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Electro-oxidation of Catechol in the presence of L-Glutamine at Different pH and Concentrations

M. A. Hafiz Mia,[1] M. A. Motin,[1,*] E. M. Huque,[2] M. Nazim Uddin,[1] P. K. Dhar[1] and M. A. Hashem[3]

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

Corresponding Author, Tel.:+88041769471, Ext. 515; Fax: +88041774403
E-Mail: abdulmotin75@yahoo.com

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Abstract- The electro-oxidation of Catechol in presence of L-Glutamine has been investigated in aqueous solution with various pH values, different electrodes and different concentration of L-Glutamine by using cyclic voltammetry, differential pulse voltammetry and controlled potential coulometry. Electrochemically generated *o*-benzoquinone (Michael acceptor) from oxidation of Catechol reacts with lower concentration of L-Glutamine as nucleophiles in the second scan of potential. The products obtained from the reaction are assumed to be 2-amino-5-((3,4-dihydroxoyphenyl)amino)-5-oxopentanoic acid that undergo electron transfer at more negative potentials than the Catechol. The effect of pH of Catechol in presence of L-Glutamine has been studied by varying pH from 5 to 11. The slope of the plots of anodic peak current, E_p against pH of Catechol-Glutamine adduct is 25.6 mV, indicates that the oxidation proceeded via the $2e^-/2H^+$ processes. The concentration effect of L-Glutamine with the fixed concentration of Catechol (2 mM) was measured from 10 mM to 100 mM. The reaction was strongly influenced by the pH as well as concentration of L-Glutamine and electrode materials. The reaction is mostly favorable in 50 mM of L-Glutamine and 2 mM of Catechol at pH 7. The behavior of the reaction is diffusion controlled followed by ECE mechanism.

Keywords- Electro-oxidation, Favorable condition, L-Glutamine, Catechol, Cyclic Voltammetry, Differential Pulse Voltammetry, Controlled potential coulometry

1. INTRODUCTION

Catechol is one of the important compounds in organic synthesis and it is produced in industrial scales as the precursor of pesticides, perfumes and pharmaceuticals [1]. The Catechol skeleton also occurs in a variety of natural products specially the antioxidant [2]. The most well-known characteristic of the Catechols is that they can be easily oxidized mainly due to their antioxidant activity and low oxidation potentials [3]. The products of oxidation are the corresponding reactive and electron-deficient *o*-quinones. One of the most successful in situ generations of reactive *o*-quinones species is the electrochemical oxidation [4]. There are many reports on electro-oxidation of Catechols to produce *o*-quinones as reactive intermediates in many useful homogeneous reactions [5].

L-Glutamine is an α-amino acid having variety of biochemical functions like protein synthesis, other of the 20 proteinogenic amino acids, Lipid synthesis, especially by cancer cells, regulation of acid-base balance in the kidney by producing ammonium, cellular energy, as a source, next to glucose, Nitrogen donation for many anabolic processes, including the synthesis of purines, Carbon donation, as a source, refilling the citric acid cycle, Nontoxic transporter of ammonia in the blood circulation. Glutamine is used to counter some of the side effects of medical treatments. For example, it is used for side effects of cancer chemotherapy including diarrhea, pain and swelling inside the mouth, nerve pain (neuropathy), and muscle and joint pains caused by the cancer drug Taxol. It is also used to protect the immune system and digestive system in people undergoing radio chemotherapy for cancer of the esophagus. Additionally, glutamine is used for improving recovery after bone marrow transplant or bowel surgery, increasing well-being in people who have suffered traumatic injuries, and preventing infections in critically ill people. Some people use glutamine for digestive system conditions such as stomach ulcers, ulcerative colitis, and Crohn's disease. It is also used for depression, moodiness, irritability, anxiety, insomnia, and enhancing exercise performance [6-8].

The electrochemical oxidation of Catechols in the presence of some other nucleophiles such as methanol, aspartic 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-hydroxy coumarin, 4-hydroxy-6-methyl-a-pyrone, 4-hydroxy-6-methyl-2-pyridone and 4-hydroxycarbostyrile were studied [9-16]. There are no papers have been published on electrochemical oxidation of Catechols with L-Glutamine. In this paper, we have studied the electrochemical properties of Catechol in presence of L-Glutamine with three different electrodes (GC, Au and Pt), wide range of concentration of L-Glutamine (50-100 mM), different pH and different scan rate.

2. EXPERIMENTAL SECTION

Catechol, L-Glutamine, acetic acid, sodium acetate, potassium chloride, sodium di hydrogen orthophosphate and di-sodium hydrogen orthophosphate were of analytical grade (E-Merck). Catechol and Catechol with L-Glutamine solutions of different concentrations were prepared in different pH by using acetate or phosphate buffer solutions. Pt and Au of 1.6 mm in diameter (BASi) and Glassy Carbon disks of 3 mm in diameter (BASi) were used as a working electrode 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 (BASi). The reference electrode was an Ag|AgCl electrode (BASi). The working electrode was then polished on this surface by softly pressing the electrode against the polishing surface in the end for 5-10 minutes. The electrode was then thoroughly washed with deionized water. At this point the electrode surface would look like a shiny mirror. The Potentiostat/Galvanostat was µStat 400 (DropSens, Spain). Nitrogen gas was bubbled from the one-compartment cell before electrochemical run.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Behavior of Catechol and L-Glutamine

Electrochemical oxidation of Catechol has been investigated by using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and controlled potential coulometry (CPC) in absence and presence of L-Glutamine. Fig. 1 (dashed line) shows the cyclic voltammogram of 2 mM Catechol of GC electrode in buffer solution of pH 7 and scan rate 0.1 V/s. The cyclic voltammogram of Catechol shows one anodic peak at (0.44 V) and corresponding cathodic peak at (0.11 V) related to its transformation to *o*-quinone and vice versa. Pure L-Glutamine is electrochemically inactive in the potential range investigated (Fig. 1, solid line). Fig. 1 (deep solid line) shows the CV of Catechol (2 mM) in the presence of L-Glutamine (50 mM) in the second scan of potential at the same condition. In the 1st scan of potential Catechol with L-Glutamine shows one anodic peak at 0.32 V and the corresponding two cathodic peaks at -0.36 V and 0.06 V respectively. Upon addition of L-Glutamine to Catechol solution, the cathodic peak C_1 decreases and a new cathodic peak C_o appears at 0.04 V. Also, in the second scan of potential a new anodic peak A_o appears and anodic peak A_1 decreases. The newly appearance of A_o and C_o peaks and decreases of A_1 and C_1 peaks and also shifting of the positions of peaks A_1 and C_1 in the presence of L-Glutamine indicates that it is due to follow up reaction of Catechol with L-Glutamine. This observation can be explained by considering nucleophilic attack of L-Glutamine to o-benzoquinone. The nucleophilic attack of L-Glutamine to o-benzoquinone reduces the o-benzoquinone concentration in reaction layer, consequently the A_1 and C_1 peaks reduces, whereas in the same time produces

Catechol-L-Glutamine adduct and consequently the peaks A_0 and C_0 appears. In the first scan of potential, the anodic peak of Catechol in presence of L-Glutamine is very similar to only Catechol. But in the second scan of potential the peak current of A_1 (deep solid line) decreases significantly compared with that of free Catechol (dashed line). The peak current for the peaks A_1 and C_1 decreased noticeably, which is indicative of a chemical reaction of L-Glutamine (2) with the *o*-quinone (1a) produced at the surface of electrode. These observations may ascribe the formation of 2-amino-5-((3,4-dihydroxoyphenyl)amino)-5-oxopentanoic acid through nucleophilic substitution reaction (Scheme 1). If the constituent is such that the potential for the oxidation of product is lower, then the further oxidation and further addition may occur [18] then the product 2-amino-5-(bis(3,4-dioxocyclohexa-1,5-dien-1-yl)amino)-5-oxopentanoic acid will be formed (Scheme 1). In the case of Catechol in presence of L-Glutamine, the oxidation of L-Glutamine substituted o-benzoquinone is easier than the oxidation of parent Catechol. This substitution product can also be attacked by L-Glutamine, however, it was not observed during the voltammetric experiments because of the low activity of *o*-quinone 4 toward 2. This behavior is in agreement with that reported by other research groups for similar electrochemically generated compounds such as Catechol and different nucleophiles [8-16,18-23]. In the absence of other nucleophiles, water or hydroxide ion often adds to the *o*-benzoquinone [24].

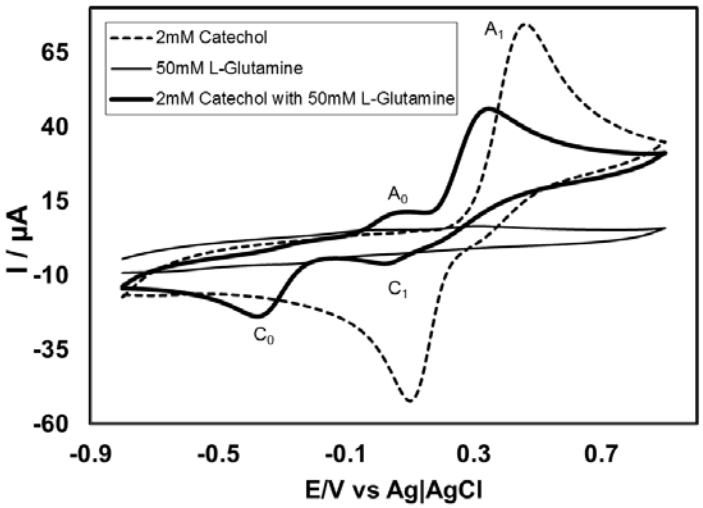
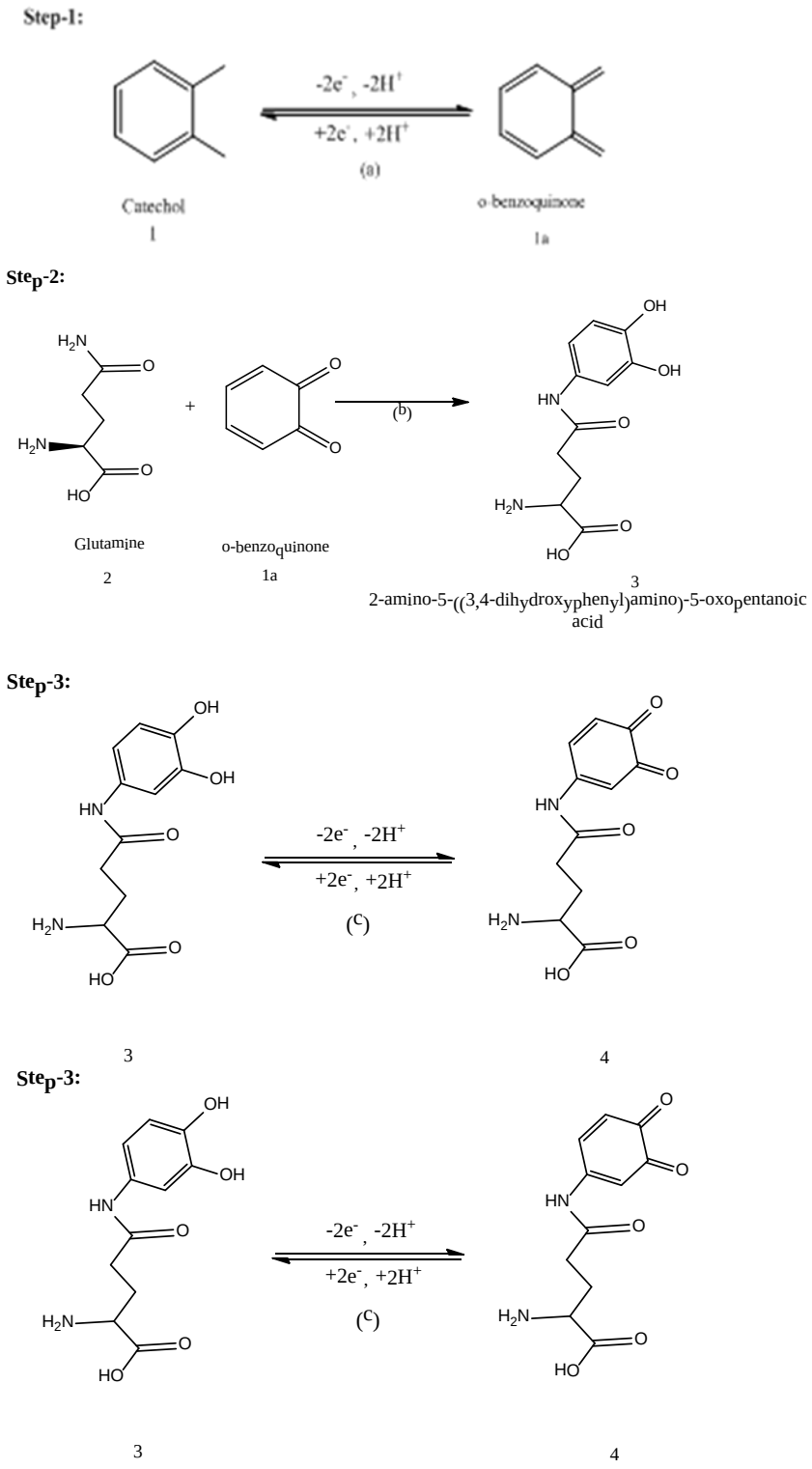


Fig. 1. Cyclic voltammogram of 2 mM catechol (dashed line), 50 mM L-Glutamine (solid line) and 2 mM catechol with 50 mM L-Glutamine (deep solid line) of Gc electrode in buffer solution (pH 7) at scan rate 0.1 V/s (2nd cycle). A_0 and A_1 is appeared anodic peak and anodic peak, C_0 and C_1 is corresponding appeared cathodic peak and cathodic peak



Scheme 1. Chemical structure of catechol and its derivatives

3.2. Effect of scan rates

Fig. 2 (a) describes the CV of second cycle of 2 mM Catechol in presence of 50 mM L-Glutamine of GC electrode in buffer solution (pH 7) at different scan rates. The peak current

of both the anodic and cathodic peaks increases with the increase of scan rate. The cathodic peaks are shifted towards left and the anodic peaks are to the right direction with increase in scan rate. Fig. 2 (b) shows plots of the anodic and cathodic net peak currents of 2 mM Catechol with 50 mM L-Glutamine for second cycle against the square-root of the scan rates where the net current means the second peak subtracted from the first one by the scan-stopped method [18]. Although the peak current increases proportionally with increasing square root of scan rates but the line is not passed through the origin, so the peak current of the reactant at each redox reaction is not controlled by purely diffusion process i.e during the reaction some surface related chemical complications are happened. As can be seen in Fig. 2a, the cathodic peak for reduction of *o*-benzoquinone is disappeared in the scan rate of 0.05 V/s. By increasing the scan rate, the cathodic peak for reduction of o-benzoquinone begins to appear and increase. The corresponding peak current ratio (I_{pa1}/I_{pc1}) *vs* scan rate for a mixture of Catechol and L-Glutamine decreases with increasing scan rate hence it is independent with any scan rate (Fig. 2c). The anodic peak current ratio (I_{pa0}/I_{pa1}) *vs* scan rate for a mixture of Catechol and L-Glutamine firstly increases and then after 0.25 V/s scan rate it is almost unchanged (Fig. 2c). On the other hand, the value of current function ($I_p/v^{1/2}$) was found to be decreased with increasing scan rate up to 0.3 V/s and after that it remains almost constant (Fig. 2c). According to the fig. 2d it can be deduced that the exponential nature of the current function versus the scan rate plot indicates the ECE mechanism for electrode process [9]. This confirms the reactivity of *o*-benzoquinone (1a) towards L-Glutamine (2) firstly increases at slow scan rate and then at higher scan rate it decreases. This behavior is in agreement with that reported by other research groups for similar electrochemically generated compounds such as catechol and different nucleophiles [9,21-23].

The existence of a subsequent chemical reaction between *o*-benzoquinone **1a** and L-Glutamine **2** is supported by the following evidence.

(i) In the presence of L-Glutamine both I_{pa1} and I_{pc1} decreases during second cycle (Fig. 1), this could be indicative of the fact that electrochemically generated *o*-benzoquinone **1a** is removed partially by chemical reaction with L-Glutamine (**2**).

(ii) Corresponding peak current ratio (I_{pa1}/I_{pc1}) varies with potential sweep rate. In this case, a well-defined cathodic peak C_1 is observed at highest sweep rate. For lower sweep rates, the peak current ratio (I_{pa1}/I_{pc1}) is less than one and increases with increasing sweep rate. This is indicative of departure from intermediate and arrival to diffusion region with increasing sweep rate [18].

(iii) Increase in the scan rate causes a decrease in the progress of the chemical reaction of 1a with 2 during the period of recording the cyclic voltammogram and therefore, decease in peak current ratio (I_{pa0}/I_{pa1}) at higher scan rate.

(iv) The current function, $I_p/v^{1/2}$ for A_1 was found to be decreased exponentially with increasing scan rate. This indicates the reaction mechanism of the system was of ECE type (Scheme 1).

From these concepts, it seems that the 1,4-Michael addition reaction of L-Glutamine(2) to *o*-benzoquinone (1a) leads to product 3.

The CV of pure Catechol in buffer solution (pH 7) at different scan rates is also observed. The proportionality of the anodic and cathodic peak current against the square-root of the scan rates suggests that the peak current of the reactant at each redox reaction is also controlled by diffusion process.

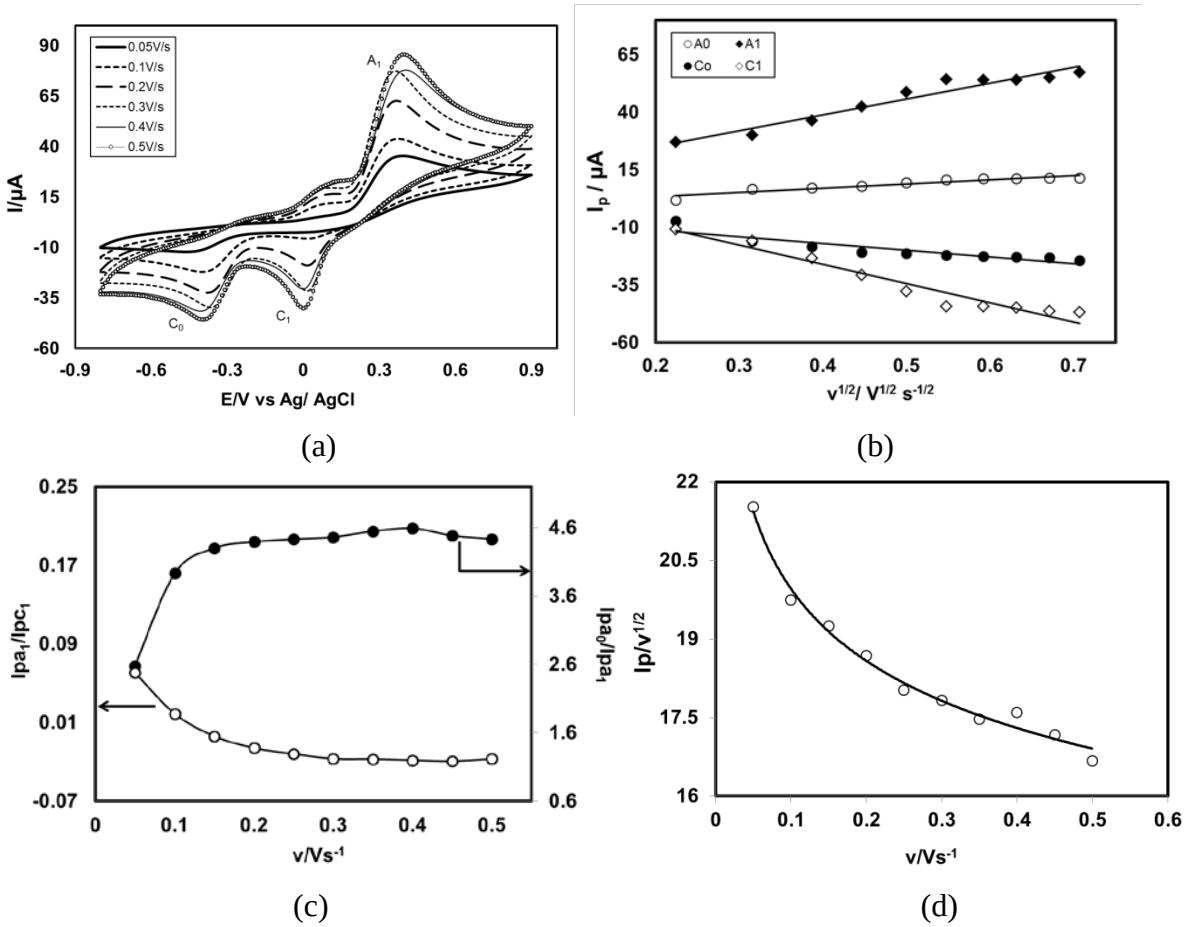


Fig. 2. a) Cyclic voltammogram of 2 mM catechol with 50 mM L-Glutamine in the second scan of potential at Gc electrode in buffer solution (pH 7) at scan rate 0.05 V/s-0.5 V/s; b) Plots of peak current *vs* square root of scan rate in the same condition. Legend shows the symbol of oxidation and reduction peaks; c) Variation of peak current ratio of corresponding peak (I_{pa1}/I_{pc1}) and anodic peak (I_{pa0}/I_{pa1}) *vs* scan rate in the same condition; d) Variation of peak current function ($Ip/v^{1/2}$) versus scan rate in the same condition.

3.3. Influence of pH

(Fig. 3a) shows the effect of pH on the electrochemical reaction of 2 mM Catechol in the presence of 50 mM of L-Glutamine has been studied through examining the electrode response in buffer solution of different pH (5 to 11) The voltammetric behavior of Catechol at pH 5, 9 and 11 in the presence of 50 mM L-Glutamine shows no new anodic peak after repetitive cycling indicating that the reaction in between *o*-benzoquinone and L-Glutamine has not occurred. This can be attributed to the fact that at lower pH, the nucleophilic property of amine groups is diminished or hindered through protonation (Fig. 3a). In the pH 7, the *o*-benzoquinone undergoes L-Glutamine attack by the amine through a 1,4-Michael addition reaction reflecting a new anodic peak appeared after repetitive cycling. Whereas, in the higher pH range (e.g., pH 9-11), the cyclic voltammograms of Catechol show irreversible behavior. It was thus suggested that the oxidation of Catechol followed by an irreversible chemical reaction with hydroxyl ion, especially in alkaline solutions [20]. However, amine in this condition can also act as nucleophiles. The peak position of the redox couple is found to be dependent upon pH.

Fig. 3 (b) shows the plot of oxidation peak potential, E_p values against pH. The slopes of the plot were determined graphically as the anodic peaks (25.6 mV/pH for second anodic peak A_1) at 0.1 V/s, which is close to the theoretical value for one step two-electron, two-proton transfer process. This indicates that both the oxidation of the Catechol and Catechol-L-Glutamine adduct proceeded via the $2e^-/2H^+$ processes (Scheme 1). This also suggests that during the reaction not only electron but also proton is released from the Catechol-L-Glutamine adduct. Other research groups also reported similar behavior for Catechol and its derivatives [17,25]. Fig. 3 (c) shows the plot of oxidation peak of A_0 which is denoted by I_p against pH of solution. From the Fig. 3(c) it is seen that the maximum peak current is obtained at pH 7. At this pH, the difference between the peak current ratio (I_{pa1}/I_{pc1}) in the presence and absence of L-Glutamine is maximum. Consequently, in this study buffer solution of pH 7 has been selected as suitable medium for electrochemical study of Catechol in the presence of L-Glutamine. This ascribed that the electrochemical oxidation of Catechol in presence of L-Glutamine is facilitated in neutral media and hence the rate of electron transfer is faster.

Differential pulse voltammetry (DPV) technique has been applied to make clearer for Catechol-L-Glutamine substitution reaction. DPV obtained for 2 mM Catechol in the presence of 50 mM L-Glutamine in second scan at different pH (5-11) was shown in Fig. 3d. In the buffer solution of pH 7, Catechol shows three well-developed wave in the presence of L-Glutamine (Fig. 3d). In pH 7, the first, second and third anodic peaks have been observed at ~-0.3 V, 0.01 V and 0.25 V respectively. Among these, peak at -0.3 V can be arising due to side reaction or polymerization (reaction scheme 1, step-4). But, in pH 5, 9 and 11 have no new anodic peak and in pH 11 of second scan of potential the first anodic peak current

intensity is very small. As can be seen three completely separated anodic peaks with high current intensity are observed in pH 7, which can be attributed to the oxidations of *o*-benzoquinone-L-Glutamine new compound and *o*-benzoquinone, respectively.

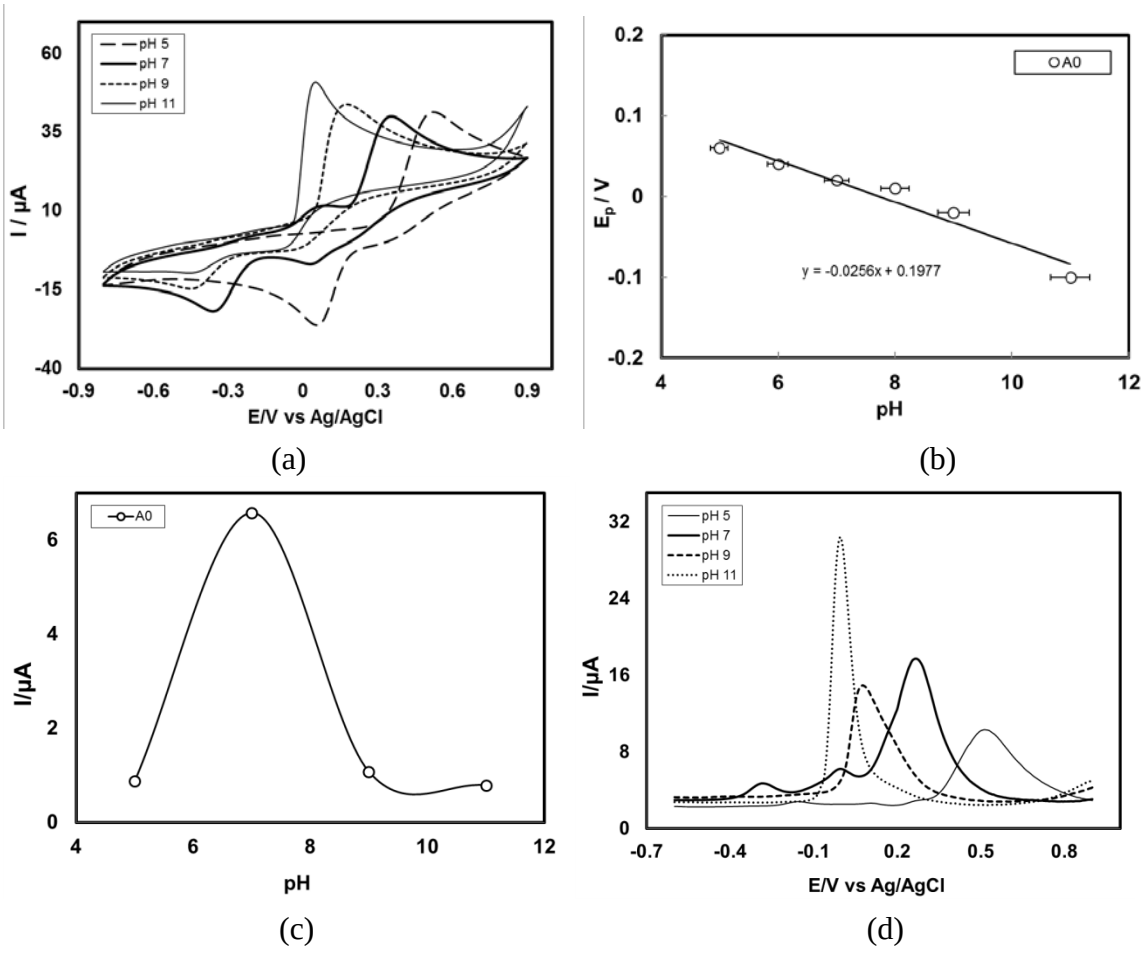


Fig. 3. a) Cyclic voltammogram of 2 mM catechol with 50 mM L-Glutamine of Gc (3 mm) electrode in different pH (5, 7, 9, and 11) at scan rate 0.1 V/s; b) Plots of peak potential *vs* pH in the same condition; c) Plots of peak current *vs* pH in the same condition; d) Differential pulse voltammogram (DPV) of 2 mM catechol with 50 mM L-Glutamine of Gc electrode in second scan of different pH (5, 7, 9 and 11) and scan rate 0.1 V/s. The meaning of symbol A_0 is similar to Fig. 1

3.4. Concentration effect of L-Glutamine

Fig. 4 (a) shows the variation of voltammogram pattern by the addition of different concentration of L-Glutamine (10, 30, 50, 70 and 100 mM) into fixed concentration of Catechol (2 mM) of GC electrode at pH 7 and scan rate 0.1 V/s. A new peak appears at -0.01 V upon addition of 2 mM L-Glutamine which suggests the formation of Catechol-L-Glutamine adduct. The net current intensity of the newly appeared anodic and cathodic peak increases with the increase of L-Glutamine composition up to 50 mM where the net current

means the peak current is measured from the baseline consideration. Further addition of L-Glutamine (>50 mM), the anodic and cathodic peak current is slightly decreased (Fig. 4b).

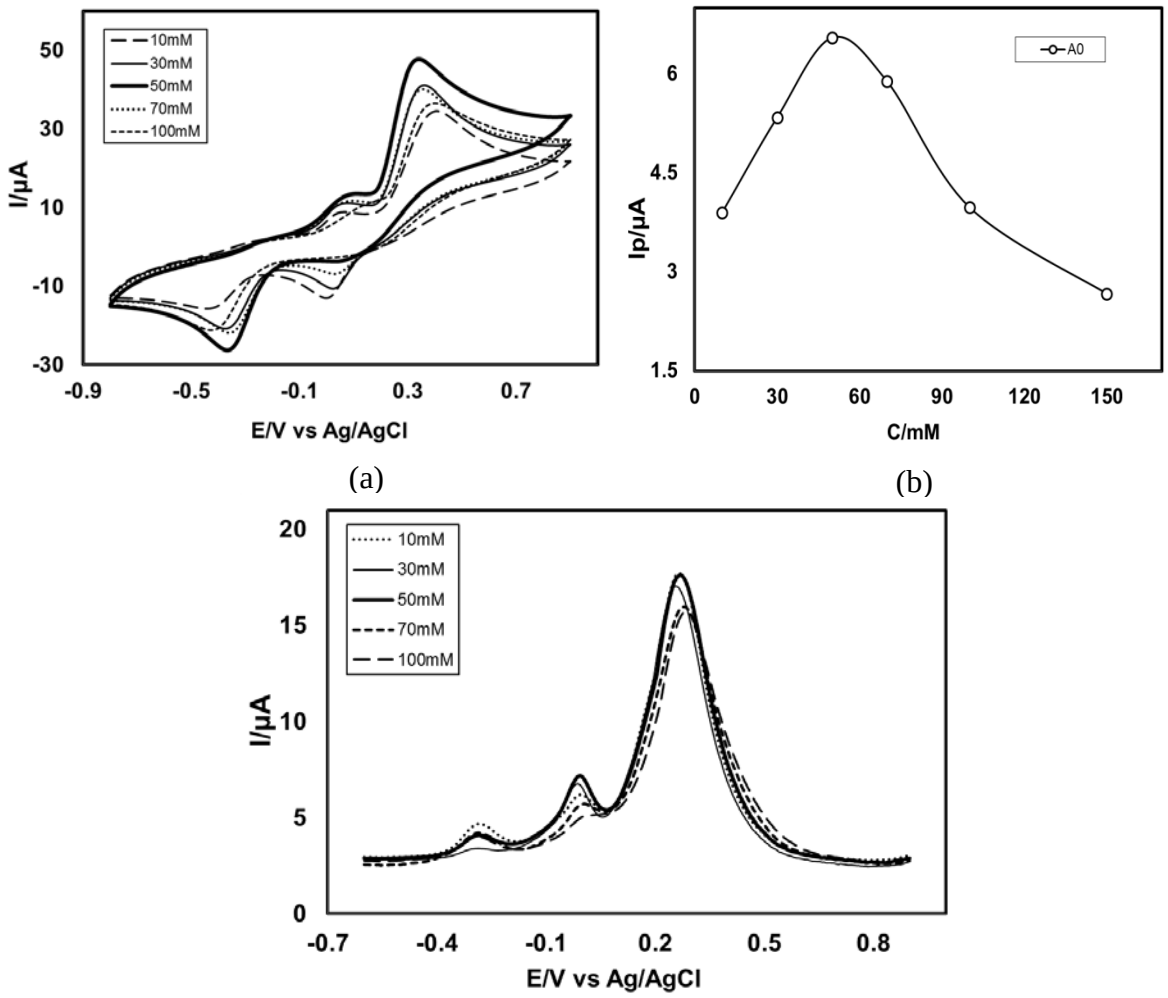


Fig. 4. a) CV of composition changes of L-Glutamine (10, 30, 50, 70 and 100 mM) with fixed 2 mM catechol of Gc electrode at pH 7 and scan rate 0.1 V/s; b) Plots of anodic peak current, *I*p *vs* concentration of (10, 30, 50, 70 and 100 mM) L-Glutamine with (fixed 2 mM catechol) in same condition. The meaning of A_0 is similar to Fig. 1; c) Differential pulse voltammogram (DPV) of composition change of L-Glutamine (10, 30, 50, 70 and 100 mM) with the fixed composition of 2 mM Catechol in second scan of pH 7 at *E*puls 0.02 V, tpuls 20 ms of Gc electrode and scan rate 0.1 Vs^{-1}

The nucleophilic substitution reaction of Catechol in presence of L-Glutamine is maximum favorable up to 50 mM of L-Glutamine at pH 7. The corresponding peak current ratio (I_{pa1}/I_{pc1}) varies with the concentration of L-Glutamine. This is related to the increase of the homogenous reaction rate of following chemical reaction between *o*-benzoquinone 1a and L-Glutamine 2 with increasing concentration of L-Glutamine up to 50 mM. At higher

concentration of L-Glutamine (>50 mM), the excess electro-inactive L-Glutamine may be deposited on the electrode surface and consequently the peak current decreased.

To understand the effect of L-Glutamine concentration on the differential pulse voltammograms of Catechol has been also employed. Fig. 4c shows DPV for 2 mM of Catechol solution containing buffer (pH 7) in the presence of various concentration of L-Glutamine from 50 mM to 100 mM at the surface of GC electrode. As indicated in this figure, there are again three separated anodic peaks appeared after addition of L-Glutamine into Catechol similar to Fig. 3d. In this case, the increasing of the concentration of L-Glutamine from 10 to 50 mM leads to increasing of first anodic peak current. For further increase of concentration from 50 to 100 mM, the first and second anodic peak current decreases gradually. In lower concentration of L-Glutamine (<50 mM), the nucleophilic substitution reaction take place in comparable degree, whereas increasing the concentration of L-Glutamine (50 mM) make favorable nucleophilic attack of L-Glutamine toward o-benzoquinone generated at the surface of electrode. For further addition of L-Glutamine (>50 mM) into Catechol solution, the excess electro-inactive L-Glutamine deposited on the electrode surface and hence the peak current decreases. Thus CV is consistent with DPV.

3.5. Effect of electrode materials

Electrochemical properties of Catechol in absence and presence of L-Glutamine has been examined by different electrodes like GC, Au and Pt at different pH. The Cyclic voltammograms of 2 mM Catechol with 50 mM L-Glutamine at GC, Au and Pt electrodes are shown in Fig. 5a.

The nature of voltammograms, the peak position and current intensity for the studied systems are different for different electrodes although the diameter of GC electrode (3 mm) is higher than Au and Pt (1.6 mm). The CV at GC electrode is significantly different based on peak current consideration from those of the Au and Pt. At the Au electrode it shows one anodic and two cathodic peaks whereas Pt electrode shows two redox pairs (-0.04/-0.28 and 0.29/0.06) similar to GC electrode (0.04/-0.36 and 0.33/0.05).

DPV technique has been employed to make clearer the better response of electrode materials in the same condition. According to Fig. 5b it can be seen that two new anodic peaks arise at -0.3 V and 0.01 V. But in Pt and Au electrode no significant new peak appears by the peak current consideration. Electrochemical properties of Catechol with L-Glutamine for example change of pH; concentration, scan rate etc. were studied in detail using Pt and Au electrodes. But among the electrodes, the voltammetric response of GC electrode was better than Pt and Au electrodes in the studied systems which are consistent with differential pulse voltammogram in Fig. 5b. Therefore, in the paper we have discussed mainly the properties of Catechol with L-Glutamine using GC electrode.

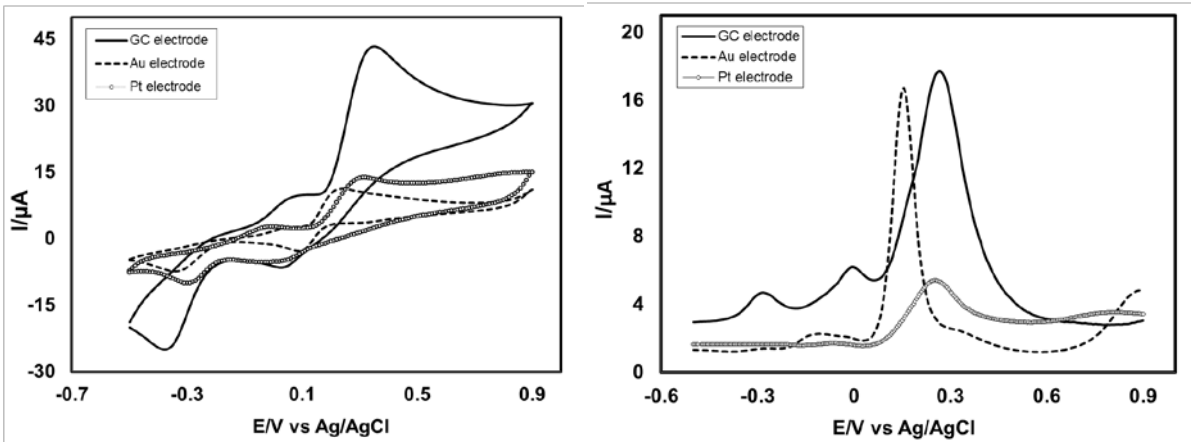


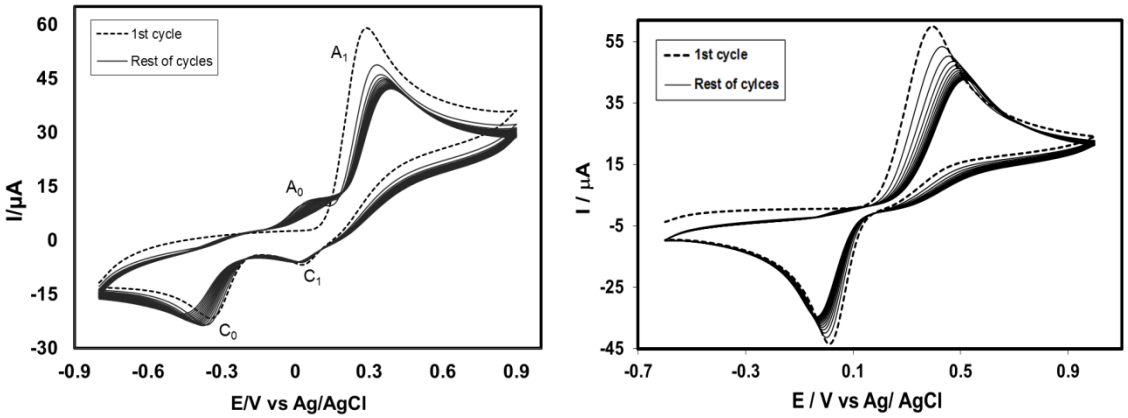
Fig. 5. a) Cyclic voltammogram (CV); b) Differential pulse voltammetry of 2 mM catechol with 50 mM L-Glutamine in Gc electrode (3.0 mm), Gold electrode (1.6 mm) and Platinum electrode (1.6 mm) at pH 7 and scan rate 0.1 V/s

3.6. Subsequent cycles of CV of Catechol-L-Glutamine

Fig. 6 (a) shows the cyclic voltammograms of the first 15 cycles of 2 mM Catechol with 50 mM L-Glutamine of GC electrode in buffer solution of pH 7 for the potential range between -0.8 V to 0.9 V at a GC electrode. The voltammogram at the 0.1 Vs^{-1} scan rates has one anodic peak at 0.28 V and two cathodic peaks at -0.36 V and 0.05 V when considered the first scan (dashed line). In the subsequent potential cycles a new anodic peak appears at 0.04 V and intensity of the first anodic peak current increased progressively on cycling but the second anodic peak current decreases and shifted positively on cycling. This can be attributed to produce the Catechol-L-Glutamine adduct through nucleophilic substitution reaction in the surface of electrode (Scheme 1). The successive decrease in the height of the Catechol oxidation and reduction peaks with cycling can be ascribed to the fact that the concentrations of Catechol-L-Glutamine adduct formation increased by cycling leading to the decrease of concentration of Catechol or quinone at the electrode surface. The positive shift of the second anodic peak in the presence of L-Glutamine is probably due to the formation of a thin film of product at the surface of the electrode, inhibiting to a certain extent the performance of electrode process. Along with the increase in the number of potential cycles the first anodic peak current increased upto 10 cycles and then the peak current almost unchanged with subsequent cycle. This may be due to the block of electrode surface by the newly formed electro-inactive species after more cycling.

Fig. 6 (b) shows the cyclic voltammograms of the first 15 cycles of 2 mM Catechol of GC electrode in buffer solution of pH 7 at a GC electrode. The voltammogram at the 0.1 Vs^{-1} scan rate has one anodic peak at 0.44 V and cathodic peak at 0.11 V (dashed line). In the subsequent potential cycles no new anodic peak appeared. This can be attributed that Catechol showed one anodic and corresponding cathodic peak related to its transformation to

o-quinone (Scheme 1). During the repetitive cycling of potential, the anodic and cathodic peak current ratio is nearly unity (Fig. 6b) that can be considered as criteria for the stability of *o*-quinone produced at the surface of electrode [25] are too slow. In other words, any hydroxylation [26-29] or dimerization [25,30] reactions are too slow that can be observed in the time-scale of cyclic voltammetry [24]. In basic solutions, the peak current ratio is less than unity and decreases with increasing of pH as well as by decreasing of potential sweep rate. These can be related to the coupling of anionic or dianionic forms of Catechols that enhanced by increasing pH with *o*-quinones (dimerization reaction) [21]. A new reduction peak appears at -0.36 V after the addition of 50 mM L-Glutamine to the solution at first cycle (Fig. 6a). Conversely, the reduction peak shifted due to Catechol species diminishes by addition of L-Glutamine. In the second scan of potential (Fig. 6a) a new oxidation peak also appears at 0.04 V which can be attributed to the oxidation of adduct formed between the *o*-benzoquinone and L-Glutamine according to Scheme 1.



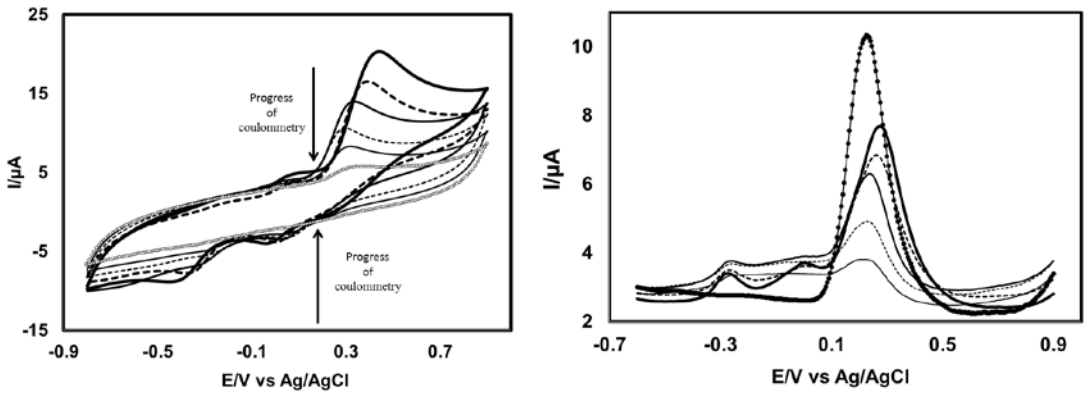
(a) (b)

Fig. 6. a) Cyclic voltammogram of 50 mM L-Glutamine with 2 mM catechol of Gc (3 mm) electrode in the buffer solution of pH 7 at scan rate 0.1 V/s (15 cycles). The appeared anodic peak current (A_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 Vs^{-1} (15 cycles)

3.7. Controlled-potential coulometry

Controlled-potential coulometry was performed in aqueous solution containing 1 mM of catechol and 25 mM of Glutamine at 0.5 V in pH 7. CV and DPV techniques were used to monitor the electrolysis progress (Fig. 7). During the time of coulometry the peaks A_0 and C_0 appeared parallel to the decrease in height of anodic peak A_1 and C_1. After some couples of hour both redox couple of appeared peak did not increase with the parallel to the decrease in height of anodic peak A_1 and cathodic peak C_1 (Fig. 7). These observations allow us to propose the pathway in Scheme 1 for the electro-oxidation of Catechol (1) in the presence of Glutamine (2). According to our results, it seems that the 1,4 addition reaction of Glutamine

to o-quinone (reaction step 2) was faster than other secondary reactions, leading to the intermediate 3.



(a) (b)

Fig. 7. a) Cyclic voltammogram and b) Differential pulse voltammogram (DPV) of 1 mM Catechol in presence of 25 mM L-Glutamine of GC electrode during controlled potential coulometry at 0.5 V in pH 7 at scan rate 0.1 V/s

3.8. Effect of deposition time

Fig. 8 shows the DPV of deposition time change (0, 10, 30, 90, 120 and 180 s) of 2 mM Catechol with 50 mM L-Glutamine of pH 7. From the Fig. 8, it is seen that the increasing of deposition time from 0 to 10 s leads to develop two new peaks and more nucleophilic attack occurs when it reaches at 30 s and consequently more Catechol-L-Glutamine adducts lead to decreasing in the concentration of o-benzoquinone and increasing in the concentration of Catechol-L-Glutamine adduct at the surface of electrode. For further increase of deposition time from 30 s to 180 s, the first anodic peak current increases and second anodic peak current decreases. This confirmed that with the increase of time decreases the concentration of *o*-benzoquinone and increases the concentration of Catechol-L-Glutamine adduct at the surface of electrode.

In this study comparatively low concentration of L-Glutamine (10-100 mM) has been used sequentially to determine the optimum condition for the nucleophilic substitution reaction of Catechol with L-Glutamine. As the reaction occurs at 50 mM concentration of nucleophiles, consequently the voltammetric peaks (CV and DPV) for adduct appeared noticeably. From the study it is understandable that L-Glutamine functions properly as a nucleophile at pH 7. When the pH is below 7, the nucleophilic activity of L-Glutamine reduces due to the protonation of amine. When pH is above 7, other nucleophiles such as –OH produce in solution, therefore, the activity of amines decreases and the oxidation of Catechol followed by an irreversible chemical reaction with hydroxyl ion [24].

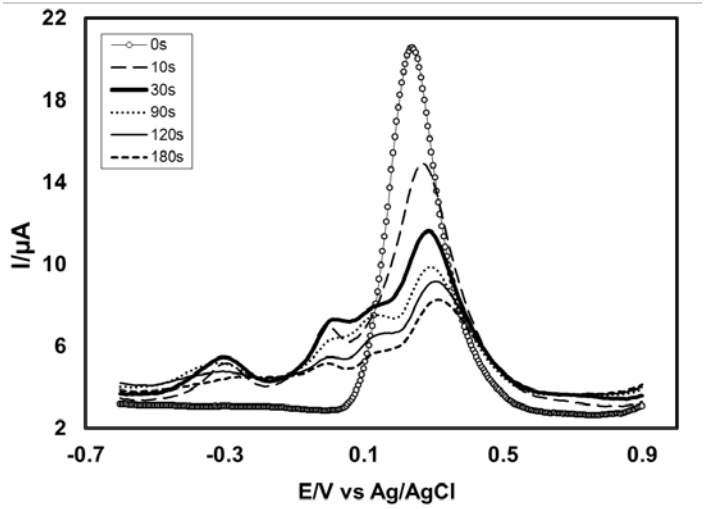


Fig. 8. Differential pulse voltammogram (DPV) of deposition time change (0, 10, 50, 90, 120 and 180 s) of 2 mM catechol with 50 mM L-Glutamine of pH 7 at *E*puls 0.02 V, tpuls 20ms and scan rate 0.1 Vs^{-1}

3.9. Spectral analysis of Catechol with L-Glutamine

The FTIR spectrum of the vibrational modes of the Catechol-Glutamine adduct, Catechol and L-Glutamine have been shown in Fig. 9.

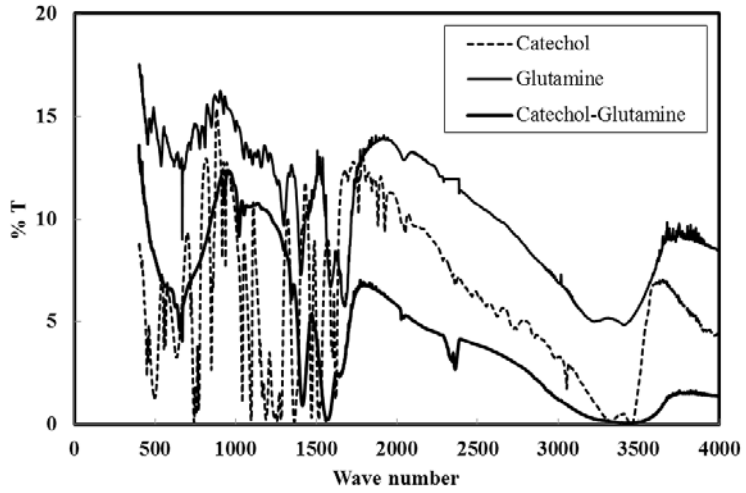


Fig. 9. Comparison of FTIR of only catechol, only Glutamine and Catechol-Glutamine adduct

The Catechol reveals the O-H stretching band at 3450 cm^{-1} whereas L-Glutamine shows a spectrum ranging 3250-3341cm^{-1} due to overlap of O-H and N-H stretching band. Peak at 1650 cm^{-1} indicates C=O stretching of –COOH group in L-Glutamine. But in case of FTIR spectrum of Catechol-Glutamine adducts, there is a significant change in finger print region (1400-600 cm^{-1}). This indicates formation of new Catechol-Glutamine adduct.

Therefore, from the above discussion it is clear that the nucleophilic substitution reaction of Catechol in presence of L-Glutamine is maximum favorable at 50 mM of L-Glutamine and at pH 7 which is consistent with both CV and DPV. All above observations can be attributed to the reaction between L-Glutamine and *o*-benzoquinone species produced at the surface of electrode, with the new anodic peak being attributed to the oxidation of newly formed *o*-benzoquinone-L-Glutamine adduct.

4. CONCLUSIONS

The oxidation of Catechol produces Michael acceptor which undergoes nucleophilic attack by L-Glutamine resulting in formation of *o*-benzoquinone-amino acid adducts. The reaction products are transferred electron at more negative potential than the Catechol. Linear relationship in between the peak current and square root of scan rate indicates the electro-oxidation of Catechol reaction is controlled by diffusion process. The nucleophilic substitution reaction of Catechol in presence of L-Glutamine is not only favored by pH media but also depends on the concentration of nucleophile, electrode materials and scan rates. This reaction is most suitable at 50 mM of L-Glutamine with 2 mM of Catechol and at pH 7 in GC electrode at 0.1 V/s scan rate. The current function curve exponentially decreases which decides the nucleophilic addition of L-Glutamine with Catechol occurs through an ECE mechanism.

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