in Table 2, the detection limit, sensitivity and the detection range of the DA determination at CB-GCE is comparable to other DA sensors. The sensitivity of CB-GCE ($0.61 \mu\text{A } \mu\text{M}^{-1}$) is consistently higher than other electrodes ($0.03\text{-}0.23 \mu\text{A } \mu\text{M}^{-1}$). The reason is likely attributed to the strong adsorption properties of CB. Crucially, the CB-GCE detection limit matches the expected concentrations of DA for real-life applications, such as the detection of DA in human plasma ($0.23 \mu\text{M}$)⁶⁴ and the human brain ($0.02\text{-}0.2 \mu\text{M}$)⁶⁵. Such competitive performance is achieved at metal-free carbon-based electrodes, thereby lowering the cost of DA detection. However, the linear detection range of DA at CB-GCE is narrow. This is not anticipated to prevent its introduction to commercial systems, as the metabolism of DA in human beings limits $[\text{DA}] < 10 \mu\text{M}$. Moreover, the recovery by the indirect detection method presented here (*via* DHI) is less affected by AA compared to other electrodes; as shown by comparing the recovery associated with LP-CPE⁶⁶, SDS-CPE⁶⁷ and

poly(9aminoacridine) modified electrodes⁶⁸ in the range of 90-110%, with the 100-102% range associated with the indirect method. The indirect method seems to minimize the influence of the AA and DA interaction.

4. Conclusion

In this work, three methods for the improved detection of an *exemplar* neurotransmitter (dopamine) in physiological conditions at diamond and glassy carbon electrodes have been investigated. Boron-doped diamond was modified by two simple methods: the addition of a layer of organic material or by hydrogenating diamond. The addition of carbon-black nanoparticles on glassy carbon was also studied. All these modifications are metal-free, cheap, and facile compared to other carbon-based materials and composites used for electroanalysis. They are also effective at improving the detection of dopamine in the presence of interferents (ascorbic acid and/or uric acid) within physiological conditions.

Modification of diamond by anodic pre-treatment in an organic medium – a phosphate buffer containing ascorbic acid – had the advantage of suppressing the detection of AA oxidation and preferentially detecting DA in solution. However, this modification was unstable (one time use only) and was found unreliable for the accurate detection of DA at low [DA]. The hydrogenation of diamond was possible by the application of small and large cathodic potentials, as well as hydrogen plasma. All three modifications shifted the AA oxidation potential to lower potentials. Large cathodic potentials ($\leq -5\text{V}$) were able to *partially* oxidize AA before DA, thereby *partially* effecting both the separation of their electrochemical signals and the elimination of DA regeneration by unoxidised AA (*via* EC' mechanism). Hydrogen plasma pre-treatments lead to the aforementioned signal separation and the *complete* elimination of the EC' mechanism. For the first time, the complete electrochemical separation of dopamine and ascorbic acid was realized at a diamond

electrode without the addition of non-diamond material, which may otherwise affect diamond electrode biocompatibility.

Modification of diamond with carbon-black nanoparticles enabled the simultaneous detection of ascorbic acid, dopamine, and uric acid significantly enhanced sensitivity of dopamine at CB-GCE. The best method to detect DA was by an unconventional route - the detection of 5,6-dihydroxyindole – made possible by the carbon-black nanoparticles. This indirect method of detection shown here works because the redox peaks of DHI are at -0.3 V at CB-GCE, significantly lower than that of DA, AA, or UA; in addition, these peaks only are observed after the oxidation of DA. These mechanistic studies enabled k_s , α and n to be calculated for DA redox and DHI. They also showed the DHI oxidation to be a 2 electron process, but less so at high pH. The amount of CB on the electrode affected the DA and DHI response and background current, which was linked to the adsorption of organic species at CB. The detection limit of DA by the direct and indirect methods was 0.06 μM and 0.013 μM , respectively. The recoveries of DA in the presence of AA and UA were improved to the 99-101% level by use of the indirect method which proves that interference of AA and UA were eliminated to a great extent. The above demonstrates that using nano-particulate carbon black at glassy carbon effects is a useful and facile modification. The potential of all the studied modifications to be of electro-analytical use is highlighted by the results within this work.

AUTHOR INFORMATION

Author Contributions

G.W. Nelson and L. Jiang are joint first authors of this publication. The work was supervised and funded by J.S. Foord. All authors contributed and approved the final version of this manuscript.

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Figure Caption

Figure 1. (A) Cyclic voltammetry in the potential range -0.25 V to 1.5V at unmodified boron-doped diamond (BDE) in 0.1 M phosphate buffer containing: (i) 0.1 mM dopamine (DA), (ii) 0.1 mM ascorbic acid (AA), (iii) 0.1 mM DA and 1 mM AA; (B) Cyclic voltammetry in the potential range -0.25 V to 1.5V at unmodified BDE in 0.1 M phosphate buffer containing varying [DA]: (i) 0.01 mM, (ii) 0.1 mM, (iii) 0.5 mM, (iv) 1 mM. *Inset:* Current (10^{-6} A) as a function of [DA]. Current response to varying [DA] is $20.5 \mu\text{A mM}^{-1}$ ($R^2 = 0.99$); (C) Cyclic voltammetry in the potential range -0.25 V to 1.5V at unmodified BDE in 0.1 M phosphate buffer containing 0.1 mM DA and varying [AA] from (i) 0 mM, (ii) 0.1 mM, (iii) 0.5 mM, (iv) 1.0 mM and (v) 1.5 mM. *Inset:* Current response of the main oxidation peak as a function of [AA] in 0.1 M phosphate buffer containing 0.1 mM DA; (D) Cyclic voltammetry in the potential range -0.25 V to 1.5V at various electrodes in 0.1 M phosphate buffer containing 0.1 mM DA: unmodified BDE (*dashed line*); BDE modified by +2VAA pre-treatment (*solid line*); (E) Cyclic voltammetry in the potential range -0.25 V to 1.5V at various electrodes in 0.1 M phosphate buffer containing 1 mM AA: unmodified BDE (*dashed line*); BDE modified by +2VAA pre-treatment without rinsing by dd-H₂O (*solid light grey line*); BDE modified by +2VAA pre-treatment followed by rinsing by dd-H₂O (*solid black line*); Successive cyclic voltammetry in potential range of -0.25 V to 1.5V at BDE electrodes modified by the +2VAA pre-treatment, exposed to solutions of 1mM AA: (F) without rinsing with water or (G) rinsed with water. The numbers 1 to 5 in these two panels refer to the number of scans.; (H) Square wave voltammetry in the potential range -0.25 V to 1.5V at +2VAA-modified BDE in 0.1M phosphate buffer containing 0.1 mM AA and a varying [DA] from 0.0 μM to 10.0 μM . Cyclic voltammetry was performed by using a scan rate of 0.1 V s^{-1} . Square-wave voltammetry (SWV) was employed the following parameters: 10 mV amplitude, 1 mV step potential, and frequency of 10 Hz.

Figure 2. Cyclic voltammetric in the potential range -0.25 V to 1.5 V of various electrodes in 0.1 M phosphate buffers containing either dopamine (DA) or ascorbic acid (AA): **(A)** boron-doped diamond electrode (BDE) pre-treated with $+2$ V (red line), -2 V (light grey), -5 V (grey), -10 V (dark grey) in 1 mM DA. Unmodified (bare) diamond (black dashed line) in 0.1 mM DA; **(B)** BDE pre-treated with $+2$ V (red line), -2 V (light grey), -5 V (grey), -10 V (dark grey) in 1 mM AA. Unmodified BDE (black dashed line) in 0.1 mM AA. **(C)** Square-wave voltammetry of variously modified BDE electrodes in the potential range -0.2 V to 0.8 V within 0.1 M phosphate buffer solutions containing 0.1 mM DA and 1 mM AA. The pre-treatments are as follows: (i) -2 V for 3 minutes in H_2SO_4 (light grey line) , (ii) -5 V for 3 minutes in H_2SO_4 (black), (iii) -35 V applied using a homemade apparatus for 3 minutes (blue), (iv) hydrogen plasma applied for 10 minutes (green); **(D)** Square-wave voltammetry of hydrogen plasma modified BDE in the potential range -0.2 V to 0.5 V within 0.1 M phosphate buffer solutions containing: (i) 0.1 mM AA (blue line), (ii) 0.1 mM DA (red), (iii) 0.1 mM DA and 0.1 mM AA (light grey), (iv) 0.1 mM DA and 1 mM AA (grey), (v) 0.1 mM DA and 5 mM AA (black). The numerals *i* and *ii* within this panel refer to the peak positions of AA and DA, respectively. Cyclic voltammetry was performed by using a scan rate of 0.1 V s^{-1} . Square-wave voltammetry (SWV) was employed the following parameters: 10 mV amplitude, 1 mV step potential, and frequency of 10 Hz.

Figure 3. XPS Survey scan of hydrogen plasma treated diamond electrode.

Figure 4: Cyclic voltammetric response of dopamine (DA) oxidation peak current ($i_{\text{pa, DA}}$) in 0.1 M phosphate buffer containing 0.1 mM DA and varying concentration of ascorbic acid (AA) at unmodified boron-doped diamond electrodes (BDE, hollow circles) and hydrogen plasma modified BDE (10 minutes, red). The responses of $i_{\text{pa, DA}}$ to varying $[\text{AA}]$ at each

type of electrode are 15.6 and 0.10 $\mu\text{A mM}^{-1}$ respectively. Cyclic voltammetry in the potential range -0.25 V to 1.5 V was performed by using a scan rate of 0.1 V s^{-1}

Figure 5. Cyclic voltammograms in the potential range -0.4 V to 0.4 V at bare glassy carbon electrode (GCE) and carbon-black modified GCE (CB-GCE) in 0.1 M phosphate buffers containing: (A) 100 μM ascorbic acid (AA), (B) 10 μM uric acid (UA), (C) 10 μM dopamine (DA) and (D) a mixture of 100 μM AA, 10 μM UA, and 10 μM DA. Cyclic voltammetry was conducted using a scan rate of 50 mV s^{-1} . The scan rate for all cyclic voltammetry experiments was 50 mV s^{-1} .

Figure 6. (A) Cyclic voltammetry in the potential range -0.6 V to 0.6 V at carbon-black modified glassy carbon electrodes (CB-GCE) in 0.1 M phosphate buffer containing 0.1 mM dopamine (DA). The first (black line) and second (red line) scans are indicated. ; (B) Cyclic voltammetry in the potential range 0 V to -0.4 V at CB-GCE in 0.1 M phosphate buffer containing varying [DA], from 0 to 0.05 mM; (C) CB-GCE modified by an applied pre-treatment (+0.4 V) for 10 seconds: data show the results of cyclic voltammetry from 0 V to -0.4 V at these modified electrodes in 0.1 M phosphate buffers containing varying [DA], from 0 mM to 0.05 mM. The scan rate for all cyclic voltammetry experiments was 50 mV s^{-1} .

Figure 7 (A) Square wave voltammetry in the potential range 0 V to +0.4 V at carbon-black modified glassy carbon (CB-GCE) in 0.1 M phosphate buffer with continual addition of dopamine (DA) such that [DA] varies between 0 μM to 10 μM ; (B) Square wave voltammetry in the potential range 0 V to -0.6 V at modified CB-GCE in 0.1 M phosphate buffer with continual addition of DA such that [DA] varied between 0 μM to 8 μM . These

particular CB-GCE were modified by the application of a +0.6 V pre-treatment for 10 s. *Inset*: Peak current as a function of [DA]. (C) Square wave voltammetry in the potential range of 0 V to +0.4 V at CB-GCE in 0.1 M phosphate buffer containing 2 mM AA, 0.1 mM UA and continual addition of DA to change [DA] from 0 to 0.1 mM. The peak labels are: (1) ascorbic acid (AA), (2) uric acid (UA), (3) dopamine (DA). (D) Cyclic voltammograms in potential range of -0.4 V to 0 V at modified CB-GCE precondition in 0.1 M phosphate buffer exposed to varying conditions: (i) the absence of both DA and AA; (ii-v) 50 μ M DA with varying [AA] from (ii) 0 mM, (iii) 0.05 mM, (iv) 1 mM and (v) 10 mM. These particular CB-GCE were modified by an applied potential (+0.6 V) for 10 s. The scan rate for all cyclic voltammetry experiments was 50 mV s⁻¹. Square-wave voltammetry (SWV) was employed the following parameters: 10 mV amplitude, 1 mV step potential, and frequency of 10 Hz

Tables

Table 1. Recovery of dopamine (DA) at carbon-black modified glassy carbon electrode

[species] / mM	Add DA / mM	Directly oxidized		via DHI	
		Found / mM	Recovery / %	Found / mM	Recovery / %
0.05 UA	0.05	0.048	96.3	0.050	99.2
1 AA	0.05	0.054	108.6	0.051	102.7
10 AA	0.05	0.056	113.5	0.050	100.1

Notes: Labels are as follows: uric acid (UA), ascorbic acid (AA), dopamine (DA), and 5,6-dihydroxyindole (DHI). All experiments conducted in 0.1 M phosphate buffer.

Table 2. Detection limit of dopamine at various sensors

Sensors	Sensitivity / $\mu\text{M } \mu\text{A}^{-1}$	Detection range / μM	Detection limit / μM	Reference
CB-GCE	0.127	0.1-20	0.06	This work (direct)
CB-GCE	0.61	0.1-40	0.013	This work (via DHI)
CNTs-La(OH) ₃		0.5-35.4	1.67	Zhang, Y. <i>et al</i> ⁶⁹
MWNTs-OMIMPF ₆	0.23	1-100	0.1	Zhao, Y. <i>et al</i> ⁴⁷
Fc@DWNTs/GCE	0.03	0.5-20	0.3	Cheng, H. <i>et al</i> ⁷⁰
Ni/Al-LDH	0.08	10-700	5.0	Zhu, Z. <i>et al</i> ⁷¹
PtAu/GCE		24-384	5.0	
TPY/PFE		0.4-160	0.2	
Au NPs	0.04	Up to 40	0.22	Raj, C. <i>et al</i> ⁷²
CILE	-	2-150	1.0	Safavi, A. <i>et al</i> ⁷³
DA-film/Au	0.06	1-60	0.2	Luczak, T. <i>et al</i> ⁵⁰

Supporting Information

The brief explanation of mechanism of indirect detection of dopamine via 5,6-dihydroxyindole at carbon black modified glassy carbon and the influence of carbon material quantity supplied as Supporting Information

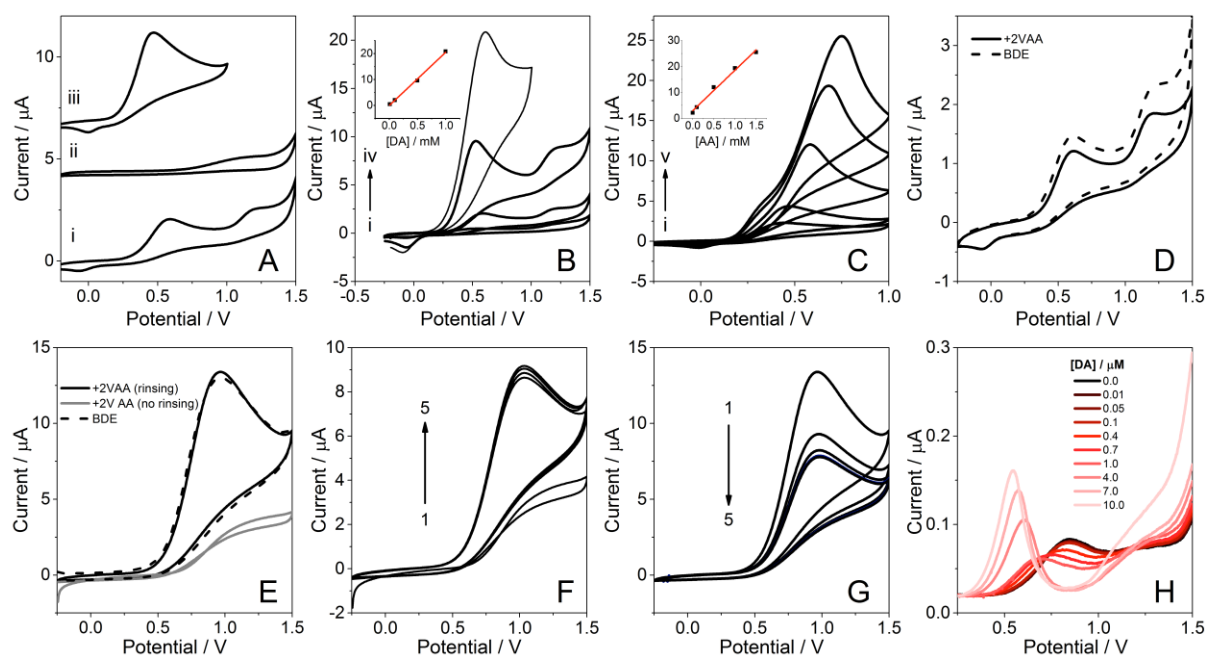


Figure 1

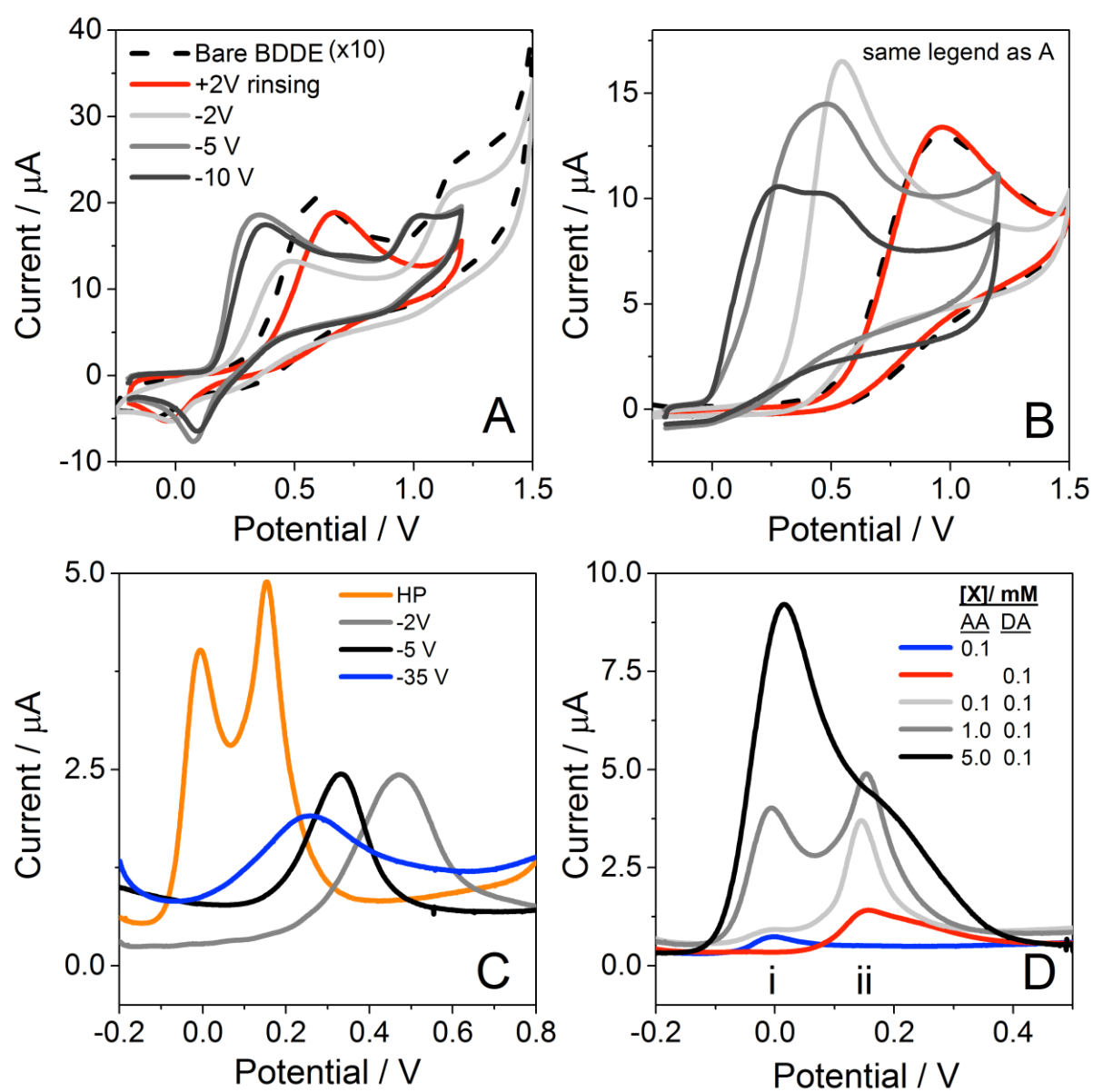


Figure 2

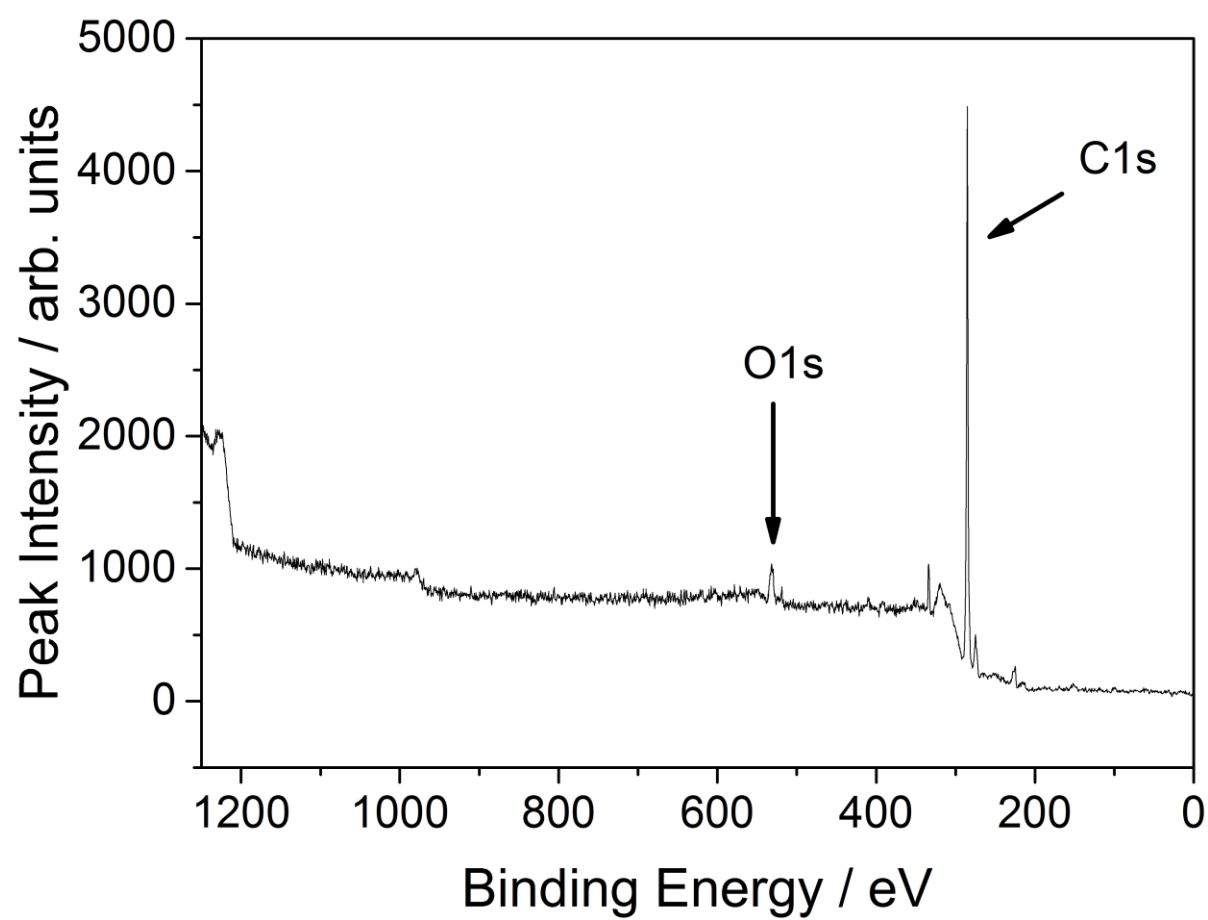


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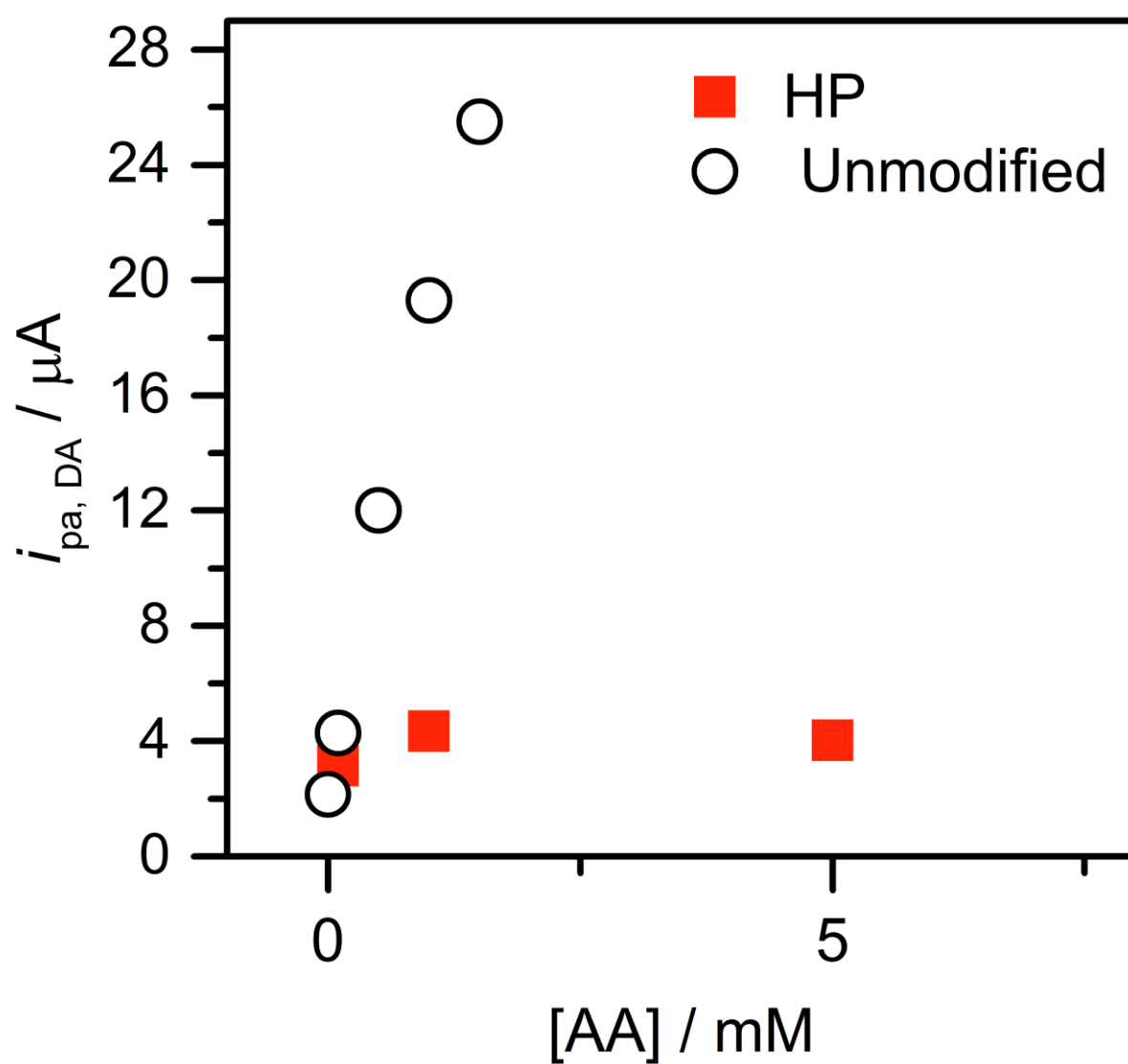


Figure 4

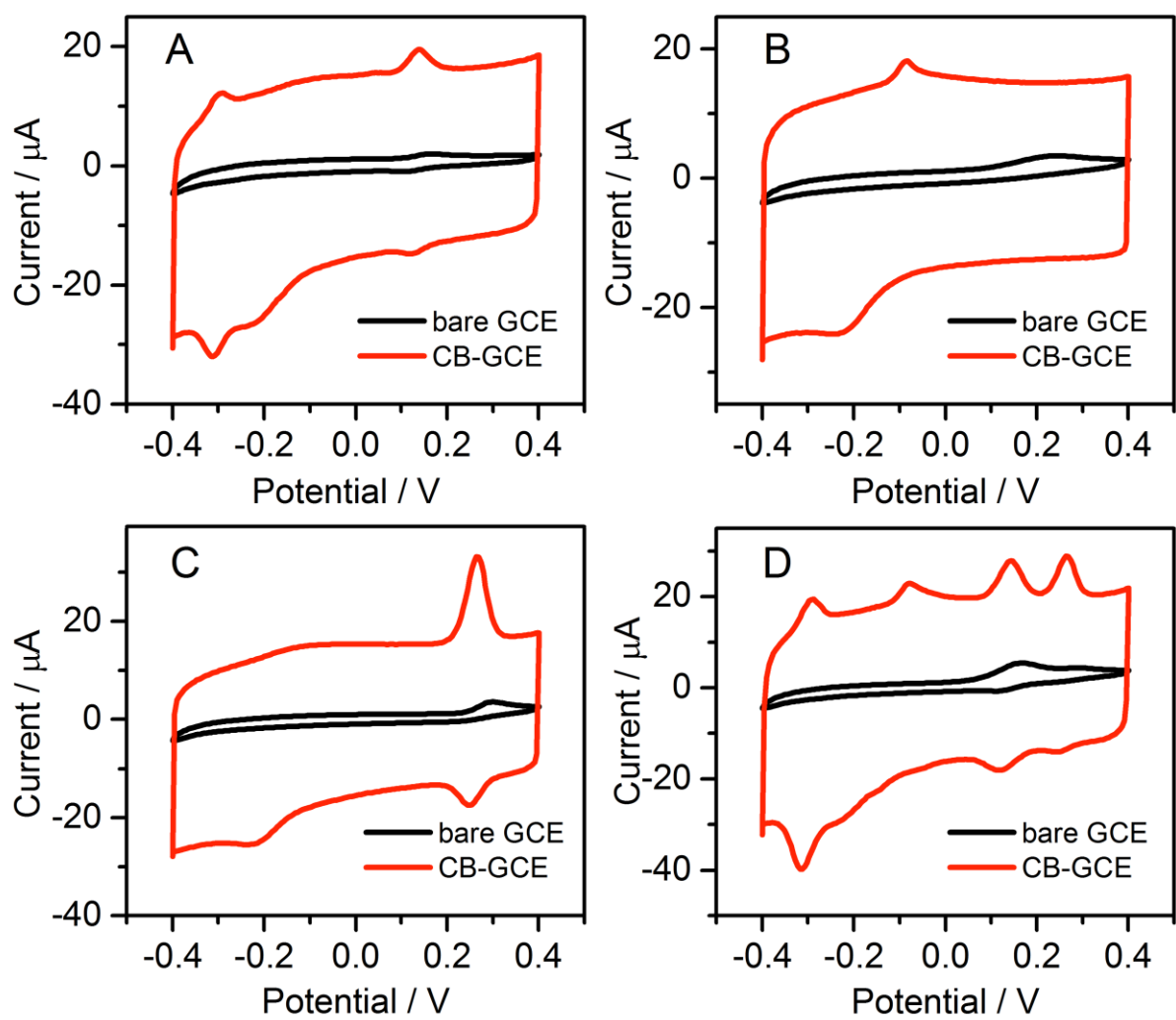


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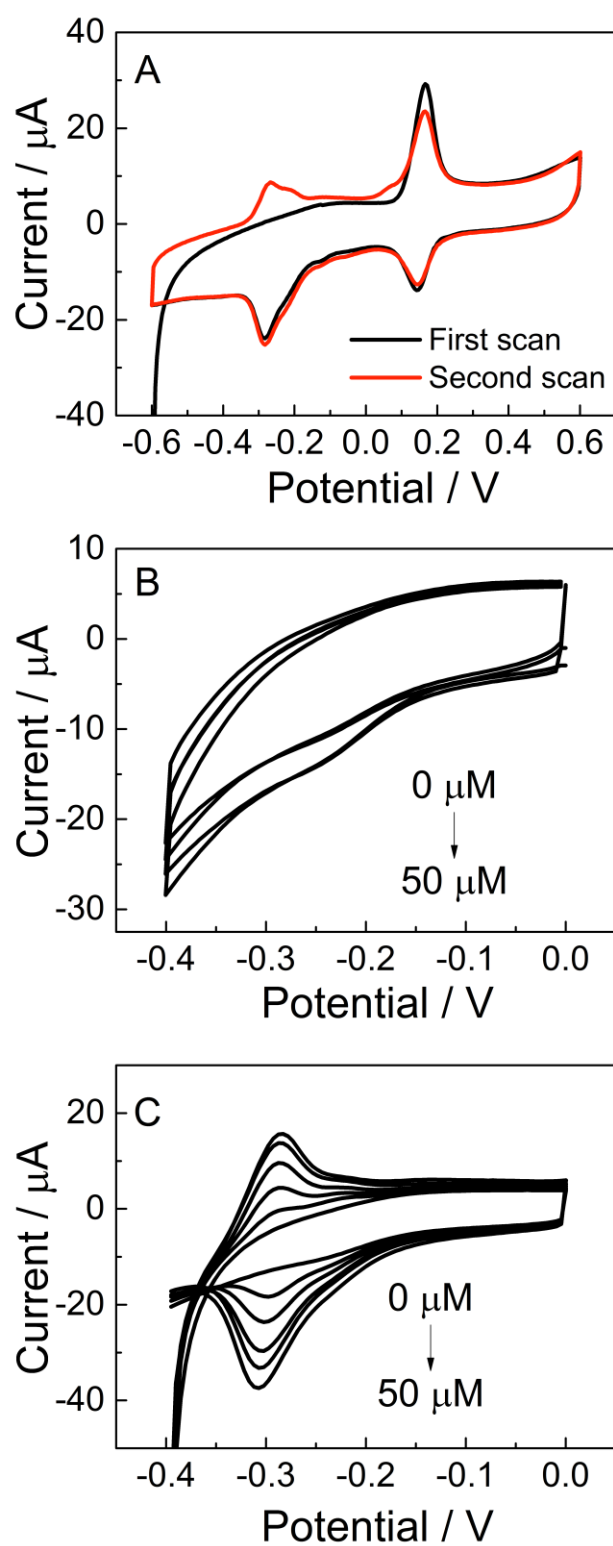


Figure 6

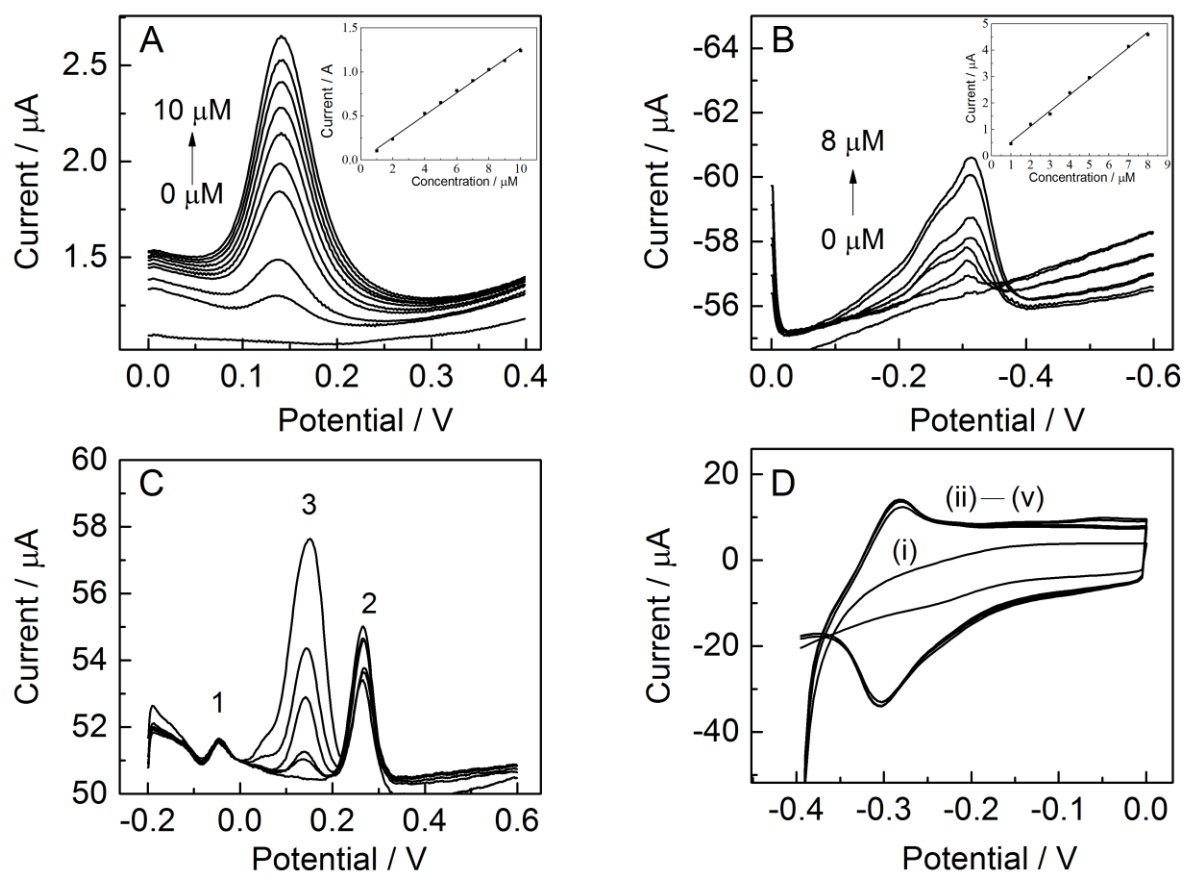


Figure 7