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Original scientific paper

Electrochemically deposited p-phenylenediamine-derived coating on graphite for nucleobase detection

Reinaldo G. Santana, Diego Haddad, Suresh R. Babu and Ana L. F. de Barros✉

Laboratory of Experimental and Applied Physics (LaFEA), Centro Federal de Educação Tecnológica Celso Suckow da Fonseca (CEFET/RJ), Av. Maracanã, 229, 20270-110, Rio de Janeiro, Brazil

Corresponding Author: ✉ana.barros@cefet-rj.br; Tel.: +55-21-2566-3179

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Abstract

A simple electrochemical sensing interface based on a p-phenylenediamine (pPD)-derived coating deposited on a graphite electrode was investigated for the individual and simultaneous detection of DNA nucleobases. The electroactive coating was obtained by cyclic potential scanning from -0.3 to +1.0 V vs. Ag/AgCl and characterized by scanning electron microscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy and Raman spectroscopy. Combined electrochemical, morphological, and spectroscopic results supported the formation of an adherent pPD-derived layer on the graphite surface, while XRD indicated preservation of the crystalline structure of the graphite substrate. Potential scan-rate (v) studies yielded a slope of approximately 0.42 for the log I_p - log v relationship, indicating contributions from charge propagation and ion transport within the deposited layer rather than a purely surface-confined process. The modified electrode exhibited concentration-dependent responses toward adenine (A), cytosine (C), guanine (G) and thymine (T), with cyclic voltammetric calibration coefficients of 0.99253, 0.98773, 0.97416 and 0.98191, respectively. Individual oxidation responses were observed at approximately 1.05, 1.38, 0.72 and 1.14 V for adenine, cytosine, guanine and thymine, respectively. In mixed solutions, the corresponding responses occurred at approximately 0.87, 1.22, 0.60 and 1.04 V, while preserving the oxidation sequence G < A < T < C. These results provide proof-of-concept evidence that a comparatively simple pPD-derived graphite interface can provide concentration-dependent nucleobase responses and electrochemical discrimination of the four DNA nucleobases in a multicomponent system.

Keywords

Electroanalysis; carbon-based electrode; polymer-modified electrode; DNA nucleobases

Introduction

DNA nucleobases are the fundamental molecular components that store and transmit genetic information. Adenine (A), cytosine (C), guanine (G) and thymine (T) form the chemical basis of DNA

and participate in fundamental biological processes, including replication and transcription [1,2]. Their electrochemical behaviour has consequently attracted considerable interest in the development of analytical approaches for DNA-related compounds and nucleobase determination [1-3]. Conventional analytical techniques used for the determination of nucleobases and related biomolecules include high-performance liquid chromatography, capillary electrophoresis, fluorescence-based methods, and mass spectrometry. Although these techniques can provide high analytical performance, they generally require specialized instrumentation and may involve relatively complex sample-preparation and analytical procedures [3,4]. Electrochemical methods therefore represent an attractive alternative because of their relatively simple instrumentation, rapid response, low operational cost, and potential for miniaturization [2-5].

The performance of an electrochemical sensing interface is strongly influenced by the physicochemical properties of the electrode surface. Accordingly, considerable effort has been devoted to the modification of conventional electrode materials with electroactive coatings capable of altering analyte-electrode interactions and the resulting electrochemical response [2-5]. Polymer-modified electrodes are particularly attractive in this context because electrochemical deposition enables the direct formation of organic layers on conductive substrates under controlled experimental conditions [4-8]. The properties of the resulting coatings depend strongly on the monomer structure, supporting electrolyte, electrode substrate, potential window and deposition conditions [5,7]. Among aromatic diamines, p-phenylenediamine (pPD) has been extensively investigated because its electrochemical oxidation can generate surface-deposited products containing oligomeric and polymeric species with different oxidation states [7,9,10]. The structure and electrochemical properties of these materials depend markedly on the synthesis conditions, and different benzenoid- and quinoid-type structural motifs have been reported for pPD-derived materials [7,10]. This versatility has motivated the investigation of poly(p-phenylenediamine) (PpPD) and related pPD-derived materials in electrochemical applications. PpPD-containing composites, including systems incorporating carbon nanotubes, reduced graphene oxide and inorganic components, have been investigated as electroactive materials and sensing interfaces [11-13]. These studies demonstrate the potential of pPD-derived materials for electrode modification while also showing that their electrochemical behaviour can be strongly affected by the accompanying substrate or nanostructured component.

Carbon-based electrodes are widely used for nucleobase sensing because of their electrochemical properties, accessibility, and compatibility with different surface-modification strategies. Huang *et al.* [14] reported the simultaneous determination of adenine and guanine using a functionalized graphene-modified electrode. Pruneanu *et al*. [15] investigated adenine and guanine oxidation using electrodes modified with graphene-platinum nanoparticle composites, whereas Wang *et al*. [16] demonstrated the simultaneous determination of adenine, guanine, cytosine and thymine using a graphene oxide/multi-walled carbon nanotube nanocomposite-modified electrode. These studies report distinguishable electrochemical oxidation responses for nucleobases at different carbon-based modified electrodes, demonstrating the feasibility of their electrochemical discrimination under the investigated experimental conditions. However, the observation of separated oxidation responses does not, by itself, establish improved oxidation thermodynamics or electron-transfer kinetics relative to unmodified graphite electrodes**.**

However, such approaches commonly incorporate graphene derivatives, carbon nanotubes, metallic nanoparticles, or combinations of nanostructured components. In this context, direct electrochemical deposition of a pPD-derived layer onto graphite provides a comparatively simple route for investigating whether an aromatic amine-derived coating alone can provide an interface suitable for nucleobase detection and discrimination, without the incorporation of additional nanostructured materials. Despite the extensive literature on PpPD and pPD-derived materials [7,10-13], their direct application on graphite for the electrochemical discrimination of all four DNA nucleobases has been

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less explored. In particular, establishing whether the individual oxidation responses of adenine, cytosine, guanine, and thymine remain distinguishable when the analytes are present simultaneously is relevant for assessing the analytical potential of such a simplified modified-electrode architecture.

Therefore, the present work investigates the electrochemical deposition of pPD-derived species onto graphite electrodes and evaluates the resulting interface for individual detection and simultaneous electrochemical discrimination of adenine, cytosine, guanine, and thymine. The modified surface was characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and Raman spectroscopy, while its electrochemical behaviour was investigated by cyclic voltammetry (CV) and chronoamperometry (CA). Particular attention was given to the scan-rate dependence of the electrochemical response, concentration-dependent nucleobase detection, and the ability of the modified electrode to distinguish the oxidation signals of all four nucleobases in mixed solutions. The proposed approach offers a simple and low-cost route to preparing graphite-based electrochemical sensing interfaces without requiring complex nanostructured materials.

Experimental

Materials and instrumentation

Graphite rods (3 mm diameter, 99.995 % purity), p-phenylenediamine (pPD, ≥99 % purity), phosphate salts, and other analytical-grade reagents were purchased from Sigma-Aldrich (USA) and used without further purification. All aqueous solutions were prepared using ultrapure water obtained from a five-stage purification system equipped with reverse osmosis, deionization, and UV sterilization units. The supporting electrolyte was prepared using KH_2PO_4 (0.062 mol L^{-1}), K_2HPO_4 (0.038 mol L^{-1}), and $NaNO_3$ (0.100 mol L^{-1}) in ultrapure water. The solution was sonicated for 30 min to ensure complete dissolution and homogenization, and the pH was subsequently adjusted to 7.0 by the addition of 0.1 mol L^{-1} NaOH.

Electrochemical measurements were performed at room temperature using an Ivium CompactStat potentiostat/galvanostat (Ivium Technologies, The Netherlands) in a conventional three-electrode electrochemical cell. The pPD-derived modified graphite electrode was used as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl (3.5 mol L^{-1} KCl) electrode as the reference electrode. Unless otherwise stated, all potentials reported in this work are referenced to Ag/AgCl.

FTIR spectra were acquired using a Cary 630 FTIR spectrometer (Agilent Technologies, USA). XRD measurements were performed using a D2 Phaser diffractometer (Bruker, Germany) and Raman spectra were acquired using an i-Raman® Plus 532H spectrometer (B&W Tek, USA). Surface morphology was investigated by SEM using a JSM-7100F microscope (JEOL, USA). These complementary techniques were employed to investigate the morphological and structural changes associated with the electrochemical deposition of pPD-derived species on the graphite surface. The pH of the solutions was measured using a SIMPLA-PH-140 digital pH meter (Lab Solutions, Brazil).

CV was employed to investigate the electrochemical deposition process, the scan-rate dependence of the modified electrode, and the individual and simultaneous responses toward the DNA nucleobases. CA was used as a complementary technique to evaluate the concentration-dependent current response of the modified electrode toward adenine, cytosine, guanine and thymine.

Electrode modification

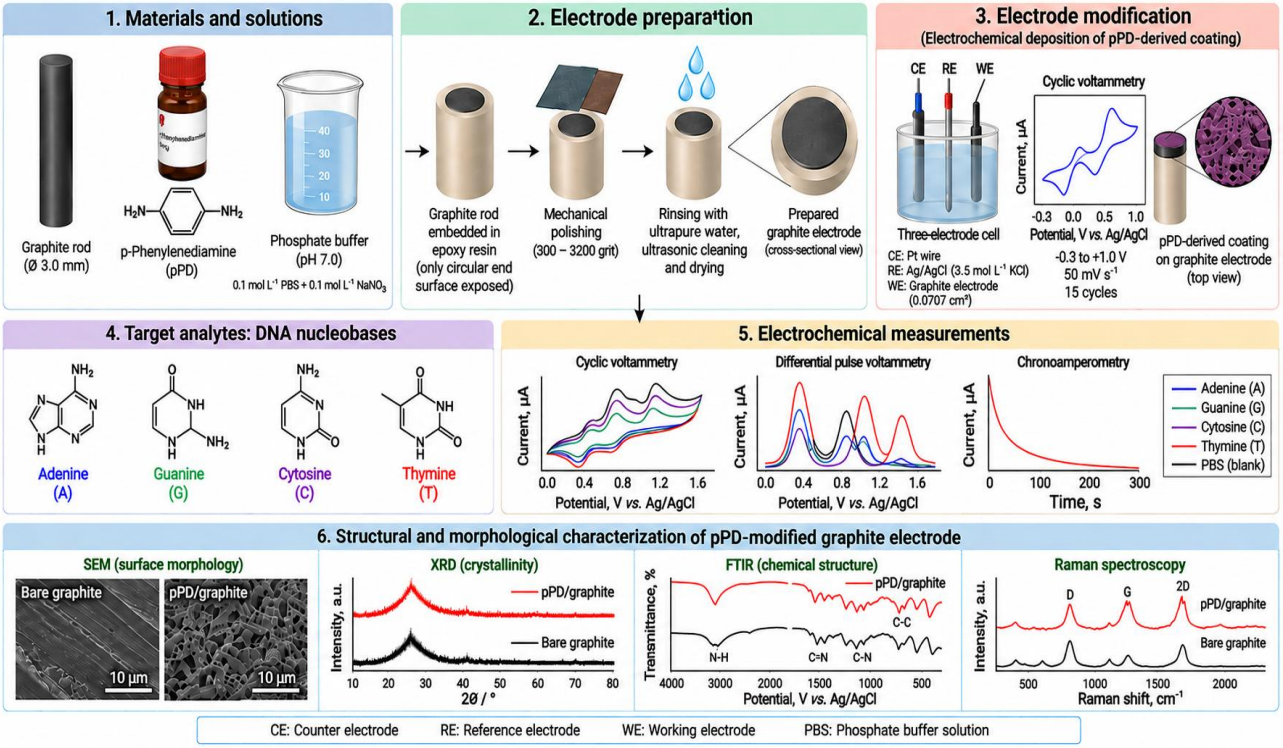
Graphite rods (3.0 mm diameter, 99.995 % purity) were cut into cylindrical segments approximately 30 mm in length and centrally embedded in electrically insulating epoxy resin, prepared using a 2:1 resin-to-hardener ratio. The epoxy completely covered the lateral surface of each graphite rod, leaving only one circular end face exposed to the electrolyte. After resin curing, the exposed graphite surface was sequentially polished using silicon carbide abrasive papers (300, 800, 1200, 2000 and 3200 grit),

followed by final polishing with a polishing cloth. The electrodes were then rinsed with ultrapure water, ultrasonically cleaned for 30 min, and dried under ambient conditions.

Encapsulation ensured that only the circular graphite surface was in contact with the electrolyte during electrochemical deposition of the pPD-derived coating and subsequent electrochemical measurements. The graphite-epoxy interface provided a fixed geometric boundary for the exposed electrode surface. Considering the nominal graphite rod diameter of 3.0 mm, the exposed geometric area was calculated as $A = \pi r^2 = \pi\ (0.15\ \text{cm})^2 = 0.0707\ \text{cm}^2$.

A freshly prepared 5 mmol L^{-1} pPD solution in 0.1 mol L^{-1} H_2SO_4 was used for electrochemical deposition. The solution was ultrasonicated for 30 min to ensure complete dissolution and homogenization. The acidic medium was employed to provide suitable conditions for the electrochemical oxidation of pPD and deposition of pPD-derived species on the graphite surface.

Electrochemical deposition was performed by cyclic voltammetry in a conventional three-electrode cell using the graphite electrode as the working electrode, a platinum wire as the counter electrode and Ag/AgCl (3.5 mol L^{-1} KCl) as the reference electrode. The potential was cycled between -0.3 and +1.0 V *vs*. Ag/AgCl at a scan rate of 50 mV s^{-1} for 15 consecutive cycles. After electrodeposition, the modified electrodes were thoroughly rinsed with ultrapure water to remove residual monomer and weakly adsorbed species, then used for morphological, structural, and electrochemical characterization. The overall procedure for electrode preparation, electrochemical modification, nucleobase detection and characterization is summarized in Scheme 1.



Scheme 1. *Schematic representation of the preparation of the pPD-derived modified graphite electrode, electrochemical deposition process, individual and simultaneous detection of DNA nucleobases and morphological and structural characterization of the modified surface*

Results and discussion

Electrochemical deposition of p-phenylenediamine-derived species on graphite

The electrochemical deposition of pPD on the graphite electrode was investigated by cyclic voltammetry over 15 consecutive potential cycles (Figure 1(a)). During the first scan, a pronounced anodic response cantered at approximately +0.45 V was observed, which is attributed to the initial

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electrochemical oxidation of pPD and the generation of reactive radical-cation species [17]. These intermediates may undergo subsequent deprotonation and coupling reactions, leading to the formation of oligomeric and polymeric species at the electrode surface. With successive potential cycling, the voltammetric profiles evolved considerably, indicating changes in the interfacial electrochemical behaviour associated with the development of a pPD-derived coating. A notable feature is the emergence of an additional redox response in the region of approximately +0.30 to 0.35 V during successive cycles. This feature may be associated with redox transitions involving electroactive units within the deposited oligomeric/polymeric layer. Its appearance suggests that the newly formed surface species contribute to the electrochemical response in addition to the initial oxidation of pPD monomers. The evolution of this feature may also reflect changes in the oxidation state and charge-compensation behaviour of the growing film [17-18]. Comparison of the first and fifteenth cycles (Figure 1(b)) reveals substantial changes in the peak currents and voltammetric profiles, consistent with progressive electrode-surface modification and the development of an electroactive pPD-derived layer. However, these changes cannot be attributed exclusively to increased polymer loading, as alterations in film redox activity, interfacial charge transfer, and transport processes may also contribute.

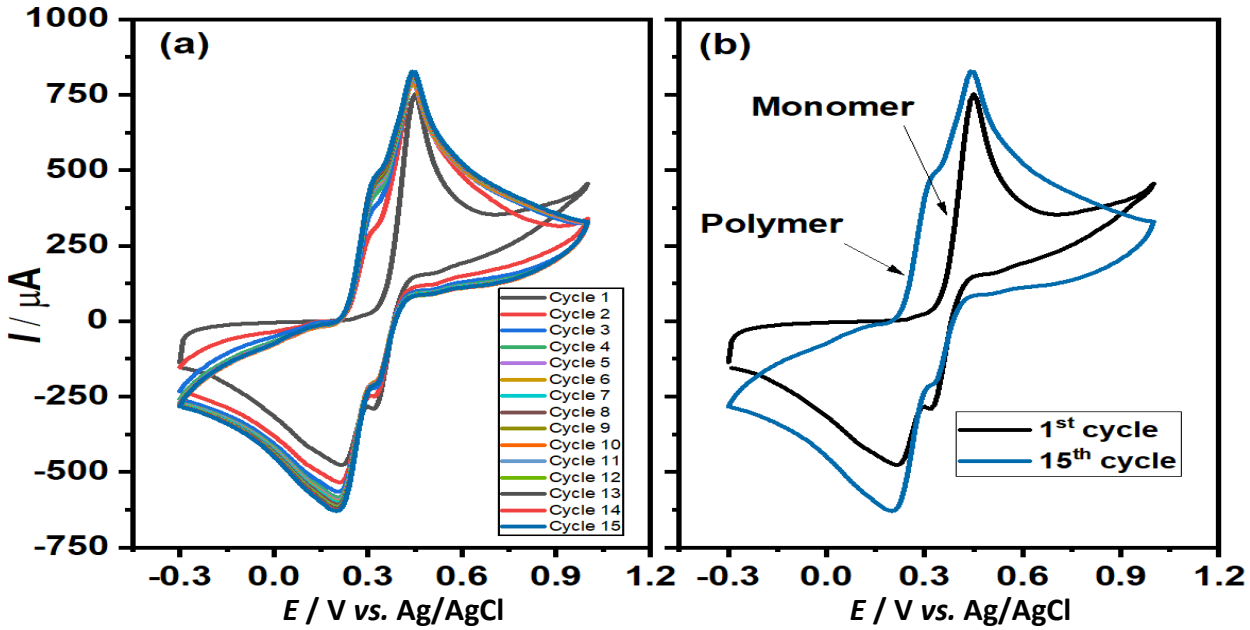

***Figure 1**. Cyclic voltammograms recorded during the electrochemical deposition of p-phenylenediamine-derived species on a graphite electrode over 15 consecutive potential cycles: (a) evolution of the voltammetric response during potential cycling and (b) comparison of the first and fifteenth cycle*

Electrochemical behaviour of the poly(p-phenylenediamine)-modified graphite electrode

The electrochemical behaviour of the bare and PpPD-modified electrodes was investigated using CV, as shown in Figure 2(a). The bare electrode exhibits anodic and cathodic peak potentials E_{pa} and E_{pc} of 0.28 and 0.18 V, with corresponding peak currents I_{pa} and I_{pc} of 30.83 and -23.67 µA, respectively. Similarly, the PpPD-modified electrode displayed anodic and cathodic peak potentials of 0.29 and 0.17 V, with peak currents of 65.18 and -47.08 µA, respectively. The higher redox peak currents observed at the PpPD-modified electrode indicate an enhanced electrochemical response compared with the bare electrode. This improvement may be attributed to the modified electrode surface and its favourable interfacial electrochemical properties. These findings demonstrate the potential of the PpPD-modified electrode for subsequent electrochemical sensing applications. The effect of potential

scan rate on the electrochemical behaviour of the PpPD-modified electrode was investigated by cyclic voltammetry over the range of 5 to 300 mV s^{-1}, as shown in Figure 2(b). An increase in scan rate resulted in a progressive increase in both anodic and cathodic peak currents, indicating a well-defined redox response of the modified electrode under different electrochemical conditions. As shown in Figure 2(c), the anodic and cathodic peak currents exhibited approximately linear relationships with the square root of the scan rate ($v^{1/2}$), with slopes of 0.01192 and -0.01072 mA (mV s^{-1})$^{-1/2}$, respectively. The corresponding coefficients of determination were 0.9988 and 0.9960. These relationships indicate a significant contribution from transport-dependent processes to the electrochemical response. However, linearity between peak current and the square root of the scan rate does not, by itself, establish a purely diffusion-controlled mechanism, particularly for an electroactive coating in which charge propagation and ionic compensation may also contribute. Therefore, the observed scan-rate dependence is interpreted as evidence of transport-influenced electrochemical behavior rather than an exclusively diffusion-controlled process.

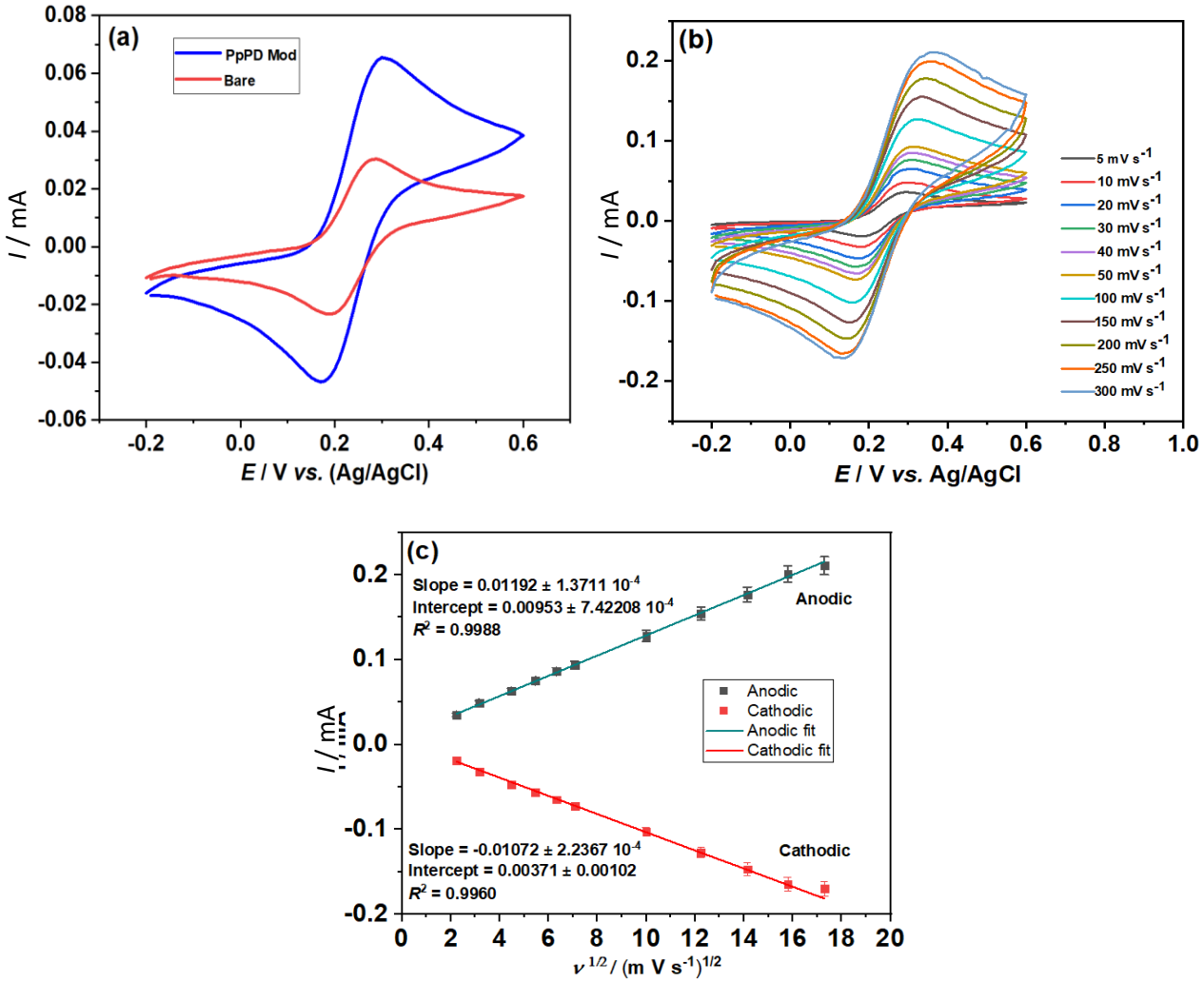

***Figure 2**. Electrochemical characterization of bare and pPD-derived modified graphite electrodes in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$: (a) cyclic voltammograms of bare and modified electrodes recorded at 20 mV s^{-1}; (b) cyclic voltammograms of the modified electrode recorded at scan rates ranging from 5 to 300 mV s^{-1}; and (c) dependence of anodic and cathodic peak currents on the square root of the scan rate, with the corresponding linear regression fits*

Morphological characterization

The surface morphology of the bare graphite electrode and the pPD-derived modified graphite electrode were investigated by scanning electron microscopy (SEM), as shown in Figure 3. The bare

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graphite surface (Figure 3(a)) exhibits the characteristic layered morphology of graphite, with well-defined flakes and exposed basal planes. Following electrochemical deposition (Figure 3(b)), a clear change in surface morphology is observed. The modified graphite surface appears more irregular and textured than the bare substrate, indicating material deposition during repeated potential cycling. These morphological changes are consistent with the formation of a surface coating on the graphite electrode. However, SEM provides morphological rather than chemical information and therefore cannot, by itself, establish the chemical composition or degree of polymerization of the deposited material. The SEM observations are consequently considered together with the Raman and FTIR spectroscopic results discussed in the following sections.

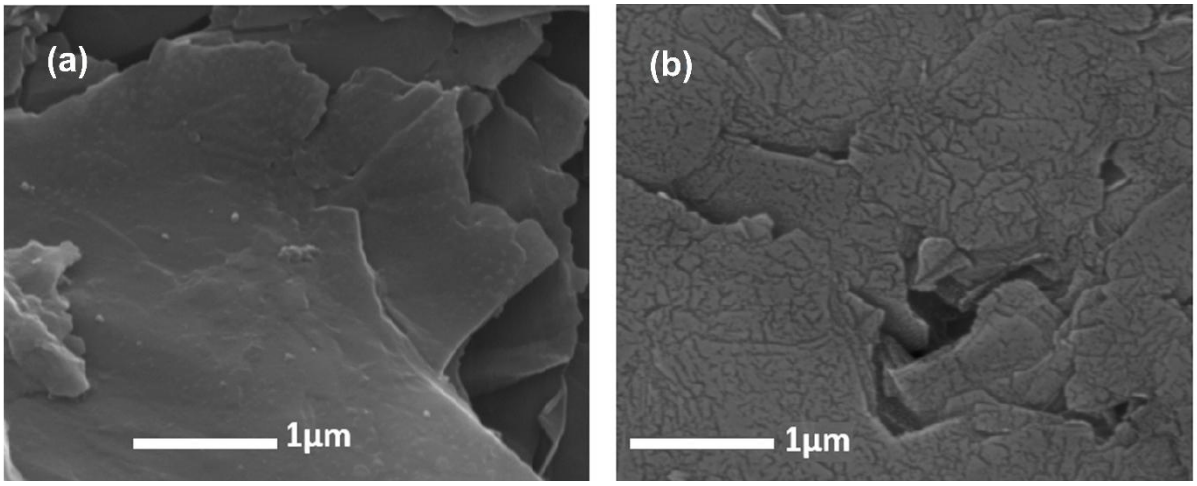

Figure 3. *SEM images of (a) the bare graphite electrode and (b) the pPD-derived modified graphite electrode, showing the morphological changes observed after electrochemical deposition*

Structural characterization by X-ray diffraction and Raman spectroscopy

The structural characteristics of the bare graphite electrode and the pPD-derived modified graphite electrode were investigated by XRD and Raman spectroscopy, as shown in Figure 4. The XRD patterns of both electrodes (Figure 4(a)) are dominated by the characteristic diffraction features of the graphite substrate. No substantial changes in peak positions are observed after electrochemical modification, indicating that the crystalline structure of the underlying graphite is essentially preserved. Differences in relative peak intensities are observed between the bare and modified electrodes; however, these variations cannot be considered direct evidence of polymer formation. No additional well-defined diffraction features attributable to the deposited organic material are clearly resolved, which may be expected for a surface layer whose diffraction contribution is weak relative to that of the highly crystalline graphite substrate.

Raman spectroscopy (Figure 4(b)) provides complementary information on the chemical changes associated with surface modification. The spectrum of the bare graphite electrode is dominated by the characteristic carbon-related bands, whereas the modified electrode exhibits additional vibrational features and changes in relative band intensities following electrochemical deposition. The most pronounced spectral modifications occur in the 1100 to 1700 cm^{-1} region. Features in the approximately 1170 to 1320 cm^{-1} region are consistent with vibrational contributions involving C-N bonds and aromatic amine-derived structures, whereas contributions in the approximately 1450 to 1550 cm^{-1} region have been associated with quinoid-type structural motifs. The spectral region around 1580 to 1620 cm^{-1} contains contributions consistent with aromatic C=C stretching vibrations. Similar vibrational assignments have been reported for poly(p-phenylenediamine) and related aromatic amine materials [7,10,19]. Taken together, the XRD and Raman results indicate that the

electrochemical deposition process preserves the crystalline structure of the graphite substrate while producing detectable changes in the vibrational characteristics of the modified surface. The Raman features are consistent with the presence of pPD-derived aromatic amine species and support the formation of the surface layer observed by SEM. However, these measurements alone do not establish a unique polymer structure or degree of polymerization. Their interpretation is therefore considered together with the FTIR results presented in the following section.

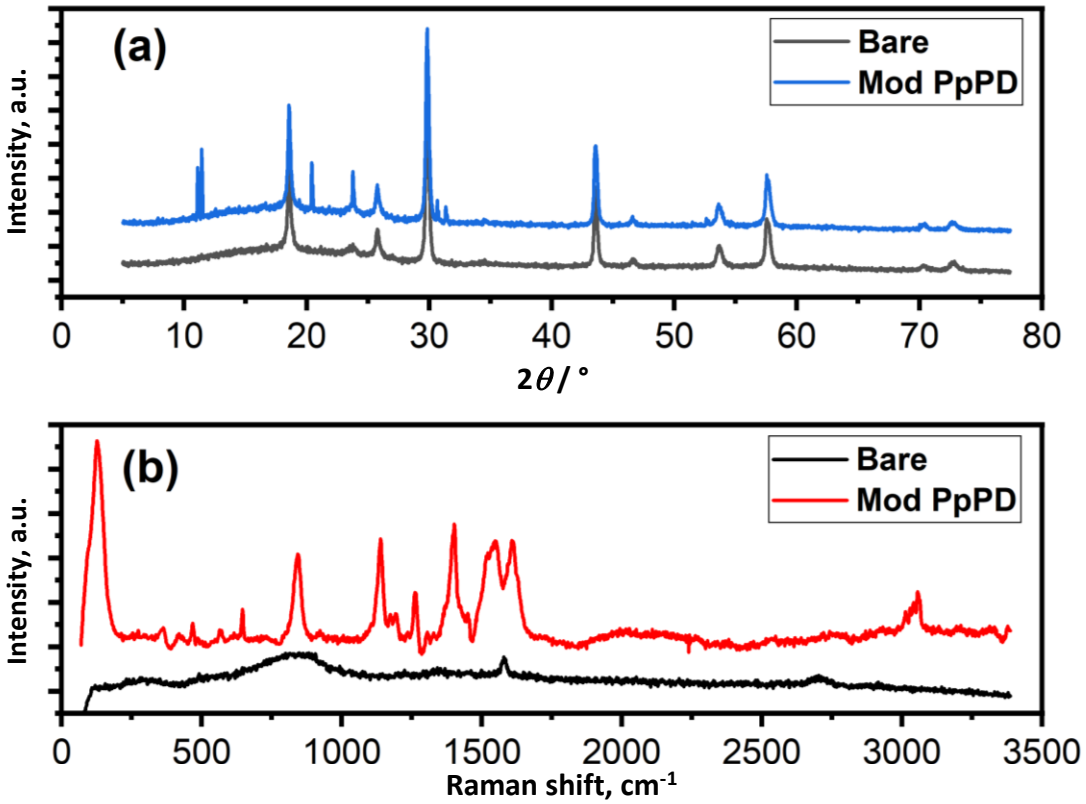

Figure 4. *Structural and spectroscopic characterization of the bare and pPD-derived modified graphite electrodes: (a) XRD patterns and (b) Raman spectra*

Fourier transform infrared spectroscopy characterization

FTIR spectroscopy was employed to investigate the chemical changes associated with the electrochemical deposition of p-phenylenediamine on the graphite surface. Figure 5 compares the spectra of the p-phenylenediamine monomer (pPD), the bare graphite electrode, and the pPD-derived modified graphite electrode. The pPD monomer exhibits characteristic vibrational features associated with aromatic amine structures. The band observed near 1630 cm^{-1} is associated with aromatic ring vibrations, whereas the features at approximately 1510 and 1442 cm^{-1} can be assigned to aromatic C=C stretching and skeletal ring vibrations. Additional bands in the 1340-1256 cm^{-1} region are consistent with C-N stretching and amine-related vibrational modes reported for phenylenediamine-derived materials and aromatic amine compounds [7,10,14,20].

Following electrochemical deposition, changes in band intensity, width, and overall spectral profile are observed for the modified electrode relative to the pPD monomer and bare graphite. Features near 1167 and 1038 cm^{-1} are consistent with changes in the vibrational environment of aromatic amine-derived and C-N-containing species [7,10,11]. A feature near 879 cm^{-1} is also observed in the spectrum of the modified electrode. However, because a contribution in this region is already present in the spectrum of the bare graphite electrode, this feature cannot be regarded

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as a unique spectroscopic marker of the deposited pPD-derived material and should therefore be interpreted only in conjunction with the overall spectral changes.

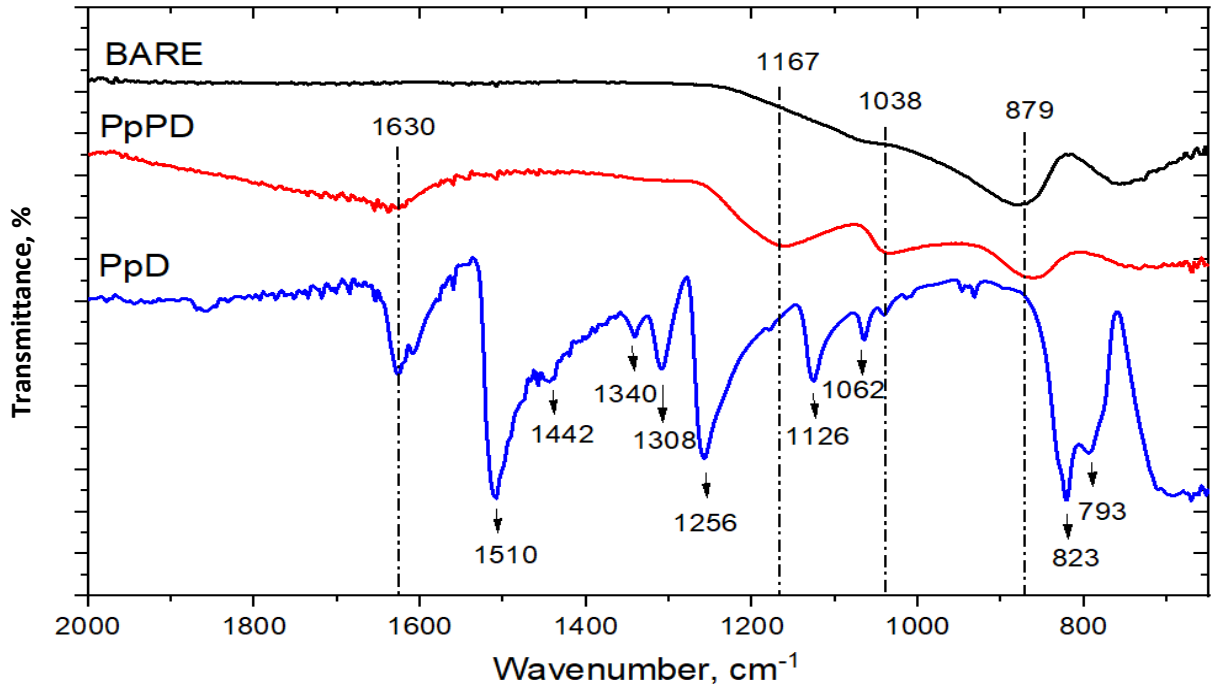

Figure 5. *FTIR spectra of the pPD monomer, bare graphite electrode and pPD-derived modified graphite electrode*

The FTIR results indicate changes in the chemical environment of the electrode surface following electrochemical deposition. The observed spectral modifications are consistent with the presence of aromatic amine-derived species containing C-N bonds and with vibrational features reported for benzenoid- and quinoid-type structures in poly(p-phenylenediamine) and related materials [7,10,20]. However, FTIR alone does not establish the degree of polymerization or oxidation state of the deposited material. When considered together with the electrochemical deposition behaviour, the morphological changes observed by SEM, and the Raman results, the FTIR data provide complementary evidence supporting the formation of a pPD-derived coating on the graphite surface.

Electrochemical detection of nucleobases

The electrochemical response of the pPD-derived modified graphite electrode toward the DNA nucleobases adenine, cytosine, guanine and thymine was evaluated by cyclic voltammetry in 0.1 mol L^{-1} PB (pH 7.0), as shown in Figure 6. Representative voltammograms obtained at selected analyte concentrations are presented in Figures 6(a), 6(c), 6(e) and 6(g), whereas Figures 6(b), 6(d), 6(f) and 6(h) show the corresponding calibration plots constructed using the complete concentration series. For quantitative analysis, the oxidation peak current (I_p) was determined after subtraction of the local voltammetric baseline. Each calibration point represents the mean of three measurements (n = 3), and the error bars represent the corresponding standard deviation.

For adenine (Figures 6(a) and 6(b)), the oxidation current progressively increased with increasing analyte concentration. The representative voltammograms shown in Figure 6(a) correspond to concentrations between 741 and 1525 µmol L^{-1}, whereas the complete concentration series was used to construct the calibration curve.

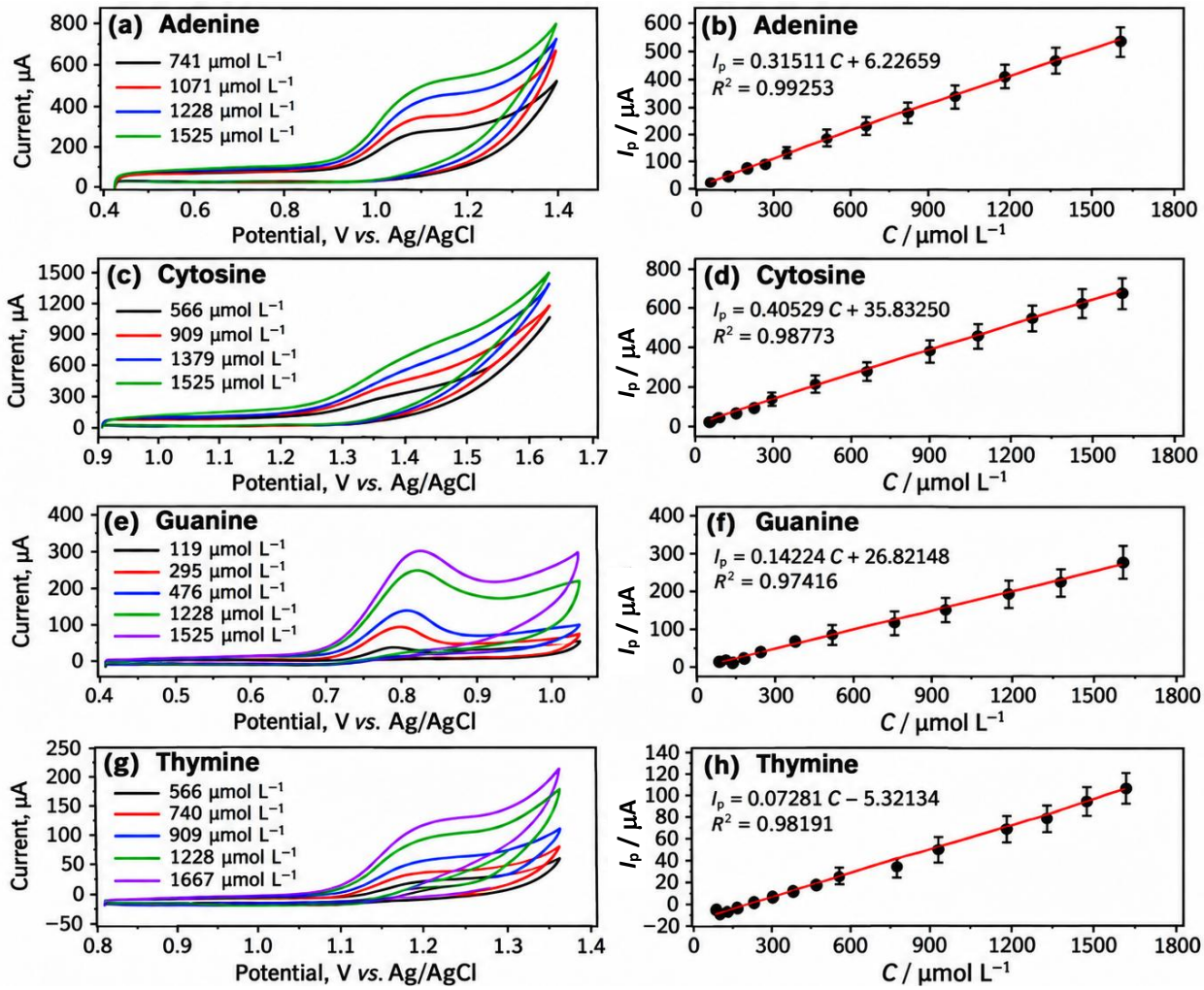

***Figure 6**. Individual electrochemical detection of adenine, cytosine, guanine and thymine using the pPD-derived modified graphite electrode. The corresponding oxidation peak potentials were approximately +1.05 V (A), +1.38 V (C), +0.72 V (G), and +1.14 V (T), all measured vs. Ag/AgCl. The voltammetric responses and corresponding calibration plots illustrate the concentration-dependent electrochemical behaviour of each nucleobase under the investigated experimental conditions*

A linear relationship between the baseline-corrected oxidation peak current and adenine concentration was obtained according to I_p = 0.31511C + 6.22659, with R^2 = 0.99253. The electrochemical response toward cytosine is presented in Figures 6(c) and 6(d). Increasing cytosine concentration resulted in a systematic increase in the oxidation response. The corresponding baseline-corrected peak currents exhibited a linear dependence on concentration, described by I_p = 0.40529 C + 35.83250, with R^2 = 0.98773.

Figures 6(e) and 6(f) show the electrochemical response toward guanine. Among the four nucleobases investigated, guanine exhibited the oxidation response at the lowest potential under the experimental conditions employed. The baseline-corrected oxidation peak current showed an overall increase with guanine concentration, and the calibration data were described by I_p = 0.14224C + 26.82148, with R^2 = 0.97416. The electrochemical behaviour of thymine is shown in Figures 6(g) and 6(h). An overall concentration-dependent increase in oxidation current was observed, and the baseline-corrected peak current followed the linear relationship I_p = 0.07281C - 5.32134, with R^2 = 0.98191.

The results demonstrate concentration-dependent electrochemical responses for the four nucleobases at the pPD-derived modified graphite electrode. The different oxidation potentials observed for adenine, cytosine, guanine and thymine also indicate that their electrochemical

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responses can be distinguished under the experimental conditions investigated. The pPD-derived surface may contribute to analyte-electrode interactions and charge-transfer processes; however, the present results do not establish the specific molecular mechanism responsible for the observed enhancement. Accordingly, these measurements demonstrate the feasibility of using the modified graphite interface for electrochemical detection of the investigated nucleobases.

Chronoamperometric response

Chronoamperometric measurements were performed in 10.0 mL of 0.1 mol L^{-1} PB (pH 7.0). Successive aliquots of the corresponding nucleobase stock solution were added at approximately 60 s intervals under continuous stirring. After each addition, the current was allowed to stabilize, and the analytical current was determined as the average current recorded during the final 5 to 10 s preceding the subsequent addition. The final nucleobase concentration after each addition was calculated considering the cumulative volume added, as shown in Figure 7.

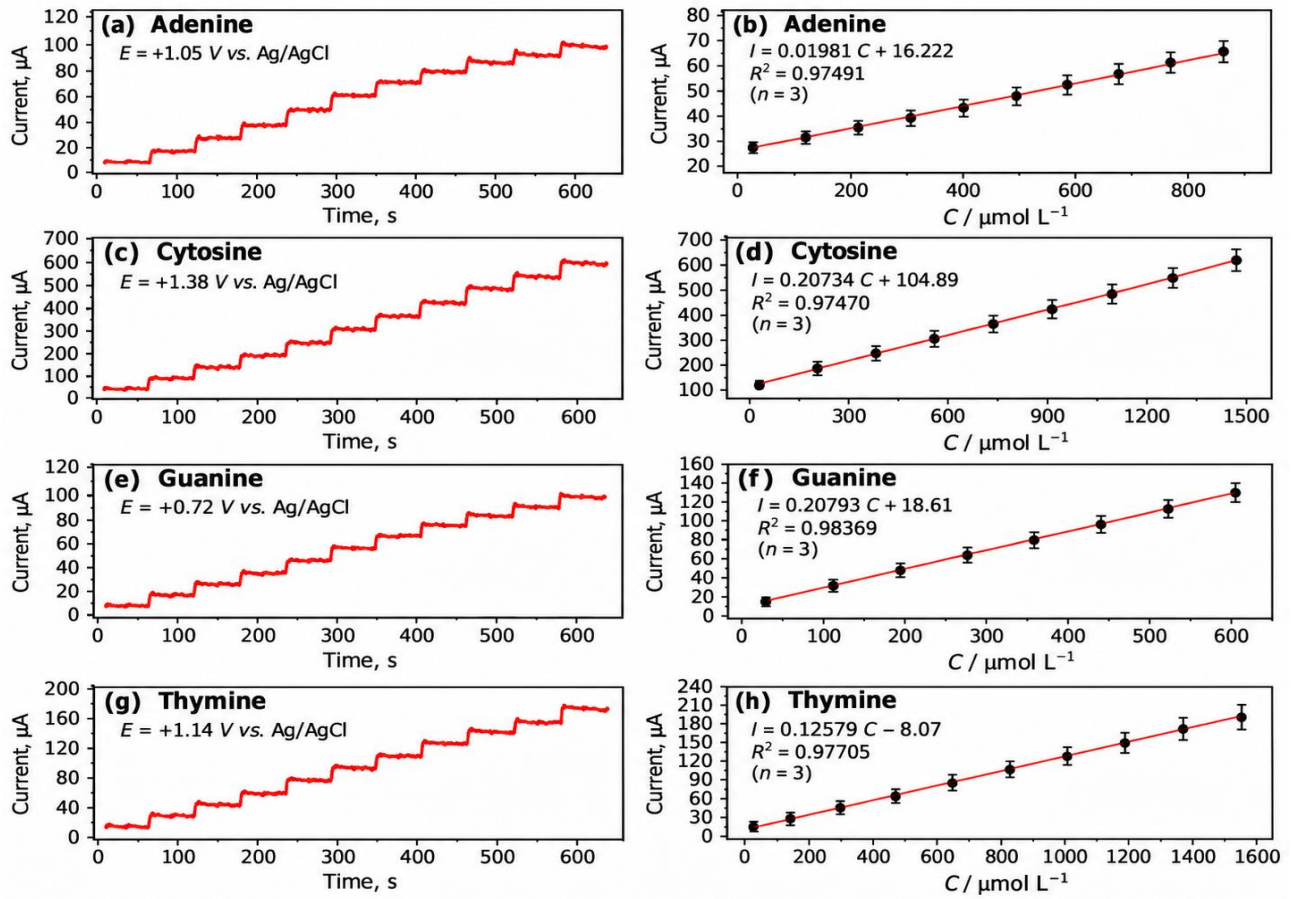

Figure 7. *Chronoamperometric responses and corresponding calibration plots obtained with the pPD-derived modified graphite electrode in 0.1 mol L^{-1} PB-(pH 7.0) for the detection of DNA nucleobases: (a,b) adenine at +1.05 V, (c,d) cytosine at +1.38 V, (e,f) guanine at +0.72 V and (g,h) thymine at +1.14 V* vs. *Ag/AgCl. Panels (a), (c), (e) and (g) show the current-time responses obtained upon successive additions of the respective nucleobases, whereas panels (b), (d), (f) and (h) show the corresponding current-concentration calibration plots. Data points represent mean values from three measurements (*n = *3), error bars represent the corresponding standard deviations, and red lines represent the linear regression fits*

The applied potentials were selected according to the oxidation responses identified by cyclic voltammetry: +1.05 V for adenine, +1.38 V for cytosine, +0.72 V for guanine and +1.14 V for thymine, *vs.* Ag/AgCl. Successive additions of each nucleobase produced stepwise changes in current, allowing the corresponding current-concentration calibration plots to be constructed. Each calibration point

represents the mean of three measurements (n = 3), and the error bars correspond to the standard deviation. In the following calibration equations, I / μA represents the measured chronoamperometric current and C / μmol L^{-1} represents the corresponding nucleobase concentration. The regression coefficients were obtained from the calibration data presented in Figure 7.

Figures 7(a) and 7(b) show the chronoamperometric response and calibration plot for adenine. The current increased with adenine concentration according to $I = 0.01981C + 16.222$ ($R^2 = 0.97491$). The current increased systematically with adenine concentration over the investigated range, showing an approximately linear concentration dependence. For cytosine the calibration relationship was $I = 0.20734C + 104.89$ ($R^2 = 0.97470$). The response toward cytosine is presented in Figures 7(c) and 7(d). At an applied potential of +1.38 V *vs*. Ag/AgCl, successive additions of cytosine similarly produced stepwise increases in current. The corresponding calibration data showed an approximately linear increase in current with increasing cytosine concentration over the investigated range.

Figures 7(e) and 7(f) show the chronoamperometric response toward guanine. At +0.72 V *vs*. Ag/AgCl, successive additions of guanine produced distinct current increments, consistent with the lower oxidation potential observed for this nucleobase in the cyclic voltammetric measurements. The calibration data followed the linear relationship $I = 0.20793C + 18.61$, with $R^2 = 0.98369$ for guanine concentration. The chronoamperometric response toward thymine is presented in Figures 7(g) and 7(h). Successive additions at +1.14 V *vs.* Ag/AgCl resulted in stepwise increases in current. The corresponding calibration data were described by $I = 0.12579C - 8.07$, with $R^2 = 0.97705$, demonstrating an approximately linear concentration dependence over the investigated range.

The chronoamperometric measurements complement the cyclic voltammetric results by demonstrating concentration-dependent current responses for adenine, cytosine, guanine and thymine at their respective applied potentials. The distinct operating potentials and current responses reflect differences in the electrochemical oxidation behaviour of the four nucleobases. Together with the CV results, these measurements support the feasibility of the pPD-derived modified graphite electrode for electrochemical detection of the investigated nucleobases under the experimental conditions employed.

Simultaneous detection of nucleobases

The simultaneous electrochemical response of the pPD-derived modified graphite electrode toward adenine, cytosine, guanine and thymine was investigated by cyclic voltammetry in 0.1 mol L^{-1} PB-(pH 7.0), as shown in Figure 8. Oxidation responses were observed at approximately 0.60, 0.87, 1.04 and 1.22 V *vs*. Ag/AgCl and were assigned to guanine, adenine, thymine and cytosine, respectively. The responses associated with guanine and adenine were comparatively well defined, whereas those attributed to thymine and cytosine occurred at more positive potentials on an increasing anodic background. The separation of these oxidation features demonstrates that the electrochemical responses of the four nucleobases can be distinguished when the analytes are present simultaneously under the investigated experimental conditions. The oxidation sequence, G < A < T < C, is consistent with differences in the electrochemical oxidation behaviour of purine and pyrimidine nucleobases reported in the literature [16,19,20].

The simultaneous measurements complement the individual CV and chronoamperometric results presented in Figures 6 and 7 and provide a proof-of-concept demonstration of electrochemical discrimination of the four nucleobases in a mixed solution. Further studies using independently varied nucleobase concentrations and potential interfering species would be required to establish quantitative selectivity for simultaneous determination in more complex matrices.

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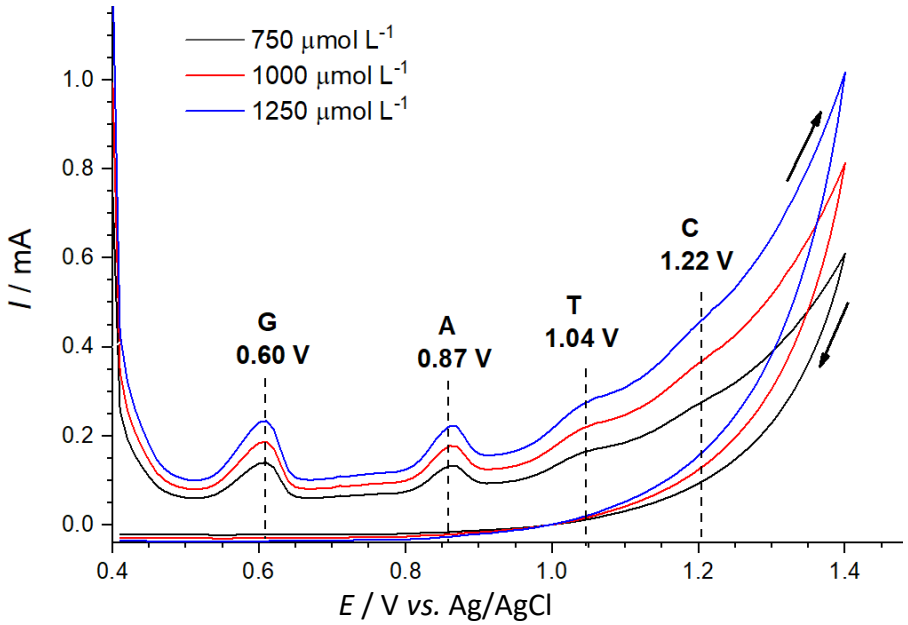

Figure 8. *Cyclic voltammetric response of the pPD-derived modified graphite electrode in the presence of a mixture containing adenine (A), cytosine (C), guanine (G), and thymine (T). The oxidation features were observed at approximately +0.87 V (A), +1.22 V (C), +0.60 V (G), and +1.04 V (T), vs. Ag/AgCl. These potentials indicate distinguishable oxidation responses under the experimental conditions investigated*

Comparison of individual and simultaneous detection and relation to previous studies

Table 1 compares the oxidation potential observed for the four nucleobases during individual and simultaneous cyclic voltammetric measurements using the pPD-derived modified graphite electrode. For all investigated nucleobases, the oxidation responses in the mixed solution occurred at less positive potentials than those observed during the corresponding individual measurements. Despite these potential shifts, the characteristic oxidation sequence was preserved, with guanine exhibiting the lowest oxidation potential, followed by adenine, thymine, and cytosine.

Table 1. *Comparison of oxidation potentials obtained for individual and simultaneous cyclic voltammetric detection of the four nucleobases using the pPD-derived modified graphite electrode*

Analyte	Individual E_p / V	Simultaneous E_p / V	ΔE_p / V
Adenine	1.05	0.87	-0.18
Cytosine	1.38	1.22	-0.16
Guanine	0.72	0.60	-0.12
Thymine	1.14	1.04	-0.10

Guanine exhibited an oxidation potential of approximately 0.72 V during the individual measurement and 0.60 V in the mixed solution, corresponding to a shift of -0.12 V. For adenine, the oxidation potential shifted from approximately 1.05 to 0.87 V (ΔE_p = -0.18 V), whereas thymine shifted from approximately 1.14 to 1.04 V (ΔE_p = -0.10 V). Cytosine exhibited the most positive oxidation response under both conditions, shifting from approximately 1.38 V in the individual measurement to 1.22 V in the mixed solution (ΔE_p = -0.16 V).

The systematic changes in oxidation potential indicate that the electrochemical environment differs when the nucleobases are present together. Such shifts may reflect changes in interfacial conditions and analyte-electrode interactions in the multicomponent system; however, the present measurements do not allow a specific mechanism to be assigned. Importantly, the oxidation order G < A < T < C was maintained in both individual and mixed measurements, enabling the electrochemical responses of the four nucleobases to remain distinguishable under the investigated conditions.

The oxidation peak potential obtained in this study should be interpreted as an experimental voltammetric response rather than a direct measure of thermodynamic oxidation potential. Although the pPD-derived modified graphite electrode exhibited distinguishable oxidation features for the four nucleobases, differences in peak potentials relative to previously reported electrodes cannot be attributed solely to improved oxidation thermodynamics or electron-transfer kinetics. Such comparisons require consideration of the reference electrode, electrolyte composition, pH, scan rate, and electrode surface properties. Furthermore, the present study did not include a systematic comparison with unmodified graphite under identical conditions. Therefore, the observed peak potential does not establish a thermodynamic advantage of the pPD-derived coating over previously reported electrode systems.

These results complement the concentration-dependent measurements presented in Figures 6 and 7 and the mixed-nucleobase response shown in Figure 8. The preservation of the oxidation sequence supports the feasibility of the pPD-derived modified graphite electrode for electrochemical discrimination of the investigated nucleobases. Nevertheless, quantitative selectivity should be established through additional experiments involving independently varied analyte concentrations and potential interfering species. Conducting polymers and carbon-based materials have been widely investigated as electrode modifiers for electrochemical sensing applications because their interfacial properties can be tailored to improve the electrochemical response of different analytes [1-6]. More elaborate sensing platforms incorporating graphene, carbon nanotubes, metal nanoparticles, and hybrid nanostructures have also been reported for the electrochemical detection of DNA nucleobases and related biomolecules [2,6,17,20].

Although these approaches can provide high analytical performance, they may require multiple modification steps or additional nanostructured components. The approach investigated here differs in that the electroactive pPD-derived coating is deposited directly onto a graphite substrate by potential cycling, without incorporating additional nanomaterials. Under the investigated conditions, the modified electrode provided concentration-dependent responses for individual nucleobases and kept their electrochemical responses distinguishable in the mixed system. Rather than establishing analytical superiority over previously reported sensors, these results demonstrate the feasibility of a comparatively simple electrode-modification strategy for nucleobase discrimination. Further optimization of the electrodeposition conditions and analytical validation, particularly regarding detection limits, selectivity, reproducibility, and performance in complex matrices, will be necessary to establish the analytical scope of the proposed platform.

Conclusions

An electroactive pPD-derived coating was successfully deposited onto graphite electrodes by cyclic potential scanning from -0.3 to +1.0 V *vs*. Ag/AgCl. The progressive evolution of the voltammetric response during electrodeposition, together with the morphological changes observed by SEM and the complementary Raman and FTIR spectral modifications, supports the formation of an adherent pPD-derived layer on the graphite surface. XRD showed that the crystalline structure of the graphite substrate remained essentially unchanged after electrode modification. Considering the potential window employed, the deposited material is more appropriately described as a pPD-derived electroactive coating that may contain oligomeric and polymeric species with different oxidation states rather than as an ideal fully oxidized PpPD structure. The scan-rate dependence provided further insight into charge transport within the deposited layer. The log I_p *vs.* log v relationship yielded a slope of approximately 0.42, indicating that the electrochemical

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response is not characteristic of a purely surface-confined process and is consistent with contributions from charge propagation and ion transport within the pPD-derived layer.

The modified graphite electrode exhibited concentration-dependent electrochemical responses toward adenine, cytosine, guanine, and thymine by both cyclic voltammetry and chronoamperometry. The CV calibration curves showed linear concentration dependences over the investigated ranges, with R^2 values of 0.99253, 0.98773, 0.97416 and 0.98191 for adenine, cytosine, guanine and thymine, respectively. These results demonstrate that the modified interface provides measurable and concentration-dependent responses toward all four DNA nucleobases under the experimental conditions investigated. An important outcome of this study was the discrimination of the four nucleobases when present simultaneously. In the mixed system, oxidation responses were observed at approximately 0.60, 0.87, 1.04 and 1.22 V *vs.* Ag/AgCl for guanine, adenine, thymine and cytosine, respectively. Although these responses were shifted toward less positive potentials relative to the corresponding individual measurements at 0.72, 1.05, 1.14 and 1.38 V, the oxidation sequence G < A < T < C was preserved. This behaviour provides proof-of-concept evidence that the four nucleobase responses can remain electrochemically distinguishable at the pPD-derived graphite interface in a multicomponent system.

The results establish a comparatively simple graphite-electrode modification strategy for electrochemical nucleobase detection without the incorporation of additional nanostructured materials. The present study demonstrates the feasibility of the pPD-derived interface for individual nucleobase detection and their discrimination in mixed solutions, while also defining the analytical aspects that require further investigation. Future work should therefore focus on rigorous determination of detection and quantification limits, electrode-to-electrode reproducibility, independently varied analyte concentrations in mixed systems, interference studies, and validation in complex or biological matrices before quantitative simultaneous nucleobase analysis and nucleic-acid-related sensing applications can be established.

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Conflict of interest*: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.*

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