*Anal. Bioanal. Electrochem.*, Vol. 10, No. 8, 2018, 974-990

**Analytical & Bioanalytical Electrochemistry**

2018 by CEE
www.abechem.com

*Full Paper*

# Electro-oxidation of Catechol in the Presence of L-Histidine at Different pH

**Md. Abdul Hafiz Mia,[1] Md. Abdul Motin[1*], and Entazul M. Huque[2]**

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

*Corresponding Author, Tel.: +88041769471-5, Ext.515; Fax: +88 041 774403
E-Mail: abdulmotin75@yahoo.com

*Received: 4 April 2017 / Received in revised form: 31 May 2018 / Accepted: 28 July 2018 / Published online: 31 August 2018*

**Abstract**- The electro-oxidation of Catechol in presence of L-Histidine has been investigated in aqueous solution with various pH values, different electrodes and different concentration of L-Histidine 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-Histidine as nucleophiles in the second scan of potential. The products obtained from the reaction are assumed to be 2-((3,4-dioxocyclohexa-1,5-dien-1-yl)amino)-3-(1H-imidazol-4-yl)propanoic acid that undergo electron transfer at more negative potentials than the Catechol. The effect of pH of Catechol in presence of L-Histidine has been studied by varying pH from 5 to 11. The concentration effect of L-Histidine with the fixed concentration of Catechol (2 mM) was measured from 2 mM to 100 mM. The reaction was strongly influenced by the pH as well as concentration of L-Histidine. The reaction is mostly favorable in 30 mM of L-Histidine and 2 mM of Catechol at pH 7. The behavior of the reaction mechanism was of ECE type.

**Keywords**- Electro-oxidation, Favorable condition, L-Histidine, Catechol, Voltammetry, Controlled potential coulometry

## 1. INTRODUCTION

In recent years, there has been a growing interest in the study of reactions between quinones produced from the oxidation of catechol and other nucleophiles due to the mechanistic and synthetic importance of these reactions [1-4]. The most well-known

characteristic of the catechol is that they can be easily oxidized mainly due to their antioxidant activity and low oxidation potentials [6]. 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 [7]. There are many reports on electrooxidation of catechol to produce *o*-quinones as reactive intermediates in many useful homogeneous reactions [2-7].

L-Histidine is an essential amino acid having many vital functions within the body. One of the most important characteristics of L-Histidine is that it can be converted into various substances, including histamine, glutamate and haemoglobin. Furthermore, it is involved in various metabolic reactions and hence ensures indirectly the oxygen supply to all the organs and tissues. The protonated form of the imidazole side chain in histidine has a p$K_a$ of approximately 6.0 [8]. Below a pH of 6, the imidazole ring is mostly protonated as described by the Henderson–Hasselbalch equation. Also, the imidazole structure can be found in many organic compounds like the in imidazole-containing protein, in pigments, and in alkaloids [9-10]. In addition, many pharmaceutical drugs are included of specifically substituted histidine [10].

On the other hand, catechol derivatives play an important role in mammalian metabolism. Many compounds of this type are known to be secondary metabolites of higher plants [11]. Thus, it was thought that synthesis of compounds with both structures of catechols and indoles would be useful from the point of view of pharmaceutical properties. The present investigation focuses on the formation of new Catechol-Histidine adduct by using volammetry and coulometry techniques at different pH, concentrations and electrodes.

The electrochemical oxidation of Catechol 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 [12-16]. However, to the best of our knowledge no papers have been published on electrochemical oxidation of catechol in the presence of L-Histidine and because of importance of aminoquinones as biologically important compounds [17] in this direction we have investigated the electrochemical properties of catechol in the presence of L-Histidine. In this paper, we have studied the electrochemical properties of Catechol in presence of L-Histidine with three different electrodes (GC, Au and Pt), wide range of concentration of L-Histidine (30-100 mM), different pH and different scan rate.

## 2. EXPERIMENTAL SECTION

Catechol, L-Histidine, 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-Histidine solutions of different concentrations were

prepared in different pH by using acetate or phosphate buffer solutions. Pt and Au disks of 1.6 mm in diameter (BASi) and Glassy Carbon disks of 3mm 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 min. 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-Histidine

Electrochemical oxidation of Catechol has been carried out with the help of cyclic voltammetry (CV), differential pulse voltammetry (DPV) and controlled potential coulometry (CPC) in absence and presence of L-Histidine. 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-Histidine 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-Histidine (30 mM) in the second scan of potential at the same condition. In the second scan of potential Catechol with L-Histidine shows two anodic peaks at 0.03 V and 0.34 V and the corresponding two cathodic peaks at -0.33 V and 0.05 V, respectively. Upon addition of L-Histidine to Catechol solution, the cathodic peak $C_1$ decreases and a new cathodic peak $C_o$ appears. 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-Histidine indicates that it is due to follow up reaction of Catechol with L-Histidine. This observation can be explained by considering nucleophilic attack of L-Histidine to o-benzoquinone. The nucleophilic attack of L-Histidine 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-Histidine 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-Histidine 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 L-Histidine (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)-3-(1H-imidazol-4-yl)propanoic 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 and further addition may occur [18]. In the case of Catechol in presence of L-Histidine, the oxidation of L-Histidine substituted *o*-benzoquinone is easier than the oxidation of parent Catechol. This substitution product can also be attacked by L-Histidine, 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 [18-20]. In the absence of other nucleophiles, water or hydroxide ion often adds to the *o*-benzoquinone [21].

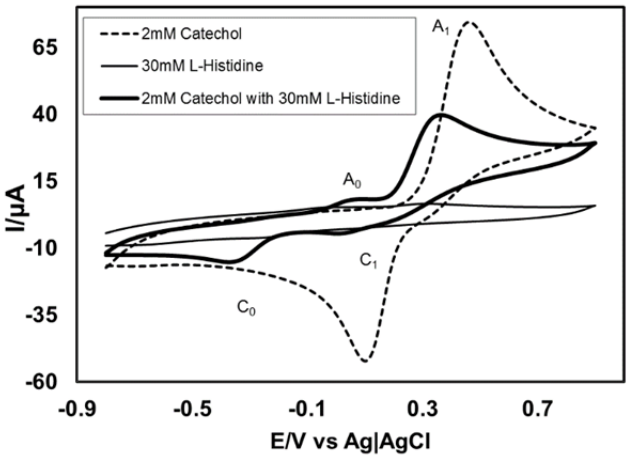
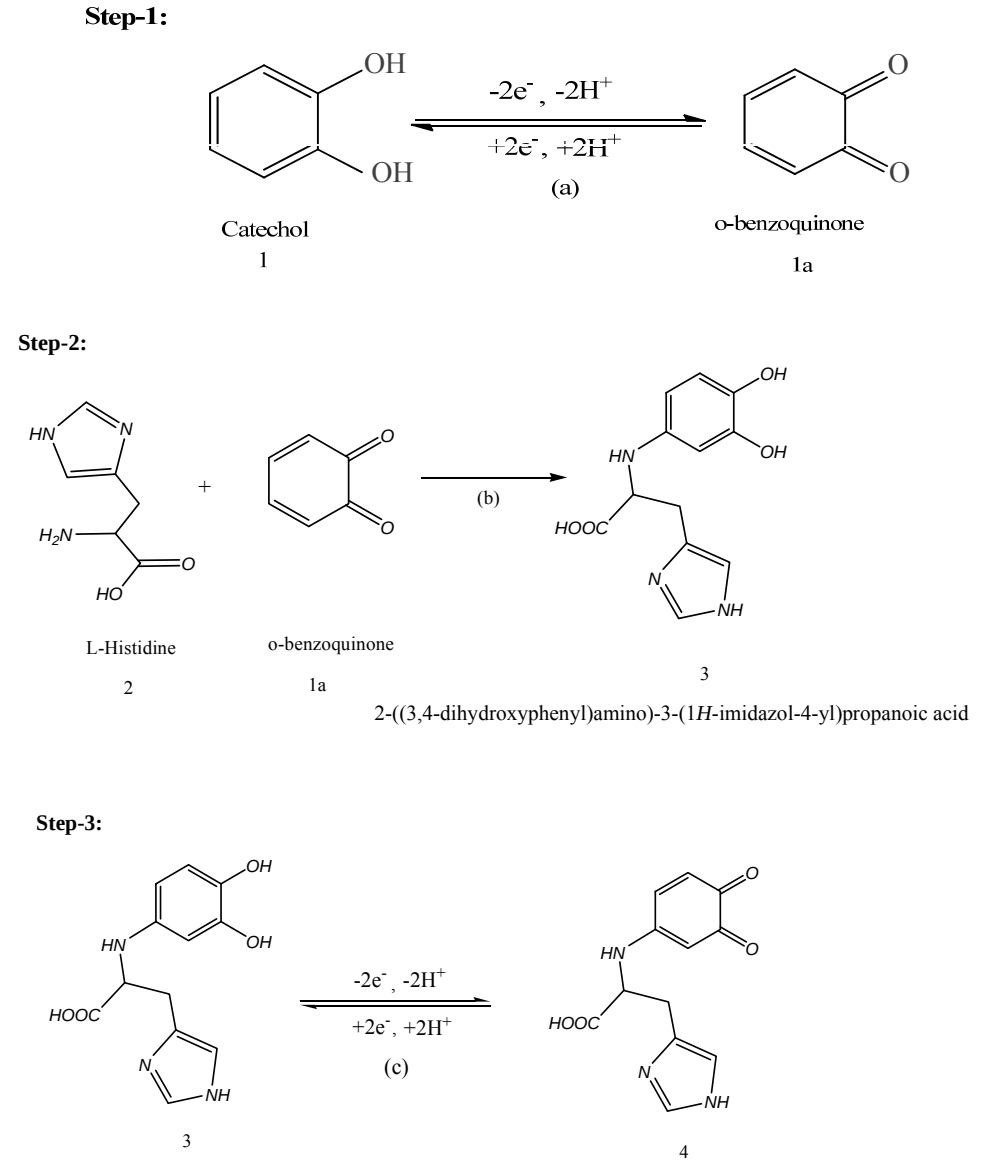



**Fig. 1.** Cyclic voltammogram of 2 mM catechol, 30 mM L-Histidine and 2 mM catechol with 30 mM L-Histidine of Gc electrode in buffer solution (pH 7) at scan rate 0.1 V/s (2nd cycle). $A_0$ and $A_1$ is appeared anodic peak and anodic peak, $C_0$ and $C_1$ is corresponding appeared cathodic peak and cathodic peak.

**Reaction scheme 1:**




## 3.2. Effect of scan rates

Fig. 2a represents the CV of second cycle of 2 mM Catechol in presence of 30 mM L-Histidine 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. 2b shows plots of the anodic and cathodic net peak currents of 2 mM Catechol with 30 mM L-Histidine 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. 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-Histidine 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-Histidine 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 (Fig. 2d). The exponential nature of the current function versus the scan rate plot indicates the ECE mechanism for electrode process [3]. This confirms the reactivity of *o*-benzoquinone (1a) towards L-Histidine (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 [2-5,21-23].

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

(i) In the presence of L-Histidine 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 L-Histidine (**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 $A_0$ 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 seems that the 1,4-Michael addition reaction of L-Histidine (2) to *o*-benzoquinone (1a) leads to product 3. The oxidation of this compound (3) is easier than the oxidation of parent molecule (1) by virtue of the presence of electron donating amine group.

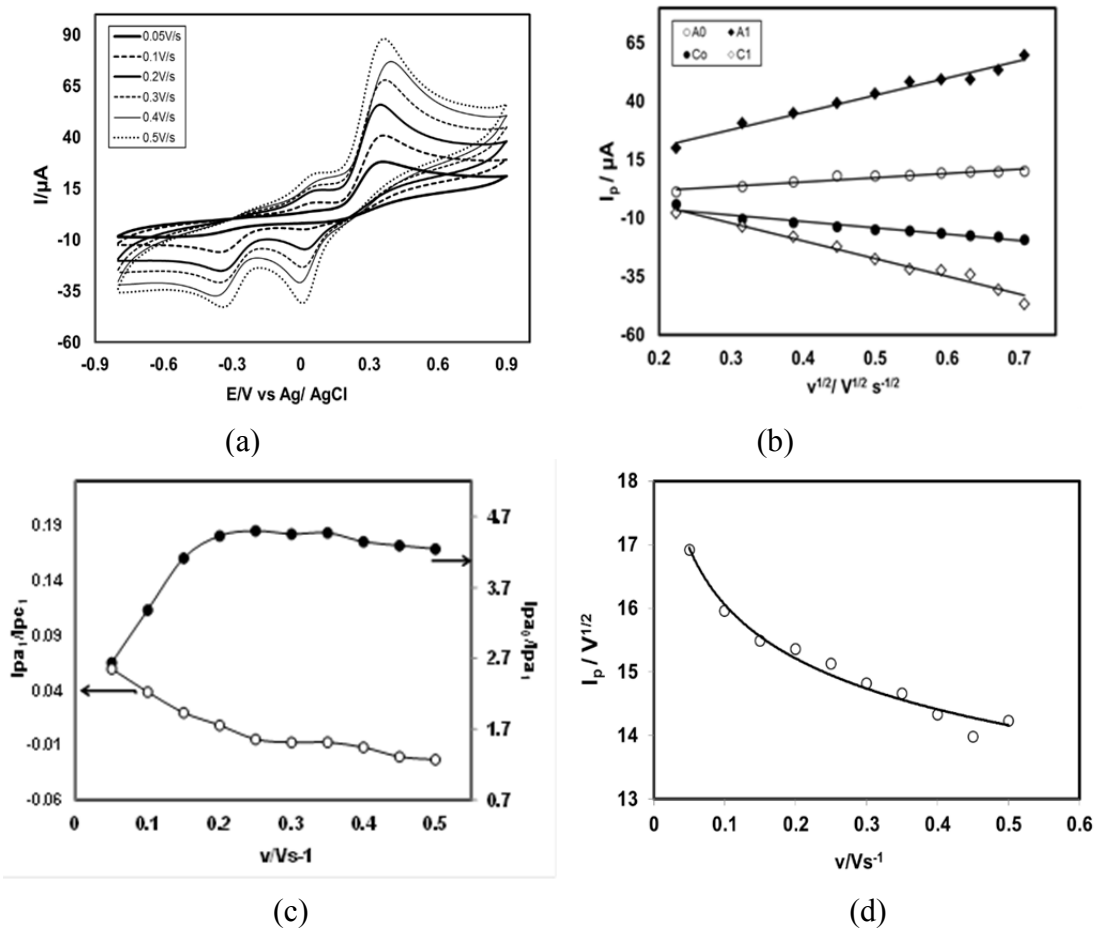



**Fig. 2.** a) CV of 2mM catechol with 30mM L-Histidine in the second scan of potential at Gc electrode in buffer solution (pH 7) at scan rate 0.05 V/s-0.5 V/s; b) Plots of peak current *vs.* square root of scan rate in the same condition. Legend shows the symbol of oxidation and reduction peaks; c) 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 ($I_p/v^{1/2}$) versus scan rate in the same condition

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.

### 3.3. Influence of pH

The influence of pH on the electrochemical reaction of 2 mM Catechol in the presence of 30 mM of L-Histidine has been studied through examining the electrode response in buffer solution of different pH (5-11) (Fig. 3a). The voltammetric behavior of Catechol at pH 5 in the presence of 30 mM L-Histidine shows that very small new anodic peak after repetitive cycling indicating that the reaction in between *o*-benzoquinone and L-Histidine is started in

very small content. This can be attributed to the fact that at pH 5, the nucleophilic property of amine groups is diminished or hindered through protonation (Fig. 3a). In the pH 7, the *o*-benzoquinone undergoes L-Histidine 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 was determined graphically as the anodic peaks (30.3 mV/pH for second anodic peak $A_1$) at 0.1 V/s, which is close to the theoretical value for two step two-electron, two-proton transfer process. This indicates that both the oxidation of the Catechol and Catechol-L-Histidine 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-Histidine adduct. Other research groups also reported similar behavior for Catechol and its derivatives [17,22]. Fig. 3c shows the plot of oxidation peak of $A_0$ which is denoted by $I_p$ against pH of solution. From the Fig. 3c 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-Histidine 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-Histidine. This ascribed that the electrochemical oxidation of Catechol in presence of L-Histidine 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-Histidine substitution reaction. DPV obtained for 2 mM Catechol in the presence of 30 mM L-Histidine in second scan at different pH (5-11) was shown in Fig. 3d. In the buffer solution of pH 5-7, Catechol shows three well-developed wave in the presence of L-Histidine. In pH 7, the first, second and third anodic peaks were found at ~ -0.30 V, -0.01 V and 0.255 V respectively. Among these, peak at -0.30 V can be arising due to side reaction or polymerization. But, in pH 9 has 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 5 and pH 7, which can be attributed to the oxidations of *o*-benzoquinone-L-Histidine new compound and *o*-benzoquinone, respectively.

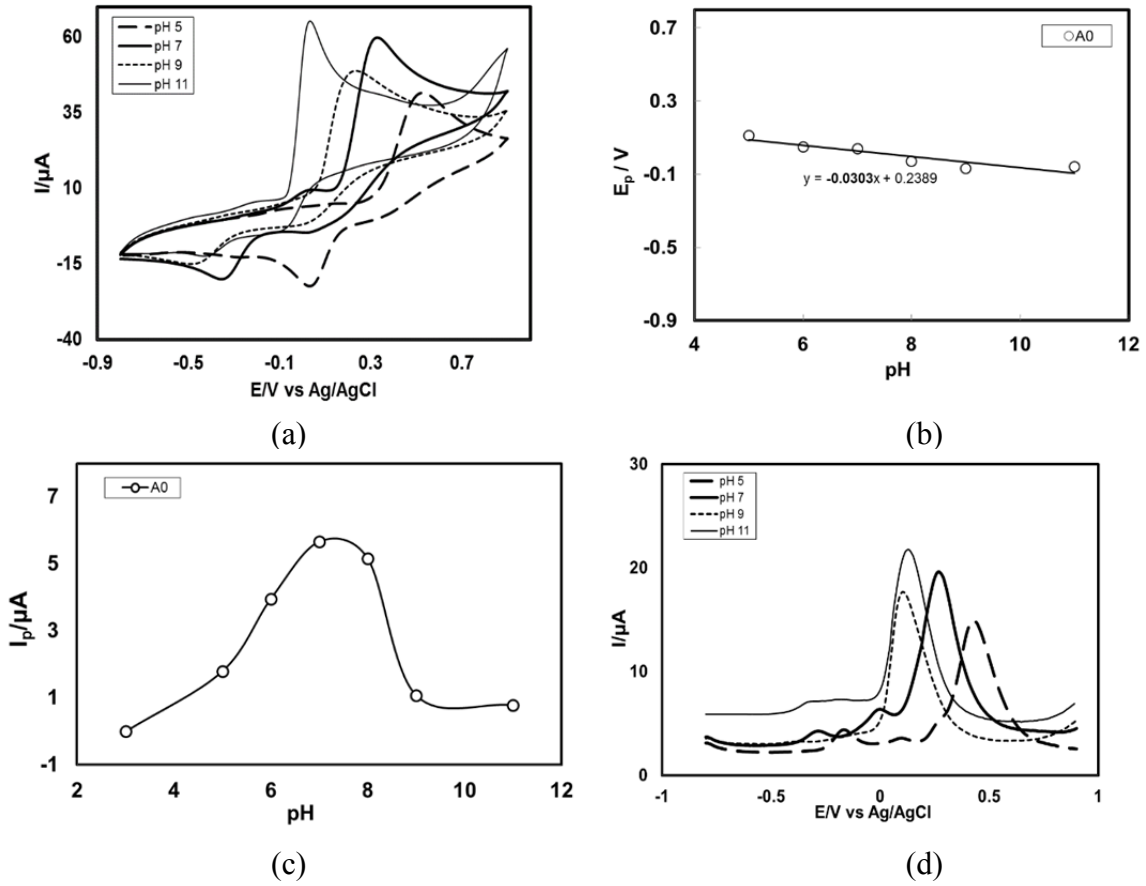



**Fig. 3.** a) CV of 2 mM catechol with 30 mM L-Histidine of Gc (3 mm) electrode in different pH (5, 7, 9, and 11) at scan rate 0.1 V/s; b) Plots of peak potential *vs.* pH in the same condition; c) Plots of peak current *vs* pH in the same condition; d) DPV of 2 mM catechol with 30 mM L-Histidine of 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-Histidine

Fig. 4a shows the variation of voltammogram pattern by the addition of different concentration of L-Histidine (2, 10, 30, 50 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-Histidine which suggests the formation of Catechol-L-Histidine adduct. The net current intensity of the newly appeared anodic and cathodic peak increases with the increase of L-Histidine composition up to 30mM where the net current means the peak current is measured from the baseline consideration. Further addition of L-Histidine (>30 mM), the anodic and cathodic peak current is slightly decreased (Fig. 4b). The nucleophilic substitution reaction of Catechol in presence of L-Histidine was maximum favorable up to 30 mM of L-Histidine at pH 7.

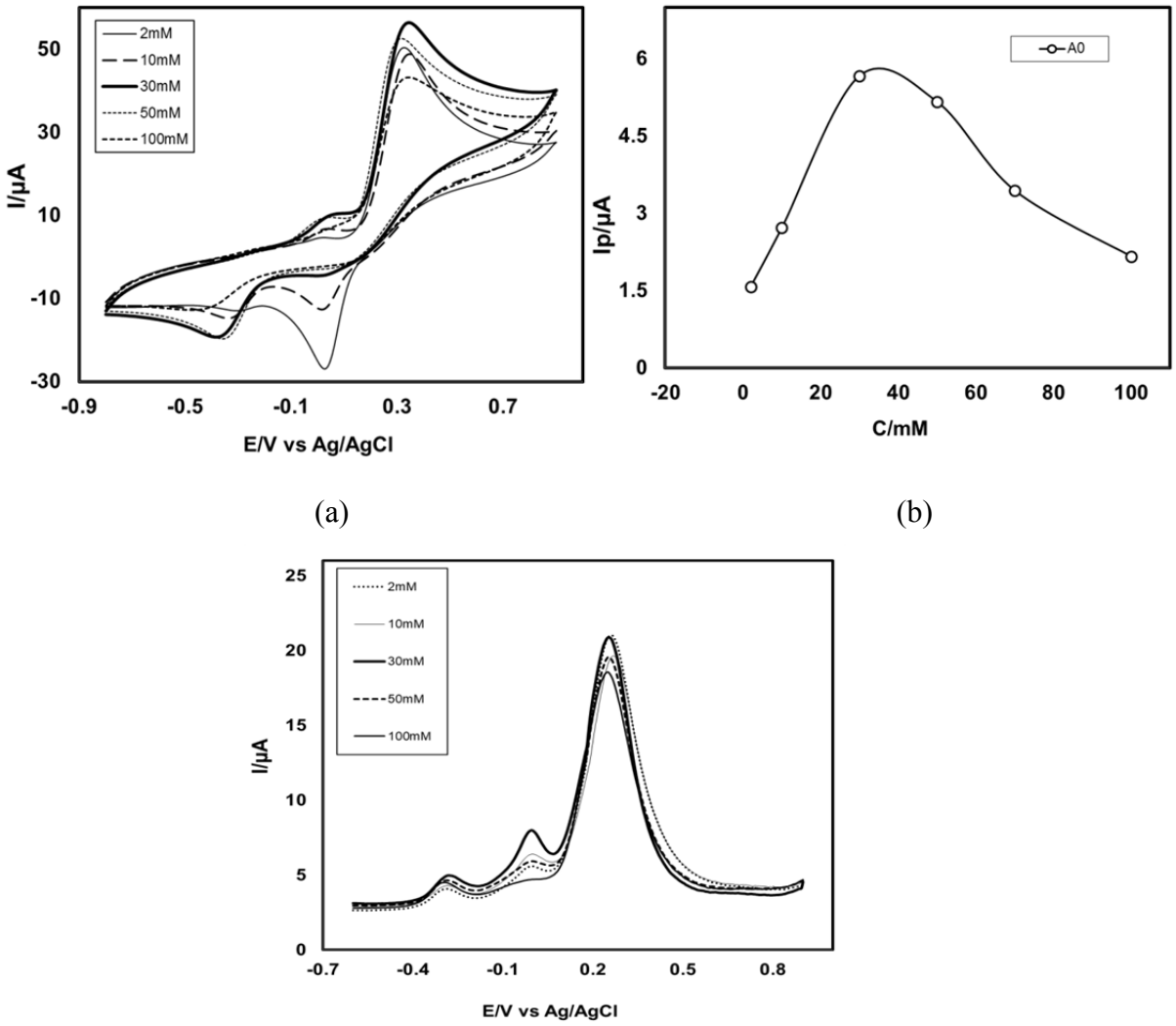



**Fig. 4.** a) CV of composition changes of L-Histidine (2, 10, 30, 50 and 100 mM) with fixed 2 mM Catechol of Gc electrode at pH 7 and scan rate 0.1 V/s; b) Plots of anodic peak current, Ip vs. concentration of (2, 10, 30, 500 and 100 mM) L-Histidine with (fixed 2 mM catechol) in same condition. The meaning of A0 is similar to Fig. 1; c) DPV of composition change of L-Histidine (2, 10, 30, 50 and 10 mM) with the fixed composition of 2 mM Catechol in second scan of pH 7 at $E_{puls}$ 0.02 V, $t_{puls}$ 20 ms of Gc electrode and scan rate 0.1 $Vs^{-1}$

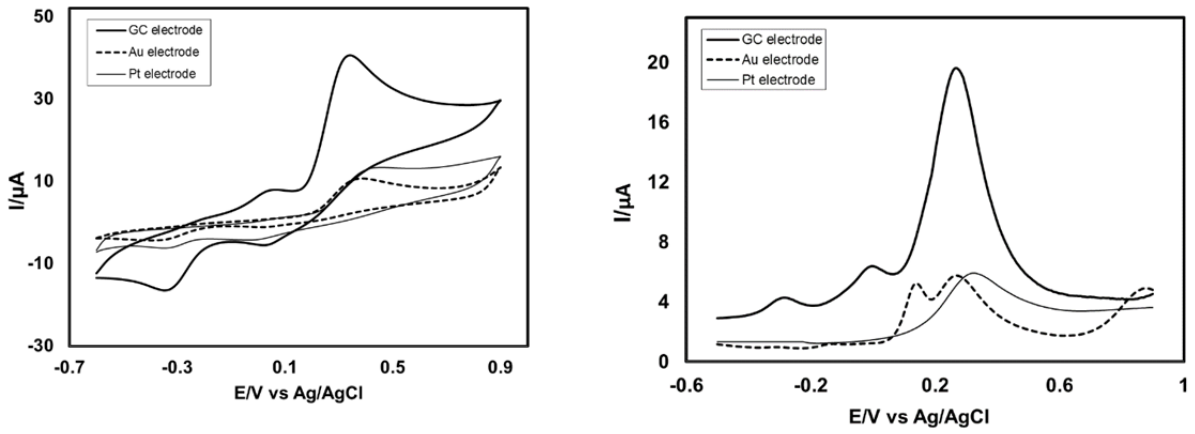
The corresponding peak current ratio ($I_{pa1}/I_{pc1}$) varies with the concentration of L-Histidine. This is related to the increase of the homogenous reaction rate of following chemical reaction between o-benzoquinone 1a and L-Histidine 2 with increasing concentration of L-Histidine upto 30 mM. At higher concentration of L-Histidine (>30 mM), the excess electro inactive L-Histidine may be deposited on the electrode surface and consequently the peak current decreased.

To understand the effect of L-Histidine 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-Histidine from 30 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-Histidine into Catechol similar to Fig. 3d. In this case, the increasing of the concentration of L-Histidine from 2 to 30 mM leads to increasing of first anodic peak current. For further increase of concentration from 30 to 100 mM, the first and second anodic peak current decreases gradually. In lower concentration of L-Histidine (<30 mM), the nucleophilic substitution reaction take place in comparable degree, whereas increasing the concentration of L-Histidine (30 mM) make favorable nucleophilic attack of L-Histidine toward o-benzoquinone generated at the surface of electrode. For further addition of L-Histidine (>30 mM) into Catechol solution, the excess electro-inactive L-Histidine 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-Histidine has been examined by different electrodes like GC, Au and Pt at different pH. The Cyclic voltammograms of 2 mM Catechol with 30 mM L-Histidine at GC, Au and Pt electrodes are shown in Fig. 5a.




(a) (b)

**Fig. 5.** a) CV b) DPV of 2 mM catechol with 30 mM L-Histidine 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 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 electrodes having two redox pair in the second scan of potential. At the Au electrode it shows one anodic and two cathodic peaks whereas Pt electrode shows one redox pair. In the case of GC we observe a new redox pair at

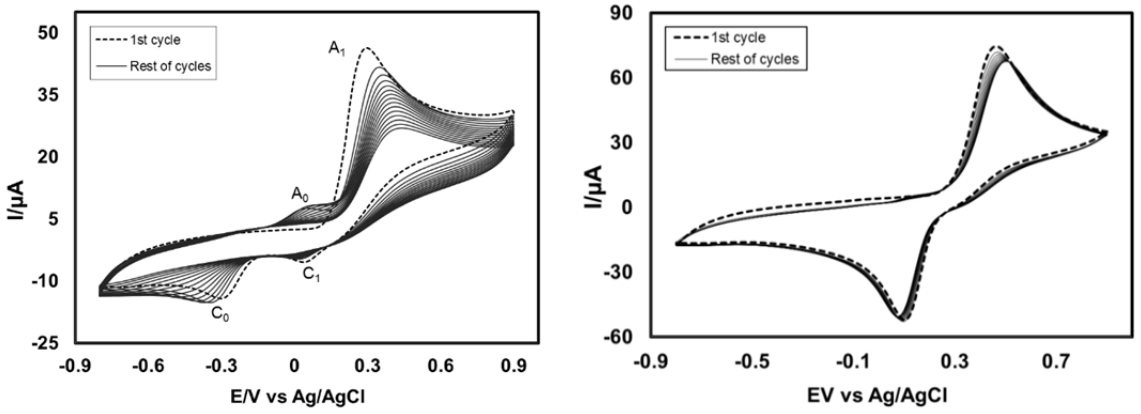
lower potential 0.03/-0.32 V which can be attributed to the redox behavior of adduct formed between the *o*-benzoquinone and L-Histidine. Electrochemical properties of Catechol with L-Histidine for example change of pH; concentration, scan rate etc. were studied in detail using Pt and Au electrodes. 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-Histidine using GC electrode.

### 3.6. Subsequent cycles of CV of Catechol-L-Histidine

Fig. 6a shows the cyclic voltammograms of the first 15 cycles of 2 mM Catechol with 30 mM L-Histidine 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 rate has one anodic peak at 0.28 V and two cathodic peaks at -0.29 and 0.04 V when considered the first scan (dashed line). In the subsequent potential cycles a new anodic peak appears at 0.03 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-Histidine 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-Histidine 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-Histidine 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 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.

Fig. 6b shows the cyclic voltammograms of the first 15 cycles of 2 mM Catechol in buffer solution of pH 7 at 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 [21] are too slow. In other words, any hydroxylation [23-26] or dimerization [22,27] reactions are too slow that can be observed in the time-scale of cyclic voltammetry [21]. 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 -0.34 V after the addition of 30 mM L-Histidine to the solution at first cycle (Fig. 6a). Conversely, the reduction peak shifted due to Catechol species diminishes by addition of L-Histidine. In the second scan of potential (Fig. 6a) a new oxidation peak also appears at 0.03 V which can be attributed to the oxidation of adduct formed between the *o*-benzoquinone and L-Histidine according to Scheme 1.




(a) (b)

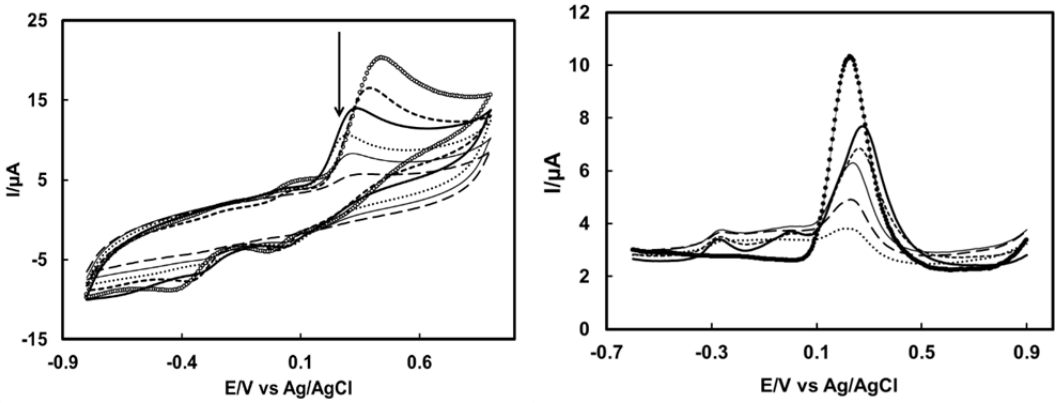
**Fig. 6.** a) CV of 30 mM L-Histidine with 2 mM catechol of Gc (3 mm) electrode in the buffer solution of pH 7 at scan rate 0.1 V/s (15 cycles). The appeared anodic peak current ($A_0$) increased with the iteration scan from the first cycle; b) CV of 2 mM Catechol in the buffer solution of pH 7 at scan rate 0.1 $Vs^{-1}$ (15 cycles)

### 3.7. Control potential coulometry studies

Controlled-potential coulometry has also been employed in aqueous solution containing 1 mM of Catechol and 15 mM of L-Histidine at 0.4 V in pH 7. The electrolysis progress was monitored by cyclic voltammetry (Fig. 7a). These figures show, during the course of coulometry the peaks $A_0$ and $C_0$ appeared and the height of the $A_0$ peak increase proportionally to the advancement of coulometry, parallel to the decrease in height of anodic peak $A_1$. All anodic and cathodic peaks disappeared after consumption of 4 electrons per Catechol.

These observations allow us to propose the pathway in Scheme 1 for the electro-oxidation of Catechol (1) in the presence of L-Histidine (2). According to our results, it seems that the 1,4 addition reaction of 2 to o-quinone (1a) (reaction (2)) is faster than other secondary reactions, leading to the intermediate 3. The oxidation of this compound (3) is 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 L-Histidine (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 L-Histidine (2).




(a) (b)

**Fig. 7.** (a) Cyclic voltammogram; (b) Differential pulse voltammogram of 1 mM Catechol in presence of 15 mM L-Histidine of Gc electrode during controlled potential coulometry at 0.4 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 30 mM L-Histidine 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 at -0.295 and -0.005 V and more nucleophilic attack occurs and consequently more Catechol-Histidine adducts lead to decreasing in the concentration of *o*-benzoquinone and increasing in the concentration of Catechol-Histidine adduct at the surface of electrode. For further increase of deposition time from 10 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-Histidine adduct at the surface of electrode.

In this study comparatively low concentration of L-Histidine (2-100 mM) has been used sequentially to determine the optimum condition for the nucleophilic substitution reaction of Catechol with L-Histidine. As the reaction occurs at 30 mM concentration of nucleophiles, consequently the voltammetric peaks (CV and DPV) for adduct appeared noticeably. From the study it is understandable that L-Histidine functions properly as a nucleophile at pH 7. When the pH is below 7, the nucleophilic activity of L-Histidine 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 [22].

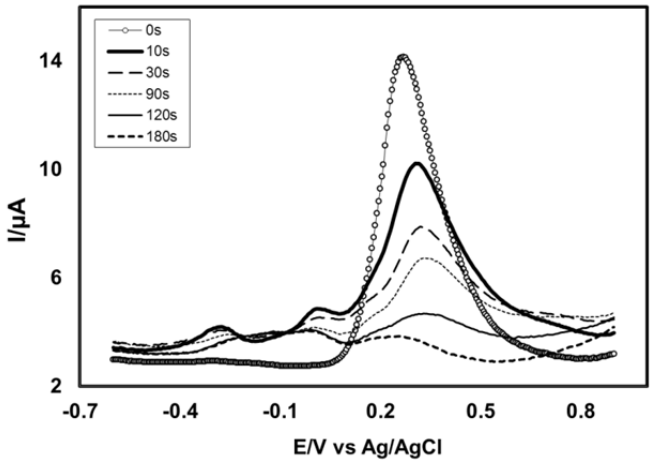



**Fig. 8.** DPV of deposition time change (0, 10, 30, 90, 120 and 180 s) of 2 mM catechol with 30 mM L-Histidine of pH 7 at $E_{puls}$ 0.02 V, $t_{puls}$ 20 ms and scan rate 0.1 $Vs^{-1}$

### 3.9. Spectral analysis of Catechol with L-Histidine

The FTIR spectrum of the vibrational modes of the Catechol-Histidine adduct, Catechol and L- Histidine has been shown in Fig. 9. The Catechol reveals the O-H stretching band at 3450 $cm^{-1}$ whereas L-Histidine shows a spectrum at 2875 $cm^{-1}$ due to overlap of O-H and N-H stretching band.

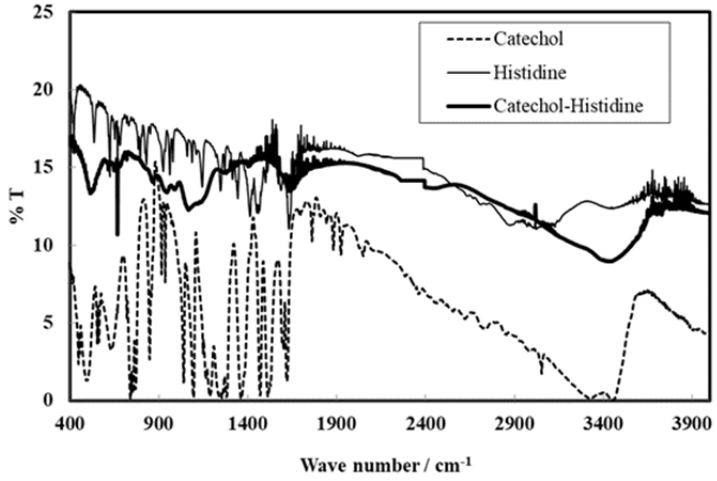



**Fig. 9.** Comparison of FTIR of only Catechol, only Histidine and Catechol-Histidine adduct

Peak at 1631 $cm^{-1}$ indicates C=O stretching of –COOH group in L-Histidine. But in case of FTIR spectrum of Catechol-Histidine adduct there is a significant change in finger print region (1000-600 $cm^{-1}$). This indicates formation of new Catechol-Histidine adduct.

Therefore, from the above discussion it is clear that the nucleophilic substitution reaction of Catechol in presence of L-Histidine is maximum favorable at 30 mM of L-Histidine and at pH 7 which is consistent with both CV and DPV. All above observations can be attributed to the reaction between L-Histidine 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-Histidine adduct.

## 4. CONCLUSION

The electro-oxidation of Catechol in the absence and presence of L-Histidine has been investigated using cyclic voltammetry, controlled potential coulometry and differential pulse voltammetry. The oxidation of Catechol produces Michael acceptor which undergoes nucleophilic attack by L-Histidine 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 1,2-dihydroxybenzene reaction is controlled by diffusion process. The nucleophilic substitution reaction of Catechol in presence of L-Histidine is not only favored by pH media but also depends on the concentration of nucleophile, electrode materials and scan rates. This reaction is maximum favorable at 30 mM of L-Histidine 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-Histidine with Catechol occurs through an ECE mechanism.

## REFERENCES

[1] L. Khalafi, and M. Rafiee, J. Hazard. Mater. 174 (2010) 801.
[2] D. Nematollahi, M. Rafiee, and L. Fotouhi, J. Iranian Chem. Soc. 6 (2009) 448.
[3] M. A. Motin, M. Nazim Uddin, P. K. Dhar, M. A. Hafiz Mia, and M. A. Hashem, Anal. Bioanal. Electrochem. 8 (2016) 505.
[4] M. A. Motin, M. Alim Uddin, M. Nazim Uddin, P. K. Dhar, M. A. Hafiz Mia, and M. A. Hashem, Port. Electrochim. Acta 35 (2017) 103.
[5] M. A. Hafiz Mia, M. A. Motin, E. M. Huque, M. Nazim Uddin, P. K. Dhar, and M. A. Hashem, Anal. Bioanal. Electrochem. 9 (2017) 597.
[6] R. H. Bisby, R. Brooke, and S. Navaratnam, Food Chem. 108 (2008) 1002.
[7] M. Rafiee, Synlett 3 (2007) 503.
[8] A. S. Prasad, J. T. Fitzgerald, B. Bao, F. W. Beck, and P. H. Chandrasekar, Ann Int. Med. 133 (2000) 245.
[9] S. Süzen, Antioxidant activities of synthetic indole derivatives and possible activity mechanisms, Heidelberg, Berlin: Springer, (2007).
[10] B. B. Aggarwal, and H. Ichikawa, Cell Cycle 4 **(**2005) 1201.
[11] AMICBASE-EssOil, Database on Natural Antimicrobials Review Science, Germany (1999–2002).
[12] A. Kiani, J. B. Raoof, D. Nematollahi, and R. Ojania, Electroanalysis 17 (2005) 1755.

[13] S. Shahrokhian, and A. Hamzehloei, Electrochem. Commun. 5 (2003) 306.
[14] D. Nematollahi, and S. M. Golabi, Electroanalysis 13 (2001) 1008.
[15] D. Nematollahi, and Z. Forooghi, Tetrahedron 58 (2002) 4949.
[16] S. M. Golabi, F. Nourmohammadi, and A. Saadnia, J. Electroanal. Chem. 529 (2002) 12.
[17] L. Papouchado, R. W. Sandford, G. Petrie, and R. N. Adams, J. Electroanal. Chem. 65 (1975) 275.
[18] P. A. Thibodeau, and B. Paquette, Free Radic. Biol. Med. 27 (1999) 1367.
[19] P. Belenky, K. L. Bogan, and C. Brenner, Trends Biochem. Sci. 32 (2007) 9.
[20] S. Mazzini, R. Monderelli, E. Ragg, and L. Scaglioni, J. Chem. Soc. Perkin Trans. 2 (1995) 285.
[21] D. Nematollahi, A. Afkhami, F. Mosaed, and M. Rafiee, Res. Chem. Intermed. 30 (2004) 299.
[22] M. D. Rayn, A. Yueh, and C. W. Yu, J. Electrochem. Soc. 127 (1980) 1489.
[23] L. Papouchado, G. Petrie, and R. N. Adams, J. Electroanal. Chem. 38 (1972) 389.
[24] L. Papouchado, G. Petrie, J. H. Sharp, and R. N. Adams, J. Am. Chem. Soc. 90 (1968) 5620.
[25] T. E. Young, J. R. Griswold, and M. H. Hulbert, J. Org. Chem. 39 (1974) 1980.
[26] A. Brun, and R. Rosset, J. Electroanal. Chem. 49 (1974) 287.
[27] D. I. Stum, and S. N. Suslov, Bio. Zika 21 (1979) 40.