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Original scientific paper

Electrooxidation of catechol in the presence of proline at different pH

Firoz Ahmed[1], Md Abdul Motin[1,✉], Md A. Hafiz Mia[1] and Md A. Aziz[2]

[1]*Department Chemistry, Khulna University of Engineering & Technology (KUET), Khulna 9203, Bangladesh*

[2]*University of Global Village (UGV), Barishal, Bangladesh*

Corresponding author: ✉motin@chem.kuet.ac.bd; Tel: +88 01920770333

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Abstract

The electrooxidation characteristics of catechol in the presence of different concentrations of proline in aqueous buffer solutions at different pH levels were examined using cyclic voltammetry, controlled potential coulometry, and differential pulse voltammetry. In the second potential scan, the reaction involving o-benzoquinone and proline occurred at higher proline concentrations. The product of catechol electrooxidation with proline is assumed to be (S)-1-(3,4-dihydroxyphenyl)pyrrolidine-2-carboxylic acid, which undergoes electron transfer at more negative potentials compared to catechol. The influence of the pH of catechol in the presence of proline was assessed by adjusting the pH of the buffer solution from 5 to 11. Both pH and proline concentration significantly affected the reaction, and the optimal conditions for this reaction were observed at a proline concentration of 150 mM and a catechol concentration of 2 mM in a buffer solution of pH 7. The reaction pathway exhibited characteristics of an electron transfer, chemical reaction and electron transfer (ECE) type, followed by a diffusion mechanism.

Keywords

Electrosynthesis, catechol-proline adduct, oxidation reaction pathway, voltammetry techniques, controlled potential coulometry

Introduction

Catechol is an important foundational compound in organic synthesis, and it is produced on an industrial scale for use as a precursor in pesticides, fragrances, and pharmaceuticals [1]. It is also present in various natural products, particularly those with antioxidant properties [2]. A notable feature of catechols is their susceptibility to oxidation, primarily because of their antioxidant capabilities and low oxidation potentials [3]. The oxidation results in the formation of reactive and electron-deficient o-quinones. Electrochemical oxidation is one of the most effective methods for generating these reactive o-quinone species [4]. Several studies have documented the electro-

oxidation of catechols to create o-quinones, which act as reactive intermediates in various favorable homogeneous reactions [5].

Proline is the only amino acid that contains a secondary amine which categorizes it as an imino acid. Additionally, it is distinctive because the alpha-amino group is directly linked to the side chain, resulting in the alpha carbon being a direct component of the side chain. Proline may act as a potential endogenous excitotoxin [6-8]. Proline unique cyclic side chain confers a notable conformational rigidity that sets it apart from other amino acids. This structural characteristic also influences the speed at which peptide bonds form between proline and different amino acids. In a peptide bond where proline is incorporated as an amide, its nitrogen does not attach to a hydrogen atom, which prevents it from serving as a hydrogen bond donor, although it can still function as a hydrogen bond acceptor [9].

The electrochemical oxidation of catechols in the presence of various nucleophiles, including aspartic acid, glutamine, sulfanilic acid, ethanol, 2-thiobarbituric acid, b-diketones, 4-hydroxy-6-methyl--2-pyrone, 2-thiouracil, dimedone, 4,7-dihydroxycoumarin, 4,5,7-trihydroxycoumarin, 4-hydroxy-6--bromocoumarin, 3-hydroxycoumarin, 4-hydroxy-6-methyl-α-pyrone, 4-hydroxy-6-methyl-2-pyridone, and 4-hydroxycarbostyrile were studied [10-22]. To the best of our knowledge, there are no existing studies on the electrochemical oxidation of catechols involving proline. In this paper, we have studied the electrochemical properties of catechol in the presence of proline with three different electrodes, a wide range of concentrations of proline, and different pH levels.

Experimental

Catechol, proline, acetic acid, sodium acetate, potassium chloride, sodium dihydrogen phosphate, and disodium hydrogen phosphate were of analytical grade (E-Merck, Germany). Distilled water was used to prepare all solutions. Phosphate buffer (PB) and acetate buffer (AB) solutions were prepared in distilled water. Solutions of catechol and catechol with proline at different concentrations were prepared at various pH levels (5-11) using acetate or phosphate buffer solutions. The target pH values were calculated using the Henderson-Hasselbalch equation, and the buffer solutions were prepared accordingly.

Platinum and gold disks with a diameter of 1.6 mm (Bioanalytical Systems, Inc.) and glassy carbon (GC) disks with a diameter of 3 mm (Bioanalytical Systems, Inc.) were used as working electrodes for voltammetry. The working electrode used in controlled potential coulometry was an assembly of three carbon rods (6 mm diameter, 4 cm length). The electrode surface was polished with 0.05 µm alumina before each run. The auxiliary electrode was a platinum coil (Bioanalytical Systems, Inc.), and the reference electrode was Ag|AgCl (3M KCl) (Bioanalytical Systems, Inc.). The working electrode was polished by gently pressing it against the polishing surface for 5-10 minutes and then thoroughly washed with deionized water. At this point, the electrode surface appeared as a shiny mirror. The potentiostat/galvanostat was the µStat 8000 (Metrohm/Drop Sens). Nitrogen gas was bubbled through the one-compartment cell before the electrochemical run.

Results and discussion

Electrochemical behaviour of catechol with proline

The electrochemical characteristics of catechol in the absence and presence of proline were investigated using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and controlled potential coulometry (CPC). Figure 1 (blue line) presents the cyclic voltammogram of 2 mM catechol on a 3 mm GC electrode in a phosphate buffer solution with pH 7 and a scan rate of 0.1 V s^{-1}. The

voltammogram reveals a single anodic peak at 0.44 V and a corresponding cathodic peak at 0.12 V, associated with the conversion of catechol to o-quinone and its reverse process.

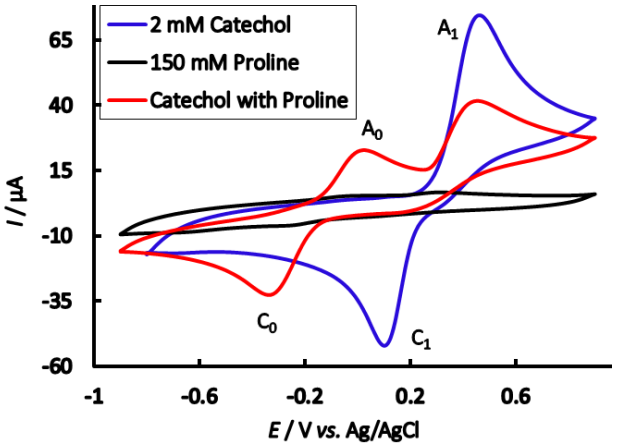
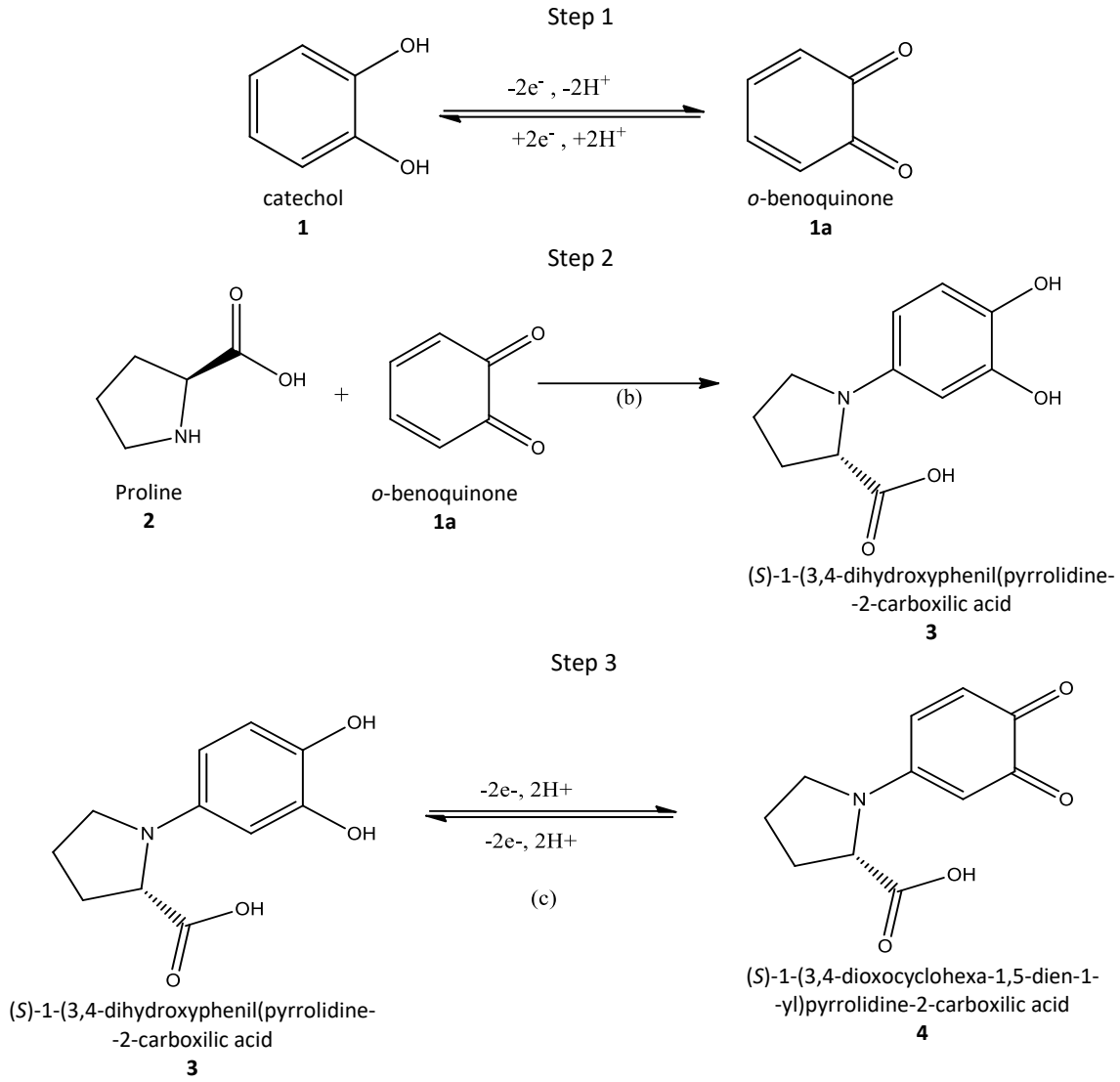


Figure 1. *Cyclic voltammograms of catechol, proline, and 2 mM catechol with 150 mM proline on GC electrode in phosphate buffer solution of pH 7, at a scan rate of 0.1 V s^{-1} (2^{nd} cycle). A_0 and A_1 are anodic peaks, and C_0 and C_1 are corresponding cathodic peaks*

Pure proline exhibits no electrochemical activity within the potential range examined (Figure 1, black line). Figure 1 (red line) presents the cyclic voltammogram (CV) of catechol (2 mM) in the presence of proline (150 mM) during the second potential scan under the same conditions. In the second potential scan, catechol combined with proline displays two anodic peaks at 0.12 and 0.44 V, along with corresponding cathodic peaks at -0.32 and 0.20 V, respectively. The appearance of A_0 and C_0 peaks, along with the reduction of A_1 and C_1 peaks and the shifting of their peak positions upon the addition of proline, suggests that these alterations are likely due to a reaction between catechol and proline. This can be clarified by exploring the nucleophilic attack of proline on o-benzoquinone. This phenomenon lowers the concentration of o-benzoquinone in the reaction layer, leading to a decrease in A_1 and C_1 peaks while simultaneously generating a catechol-proline adduct, which results in the appearance of peaks A_0 and C_0. During the initial scan of the potential, the anodic peak of catechol with proline resembles that of pure catechol. However, in the subsequent potential scan, the peak current of A_1 and C_1 (depicted by the red line) shows a notable decrease compared to free catechol (shown by the blue line).

The peak current ratio for peaks A_1 and C_1 (I_{pa_1}/I_{pc_1}) showed a significant decrease with cycling, suggesting a chemical reaction between proline and the o-quinone generated on the electrode surface (step 1 in Scheme 1). This could point to the formation of (S)-1-(3,4-dihydroxyphenyl)pyrrolidine-2-carboxylic acid *via* a nucleophilic substitution reaction (step 2 in Scheme 1). This behaviour aligns with previous findings regarding the electrochemical oxidation of catechols when combined with aspartic acid, glutamine, and sulfanilic acid [23-25]. When the oxidation potential of the resulting product is relatively low, further oxidation tends to be reduced, which allows for additional oxidation and integration of other elements [26].

Specifically, in the case of catechol interacting with proline, the oxidation of the proline-substituted o-benzoquinone (step 3 in Scheme 1) occurs more readily than that of the original catechol. While this substitution product can be further attacked by proline, such reactions were not observed in the voltammetric studies due to the low reactivity of o-quinone **4** towards **2**. Similar compounds generated electrochemically, like catechol and various nucleophiles, have also been documented [10-23,26]. When there are no other nucleophiles present, water or hydroxide ions typically react with o-benzoquinone [27].



Scheme 1. *Reaction steps of catechol oxidation in presence of proline*

In Figure 2a, the cyclic voltammograms (CV) of the second cycle for 2 mM catechol in the presence of 150 mM proline at the GC (3 mm) electrode in a buffer solution (pH 7) are presented at various scan rates. As the scan rate increases, the anodic and cathodic peak currents increase to 0.3 V s^{-1} for A_0 and C_0. Additionally, the cathodic peaks shift to the left, while the anodic peaks move to the right with the increasing scan rate. However, at scan rates of 0.4 to 0.5 V s^{-1}, the anodic peak current for A_0 and C_0 decreased while the A_1 anodic peak current increased. At this point, the anodic peak potential continued to move to the right, whereas the cathodic peak potential shifted left. It was noted that the cathodic peak related to the catechol and o-benzoquinone redox reaction was quite small at the investigated scan rates, likely due to the rapid chemical reaction between proline and o-benzoquinone.

Figure 2b shows the plots of the net anodic and cathodic peak currents for 2 mM catechol with 150 mM proline during the second cycle, plotted against the square root of the scan rates. Here, the net current is calculated as the second peak minus the first one based on the scan-stopped method [28]. Although the peak current of A_1 and C_1 increases proportionally with the square root of scan rates, these lines do not pass through the origin. This indicates that the pure diffusion process does not fully control the peak current of the reactant at each redox reaction, suggesting that some surface-related chemical complications occur during the reaction.

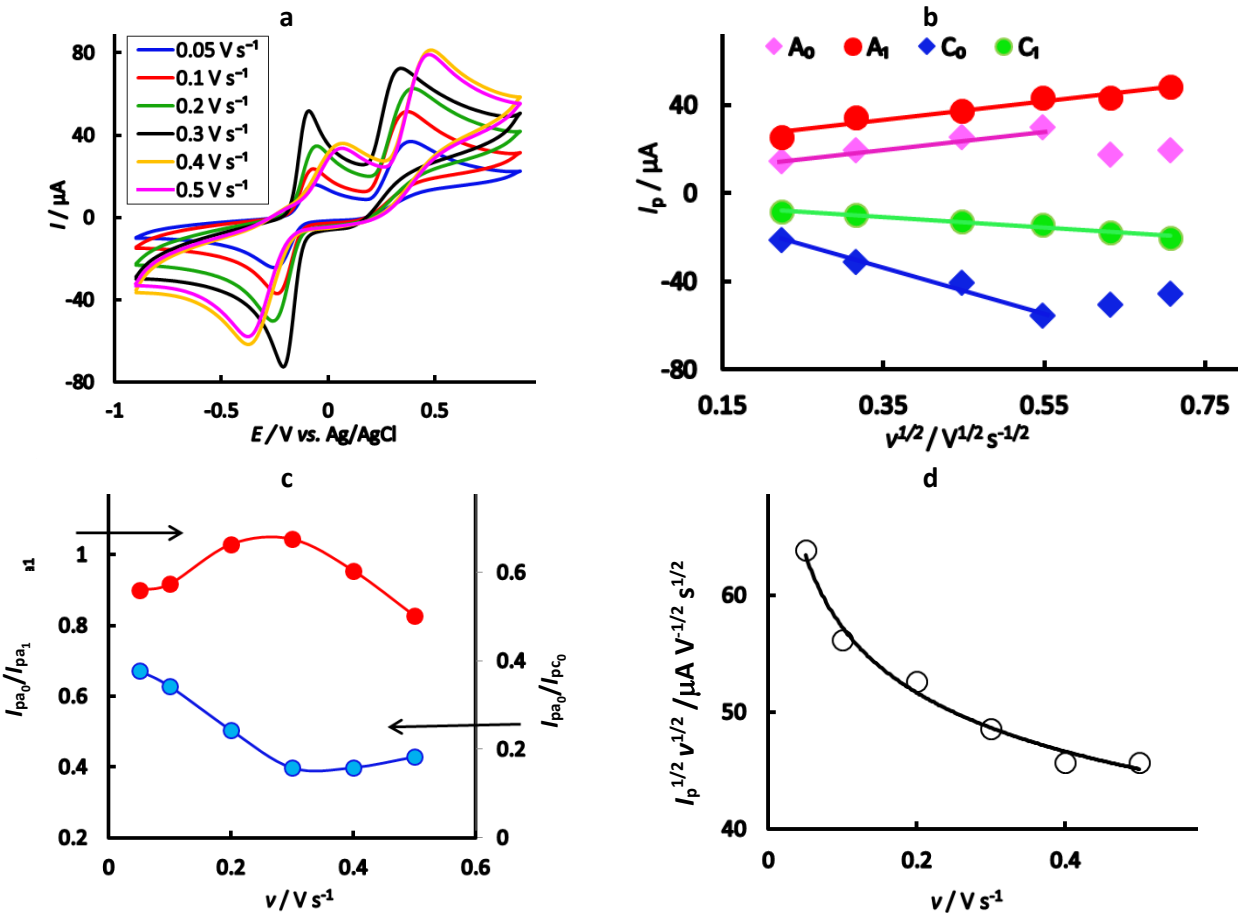


Figure 2. *a) cyclic voltammograms of 2 mM catechol with 150 mM proline in the second scan of potential at the GC electrode in a buffer solution of pH 7 at different scan rate; b) plots of peak current vs. square root of scan rate in the same conditions. Legend shows the symbols of oxidation and reduction peaks; c) variation of peak current ratio of corresponding peak (I_{pa_0}/I_{pc_0}) and anodic peak (I_{pa_0}/I_{pa_1}) vs. scan rate in the same conditions; d) current function ($I_p/v^{1/2}$) vs. scan rate*

For A_o and C_o, the peak current increases proportionally up to 0.3 V s^{-1} with increasing square root of scan rates. The anodic peak current ratio (I_{pa_0}/I_{pa_1}) for the catechol-proline mixture initially increased with rising scan rates up to 0.3 V s^{-1} and then decreased (Figure 2c). Correspondingly, the peak current ratio (I_{pa_0}/I_{pc_0}) also decreased with increasing scan rates initially but then increased after reaching 0.3 V s^{-1} (Figure 2c). Additionally, the current function value ($I_p/v^{1/2}$) was observed to decrease with rising scan rates (Figure 2d). The exponential trend observed in the current function versus scan rate graph suggests an ECE (electrochemical-chemical-electrochemical) mechanism for the electrode process [13,14,26]. This indicates that the reactivity of o-benzoquinone (**1a** in Scheme 1) with proline (**2** in Scheme 1) firstly increases at lower scan rates but decreases at higher scan rates.

The evidence supporting a subsequent chemical reaction between o-benzoquinone **1a** and proline **2** (step 2 in Scheme 1) includes the following points:

i. In the presence of proline, both the anodic peak current (I_{pa_1}) and cathodic peak current (I_{pc_1}) decrease during the second cycle (see Figure 1), suggesting that the electrochemically generated o-benzoquinone **1a** is partially consumed through a chemical reaction with proline **2**.

ii. The ratio of the peak currents (I_{pa_0}/I_{pc_0}) decreases as the potential sweep rate increases up to 0.3 V s^{-1}, after which it begins to rise gradually. This suggests that at lower scan rates, a greater accumulation of cathodic species occurs, whereas at higher scan rates, anodic species are more prevalent.

iii. The peak current ratio (I_{pa_0}/I_{pa_1}) shows an initial increase followed by stabilization. An increase in the scan rate leads to a reduction in the extent of the chemical reaction between **1a** and **2** while the cyclic voltammogram is being recorded [26].
iv. The current function, represented as $I_p/v^{1/2}$, decreases exponentially with increasing scan rate. This finding suggests that the reaction mechanism involves a sequence of electron transfer, a chemical reaction, followed by another electron transfer type (see Scheme 1) [13,14].

Based on these results, it appears that the 1,4-Michael addition reaction of proline 2 to o-benzoquinone **1a** produces compound **3**. The oxidation of this product **3** is facilitated compared to the oxidation of the parent molecule **1** due to the presence of the electron-donating amine group.

The cyclic voltammogram of pure catechol was also observed in a buffer solution of pH 7 by varying scan rates. The linear relationship between the anodic and cathodic peak currents versus the square root of the scan rates indicates that the peak current of the reactant during each redox reaction is influenced by the diffusion process.

Influence of pH

The electrochemical properties of catechol were investigated both in the presence and absence of proline by analysing the electrode response in buffer solutions with varying pH levels from 5 to 11. CV was conducted to examine the oxidation of 2 mM catechol at a scan rate of 0.1 V s^{-1} across different pH values. In a buffer solution at pH 7, catechol produced a distinct reversible wave. The anodic peak potential for catechol shifted to the left as the pH increased. The electrochemical reaction involving catechol at pH 7 is characterized as a two-proton, two-electron transfer process (as illustrated in Scheme 1) [29,30].

The cyclic voltammogram of 2 mM catechol in the presence of 150 mM proline was analyzed using a 3 mm glassy carbon electrode in buffer solutions at pH levels ranging from 5 to 11 (see Figure 3a). At pH 5 to 7, the voltammetric behaviour of catechol indicates that new anodic and cathodic peaks developed upon second cycling, suggesting that the reaction between o-benzoquinone and proline occurred (Figure 3a). At pH 7, the peak currents for both the anodic peak (A_0) and the cathodic peak (C_0) are greater than those observed at pH 5. In contrast, at higher pH values (specifically between pH 9 and 11), the cyclic voltammograms of catechol exhibit irreversible characteristics. In the basic medium, OH^- acted as a stronger nucleophile than proline; it underwent a homogeneous chemical reaction with o-benzoquinone. This homogeneous reaction was too fast that it cannot be observed in the time scale of cyclic voltammetry. This suggests that in alkaline environments, the oxidation of catechol proceeds by undergoing an irreversible chemical reaction with hydroxide ions [30].

In neutral media, o-benzoquinone can react with the amine group *via* a nucleophilic substitution mechanism through a 1,4-Michael addition, resulting in the appearance of a new anodic peak in voltammetric cycling. The position of the redox couple peak varies with changes in pH.

Figure 3 b illustrates the relationship between oxidation peak potential (E_p) and pH. The slopes of the plot were determined graphically as the anodic peaks (39.0 and 32.0 mV/pH for anodic peaks A_1 and A_0) at 0.1 V s^{-1}, which aligned closely with the theoretical expectation for a process involving two electrons and two protons. This finding suggests that the oxidation processes of both catechol and the catechol-proline adduct proceed through the $2e^-/2H^+$ mechanism (refer to Scheme 1). It also implies that during the reaction, protons, as well as electrons, are released from the catechol-proline adduct. Other researchers have observed similar behaviours in studies of catechol and its derivatives [12-14,22,31]. In both acidic and basic mediums, the peak current ratio decreased.

The plot of oxidation peak (A_0) current (I_p) versus pH of the buffer solution is shown in Figure 3c. The data indicates that the peak current reaches its maximum at pH 7. At this pH level, the difference in the peak current ratio (I_{pa_0}/I_{pc_1}) between the presence and absence of proline was at its highest. This suggests that the electrochemical oxidation of catechol with proline is more effectively facilitated in neutral conditions, accelerating the rate of electron transfer. Therefore, this study adopts a buffer solution with a pH of 7 as the optimal medium for the electrochemical analysis of catechol in the presence of proline.

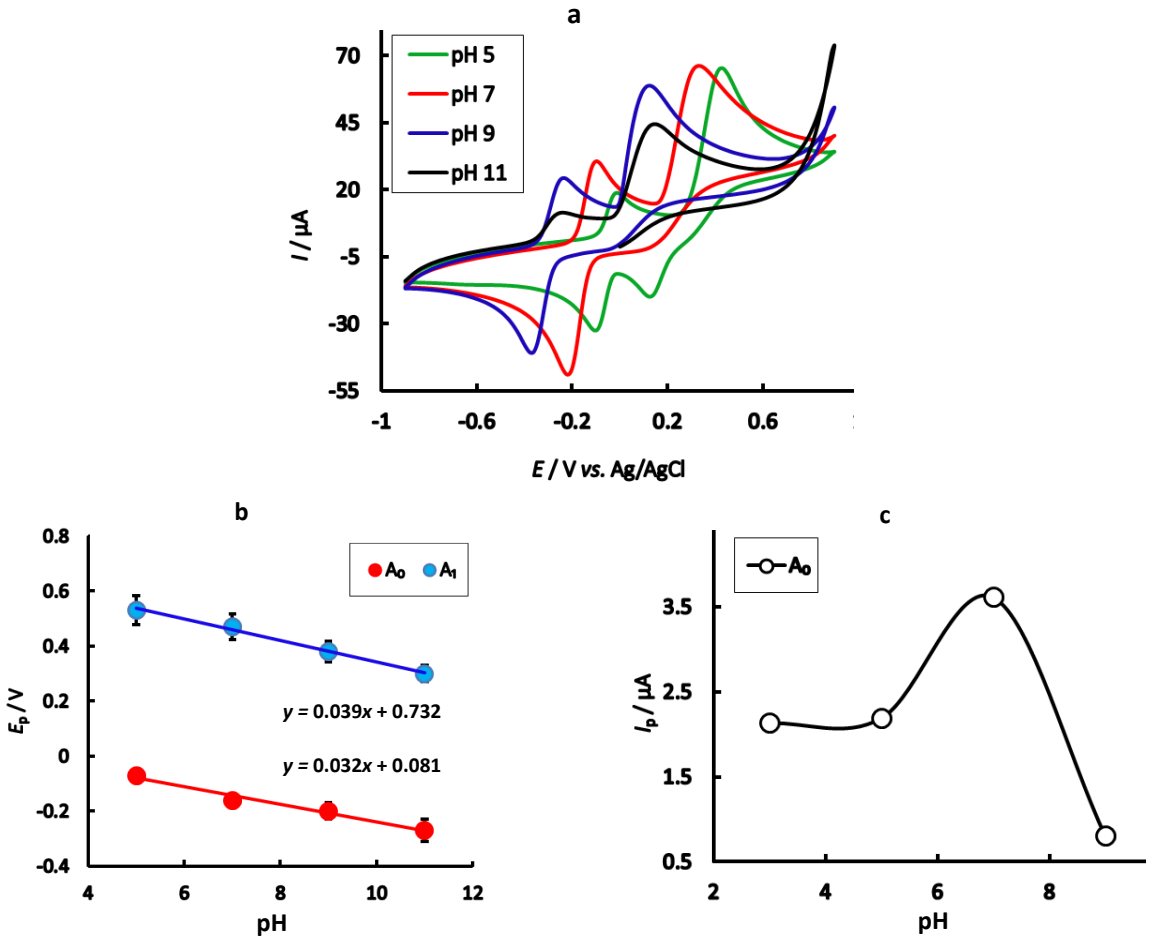


Figure 3. *a) Cyclic voltammograms of 2 mM catechol with 150 mM proline of GC (3 mm) electrode in buffer solutions of different pH, at a scan rate 0.1 V s^{-1}; b) plots of peak potential vs. pH in the same conditions; c) plots of peak current vs. pH in the same conditions. The meaning of symbols A_0 and A_1 is like in Figure 1*

Concentration effect of proline

Figure 4a illustrates the changes in the voltammogram pattern by incorporating varying concentrations of proline (50, 100, 150, 200, and 250 mM) into a constant concentration of catechol (2 mM) of the glassy carbon electrode in a buffer solution of pH 7 and a scan rate 0.1 V s^{-1}. The net current intensity of the newly observed anodic and cathodic peaks increases as the proline concentration rises up to 150 mM. However, with further increases in proline concentration (beyond 150 mM), there is a decrease in the anodic and cathodic peak currents (see Figure 4b). At these higher proline concentrations (>150 mM), the excess amount of electro-inactive proline may accumulate on the electrode surface, leading to a reduction in peak current. This suggests that the nucleophilic substitution reaction of catechol in the presence of proline was most favourable at a concentration of up to 150 mM proline at pH 7.

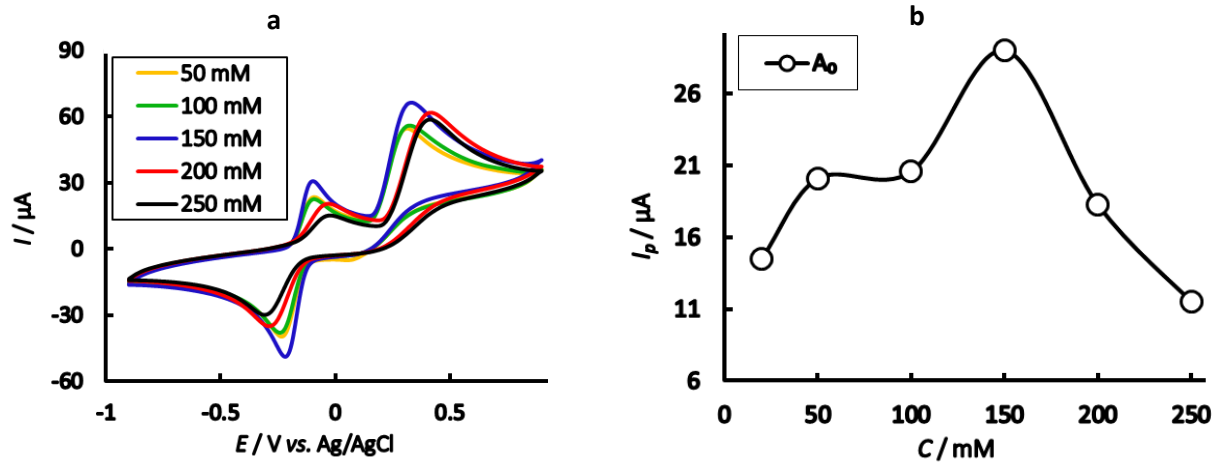


Figure 4*. a) Cyclic voltammograms of composition changes of proline with fixed 2 mM catechol at GC electrode in buffer solution at pH 7, and scan rate 0.1 V s^{-1}; b) plots of anodic peak current vs. concentration of proline with fixed 2 mM catechol in the same conditions. The meaning of A_0 is like in Figure 1*

Effect of electrode materials

The electrochemical characteristics of catechol were investigated both in the absence and presence of proline using various electrodes, including glassy carbon, gold, and platinum, under different pH conditions. The CV of 2 mM catechol in the presence of 150 mM proline at the GC, gold and platinum electrodes in buffer solution of pH 7 and a scan rate of 0.1 V s^{-1} are presented in Figure 5a. The characteristics of the voltammograms, including peak positions and current intensities, vary across the different electrodes despite a larger diameter of the GC than gold and platinum electrodes.

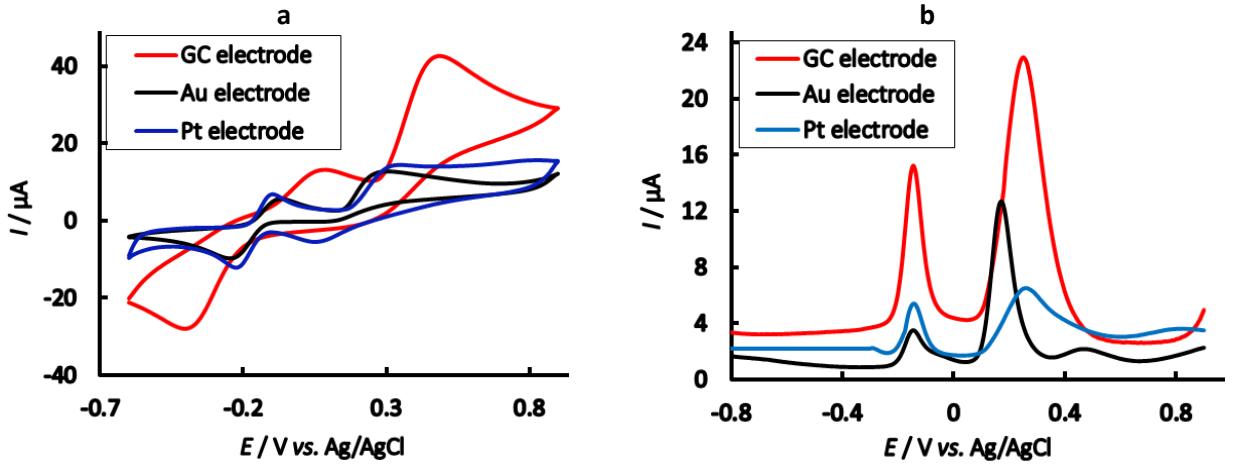


Figure 5. *a) Cyclic voltammograms and b) differential pulse voltammograms of 2 mM catechol with 150 mM proline at GC electrode (3.0 mm) gold electrode (1.6 mm) and platinum electrode (1.6 mm) in buffer solution of pH 7 and scan rate 0.1 V s^{-1}*

The electrochemical characteristics of catechol in the presence of proline, including variations in pH, concentration, and scan rate, have been extensively studied using platinum and gold electrodes. In the second potential cycle, all electrodes show a new oxidation and reduction peak at a lower oxidation potential. This phenomenon is likely due to the oxidation of the adduct formed between o-benzoquinone and proline. GC, Pt, and Au electrodes show two redox couples of adducts at 0.05/-0.38, -0.12/-0.21 and -0.08/-0.21 V, respectively. The DPV of 2 mM catechol in the presence of 150 mM proline at GC, gold, and platinum electrodes at pH 7 and a scan rate of 0.1 V s^{-1} are also

presented in Figure 5b. The DPV response is similar to the CV response. However, the GC electrode demonstrated notably better voltammetric responses than other evaluated electrodes. As a result, this paper concentrates primarily on the properties of catechol with proline when utilizing the GC electrode.

Subsequent cycles of CV of catechol-proline derivative

Figure 6a illustrates the cyclic voltammogram of the initial 15 cycles of 2 mM catechol in the presence of 150 mM proline in a buffer solution of pH 7 buffer solution using a GC (3.0 mm) electrode, within a potential range from -0.9 to 0.9 V. The first cycle revealed an anodic peak at 0.38 V and a corresponding cathodic peak at -0.29 V, as marked by the red line, with a scan rate of 0.1 V s^{-1}. In the following cycles, a new anodic peak emerged around 0.02 V, while the current of the initial anodic peak steadily increased with cycling. In contrast, the current for the second anodic peak decreased and shifted positively. This behaviour is attributed to the formation of a catechol-proline adduct, which resulted in a gradual reduction in the redox couple height of catechol due to a nucleophilic substitution reaction occurring at the electrode surface (refer to Scheme 1). The gradual decline in the height of the peaks associated with the oxidation and reduction of catechol during cycling can be attributed to the increased formation of the catechol-proline adduct. This results in a reduced concentration of catechol or quinone available at the electrode surface. Over the first ten cycles, the first anodic peak current increases but is stabilized with subsequent cycles, likely due to the buildup of newly formed electro-inactive species blocking the electrode surface after extensive cycling.

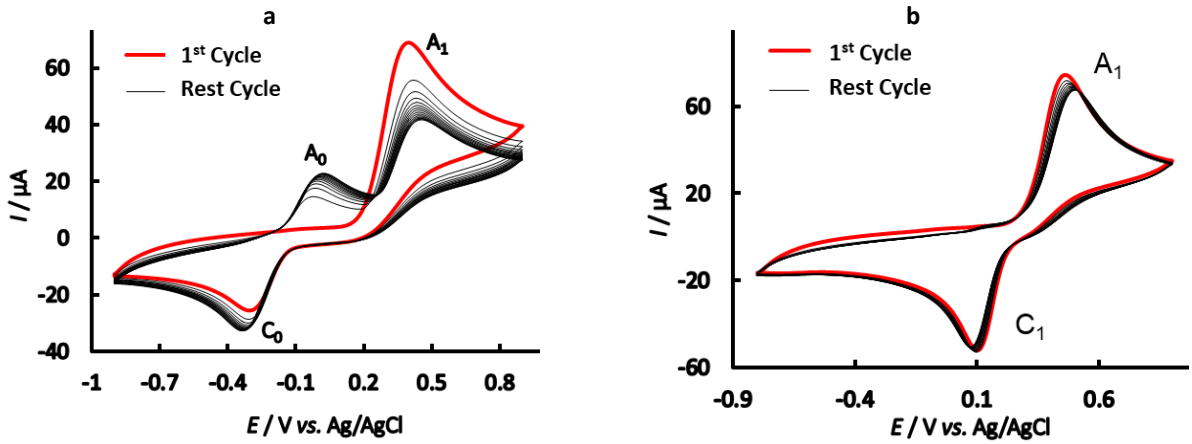


Figure 6. *a) Cyclic voltammograms of 150 mM proline with 2mM catechol on GC (3 mm) electrode in the buffer solution of pH 7 at scan rate 0.1 V s^{-1} (15 cycles). The anodic peak current (A_0) and cathodic peak current (C_0) increased with the iteration scan from the first cycle; b) CV of 2mM catechol in the buffer solution of pH 7 at scan rate 0.1 V s^{-1} (15 cycles). The first cycle is denoted by red line and the rest of the cycles by black lines*

In Figure 6b, the cyclic voltammograms of the first 15 cycles of 2 mM catechol on the GC (3 mm) electrode in a pH 7 buffer solution are displayed. At a scan rate of 0.1 V s^{-1}, one anodic peak appeared at 0.43 V, along with a cathodic peak at 0.11 V (red line), and no new anodic peak emerged in later cycles. This observation suggests that catechol primarily displayed one anodic and corresponding cathodic peak associated with its conversion to o-quinone (as shown in Scheme 1). Throughout the repetitive cycling of potential, the ratio of anodic to cathodic peak currents is nearly equal (illustrated in Figure 6b), which can be interpreted as an indicator of the stability of the o-quinone formed on the electrode surface [29]. This implies that reactions such as hydroxylation or dimerization [32,33] occur at a rate that is too slow to be detected within the time frame of cyclic voltammetry [29].

Following the addition of 150 mM proline during the first cycle (Figure 6a), no new reduction peak is evident; rather, the reduction peak shifted due to a decrease in catechol species caused by proline. In the second potential scan (Figure 6a), a new oxidation peak around -0.01 V appeared, likely corresponding to the oxidation of an adduct formed between o-benzoquinone and proline, as illustrated in Scheme 1.

Controlled-potential coulometry was conducted in an aqueous solution containing 1 mM catechol and 75 mM proline at 0.5 V in pH 7. The progression of the electrolysis was monitored using cyclic voltammetry and differential pulse voltammetry (Figure 7). As illustrated in Figure 7, during the coulometric process, peaks A_0 and C_0 appeared, but the height of peaks A_0 and C_0 did not increase in direct proportion to the progress of the coulometry, coinciding with a reduction in height for both the anodic peak A_1 and the cathodic peak C_1 (the meaning of symbols A_0, C_0, A_1 and A_2 is similar to Figure 1). Ultimately, all anodic and cathodic peaks disappeared after the consumption of 4 electrons per molecule.

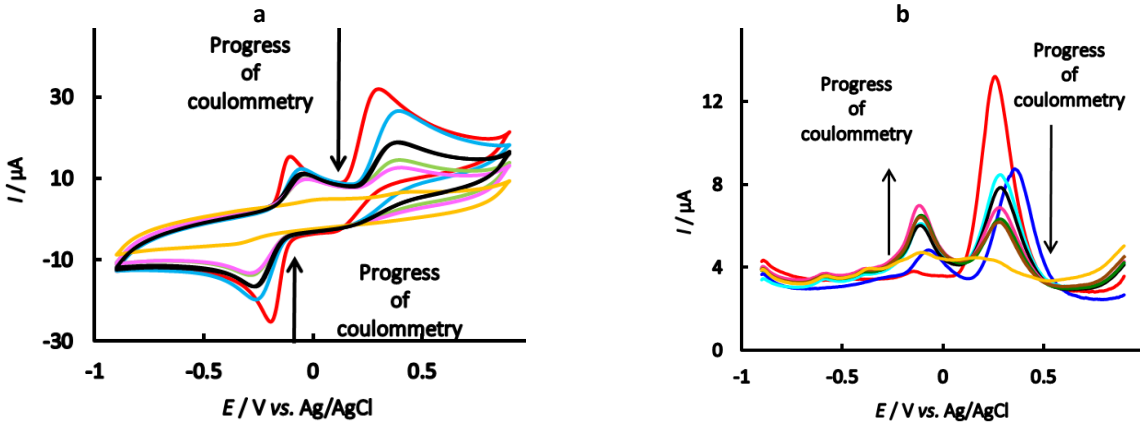


Figure 7. *a) Cyclic voltammograms and b) differential pulse voltammograms (taken after a 30-minute interval) of 1 mM catechol in the presence of 75 mM proline of GC electrode in buffer solution, pH 7 during controlled potential coulometry at 0.5 V and scan rate 0.1 V s^{-1}*

These observations enable us to suggest the pathway illustrated in Scheme 1 for the electro-oxidation of catechol **1** in the presence of proline **2**. These findings indicate that the 1,4 addition reaction of proline 2 to o-quinone **1a** occurs more rapidly than other secondary reactions, resulting in the formation of intermediate **3**. The oxidation of this intermediate **3** is more favourable than that of the original starting molecule **1** due to the presence of an electron-donating group. Similarly, o-quinone **4** can also be attacked by proline **2** at the C-5 position. Nevertheless, no excessive reaction was detected during voltammetric experiments, likely due to the relatively low reactivity of o-quinone **4** towards the 1,4 (Michael) addition with proline **2**.

Differential pulse voltammetry

In Figure 8, a DPV of 2 mM catechol in the presence of 150 mM proline in a second scan at different pHs (5 to 11) was exhibited. In a buffer solution with a pH of 7, catechol exhibited two distinct peaks when proline was present (Figure 8). In neutral media, the first and second anodic peaks were shown at -0.15 V and 0.24 V, respectively. In the second scan of potential, the anodic peak current intensity observed at pH 5, 9 and 11 is notably low. The DPV voltammogram is consistent with CV. From Figures 3 and 8, it was seen that two distinct anodic peaks exhibiting high current intensity were detected at pH 7 due to the oxidation of the o-benzoquinone-proline derivative. Product **3** can also be embattled by proline, but this reaction is not detected during the differential pulse voltammetry analysis.

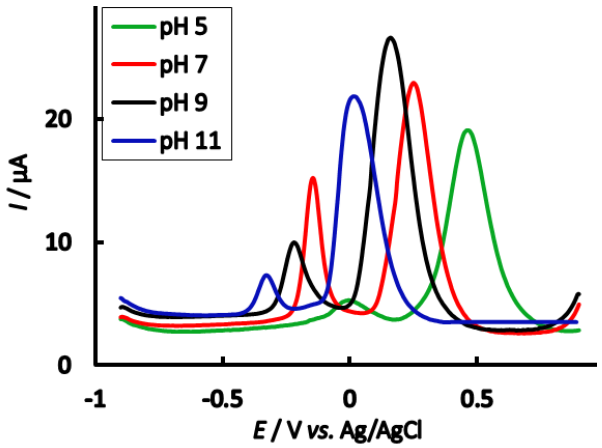


Figure 8. *Differential pulse voltammograms in the second scan of 2 mM catechol with 150 mM proline of GC electrode at different pH levels of buffer solution and scan rate 0.1 V* s^{-1}

Effect of deposition time in DPV

Figure 9 illustrates differential pulse voltammetry (DPV) responses for various deposition times (0, 10, 60, 120, 150 and 240 seconds) using a solution of 2 mM catechol and 150 mM proline in a buffer solution at pH 7.

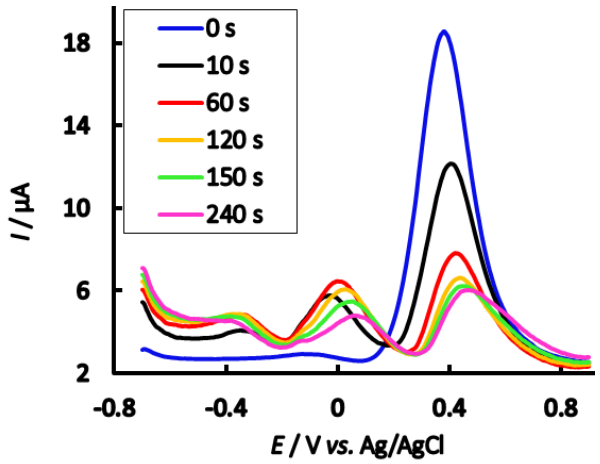


Figure 9*. Differential pulse voltammograms of 2 mM catechol with 150 mM proline in buffer solution of pH 7 for various deposition time changes at* E_{puls} *0.02 V,* t_{puls} *20 ms and scan rate 0.1 V* s^{-1}

It is shown that an increase in deposition time results in the appearance of a significant new peak at -0.02 V. A rise in deposition time by 10 seconds promotes nucleophilic attack, resulting in the formation of more catechol-proline adduct at the electrode surface. This leads to a decrease in the concentration of o-benzoquinone and an increase in the concentration of the catechol-proline adduct. Maximum peak intensity was achieved at a 60-second deposition time. However, when the deposition time extends beyond 60 to 240 seconds, both the first and second anodic peak currents diminish. It can be inferred that longer deposition times decrease the concentration of o-benzoquinone.

The effect of proline concentration on catechol was also examined using DPV (Figure 10). The study involved 2 mM catechol with proline concentrations ranging from 50 to 250 mM at pH 7. As shown in a previous CV shown in Figure 1, two distinct anodic peaks were observed with the addition of proline to catechol. Increasing the concentration of proline up to 150 mM resulted in a rise in the current of the first anodic peak. However, when the concentration was increased further, from 200 to 250 mM, a gradual decrease in all anodic peak currents was noted. At lower proline concentrations (<140 mM), the nucleophilic substitution reaction occurred to a similar extent, but as the concentration of proline

exceeded 140 mM, the nucleophilic attack on the o-benzoquinone generated at the electrode surface became more favorable. Further addition of proline beyond 150 mM led to an accumulation of excess electroinactive proline on the electrode's surface, resulting in a reduction in peak current.

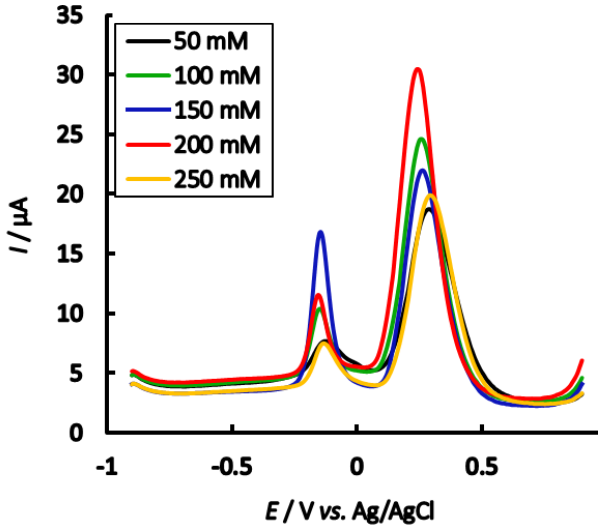


***Figure 10**. Differential pulse voltammograms of 2 mM catechol at different proline concentrations in the second scan of pH 7 at E_{puls} 0.02V, t_{puls} 20 ms of GC electrode and scan rate 0.1 V/s*

Spectral analysis of catechol with proline

The FTIR spectra of the catechol-proline adduct were taken over the wavenumber range of 400 to 4000 cm^{-1}. The FTIR spectrum of the catechol-proline adduct has been shown in Figure 11.

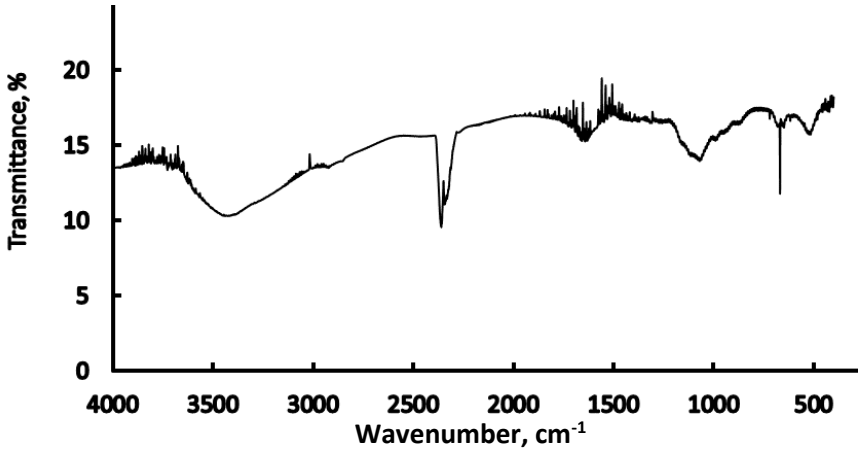


***Figure 11**. FTIR spectrum of catechol-proline adduct*

Catechol displays an O-H stretching band at 3450 cm^{-1}, while proline exhibits a broad spectrum at 3380 cm^{-1}caused by the overlap of O-H and N-H stretching bands. In the catechol-proline adduct, the absorption peak corresponding to the broad O-H stretching vibration occurs at 3350 cm^{-1}. For the catechol-proline adduct, the peak intensity for N-H stretching decreases, and a significant change in fingerprint region is observed. Similar behaviours have been noted in studies of catechol and its derivatives [13]. This also verified the formation of a catechol-proline adduct.

Based on the preceding analysis, it is evident that the nucleophilic substitution reaction of catechol and proline is most favourable at a concentration of 150 mM proline and 2 mM of catechol and a pH of 7 at the GC electrode. This finding aligns with the results obtained from both cyclic voltammetry and differential pulse voltammetry.

Conclusions

The electrochemical behavior of catechol, both in the absence and presence of proline, was examined using cyclic voltammetry, differential pulse voltammetry, controlled potential coulometry, and FTIR spectroscopy. To determine the optimum reaction conditions of catechol and proline, an investigation was conducted to examine the effects of pH level, electrode materials, and solution concentration on the reaction. The oxidation of catechol leads to the formation of o-benzoquinone, which is subsequently attacked by proline. The products generated from the reaction are accomplished by transferring electrons at a more negative potential than catechol. The oxidation reaction of catechol-proline adducts is produced *via* the one-step $2e^{-}/2H^{+}$ process. The voltammetric performance of the glassy carbon (GC) electrode is better than that of gold (Au) and platinum (Pt) electrodes. The peak current for the catechol-proline adduct during each redox reaction is governed by a diffusion process. The nucleophilic reaction involving of catechol in the presence of proline is most favourable at a concentration of 2 mM catechol and 150 mM proline and at pH 7 on a glassy carbon electrode. The catechol-proline adduct was electrochemically synthesized during coulometry, supported by FTIR spectrum. The nucleophilic addition reaction of proline with catechol occurs *via* an electron transfer, chemical reaction and electron transfer mechanism.

Conflict of interest: *All authors declare no conflicts of interest*

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