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Voltammetric Electro-synthesis of Catechol-Aspartic Acid Adduct at Different pHs and Concentrations

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Abstract- Electrochemical oxidation of catechol generates o-benzoquinone which acts as Michael acceptor and its reaction has been studied in the presence of different concentration of aspartic acid in aqueous solution with various pH values, different electrodes using cyclic voltammetry, controlled potential coulometry and differential pulse voltammetry. In this investigation aspartic acid behaves as a nucleophile and undergoes 1,4-Michael addition reaction with catechol. The participation of reaction of o-benzoquinone with aspartic acid at moderately higher concentration of nucleophiles in the second scan of potential was observed. The products synthesized from the reaction are assumed to be 2-((3,4-dioxocyclohexa-1,5-dien-1-yl)amino)succinic acid that undergo electron transfer at more negative potentials than the catechol's. The effect of pH on catechol in presence of aspartic acid was studied by varying pH from 5 to 11. The concentration effect of nucleophiles with the fixed concentration of catechol (2 mM) was measured from 30 mM to 100 mM. The reaction was strongly influenced by the pH as well as concentration of aspartic acid. This nucleophilic addition reaction was mostly suitable in 70 mM of aspartic acid and 2 mM of catechol at pH 7. The behavior of the reaction was of ECE type followed by diffusion mechanism.

Keywords- Electro-oxidation, Favorable condition, Aspartic acid, Catechol, Voltammetry, Controlled potential coulometry

1. INTRODUCTION

Catechol, also known as 1,2-dihydroxybenzene, is an organic compound with the molecular formula $C_6H_4(OH)_2$. This colorless compound occurs naturally in trace amounts. Approximately 50% of synthetic catechol is consumed in the production of pesticides, the remainder being used as a precursor to fine chemicals such as 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. There are many reports on electro-oxidation of catechols to produce *o*-quinones as reactive intermediates in many useful homogeneous reactions [4].

Aspartic acid (abbreviated as Asp) is an α-amino acid that is used in the biosynthesis of proteins. Aspartate is non-essential in mammals, being produced from oxaloacetate by transamination. It carries reducing equivalents in the malate-aspartate shuttle, which utilizes the ready inter-conversion of aspartate and oxaloacetate, which is the oxidized (dehydrogenated) derivative of malic acid. Aspartate donates one nitrogen atom in the biosynthesis of inosine, the precursor to the purine bases. In addition, aspartic acid acts as hydrogen acceptor in a chain of ATP synthase [5].

The electrochemical oxidation of catechols in the presence of some other nucleophiles such as methanol, 4-hydroxycoumarin, 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 [8–17]. However, aspartic acid and catechol are biologically important compounds in this direction, therefore, it demand detail electrochemical studies of catechol in the presence of aspartic acid. To our knowledge, the electrochemical oxidation of catechol with aspartic acid at different pH, different scan rate and different concentration has not been analyzed before this work. Cyclic voltammetry, controlled potential coulometry and differential pulse voltammetry were used in this work.

2. EXPERIMENTAL SECTION

Catechol, aspartic acid, acetic acid, sodium acetate, potassium chloride, sodium di hydrogen ortho phosphate and di-sodium hydrogen ortho phosphate were of analytical grade (E-Merck). Catechol and catechol with aspartic acid solutions of different concentrations were prepared in different pH by using acetate or phosphate buffer solutions. Platinum and gold disks 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 (6mm 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 Aspartic acid

Cyclic voltammetry (CV), controlled potential coulometry (CPC) and differential pulse voltammetry (DPV) were used for the investigation of electrochemical behavior of catechol in absence and presence of aspartic acid.

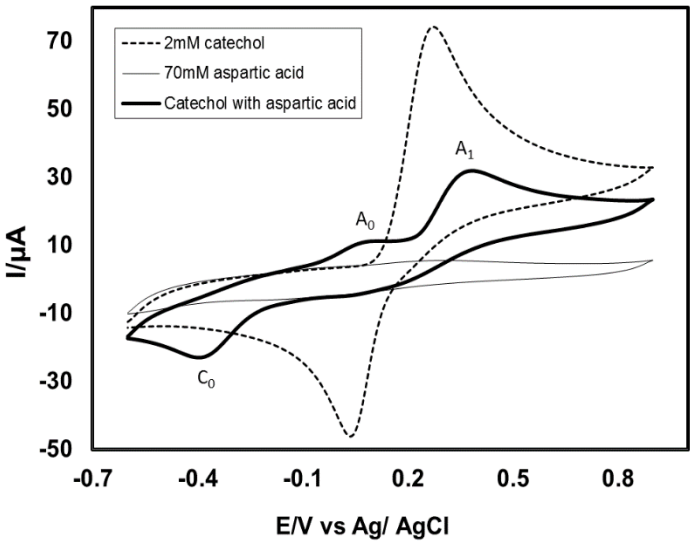


Fig. 1. Cyclic voltammogram of 2 mM catechol (dashed line), 70 mM aspartic acid (solid line) and 2 mM catechol with 70 mM aspartic acid (deep solid line) of GC electrode in buffer solution (pH 7) at scan rate 0.1 V/s (2nd cycle). A_0 and A_1 is appeared anodic peak and anodic peak, C_0 is corresponding cathodic peak

Fig. 1(dashed line) shows the cyclic voltammogram of 2 mM catechol of GC (3 mm) electrode in buffer solution of pH 7 and scan rate 0.1 V/s. The cyclic voltammogram of catechol shows one anodic peak at (0.26 V) and corresponding cathodic peak at (0.05 V)

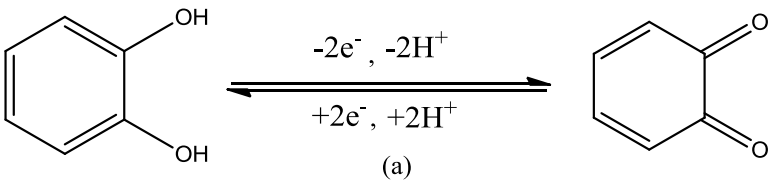
related to its transformation to *o*-quinone and vice versa. Pure aspartic acid is electrochemically inactive amino acid hence no redox peak is observed in the potential range investigated (Fig. 1, solid line). Fig. 1 (deep solid line) shows the CV of catechol (2 mM) in the presence of aspartic acid (50 mM) in the second scan of potential at the same condition. After the first scan of potential, Michael acceptor quinone reacts with aspartic acid hence two anodic peaks at 0.06 V and 0.38 V and a cathodic peak at -0.38 V were observed. Upon addition of aspartic acid to catechol solution, the anodic peak A_1 and cathodic peak C_1 decreases and newly appeared anodic peak A_0 cathodic peak C_o were increased gradually. This increase and decrease of anodic and cathodic peak was due to follow up reaction of catechol with aspartic acid. This phenomenon can be explained by the fact of nucleophilic attack of aspartic acid to o-benzoquinone. Due to the conduction of nucleophilic substitution reaction of aspartic acid to Michael acceptor reduces the o-benzoquinone concentration in reaction layer, consequently the A_1 and C_1 peaks reduces, whereas in the same time produces catechol-aspartic acid adduct and consequently the peak A_0 appear. In the first scan of potential, the anodic peak of catechol in presence of aspartic acid 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 ratio for the peaks A_1 and C_1 (I_{Pa1}/I_{pc1}) decreased noticeably, which is indicative of a chemical reaction of aspartic acid (2) with the o-quinone (1a) produced at the surface of electrode. These observations may ascribe the formation of 2-((3,4-dioxocyclohexa-1,5-dien-1-yl)amino)succinic acid through nucleophilic substitution reaction (Scheme 1). If the constituent is such that the potential for the oxidation of product is lower, then further oxidation of the product is lower, the further oxidation and further addition may occur [18]. According to this concept it can be said that the oxidation of catechol-aspartic acid adduct is easier than the oxidation of parent catechol and this substituted product can be further attacked by aspartic acid. However, it was not observed in cyclic voltammogram because of the low activity of o-quinone 4 toward 2 but a slight signal was seen at differential pulse voltammogram. This behavior is in agreement with that reported by other research groups for similar electrochemically generated compounds such as catechol and different nucleophiles [8-16,18-20]. In the absence of other nucleophiles, water or hydroxide ion often adds to the o-benzoquinone [21].

Fig. 2 (a) shows the CV of second cycle of 2 mM Catechol in presence of 70 mM aspartic acid of GC (3 mm) electrode in buffer solution (pH 7) at different scan rates. The peak current of both the peaks were increased with the increase of scan rate. The cathodic peaks were shifted towards left whereas the anodic peaks were moved to the right direction with increase in scan rate. Fig. 2 (b) showed plots of the anodic and cathodic net peak currents of 2 mM catechol with 70 mM aspartic acid 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]. The nearly proportionality of the anodic and the cathodic peaks suggests that the peak current of the reactant at each redox reaction is controlled by diffusion process. It can be seen in Fig. 2a, the cathodic peak for reduction of o-benzoquinone was almost disappeared in the scan rate of 0.05 V/s.

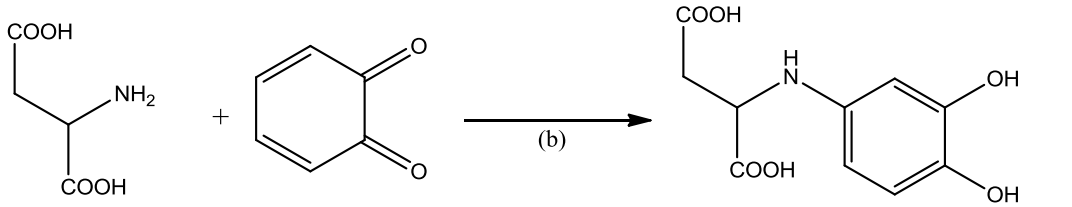
Step-1:



Catechol
1

o-benzoquinone
1a

Step-2:

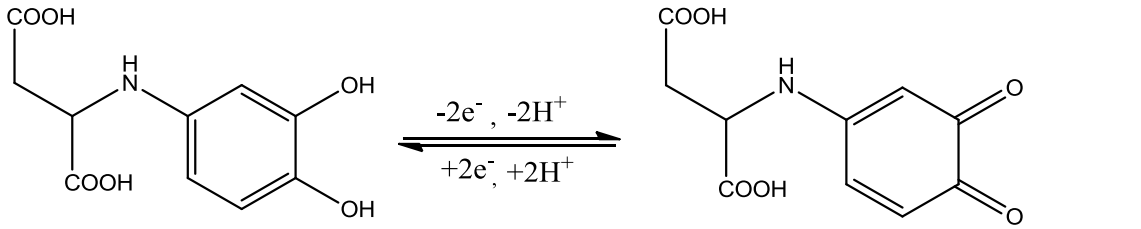


Aspartic acid
2

o-benzoquinone
1a

2-((3,4-dihydroxyphenyl)amino)succinic acid
3

Step-3:



2-((3,4-dihydroxyphenyl)amino)succinic acid
3

2-((3,4-dioxocyclohexa-1,5-dien-1-yl)amino)succinic acid
4

Scheme 1. Substitution reaction of catechol with aspartic acid

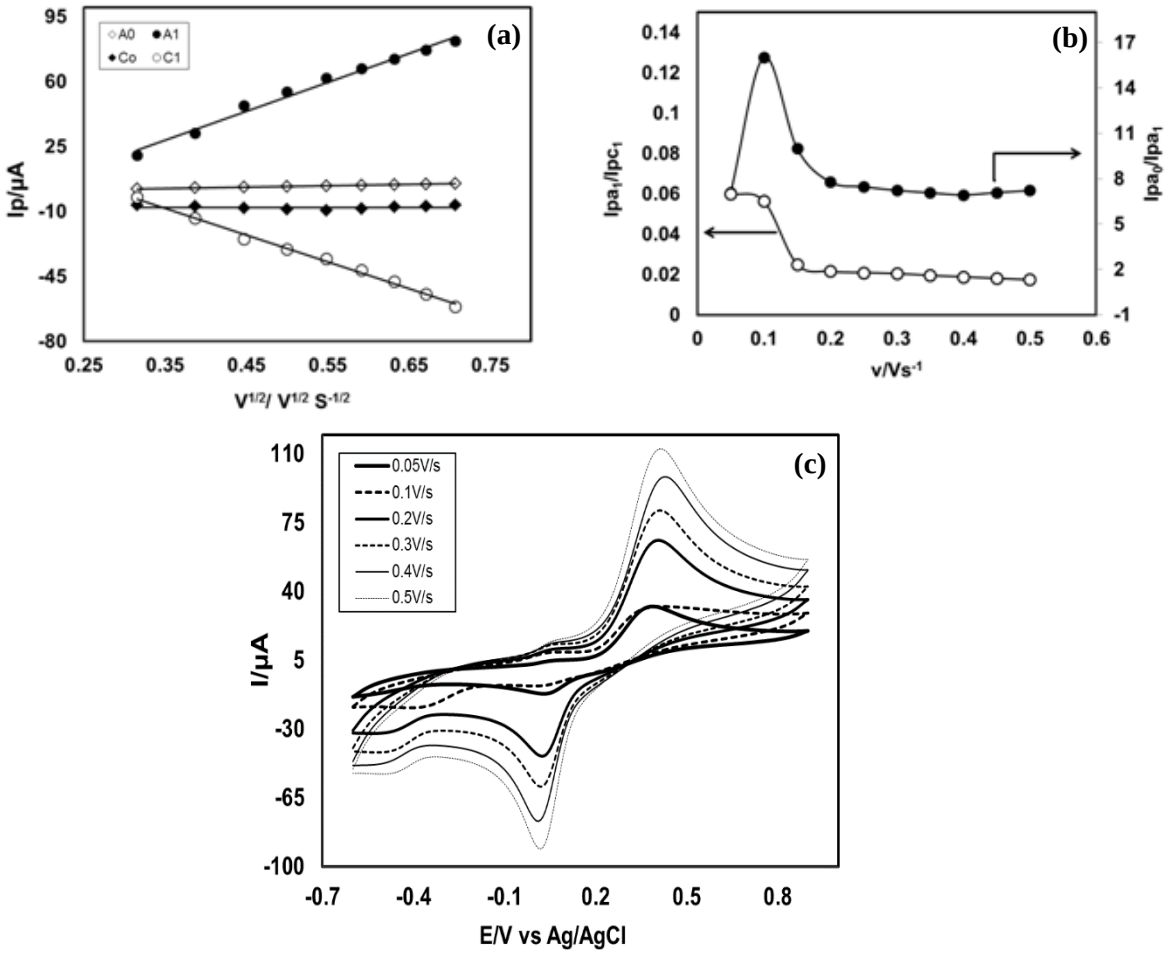


Fig. 2. a) Cyclic voltammogram of 2 mM catechol with 70 mM aspartic acid in the second scan of potential at Gc electrode in buffer solution (pH 7) at scan rate 0.05 V/s to 0.5 V/s; b) Plots of peak current *vs.* square root of scan rate in the same condition. Legend shows the symbol of oxidation and 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

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 aspartic acid decreases with increasing scan rate firstly and then after 0.2 V/s, it was almost unchanged (Fig. 2c). The anodic peak current ratio (I_{pa0}/I_{pa1}) *vs.* scan rate for a mixture of catechol and aspartic acid was firstly increased and then after 0.1 V/s scan rate the peak current was started to decrease gradually and after 0.2 V/s it remains constant in (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. 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 aspartic acid (2) firstly increases at slow scan rate and then at higher scan rate it decreases.

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

(i) In the presence of aspartic acid both I_{pc1} and I_{pa1} 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 aspartic acid (**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. 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).

According to the results, it was assumed that aspartic acid (2) undergoes the 1,4-Michael addition reaction with o-benzoquinone (1a) leads to product 3. The oxidation of this compound (3) was observed easier than the oxidation of parent molecule (1) by virtue of the presence of electron donating amine group.

The Cyclic voltammogram of pure Catechol was also observed in buffer solution (pH 7) by varying scan rates. 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.

3.2. Influence of pH

Influence of pH on the electrochemical response of catechol was studied in the presence of aspartic acid in buffer solution of different pH at scan rate 0.1 V/s. Catechol gave a well-developed reversible wave in the buffer solution of pH 7. The anodic peak potential of catechol shifted towards left with the increase of pH. The electrochemical reaction of catechol occurring at pH below 7 is a two-proton, two-electron transfer process (Scheme 1). This behavior is in agreement with that reported by other research groups for catechol and its derivatives [19,20].

Cyclic voltammogram of catechol in presence of 70 mM aspartic acid of GC (3 mm) electrode was studied at pH from 5 to 11 (Fig. 3a). The voltammetric behavior of catechol at pH 5, 9 and 11 in the presence of 70 mM aspartic acid there is no new anodic peak appeared. This indicates that the reaction between o-benzoquinone and aspartic acid at pH 5, 9 and 11 is not occurred. This can be attributed to the fact that at pH 5 or lowers, the nucleophilic property of amine group was diminished through protonation and hence exist as zwitter ion

(Fig. 3a). At the pH 7 the zwitter ionic form of amino acid was deprotonated and at this situation amino group was susceptible for the nucleophilic attack. In neutral medium o-benzoquinone undergoes nucleophilic substitution by the amine through a 1, 4-Michael addition reaction reflected that voltammetric new anodic peak appeared after repetitive cycling. Whereas, in the basic medium the oxidation of catechol followed by an irreversible chemical reaction with hydroxyl ion, especially in alkaline solutions [20]. The peak position of the redox couple is found to be dependent upon pH.

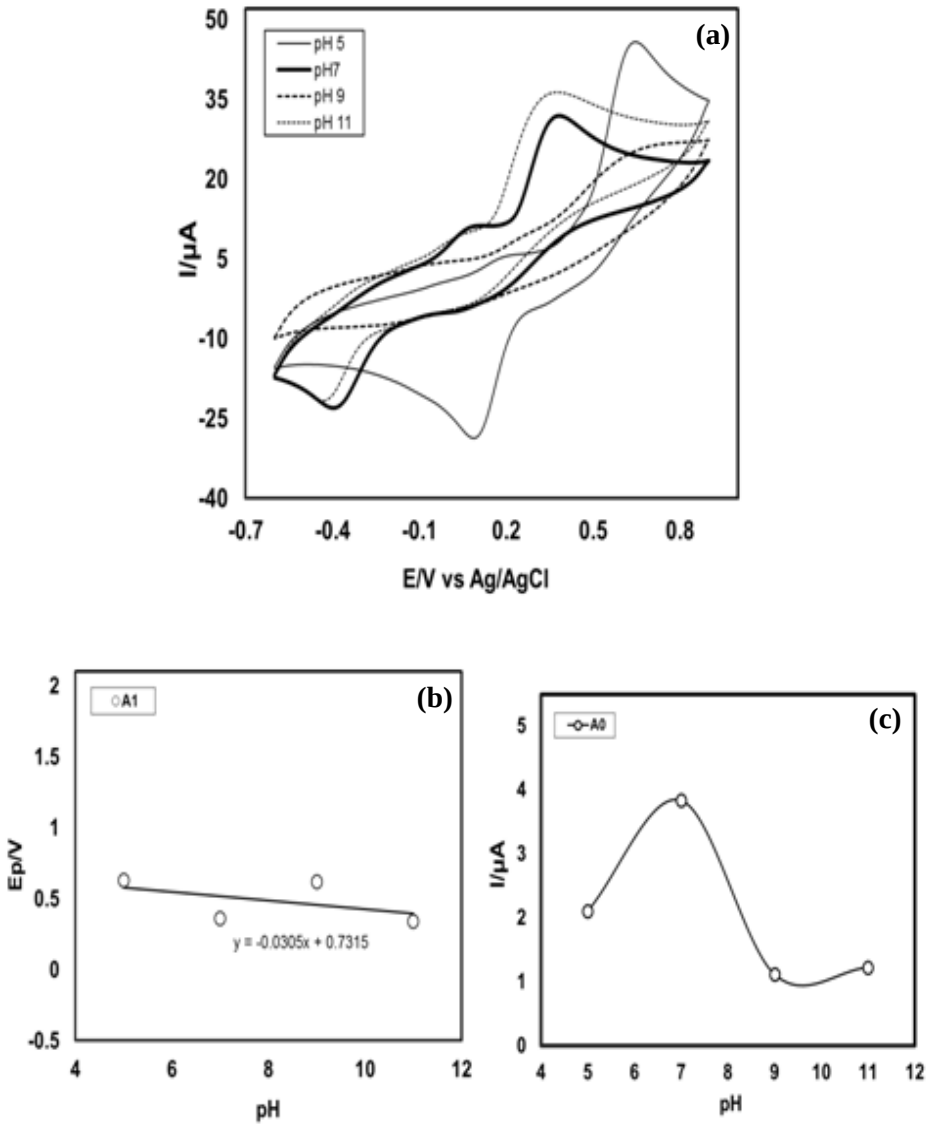


Fig. 3. a) Cyclic voltammogram of 2 mM catechol with 70 mM aspartic acid of GC (3 mm) electrode in different pH (5, 7, 9 and 11) at scan rate 0.1 V/s; b) Plots of peak potential *vs* pH in the same condition; c) Plots of peak current *vs* pH in the same condition. The meaning of symbol A_0 and A_1 is similar to Fig. 1

Fig. 3 (b) shows the plot of oxidation peak potential, E_p values against pH. The slope of the plot was 30.5 mV/pH for second anodic peak A_1. This suggested that the oxidation of the catechol proceeded via the $2e^-/2H^+$ processes (scheme 1) and during the course of reaction not only electron but also proton were released from the catechol. Other research groups also reported similar behavior for catechol and its derivatives [17,27]. The peak current of the redox couple also was found to be dependent upon pH. Fig. 3 (c) showed the plot of oxidation peak (A_0) current, I_p against pH of solution. From the Fig. 3(c) it was seen that the maximum peak current was obtained at pH 7. At this pH, the difference between the peak current ratio (I_{pC0}/I_{pA0}) in the presence of aspartic acid was maximum. Consequently, pH 7 was selected as optimum condition for electrochemical study of catechol, at which the electro oxidation was facilitated in neutral media and hence the rate of electron transfer was faster. The peak current decreased in both acidic and basic medium. This can be related to protonation of amine or formation of hydroxyl ion that inactivation of it towards Michael addition reaction with o-benzoquinone (2a). Thus the coupling reaction was highly pH dependent and the best condition was neutral medium.

3.3. Concentration effect of aspartic acid

Fig. 4 (a) shows the CV of composition changes of aspartic acid (30, 50, 70, 80 and 100 mM) with fixed 2 mM catechol of GC electrode at pH 7 and scan rate 0.1 V/s. Upon addition of aspartic acid the anodic peaks shifted positively and a new peak was appeared at ~0.06 V which suggested the nucleophilic attack of aspartic acid on catechol that is to be said the formation of catechol-aspartic acid adduct.

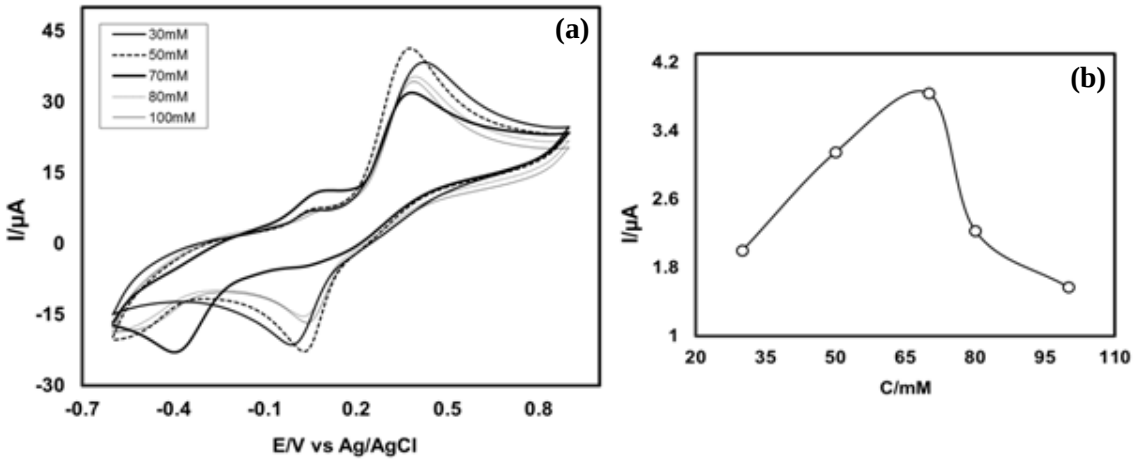


Fig. 4. a) CV of composition changes of aspartic acid (30, 50, 70, 80 and 100 mM) with fixed 2 mM catechol of GC electrode at pH 7 and scan rate 0.1 V/s. b) Plots of anodic peak current, I_p *vs* concentration of (30, 50, 70, 80 and 100 mM) aspartic acid with (fixed 2 mM catechol) in same condition. The meaning of A_0 is similar to Fig. 1

The peak current intensity of the newly appeared anodic and cathodic peak was increased with the increase of aspartic acid concentration up to ~70 mM. After addition of more (>50 mM), the anodic and cathodic peak current was started to be decreased (Fig. 4b). The nucleophilic substitution reaction of catechol in presence of aspartic acid was maximum favorable up to 70 mM of aspartic acid at pH 7.

The corresponding peak current ratio (I_{pc1}/I_{pa1}) changes with the concentration of aspartic acid. This was related to the increase of the homogenous reaction rate of following chemical reaction between o-benzoquinone 1a and aspartic acid 2 with increasing concentration of aspartic acid up to 70 mM. At higher concentration of aspartic acid (>70 mM), the excess electro-inactive aspartic acid may be deposited on the electrode surface and consequently the peak current decreased.

3.4. Effect of electrode materials

Effect of electrode materials on electrochemical properties of catechol in presence of aspartic acid was studied with the help of both cyclic voltammetry (CV) and differential pulse voltammetry (DPV) by using different electrodes like GC, Au and Pt. The Cyclic voltammograms of 2 mM Catechol with 70 mM aspartic acid at different electrodes such as GC, Au and Pt electrodes are shown in Fig. 5.

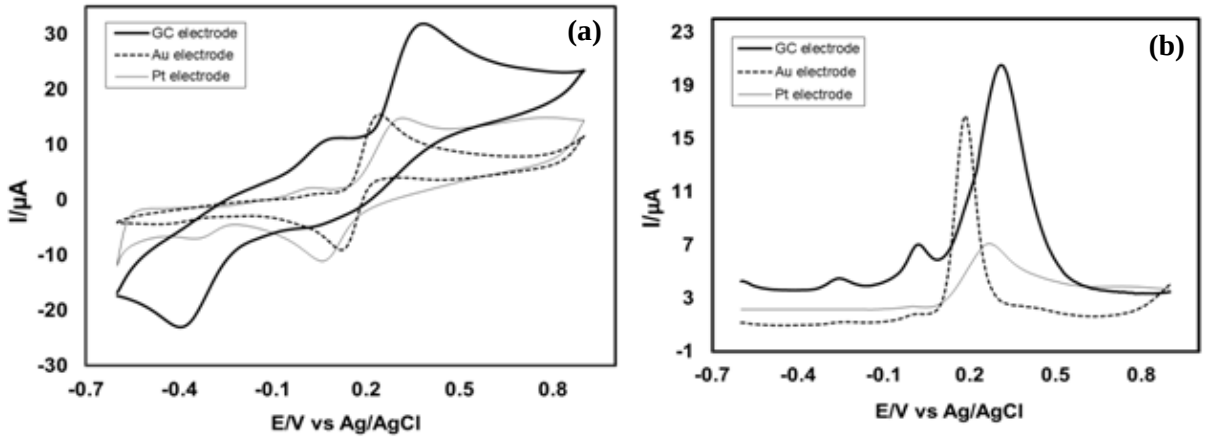


Fig. 5. a) Cyclic voltammogram (CV) and b) Differential pulse voltammogram (DPV) of 2 mM catechol with 70 mM aspartic acid in Gc electrode (3.0 mm), Gold electrode (1.6 mm) and Platinum electrode (1.6 mm) at pH 7 and scan rate 0.1 V/s

The nature of voltammograms, the peak position and current intensity for the studied systems were different for different electrodes although the diameter of GC electrode (3 mm) was higher than Au and Pt (1.6 mm). The CV and DPV at Au electrode were significantly different from those of the GC and Pt electrodes based on the appeared peak current

consideration (Fig. 5). GC and Pt electrodes show newly appearing two redox couple of adduct at 0.06/-0.38 V and -0.01/-0.31 V respectively whereas in Au electrode no appearing peak was observed. Among GC, Pt and Au electrodes, the peak current and voltammetric response of GC electrode was found to be much better than Pt and Au electrodes under investigation of electrochemical properties. Therefore, GC electrode was selected for the investigation of electrochemical properties.

3.5. Subsequent cycles of CV of Catechol-aspartic acid

Fig. 6 shows the cyclic voltammogram of the first 15 cycles of 2 mM Catechol with 70 mM aspartic acid in buffer solution of pH 7 for the potential range between -0.6 V to 0.9 V at a GC (3.0 mm) electrode. The voltammogram of first scan at 0.1 Vs^{-1} has one anodic peak at 0.29 V and corresponding cathodic peak at -0.29 V (dashed line). In the subsequent potential cycles a new anodic peak appeared at ~0.06 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 can be explained by the fact that the catechol-aspartic acid adduct was formed and consequently the height of redox couple of catechol was decreased successively through nucleophilic substitution reaction in the surface of electrode (Scheme 1).

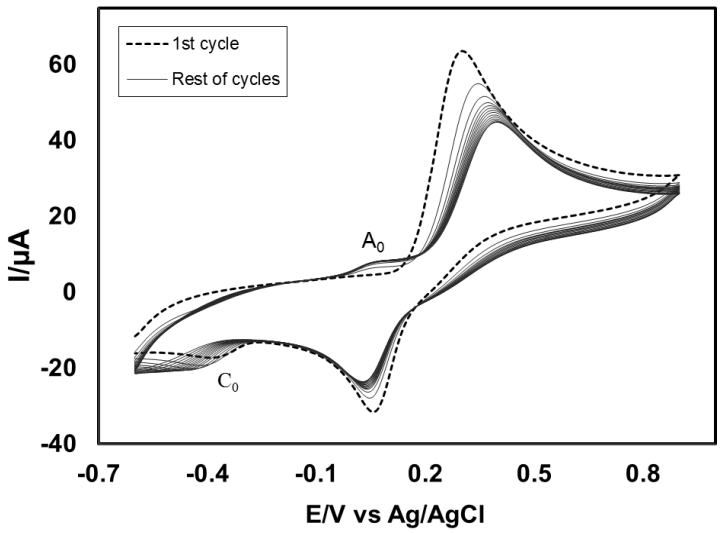


Fig. 6. Cyclic voltammogram of 70 mM aspartic acid with 2 mM catechol of GC (3 mm) electrode in the buffer solution of pH 7 at scan rate 0.1 V/s (15 cycles). The appeared anodic peak current (A_0) and cathodic peak current (C_0) increased with the iteration scan from the first cycle. First cycle marked by dashed line and the rest of the cycles by solid line

The positive shift of the second anodic peak in the presence of aspartic acid was 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 up to 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.

No new anodic peak appears at after the addition of 70 mM aspartic acid to the solution at first cycle (Fig. 6). Conversely, the reduction peak shifted due to catechol species diminishes by addition of aspartic acid. In the second scan of potential (Fig. 6) a new oxidation peak also appeared at ~0.06 V which could be attributed to the oxidation of adduct formed between the o-benzoquinone and aspartic acid according to Scheme 1. The product 3 was further confirmed by FTIR in the next section.

3.6. Controlled-potential coulometry

Controlled-potential coulometry was performed in aqueous solution containing 1 mM of catechol and 35 mM of aspartic acid at 0.45 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 aspartic acid (2). According to our results, it seems that the 1,4 addition reaction of 2 to o-quinone (1a) (reaction (2) was faster than other secondary reactions, leading to the intermediate 3.

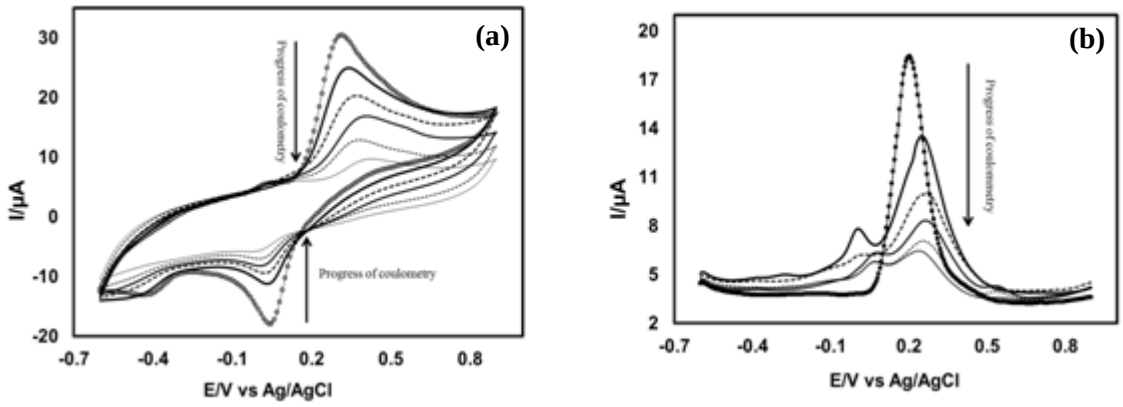


Fig. 7. a) Cyclic voltammogram and b) Differential pulse voltammogram (DPV) of 1mM Catechol in presence of 35 mM aspartic acid of GC electrode during controlled potential coulometry at 0.45 V in pH 7 at scan rate 0.1 V/s after consumption of 0-100 C

The oxidation of this compound (3) was easier than the oxidation of parent starting molecule (1) by virtue of the presence of electron-donating group. Like o-quinone**1a**, o-quinone **4** can also be attacked from the C-5 position by aspartic acid (2). However, no over reaction was observed during the voltammetric experiments because of the low activity of the o-quinone **4** toward 1,4-(Michael) addition reaction with aspartic acid (2).

3.7. Differential pulse voltammetry

Differential pulse voltammetry (DPV) technique was used to make clearer for the nucleophilic substitution reaction of aspartic acid on catechol. DPV obtained from 2 mM catechol in the presence of 70 mM aspartic acid in second scan at different pH (5-11) was shown in Fig. 8. In the buffer solution of pH 7, catechol gave three well-developed wave in the presence of aspartic acid (Fig. 8). In neutral media, the first, second and third anodic peaks were showed at ~ -0.28 V, 0.0V and 0.28V respectively. According to our observation peak at -0.28 V may be due to the nucleophilic attack on newly appeared adduct which had been reported by a group of research worker [18]. As can be seen two completely separated anodic peaks with high current intensity are observed in pH 7, which can be attributed to the oxidations of o-benzoquinone - aspartic acid new compound and catechol, respectively.

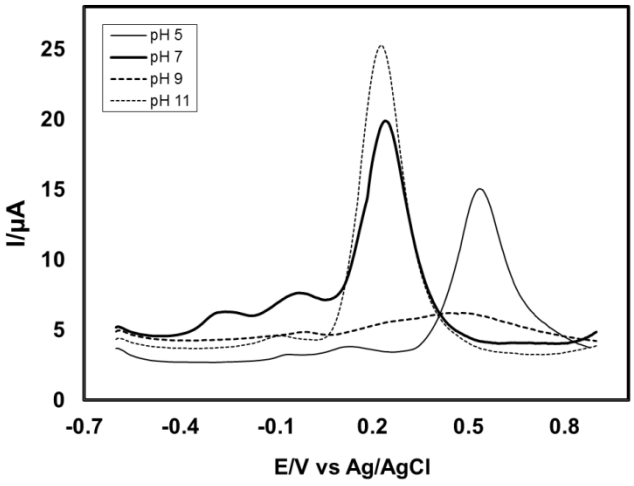


Fig. 8. Differential pulse voltammogram (DPV) of 2 mM catechol with 70 mM aspartic acid of GC electrode in second scan of different pH (5, 7, 9 and 11) and scan rate 0.1 V/s

Fig. 9 shows the DPV of deposition time change (0, 30, 90, 120, 180 and 240 s) of 2 mM catechol with 70 mM aspartic acid of pH 7. According to this figure, it is seen that the increasing of deposition time leads to develop a new anodic peak at 0.0 V. When the deposition time increases 30 s, more nucleophilic attack occurred and consequently more

catechol-aspartic acid adduct was formed which leads to decreasing in the concentration of o-benzoquinone and increasing in the concentration of catechol-aspartic acid adduct at the surface of electrode. Maximum peak intensity was obtained up to 30 s. For further increase of deposition time from 90 s to 240 s, both first and second anodic peak current decreases. This confirmed that with the increase of time decreases the concentration of o-benzoquinone.

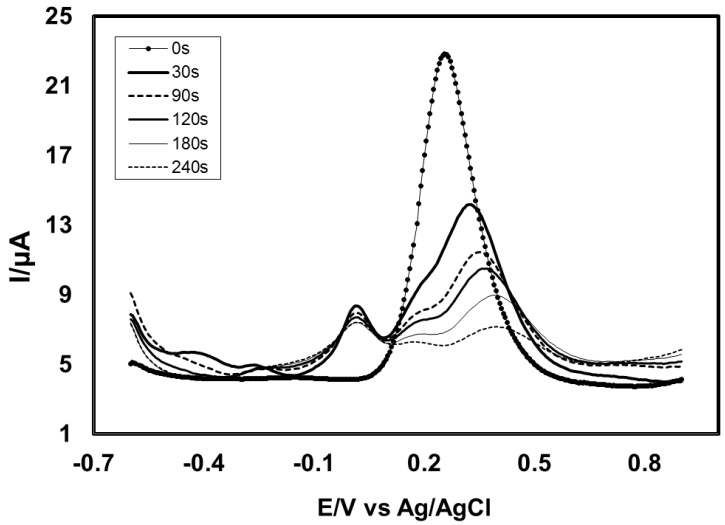


Fig. 9. Differential pulse voltammogram (DPV) of deposition time change (0, 10, 30, 60, 90,120 and 240 s) of 2 mM catechol with 70 mM aspartic acid of pH7 at *E*puls 0.02 V, tpuls 20 ms and scan rate 0.1 Vs^{-1}

DPV of 2 mM catechol with 30 to 100 mM of aspartic acid at pH 7 was studied. We observed again three separated anodic peaks appeared after addition of aspartic acid into catechol similar to Fig. 8. In this case, the gradual increasing of the concentration of aspartic acid up to 70 mM leads to increasing of first anodic peak current. For further increase of concentration from 80 to 100 mM, all anodic peak decreases gradually. In lower concentration of aspartic acid (<60 mM), the nucleophilic substitution reaction take place in comparable degree, whereas increasing the concentration of aspartic acid (>60 mM) make favorable nucleophilic attack of aspartic acid toward o-benzoquinone generated at the surface of electrode. For further addition of aspartic acid (>70 mM) into catechol solution, the excess electroinactive aspartic acid deposited on the electrode surface and hence the peak current decreases.

In this study 30-100 mM concentrated aspartic acid was used to determine the optimum condition for the nucleophilic substitution reaction on catechol. As the reaction was occurred at moderately high concentration of nucleophiles, consequently the voltammetric peaks (CV and DPV) for adduct appeared noticeably. In contrast, comparatively low concentration of

aspartic acid was not favorable for the study of electrochemical oxidation of catechol because the appearing peak was not so prominent.

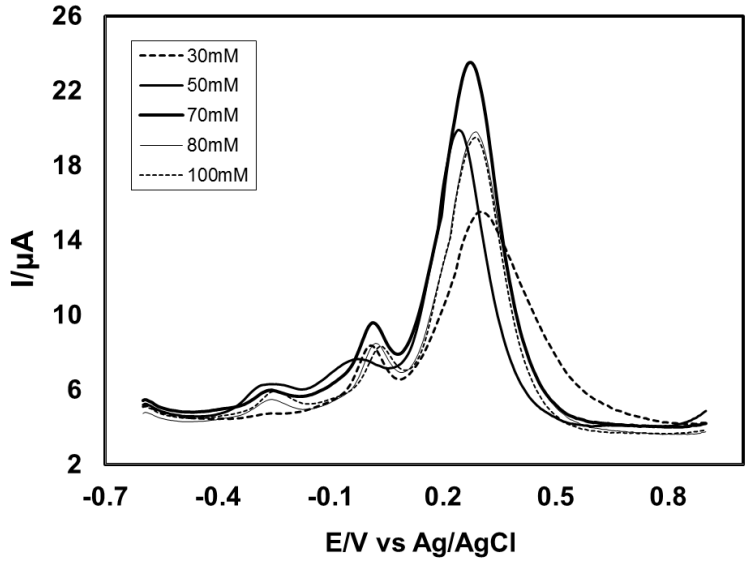


Fig. 10. Differential pulse voltammogram (DPV) of composition change of aspartic acid (30, 50, 70, 80 and 100 mM) with the fixed composition of 2 mM Catechol in second scan of pH 7 at *E*puls 0.02 V, tpuls 20 ms of GC electrode and scan rate 0.1 Vs^{-1}

From the experimental study it is noticeable that aspartic acid acts properly as a nucleophile at pH 7. When the pH is below 7 that is at acidic media, the nucleophilic activity of aspartic acid reduces due to the protonation of amine. Whereas at basic condition, 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 [27].

Therefore, from the above discussion it is clear that the nucleophilic substitution reaction of catechol in presence of aspartic acid is maximum favorable at 70 mM of aspartic acid and at pH 7 which is consistent with both CV and DPV. All above observations could be attributed to the reaction between aspartic acid 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- aspartic acid adduct.

3.8. IR-spectroscopy

The FTIR spectral assignments of the vibrational modes of the catechol-aspartic acid adduct, aspartic acid and catechol were studied. The aspartic acid showed the OH stretching band at 3450 cm^{-1} and N-H stretching band at 2911 cm^{-1} and. Catechol showed O-H stretching at 3421 cm^{-1}. The absorption peaks due to the O-H broad stretching vibration was

appeared at 3444 cm^{-1}, N-H stretching band at 3118 cm^{-1}, C=O stretching at 1624 cm^{-1} and aromatic C=C stretching at 1577 cm^{-1} for the catechol- aspartic acid adduct. In adduct the peaks at finger print region are different from pure catechol and aspartic acid.

4. CONCLUSION

Cyclic voltammetry, controlled potential coulometry and differential pulse voltammetry were employed to investigate the electrochemical behavior of catechol in the absence and presence of aspartic acid. Michael acceptor was obtained by the electro-oxidation of catechol which was susceptible for nucleophilic attack by aspartic acid. The reaction products transferred electron at more negative potential than the catechol. The peak current of the catechol- aspartic acid adduct at each redox reaction was controlled by diffusion process. The nucleophilic substitution reaction of catechol in presence of aspartic acid was maximum favorable at 70 mM of aspartic acid and at pH 7 in GC electrode. In this condition it can be deduced that the nucleophilic addition of aspartic acid occurs through an ECE mechanism.

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