**Synthesis, Follow-Up, and Characterization of Polydopamine-like Coatings Departing from Micromolar Dopamine-o-Quinone Precursor Concentrations**

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**ABSTRACT:** The understanding of oxidized species derived from the neurotransmitter dopamine (DA) is a relevant topic for both the medical field (Parkinson’s disease) as well as for the field of materials science where the formation process of polydopamine (PDA) films is an active area of research. Polymers that interact strongly with almost all surfaces but have a low electrical conductivity have been obtained by the chemical oxidation of DA. Since electrical conductivity is a desired property for several applications, deposition alternatives such as electrochemical PDA synthesis have been proposed, but the results are still insufficient. In this context, we propose a new PDA chemical–electrochemical deposition process on glassy carbon electrodes. The chemical oxidation step that converts dopamine into dopamine-o-quinone previous to the electrochemical deposition was crucial to decrease the precursor concentration to the micromolar range. The PDA-like films synthesized by this method had high adhesion and low charge-transfer resistance, which was evidenced by impedance measurements and the successful electrodeposition of a polypyrrole coating on top of a PDA-like film. In addition, we observed that anodization of GC surfaces increases sensitivity toward six electroactive couples derived from DA oxidation in the pH regimes studied. These results show the complexity of the intermediates formed during the electrochemical polymerization of PDA.

**INTRODUCTION**

Dopamine (DA) is a neurotransmitter that affects both the central nervous system (CNS) and the periphery. In the CNS, dopamine regulates voluntary movement, feeding, reward, addiction, learning, and sexual behavior among others,1−5 while in the periphery, DA is involved in cardiovascular, renal, hormonal, and retinal functions.6−8 In vivo, dopamine can be oxidized and form different toxic compounds like aminochrome (AC) and 5,6-dihydroxyindole (DHI). These in turn can polymerize forming polydopamine, and by autophagocytosis and complex chemical interaction with biomolecules end up producing the nondegradable pigment known as neuromelanin.9,10 (Scheme 1). The oxidized species derived from DA have been implicated in cellular dysfunction caused by the inhibition of ATP synthesis, proteolysis impairment, and reactive oxygen species (ROS) control system deregulation.6,11−16 In addition to the covalent inactivation of several enzymes, both the AC and the DHI can interact with the protein α-synuclein and promote its aggregation, which is a classical pathological marker in Parkinson’s disease.17−19 The role of DA oxidized products in Parkinson’s disease has been further established by the development of a murine model using intracerebral injection of aminochrome.20,21 Although DA and AC have been studied electrochemically in different types of electrodes for decades, the simultaneous determination of DA, AC, and DHI is barely reported. Besides, quantification of those species relies completely on spectrophotometrical methods, which limits in vivo applications.22−24 The lack of knowledge on DHI electrochemistry due to its...
hard preparation and short shelf life can lead to a misidentification of the compound using an exclusion logic. Since the classical DA oxidation mechanism includes three canonical redox pairs (Scheme 1), any other pair that is not dopamine or aminochrome is identified as the DHI. For example, Jiang et al. identified the DHI as a peak that is rather the aminochrome or overlaps with the AC signal, while Yang’s group identified the DHI as a signal that appears very close to the dopamine oxidation peak. It is a very unlikely situation that the DHI signal is located in such different positions since the DA to AC peak separation is about 400 mV on the bare GCE. In addition, it has been shown that DA can lead to many other compounds when chemically oxidized, and some of them can be embedded in the polymeric matrix of PDA polymers.0,28 Thus, observing other electroactive pair during dopamine detection in neutral or mildly alkaline media does not imply necessarily the presence of DHI. The difficulty of working with the DHI previously reported by Horak and Weeks can be overcome by a simple sample storage strategy proposed by Bruning et al., a method that will be implemented with some modifications in this work.25,29

Since DA oxidation can lead to a polymer with high adhesion and easy chemical modification, the understanding of oxidative derivatives of DA is not only of medical interest but is also relevant in the field of materials science.30,31 The rediscovery of PDA as a bioinspired material from the adhesive proteins of mussel leads to an explosion of applications in the field of energy (batteries, capacitors, and catalysts), biomedical sciences (cell culture, tissue engineering, and antimicrobials), environmental engineering (water purification, separation of heavy metals, and separation of oil and water), and analytical chemistry (development of sensors).32,33 PDA coating has emerged as a quick, cheap, and almost universal surface modification strategy. The chemical deposition of PDA has been broadly studied including synthesis conditions such as oxidant type, oxidant-to-DA proportion, type of buffer strategy. The chemical deposition of PDA has been broadly studied including synthesis conditions such as oxidant type, oxidant-to-DA proportion, type of buffer, and pH. Tuning synthesis conditions including the use of other catechol analogues has allowed the achievement of materials with different properties of wettability, cell adhesion, composition, porosity, and thickness.34–36 Yet, the polymers obtained by chemical synthesis tend to be electrical insulators, which limits their applications in certain fields.37,38 Electrochemical methods have been recently explored as an alternative where film thickness and electrical conductivity can be controlled better compared to chemical deposition. Even though the current methodologies use high dopamine concentrations (≥2 mg/mL, 10.6 mM) and require very slow scan rates (10 mV/s CV) to allow DA cyclization, the electrical conductivity of the obtained deposits is still low.37,39

Catechol group oxidation kinetics is fast when sodium periodate (NaIO₄) is used to oxidize DA in acidic conditions, while the amino group is insensitive to oxidation in aqueous media.40–43 In addition, DA solubility in 0.1 M HCl is high, and the cyclization of DOQ to form AC is a very slow process at this pH. These conditions secure a very high yield of DOQ that can later be used as the precursor for the PDA formation at different pH solutions. As DOQ is the main precursor for the formation of AC and DHI, we suggest that a prior total oxidation of DA into DOQ in micromolar concentrations could be equivalent or even higher to the surface DOQ concentrations generated by the previous slow scan electrochemical methods. Therefore, we propose that when a chemical method (DA oxidation with NaIO₄ in acidic media) is combined with an electrochemical deposition (CV), the scan rate can be shifted up and the precursor concentration reduced. A reduction in the concentration of the precursor lessens the peak width of the different intermediates and thus could allow an electrochemical follow-up of the electroactive intermediates generated during PDA polymer formation.

Unmodified carbon surfaces vary greatly with their origin, and this heterogeneity combined with the effects of cleaning methods provides a final electrochemical response, which is often poor in terms of reproducibility. Electrochemical pretreatment of glassy carbon electrodes (GCEs) has proven to be a cheap and quick surface treatment for the detection of catecholamines, yielding higher reproducibility as well.44,45 For example, the oxidation of the GCE in acidic and neutral pH by either cyclic voltammetry or constant amperometry provides high peak-to-peak separation between ascorbic acid (AA) and DA with a simultaneous increase in sensitivity. Nevertheless, under such conditions, the electrodes are affected by the formation of an oxide film that strongly increases electrode capacitive currents.44,46,47 Kiema et al. described a short anodization cleaning procedure in basic conditions, in which all alumina traces were eliminated and almost no oxidation occurred.48 Under these conditions, we expect to prevent large capacitance changes and gain sensitivity and selectivity during the simultaneous determination of DA, AC, and DHI occurring during the electrochemical formation of PDA polymers. In this work, the electrode cleaned in this way is called “anodized”.

We combine an electrochemical cleaning procedure of the GCE with a novel PDA synthesis proposal so that different electroactive couples can be detected simultaneously during the polymerization process. Our chemical—electrochemical approach is not limited by slow scan rates, is applicable with very small precursor concentrations (micromolar range), and permits the synthesis of adherent and conductive materials. In addition, we study the electrochemistry of DHI and 6-hydroxydopamine (6-OHDA) on the GCE for later association with the polymer signals.

**RESULTS AND DISCUSSION**

**Comparison of GCE Cleaning Treatments on DA Electrochemistry.** DA electrochemistry at the GCE cleaned by three methodologies (conventional polishing, soaking in isopropanol/activated carbon (IPA), and anodization) was compared (Figure 1). Soaking a GCE in isopropanol purifed with activated carbon had been previously reported to reduce peak-to-peak separation (∆E = Ecathodic − Eanodic) in DA solutions and to produce a negative shift on the AA signal as a result of the polar impurity removal from the electrode surface.49 We found similar results with values of ∆E (DA) as low as 50 mV at pH 5.0. However, IPA treatment was poorly reproducible in terms of peak currents and potentials, and thus, no significant differences were found compared to the conventional cleaning method (Figure 1). Surface poor reproducibility coming from cleaning has been previously recognized to be dependent on assorted factors such as the size of alumina particles, the condition of the cleaning plate, and even the alumina supplier.51,52 In contrast, the electrode cleaned by anodization was capable of reducing ∆E (DA) to an average value of 24 mV, caused a 2.5-fold increment in DA current compared to the conventional method and showed a high reproducibility in the peak position (Figure 1). A ∆E...
on carbon fiber and show that this delicate anodization of the GCE promotes DA adsorption, probably due to greater removal of impurities compared to the conventional cleaning. The GCE is used as a model system for many measurements, in particular a model for carbon fibers. The results presented here suggest that the anodized GCE can be used as a better model system for the DA–DOQ reaction where the electrochemical process resembles more the adsorption occurring at carbon fibers.

**Sensitivity of Anodized GCE toward Multiple Organic Molecules.** Since GCE anodization increased DA and AC currents, it is interesting to assess if this phenomenon could also occur with other organic molecules. We evaluated the total cathodic charge obtained from differential pulse voltammetry (DPV) with a variety of organic molecules such as methylene blue (MB), epinephrine (EP), 3,4-dihydroxyphenylacetic acid (DOPAC), ascorbic acid (AA), uric acid (UA), catechol, and 1,3,4-dihydroxyphenylalanine (L-DOPA). L-DOPA is used as an example in Figure 2 to show that GCE anodization promotes adsorption and also boosts sensitivity. A summary of the results obtained from all the analytes is shown in Figure S5.

(DA) of 24 mV indicates that the surface is not only electrocatalytic (ideal Nernstian behavior) but also promotes the adsorption of DA to the anodized GCE surface, in which case, totally symmetric peaks are expected (Figures S4 and S5). In sum, anodization was the assayed method with the highest reproducibility and sensitivity for DA determination at pH 5.0.

DA at the anodized GCE followed the classical oxidation mechanism, which involves the exchange of two protons and two electrons and results in the formation of DA-quinone (DOQ) as a final product (Figure S1 and S2). A second electrochemical couple corresponding to the oxidation of leucoaminochrome into aminochrome (AC) was also observed (Figures S1 and S3). As expected, AC currents augmented with pH because of the amino group deprotonation and the cyclization rate relationship with pH (Figure S3). Although the second electrochemical couple was observed at the conventional and the anodized surfaces (Figure S1) at pH values between 6 and 8, the peak-to-peak separation values were smaller after anodization (33 mV vs 21 mV; Figure S1), suggesting analyte adsorption. Adsorption of DA and desorption of DOQ define the voltammogram shape at carbon fiber microelectrodes. DA is adsorbed more strongly than DOQ to the carbon fiber material, resulting in peak current asymmetries at neutral pH. When DA was cycled at several scan rates on GCEs at pH 1 or 5, we observed different electrochemical phenomena depending on the cleaning procedure (Figures S4 and S5). DA electrochemistry at the conventionally cleaned GCE was primarily diffusion controlled, while at the anodized GCE DA adsorption was promoted. This change in electrochemical behavior is evidenced by the higher slope values in the Ln[lox] versus Ln[scan rate] graphs obtained from anodization. In contrast, ferricyanide electrochemical behavior was independent of the cleaning method (Figure S4). These results resemble DA and DOQ adsorption

**Figure 1.** Comparison of different GCE cleaning methods on DA sensitivity and reversibility. (a) Cyclic voltammograms of 100 μM DA in 0.1 M PBS (pH = 5.0, 100 mV/s) at the GCE cleaned by three methods: conventional, isopropanol with activated carbon (IPA/AC), and anodization. (b) DA oxidation currents and (c) ΔE values obtained from the three methodologies were compared (Kruskal–Wallis test and post-hoc Tukey test). Nine replicates were taken from each treatment. Error bars = 2 SEM. Asterisk symbol denotes that differences were considered significant when p < 0.05.

**Figure 2.** (a) L-DOPA electrochemical behavior at the GCE. CV of 100 μM L-DOPA in H2SO4 (pH 1.0) at increasing scan rates (10, 25, 50, 75, 100, and 150 mV/s). Notice how GCE anodization improves reversibility and adsorption compared to GCE conventional cleaning. (b) DPV of 100 μM L-DOPA in H2SO4 (pH 1.0), charge had a 3.1-fold increment.

This sensitivity rise observed at the anodized GCE may be explained by a larger electrochemical active area of the anodized surface. To test this hypothesis, the electroactive areas of the conventional GCE and anodized GCE were determined and compared by chronoamperometry of ferricyanide (Figure S6). Certainly, anodization increased the electrode active area by 20% (1.19-fold increase), which coincides with the AA but not with DA, MB, EP, catechol, L-DOPA, or UA charge increments that were substantially higher (Figure S5). The results suggest that the exposed carbon surface may interact by π–π interactions with planar organic molecules during adsorption (DA, MB, DOPAC, EP, catechol, L-DOPA, and UA). Additionally, MB, which was slightly adsorbed to the conventionally cleaned GCE, had a remarkable

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adsorption behavior after anodization (Figure S5). In sum, anodization provides the cleanest carbon surface to which high affinity molecules, such as DA, EP, catechol, DOPAC, MB, L-DOPA, and UA, can adsorb better. This is in accordance with the results of Nagaoka and Yoshino who attributed the catalysis of catechols at anodized GCE surfaces to specific interactions such as π−π-triggered adsorption, and therefore, it is very sensitive to the electronic structure of the adsorbed molecule.57 Besides the increments in sensitivity, we also evaluated whether anodization had or not had an effect on peak potential reproducibility. As it can be seen in Table S5, the F test was significant for all the molecules tested. Since the variation coefficients were always smaller at the anodized surfaces and the F tests were significant, we can conclude that anodization significantly reduced the variability in peak potential. In sum, GCE anodization does not only increase electrode sensitivity but also peak potential reproducibility.

Increments in sensitivity could be the result of surface oxides. Short anodization in alkali has been reported to be mild, showing little surface oxidation and is accompanied by superficial carbon dissolution.57−59 It was previously shown by Kiema et al. that a light anodization (0.1 M NaOH for 10 s at 1.8 V) results in a small increment in the O/C ratio measured by XPS (4%) and causes a minor effect on the kinetic constant k5 of the inner sphere Eu3/2 redox couple.48 We have also observed that just a slight increment in charge-transfer resistance occurs upon anodization (Figure S7). Altogether, XPS and electrochemistry (CV and EIS) results point out that mild anodization in alkali does not significantly oxidize the GC surface, and therefore, surface oxides are not responsible for GCE sensitivity. Instead, the mechanism by which short alkali anodization increases sensitivity seems to be alumina clearance and carbonyl group exposure (Figures S8, S9, and S10).48 Anodization eliminates alumina residues and exposes carbonyl groups ideal for catechol and catecholamine interactions,60 which favors adsorption and a signal increment due to the autocatalytic effect of catecholamines on their own oxidation.61 Our suggested mechanism is congruent with data published by Compton et al. on alumina-mediated catechol electrocatalysis at low pH. Catechol is first adsorbed on 1 μM of alumina particles and then alumina oxygenated groups serve as proton acceptors, promoting catechol oxidation electrocatalysis.62 Similarly, exposed carbonyl groups on the anodized GCE could not only serve as interaction sites for adsorption but also as proton acceptors, promoting DA electrocatalysis.

Summarizing, the high sensitivity and reproducibility in redox signals from the multiple organic molecules assayed at the anodized GCE could be related to the elimination of alumina impurities, which led to the exposure of oxygenated groups. This cleaner surface allows the adsorption of chemically alike compounds such as DA, EP, MB, and UA through π−π-triggered interactions as well as hydrogen bonding with the oxygenated groups with the resulting effect on sensitivity.

Identification of Six Electroactive Couples Derived from DOQ at Anodized GCE. The results shown above indicate that the anodized GCE could be an ideal surface for the detection of the chemical intermediates formed upon DA oxidation. Acid DOQ was dissolved in PBS buffer at different pH values, and the appearance of electroactive couples was followed by DPP and CV every 2 min (Figure 3 and Figures S11 and S12). The formation of DOQ was first confirmed by UV−vis spectroscopy; while the DA signal at 280 nm disappeared upon the addition of NaIO4 the DOQ typical band appeared (395 nm band; Figure S13). When DOQ was diluted in PBS (pH 4.0 or 5.0), the main band centered at 395 nm remained, and as expected, the solutions were yellow; although when the quinone was dissolved in PBS (pH 6.0 or 7.2), the solutions became orange-red, the band centered at 7.2 nm remained, and as expected, the solutions were yellow; acid DOQ at Anodized GCE.
evidenced by the 300 nm band and its 280 nm associated shoulder, yet the broad peak of AC in this region interferes (Figure S15). The oxidized DHI-quinone was not present at any pH (Figure S14) as expected from its low stability. The spectrophotometric results were confirmed by HPLC experiments of DOQ diluted at pH 5.0 and 7.2 (Figures S15 and S16). Blank CV of iodate and periodate solutions was also carried out to assure that no voltammetric peaks would derive from the oxidant or their products (Figure S17). The DOQ was also tested on activated gold and platinum electrodes in PBS (pH 5.0) to show that the multiple couples can only be observed at the anodized GCE (Figure S18).

As it can be seen in Figure 3, five oxidation peaks could be observed at pH 5.0 after DOQ dilution. From the DPV of pure dopamine, we assigned peak e to DA oxidation (Figure 4).

Similarly, the leucoaminochrome–AC couple could be identified from the DPV of pure dopamine, yet peaks a and b were too close to make any assignment (Figure 4). Comparable results were obtained at pH 4.0, although peak intensities were smaller and only four couples were present (Figure S11). When the DOQ was dissolved in PBS at more basic pH (6.0 and 7.2), a new electrochemical couple (f) located very close to the dopamine signal became clear. The position of peak f matched the position of pure DHI (Figures 3 and 4). In the acidic regime (pH 4.0 and 5.0), the DA signal decreased as time progressed with the concomitant increment in the intensities of the other signals. In contrast, at pH 6.0 and 7.2, the DA signal decreased in the first 2 min but later increased. The increment in the DA signal at pH 7.2 coincided with a reduction of the leucoaminochrome signal, which may correspond to a DA regeneration mechanism where the DOQ is reduced back to DA by the coupled oxidation of leucoaminochrome to produce AC. As a closing remark regarding this topic, we want to emphasize that the electrochemical assignment of DHI should be performed using the standard to avoid misidentification. From our experiments, the DHI oxidation peak appears 80 mV to the left of the dopamine oxidation signal, and these results are congruent with the report of Yang et al. using carbon nanotubes. Interestingly, a very simple anodization procedure of the GCE is sufficient to attain high catalytic activities toward catecholamines and their oxidation derivatives (AC, DHI, and 6-OHDA), as shown here. The electrochemical formation of the aminochrome-leucoaminochrome couple at carbon electrodes is a well-known phenomenon, even though emerging biochemical knowledge related to oxidation products of DA with cell damage through mitochondrial, proteosomal, cytoskeletal, and autophagy dysfunctions in Parkinson’s disease has relied only on UV spectroscopy. As electrochemical sensors can be miniaturized to detect real-time local changes in concentration and the AC and DHI were clearly identified at the anodized GCE, we suggest that the simultaneous detection of aminochrome, DA, and DHI could be addressed electrochemically in vivo.

The results show the simultaneous electrochemical detection of more than three electroactive couples during DA oxidation with NaIO₄. To the best of our knowledge, this is the first time that these results have been presented. Three of them are the conventionally accepted couples for the DA system (Scheme 1), although other three couples were formed depending on the pH. From previous chromatography-based studies, one may suggest the presence of 6-hydroxydopamine (6-OHDA) and norepinephrine (NE). If norepinephrine indeed forms, then it is plausible that the peak close to the AC (a and b) could be the noradrenochrome. In this context, we decided to determine the oxidation peak position of 6-OHDA by DPV to try to assign either peak c or d. The peak position of the 6-OHDA standard corresponded to peak c in the mixture (Figures 3 and 4), leaving only peak d unidentified. 6-OHDA can be formed by DOQ hydroxylation in acidic media (pH < 6.0). Under these conditions, the amino group of dopamine is mainly protonated and water can attack C6 double bond in the ring, which later suffers from a proton rearrangement to gain aromaticity (Figure S19).

Electrochemical Modification of Glassy Carbon Surfaces Using DOQ as the Precursor. The electrochemical modification of the GCE from DOQ solutions at six different pH values (4.0, 5.0, 6.0, 7.2, 8.5, and 10.0) was assayed (Figure S20). An apparently covalent modification (resistant to 10 min of sonication) was obtained at all the pH evaluated and evidenced by a common electroactive peak, which was pH sensitive (Figure S21). A catechol to quinone conversion is very likely to be responsible for the observed peak, which followed an adsorption electrochemical behavior (Figures S21 and S22). A second redox pair about 0.35 V (close to the DA/DHI signal) was clearly identifiable in the
deposit synthesized at pH 8.5. That signal was also present when pH values of 7.2 and 10.0 were used during the synthesis but with modest intensities (Figure 5 and Figure S23). We further sonicated the deposits synthesized at pH 8.5 during 25 min. The peak that appeared at 0.35 V falls off when subjected to multiple rounds of sonication; this detachment did not occur to the compound responsible for the principal peak, which seems to be covalently bound to the GC surface (Figure S23). Our hypothesis is that at low pH values, a covalent surface modification by compound d occurs (pH 4.0, 5.0, and 6.0), and when the pH is raised and DHI is formed abundantly, a mixed material regarding polydopamine (PDA) could be formed (pH 7.2, 8.5, and 10.0). Interestingly, DHI by itself produces a broad electroactive peak that lays just below peak d (Figure 4), and this may explain the high electric resistance and peak area observed when pH 7.2 is used during the synthesis (Figure S23). Altogether, these results coincide with the idea of the mixed nature of PDA polymers, whose structure is held by both covalent cross-links and very tight noncovalent interactions. In our particular case, covalent cross-linking to the GCE surface apparently contributes to its strong adhesion as well.72

The presence of a pH-dependent electroactive peak makes the material ideal for applications in pH sensing. Indeed, Amiri et al. have electrochemically synthesized thin PDA polymers on the GCE for pH sensing purposes.73 In Amiri’s work, the indole couple (peak f) is used as the sensing couple; here, we present couple d as an alternative for pH determination. The position of the main electroactive peak (d) had a linear dependence with pH regardless of the pH of synthesis. For example, the oxidation peak of PDA7.2 followed a linear Nernstian dependence with pH with a sensitivity of 59.9 ± (0.53) mV per pH unit (Figure S21). As an approximation to the deposition on GC surfaces, PDA electrodeposition was performed on polycrystalline gold electrodes for C-AFM measurements. The maps showed a decrease in surface current associated with lower conductivity at the PDA-modified electrode. The images also indicate that the coating was evenly distributed over the entire surface. The small difference in the root-mean-square roughness may be an indication of the low thickness of the PDA coating (11.6892 nm for Au and 14.4833 nm for PDA-coated Au) (Figure S24). However, it should be noted that this approach differs significantly from deposition on GC as the covalent bonds of compound d may not form on this substrate.

A comparison between the intensities of the principal peaks of the polymers as a function of synthesis pH showed an inverted U behavior, with the maximum value obtained at pH 7.2 (Figure S22). The intensity of the peak could be related to a difference in the film thickness. Thicker films with the same material resistivity are expected to have higher \( R_\text{ct} \) values in EIS experiments.74 Indeed, a linear correlation between anodic peak area and \( R_\text{ct} \) was observed (Figure S25). From this relationship, we can suggest that the thicker film was obtained at pH 7.2 and the thinner one at pH 4.0. A recent work where polymers from DHI were synthesized showed a similar pattern. Synthesis at pH 5.0 formed uniform DHI films of 5.7 nm in thickness, and at pH 7.0, the thickness increased to 7.3 nm, but when more alkaline pH (8.5) was used, the deposition was irregular with almost no polymer being formed.72 We also observed smaller principal peaks at pH 8.5 and 10.0, yet the polymers formed as evidenced by EIS and CV. It has been proposed that DHI self-aggregation in solution at alkaline pH competes with polymer formation at the surface.72,75 Our chemical–electrochemical deposition results are in agreement with previous results obtained by chemical deposition of DHI, yet a different intermediate is responsible for the electroactivity of the main component in our polymers. The ellipsometry technique could later be used to evaluate the thickness of the coatings.35

Very interestingly, the surface coating of the GCE had no significant effect on the FeCN\(_n\) signals (Figure S22) even when basic pH values were used during the synthesis. These results are in contrast to previous reports on electrochemical PDA deposition departing from 0.5 mg/mL DA by CV at a 10 mV/s scan rate. They report that synthesis with 70 CV cycles in Tris buffer (pH 8.5) produced very resistant polymers capable of blocking FeCN\(_n\) electroactivity.39 The EIS characterization of our polymers showed resistance values between 30- and 70-fold lower compared to the previous PDA polymers synthesized by Ball. For example, at pH 8.5, we obtained a charge-transfer resistance of 1186 ± 494 \( \Omega \cdot \text{cm}^2 \) \( (n = 3) \), compared to the 61.340 ± 1400 \( \Omega \cdot \text{cm}^2 \) reported pre-

Figure 5. (a) Electrochemical modification of the GCE from 20 \( \mu \text{M} \) DOQ. Formation of PDA by CV in PBS (pH 7.2; 235 cycles). (b) CV in PBS (pH 4.0) of the resulting compound synthesized at pH 7.2 and 8.5 after 10 min of sonication, notice the two remaining electroactive couples. (c) EIS characterization of the bare GCE and the coatings synthesized at different pH values (4.0, 5.0, 6.0, 7.2, 8.5, and 10.0). The equivalent circuit used to fit the data is presented in Figure S22.
viously.37,39 The low resistances and the permeability toward ferricyanide suggest that the deposits obtained from the micromolar DOQ synthesis solution are thinner than 30 nm, and still the possibility of differences in the coating surface and/or differences in resistivity among materials exists.39

Finally, we compare the purely chemical PDA deposition method with the chemical—electrochemical method described herein. First of all, DA autoxidation leading to PDA formation requires of a minimum DA concentration of 1 mM.75 We have seen that when DA is previously oxidized to DOQ and then electrodeposited, concentrations of 20 μM are enough to attain highly adhesive mixed deposits (2 electroactive couples that resist 10 min of sonication). This is in accordance with d’Ischia et al’s work, where periodate addition accelerated PDA deposition during the first hours of film formation.77 d’Ischia et al proposed a two-step mechanism for PDA formation: during the first step free amines cross-link with quinone forms, and these cross-linked oligomers would confer the adhesive properties; in the second step, the polymer starts its growth incorporating DHI, DHI-containing oligomers, DA, AC, and all the other intermediates from the mixture.75 This mechanism explains why at low DA concentrations no adhesive polymer is formed. As DHI is favored over free amine—quinone cross-linking at low DA concentration, then eumelanin materials with low adhesion are produced. By selecting cyclic voltammetry as the electrodeposition technique, we assured that both the oxidized and the reduced forms of the different couples were present at the interface. Therefore, even when we depart from the chemically synthesized DOQ, once the electrode is subjected to reducing potentials, DA is electrochemically generated at the interface where the deposition process occurs. Moreover, we have shown that 6-OHDA is formed during the synthesis, which is a source of free amines for the initial deposition process. It is worth exploring in future work the chemical deposition of PDA films from DOQ. Our results suggest that film thickness could be better controlled by using DOQ as the precursor. By finely tuning DOQ concentration, pH, and deposition times, thin adhesive polymers (low electrical resistance) could be produced.

Chemical—Electrochemical Polymer Synthesis Method Applied to Other Catecholamines. A major advantage of the synthesis method proposed here is the low concentration of precursor employed. To show that this method can be generalized, EP and L-DOPA were used as precursors. As shown in Figure 6, EP also exhibits the formation of multiple electroactive couples at pH 4.0. After 235 cycles at 300 mV/s, an electroactive deposit formed on the GCE surface, which seemed to be covalently attached since it resisted multiple rounds of sonication similar to the behavior of PDA-like polymers. The obtained deposit also allowed FeCN₆ oxidation and reduction and presented low Rₛ resistance evaluated by EIS (Figure S26). Similarly to PDA, the principal peak position from the obtained materials using EP as a precursor was dependent on the pH. The oxidation peak showed a sensitivity of 64.01 mV ± (0.46) per unit of pH, which strengthens the idea that these materials are suitable for applications in pH sensing (Figure S26).

Oxidized L-DOPA solution turned red quickly when dissolved in PBS (pH = 4.0), which resembled EP-o-quinone cyclization kinetics more than DOQ cyclization. Only two electroactive couples appeared during the synthesis (L-DOPA couple and its associated AC). It is well documented that DHI can be produced from oxidation and further decarboxylation of L-DOPA,76 and actually, electric properties of synthetic eumelanin have been studied on the polymers derived from L-DOPA.77 DHI was not produced under the conditions studied (Figure S27). After 10 min of sonication, all the physisorbed species were eliminated and no differences between the voltammograms before and after the synthesis were observed. Thus, the polymerization was unsuccessful when L-DOPA was used as a precursor (Figure S27). Mechanistically speaking, this finding suggests that the electrochemical polymerization of catecholamines requires multiple intermediates (d/f) and that the aminochrome-like forms are not enough to promote the formation of highly adhesive polymers on the GC surface.

Applications on Hybrid Materials. The properties of catechol, catecholamines, and aminomalonitrile-based films synthesized by electrochemical methods have been recently reviewed. The high adhesion of the films is a common feature, yet the low conductivity is a limiting factor for numerous applications.77 Taking advantage of the low charge-transfer resistances of the deposits obtained, we tried to polymerize the conductive polymer polypyrrole (PPy) by a galvanostatic method on top of a PDA-like film. Since the PPy polymerization process requires pyrrole oxidation, it is decisive to have a conductive substrate for the process to occur. The synthesis of PPy was possible both on the bare GCE as well as on top of the PDA-like coating synthesized at pH 7.2 (Figure 7 and Figure S28). PPy on the GCE is easily identifiable by its orange color, which contrasts with the typical mirror appearance of the GC substrate (Figure 7 and Figure S26). Normally, PPy is described as a blue-gray polymer when oxidized and yellow-green in the neutral form.78 The synthesis conditions allowed the formation of very thin films (50 to 100 nm estimated from SEM microscopy), which may explain the color observed under oxidation conditions (air; Figure S28). In addition, the polymer became greenish after CV (data not shown), which is characteristic of PPy depending on the oxidation level.78 We also evaluated the presence of the PPy electrochemically by CV in 0.5 M LiClO₄/acetonitrile. As it can be seen in Figure 7, the...
biocompatibility of PDA. Possibility to control PDA electric properties, summed with the chemical composition of the substrates.79,80

**CONCLUSIONS**

In the first place, GCE anodization cleaned and activated the carbon surface, allowing higher sensitivity toward multiple organic compounds including the intermediates formed during PDA synthesis. Thus, short anodization in alkali of the GCE is an adequate method to follow up reactions involving multiple organic electrochemically active intermediates, in particular the complex oxidation of catecholamines. The identification of different chemical intermediates depending on the pH suggests that the effect of pH during PDA synthesis does not only influence the kinetics of polymerization but also could affect the chemical composition of the final polymers. To sum up, our work provides a semiquantitative analytical technique to control PDA electric properties, summed with the biocompatibility of PDA films, makes PDA an ideal material for applications such as bioelectrodes and cell growth substrates.79,80

**EXPERIMENTAL SECTION**

**Reagents and Chemicals.** Dopamine hydrochloride, 6-hydroxydopamine hydrochloride, ascorbic acid, uric acid, monobasic phosphate, dibasic phosphate, sodium chloride, potassium chloride, DOPAC, and l-DOPA were all Sigma-Aldrich analytical grade products and were used as received. Potassium ferricyanide, sodium periodate, and epinephrine were purchased from Alfa Aesar and methylene blue from Merck. All solutions were prepared using Milli-Q type 1 deionized water (18.2 MΩ cm⁻¹) with a TOC content less than 2 ppm. Electrochemical experiments were carried out with freshly prepared solutions that were previously deoxygenated under a nitrogen stream (5 min) and kept under constant nitrogen flow at 20 °C (±2 °C) during the measurement, unless otherwise stated. All solutions were kept in the dark when not in use. DA- o-quinone (DOQ) was prepared by mixing 0.1 M HCl solutions of DA hydrochloride (40 mM) and NaIO₄ (40 mM) in a 1:1 proportion. The resulting 20 mM mixture was used as a concentrated stock and diluted in PBS to reach a final concentration of 20 μM in most cases. DA and NaIO₄ were stable in acidic media and thus prepared weekly, while DOQ solutions were used only once immediately after preparation (5 min after). 5,6-Dihydroxyindole was synthesized by oxidation, cyclization, and decarboxylation of l-DOPA using K₃Fe(CN)₆ as the oxidant.76

The product was extracted with ethyl acetate and recrystallized from hexane as described by Chai et al.72 The white product was characterized by 1H NMR and 13C NMR. 1H NMR (400 MHz, methanol- d₄): δ 6.96 (d, J = 3.1 Hz, 1H), 6.88 (s, 1H), 6.79 (s, 1H), 6.18 (d, J = 3.1 Hz, 1H). 13C NMR (101 MHz, methanol-d₄): δ 142.2, 139.9, 130.8, 122.0, 121.0, 104.1, 100.1, 96.5 (Figure S29). DHI stock solution (20 mM) in DMSO or ethyl acetate (DHI was more stable in this solvent) was aliquoted and stored at −80 °C for further use.79

**Instrumentation and Electrochemical Measurements.** The electrochemical measurements were made using an Autolab compact line potentiostat/galanostat PGSTAT101. A typical three-electrode cell was used in all the experiments, which consisted of a glassy carbon electrode (BASI MF-2012, 3 mm in diameter), a platinum wire, and a Ag/AgCl (BASI RSB) electrode as the working electrode, counter electrode, and reference electrode, respectively. The working electrode was hand polished under alumina slurries prepared with 1.0, 0.3, and 0.05 μm of alumina powders (Electron Microscopy Sciences) on a Buehler MicroCloth (PN: 40-7212) for 2 min and then sonicated in Milli-Q water during 2, 2 and 5 min after each alumina cleaning respectively (conventional cleaning). The conventional cleaning results were compared with cleaning in isopropanol/activated carbon and with electrochemical cleaning (anodized). Briefly, the electrode was...
conventionally polished and then further sonicated in isopropanol/activated carbon (IPA) during 5 min and left in the solvent mixture for another 15 min without sonication.\textsuperscript{50,55} Alternatively, the electrode was conventionally polished and then anodized in a 0.1 M NaOH solution at 1.8 V for 10 s.\textsuperscript{48}

Differential pulse voltammograms for charge determination of multiple organic compounds were recorded at 50 mV/s.\textsuperscript{81} In the case of the simultaneous determination of DA oxidation products, a lower amplitude was chosen to favor selectivity: a modulation amplitude of 5 mV, a modulation time of 0.15 s, an interval time of 0.2 s, and a voltage step of 15 mV with a resulting scan rate of 7.6 mV/s. Charge-transfer resistance after PDA deposition was determined by impedance spectroscopy (EIS) using aqueous solution containing K\textsubscript{3}Fe(CN)\textsubscript{6} and K\textsubscript{4}Fe(CN)\textsubscript{6} 5.0 mM each in KCl 0.5 M and a Gamry 600 of 40 mM NaIO\textsubscript{4}, and also dissolved in 0.1 M HCl. The 20 was prepared in 0.1 M HCl, then mixed with an equal volume of 0.1 M PBS (pH = 4.0) with 3% methanol at a flow rate of 4.5 mL/min was used for chromatographic separation with a reversed-phase column as the stationary phase (250 mm in length, 10 mm in diameter, C-18, 5 μm).

PDA and Polypeepinephrine Electrochemical Synthesis. A stock solution (40 mM) of either DA or epinephrine (EP) was prepared in 0.1 M HCl, then mixed with an equal volume of 40 mM NaIO\textsubscript{4} and also dissolved in 0.1 M HCl. The 20 mM mixture was left reacting during 5 min at room temperature. The quinone stock solution was diluted in PBS before the synthesis (20 μM). The polymers were deposited on the anodized GCE by CV using a fixed potential window from −0.55 to 0.6 V, 235 cycles (30 min), and a scan rate of 300 mV/s. This unusually high speed was chosen to have a readout of the intermediates that could have short life times during PDA formation. In addition, this high speed allows to prove the concept that a prior chemical oxidation of DA into DOQ permits electrochemical deposition at high speeds, contrary to the slow speed limited electrochemical methods used until today. The obtained deposits were sonicated in water during 10 min before further characterization.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00676.

Mechanism of DA electrochemical oxidation; electron–proton relationship in electrochemical oxidation of DA at the conventionally cleaned GCE and anodized GCE; DA oxidation currents as a function of pH at conventional versus anodized GCE; DA adsorption behavior at the anodized GCE in acidic media contrasted to the diffusion control of ferricyanide; adsorption behavior of multiple organic molecules at the anodized GCE; area determination by chronoamperometry; EIS to assess surface oxidation depending on the cleaning method; SEM microscopy of the anodized GCE; intrinsic GCE redox couple exposed by anodization; pH dependence of intrinsic GCE redox couple; DA oxidation intermediates followed by DPV at pH 4.0 and 6.0; DA oxidation intermediates followed by CV at pH 4.0, 5.0, 6.0, and 7.2; DA oxidation with NaIO\textsubscript{4} in HCl 0.1 M followed by UV–vis spectroscopy; DA oxidation with NaIO\textsubscript{4} in PBS at different pH values followed by UV–vis spectroscopy; HPLC of DOQ dissolved in PBS 0.1 M pH 7.2; HPLC of DOQ dissolved in PBS 0.1 M pH 5.0; electroactivity of iodate and periodate on the GCE at different pH values; DOQ in PBS (pH = 5.0) at gold and platinum electrodes; proposed synthesis mechanism for 6-hydroxydopamine; PDA synthesis at different pH values; Nernstian pH dependence of PDA7.2 polymer electroactive signal; electrochemical characterization of PDA films synthesized at different pH values; material nature depending on the synthesis pH regime; PDA chemical–electrochemical deposition on gold electrodes; correlation of the anodic peak area and the resistance to charge transfer (R\textsubscript{ct}); characterization of the PEP film synthesized at pH 4.0; L-DOPA does not modify the GCE under the chemical–electrochemical method conditions; morphology of PPy electrodeposited at the bare GCE; and DHI NMR characterization (PDF)

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A.M.J. designed and performed the experiments, as well as helped in writing the manuscript. R.B.-G. contributed in doing some of the experiments and graphs. M.T.C. designed the experiments and wrote the manuscript. All the authors contributed in reviewing the final version of the article.

Notes
The authors declare no competing financial interest.

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