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Electrochemical Assessment of EC and ECE Mechanisms for Caffeic Acid in the Presence of Aromatic Amines

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In this work, electrochemical behavior of caffeic acid (CA) in the absence and presence of aromatic amines such as 4-amino-1,3-dimethyluracil (4A-DMU), *p*-toluidine (*p*-TI) and sulfacetamide (SA) has been investigated using cyclic voltammetry technique in 0.15 M sodium acetate solution/ethanol (80:20, v/v) mixture. The effect of different parameters such as concentration and scan rate indicated that the oxidation mechanism of caffeic acid (CA) in the presence of aromatic amines can be EC and ECE. At the working electrode surface, CA is oxidized to corresponding *o*-benzoquinone (CA_{OX}) through two electrons and two protons process. The Michael-type addition reaction has occurred between *o*-benzoquinone and aromatic amines. In the second cycle, compared to CA oxidation peak, a new oxidation peak appear in negative potentials because of the electron-donating properties of amines. The cyclic voltammetry technique can recognize chemical and electrochemical processes in solution and electrode surface, respectively.

Keywords: Electrochemical behavior, Cyclic voltammetry, Green chemistry, Caffeic acid, Aromatic amines, ECE mechanism

INTRODUCTION

Caffeic acid (CA), 3,4-dihydroxycinnamic acid, is a phenylpropanoid compound found in a wide spectrum of vegetables and foods [1,2]. Pharmacological aspects of CA such as anti-inflammatory [3,4], antibacterial [5], antimicrobial [6], anti-atherosclerotic [7], antiproliferative [8], immunostimulatory [9] and cardioprotective [10] activity are discovered in biological samples. Antioxidant properties of CA can help scavenge reactive oxygen and nitrogen species (ROS and RNS), therefore act as a hindrance in the oxidative stress process [11,12]. Electrochemistry is a powerful approach to study the oxidation mechanism of organic compounds in aqueous and non-aqueous solution. Disproportionation, comproportionation, cleavage, hydrolysis and dimerization process can be identified and recognized by electrochemical study. Different types of mechanisms such as EC, ECE,

CE, ECE-DISP, EC-ECECC have been identified by electrochemical techniques [13-19]. The most common method for identifying the mechanism of reactions at the electrode surface is cyclic voltammetry and changes in its parameters can determine the reaction mechanism. *o*-diphenols have low oxidation potential and easily oxidized at the electrode surface. The result of this electron/proton transfer process is the generation of active compounds (*o*-benzoquinones) [20-22]. To the best of our knowledge, the nucleophilic addition of aromatic amines to Michael acceptors leads to synthesis of a variety of *N*-phenylquinoneimines derivatives [13,18]. In this way, electrochemical behavior of CA is carried out in the absence and presence of 4-amino-1,3-dimethyluracil (4A-DMU), *p*-toluidine (*p*-TI), and sulfacetamide (SA) in aqueous solution and room temperature. In acidic media, the NH₂ functional group is protonated and its nucleophilic feature decreases. In contrast, the nucleophilic power of aromatic amines enhances with increasing pH. In this work, the effect of concentration and scan rate is performed on electrochemical

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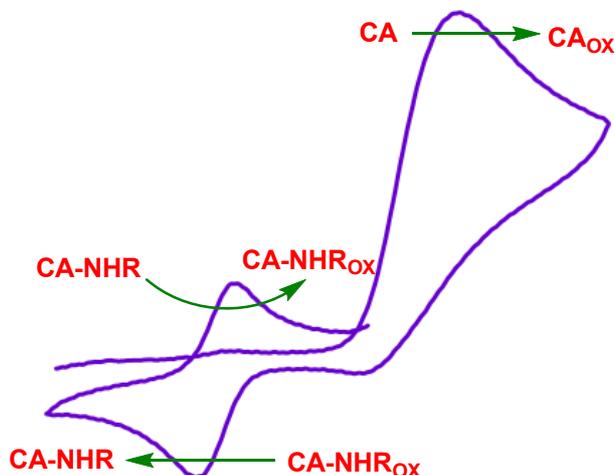


Fig. 1. The representative pattern of ECE mechanism for CA in the presence of aromatic amines.

response. A general pattern of ECE mechanism of CA in the presence of amines is illustrated in Fig. 1.

EXPERIMENTAL

Cyclic voltammetry and differential pulse voltammetry were performed using an Autolab model PGSTAT 204 potentiostat/galvanostat. The working electrode used in the voltammetry experiment was a glassy carbon disc (1.8 mm diameter) and platinum wire was used as a counter electrode. The working electrode potentials were measured *versus* Ag/AgCl (all electrodes from AZAR electrode). Caffeic acid (CA), 4-amino-1,3-dimethyluracil (4A-DMU), p-toluidine (p-TI), sulfacetamide (SA), phosphoric acid, acetic acid, sodium carbonate salt and NaOH were obtained from commercial sources without further purification.

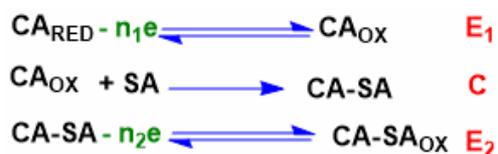
RESULTS AND DISCUSSION

Electrochemical Investigation of CA in the Presence of Sulfacetamide (SA) (ECE Mechanism)

Electrochemical oxidation of CA (1 mM) in the absence and presence of SA (1 mM) was performed in 20/80 ethanol/sodium acetate (0.15 M) aqueous solution at a glassy carbon electrode. As shown in Fig. 2, cyclic voltammogram of CA indicates a reversibility behavior (A_1/C_1 peak) at 0.12 and 0.08 V *vs.* Ag/AgCl in the absence

of SA, but in the presence of SA, the current of cathodic peak (C_1) decreases and a new cathodic peak (C_2) is observed in negative potentials. With increasing concentration of SA (3 and 5 mM), the current of C_1 and C_2 peaks was decreased and increased, respectively. In the second cycle, a new oxidation peak (C_2) at potential near -0.075 V *vs.* Ag/AgCl was formed and with increasing concentration of SA, its current was increased (Fig. 2 inset). As a result, this redox couple (A_2/C_2) is corresponding to oxidation and reduction of amine compound (CA-SA) (Scheme 2). Another approach to demonstrate the ECE mechanism, normalized cyclic voltammograms ($I/v^{1/2}$ *vs.* potential) of CA in the presence of SA, has been carried out in different scan rates and the results show that the anodic peak (A_1) decreased with increasing scan rate (Fig. 3). In fact, in higher scan rate, the opportunity is not given for the second electrochemical reaction (E_2) and the number of apparent electrons is equal to n_1 [23], while, this value is $n_1 + n_2$ for lower scan rate (Scheme 1).

To obtain more information about the oxidation mechanism of CA in the presence of SA, we performed controlled potential coulometry of 0.25 mmol CA in the presence of 0.25 mmol of SA in water/ethanol (80/20) solution containing 0.15 M sodium acetate at room temperature (Fig. 4). The recorded CVs during the coulometry are shown in Fig. 4. The CVs show that both



Scheme 1. The schematic representative of ECE mechanism.

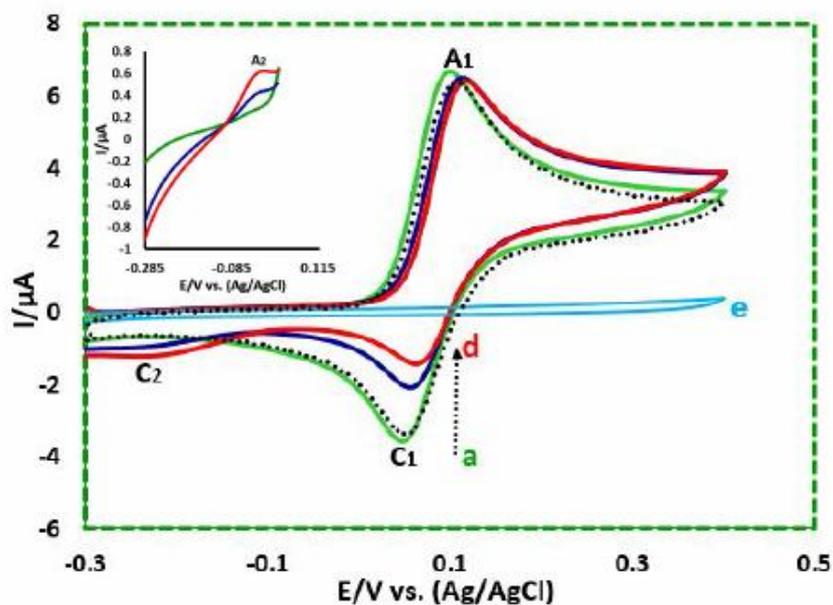


Fig. 2. Cyclic voltammograms of 1.0 mM CA: a) in the absence of SA, in the presence of b) 1.0 mM SA, c) 3.0 mM SA, and d) 5.0 mM SA. e) CV of 1.0 mM SA. (Inset) CVs of second cycle in 1.0, 3.0 and 5.0 mM concentration of SA. Solvent: water/ethanol (80/20) solution containing 0.15 M sodium acetate. Temperature: 25 ± 1 °C. Scan rate: 50 mV s^{-1} .

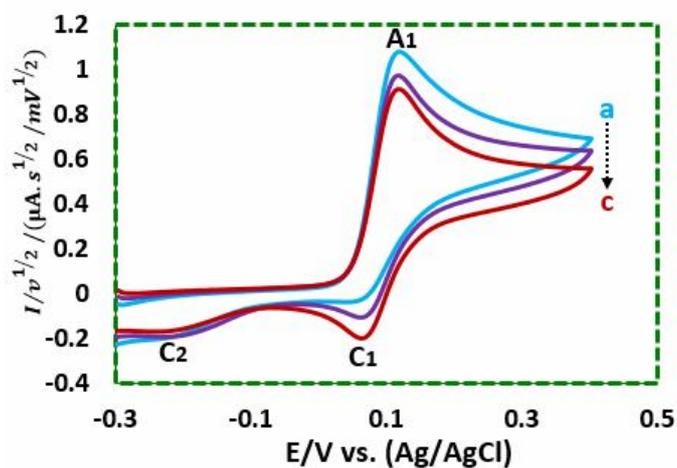


Fig. 3. Normalized cyclic voltammograms of 1.0 mM CA in the presence of 1.0 mM SA at various scan rates. Scan rates from a to c are: 10, 25 and 50 mV s^{-1} .

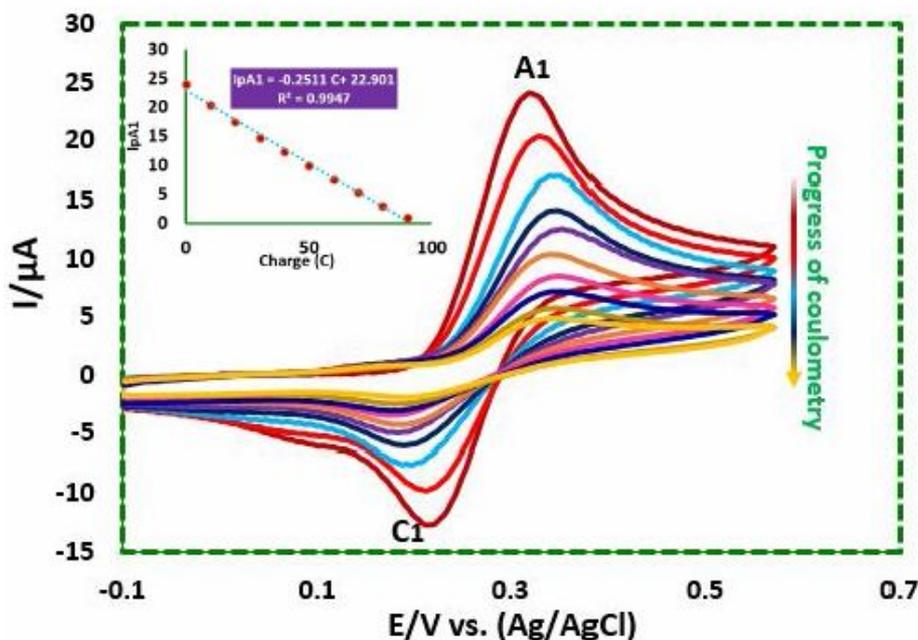


Fig. 4. CVs of CA (0.25 mmol) in the presence of SA (0.25 mmol) during controlled-potential coulometry at +0.30 V vs. Ag/AgCl in water/ethanol (80/20) solution containing 0.15 M sodium acetate. Temperature: 25 ± 1 °C, Scan rate: 50 mV s^{-1} . After consumption of 0, 10, 20, 30, 40, 50, 60, 70, 80 and 96 C. Inset: Variation of peak current (I_{pA1}) vs. charge consumed.

anodic (A_1) and cathodic (C_1) peaks decrease with the progress of coulometry and after passing about $4e^-$ per CA, the CA oxidation peak was disappeared. This data show that two electrochemical process occur for oxidation of CA in presence of SA.

On the basis of the results, CA is oxidized to *o*-benzoquinone form (CA_{OX}) with two electrons and two protons process. In solution, the Michael-type addition reaction occurred between *o*-benzoquinone and SA, then, after aromatization, the amine derivative (CANH) was formed. The oxidation potential of CANH is lower than that of CA, because of the electron-donating property of SA. Therefore, oxidation of CANH occurred in the second cycle and $CANH_{OX}$ was obtained at a glassy carbon solution (Scheme 2).

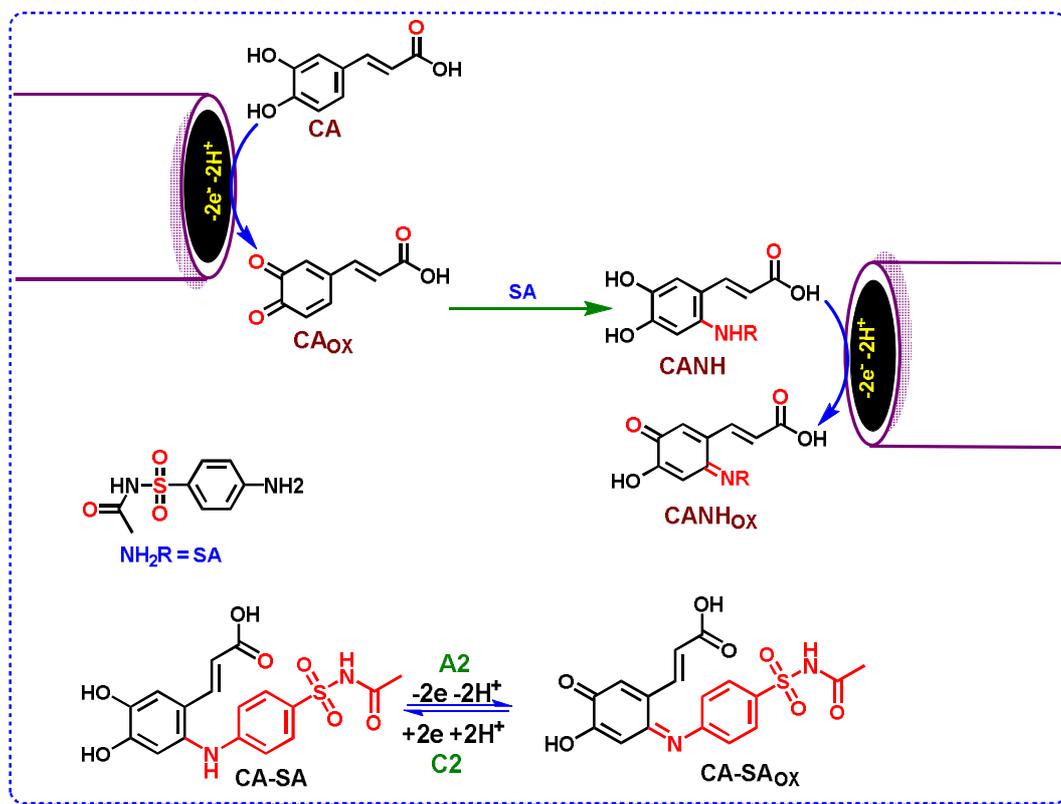
Electrochemical Investigation of CA in the Presence of 4-Amino-1,3-dimethyluracil (4A-DMU) (EC Mechanism)

In another study, electrochemical oxidation of CA was

performed in the absence and presence of 4-ADMU at the glassy carbon electrode in water/ethanol (80/20) solution containing 0.15 M sodium acetate solution (Fig. 5). Similar to the previous study, the current of cathodic peak was decreased with increasing the 4-ADMU concentration (decrease I_{pc}/I_{pa}). Also, no anodic peak was observed in the second cycle, therefore, the oxidation mechanism of CA in the presence of 4A-DMU is EC. The normalized cyclic voltammograms of CA in the presence of 4A-DMU were obtained in different scan rates at room temperature (Fig. 6). This investigation has a remarkable effect on cathodic current, and with decreasing scan rate, I_{pC1} decreases.

Electrochemical Investigation of CA in the Presence of *p*-toluidine (*p*-TI) (ECE Mechanism)

Another electrochemical approach that indicated ECE mechanism is voltammetric study of CA in the presence of *p*-toluidine (*p*-TI) in 0.15 M sodium acetate aqueous solution/ethanol (80:20, v/v) mixture at the glassy carbon electrode (Fig. 7). As shown in Fig. 7, the elimination and



Scheme 2. The details of the oxidation mechanism of CA in the presence of SA

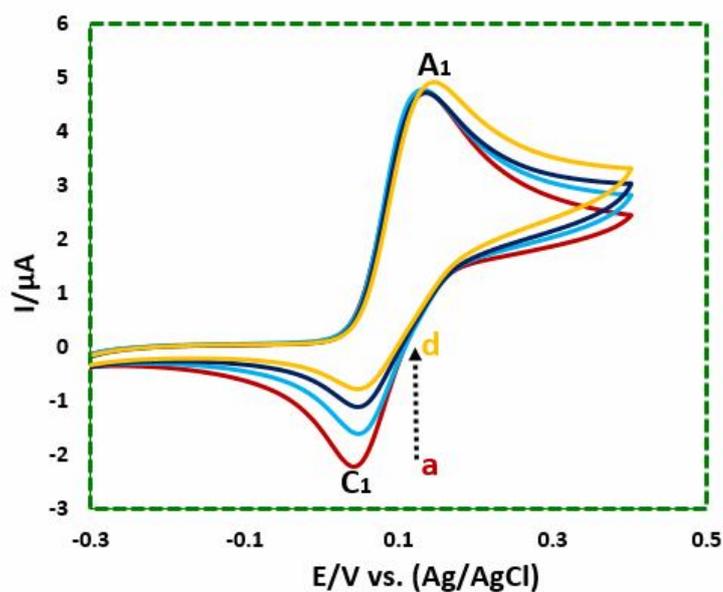


Fig. 5. Cyclic voltammograms of CA (1 mM) in the absence (a) and presence of 4A-DMU at different concentrations (b-d: 1, 3 and 5 mM) in 0.15 M sodium acetate aqueous solution/ethanol (80:20, v/v) mixture at the glassy carbon electrode. Temperature: 25 ± 1 °C.

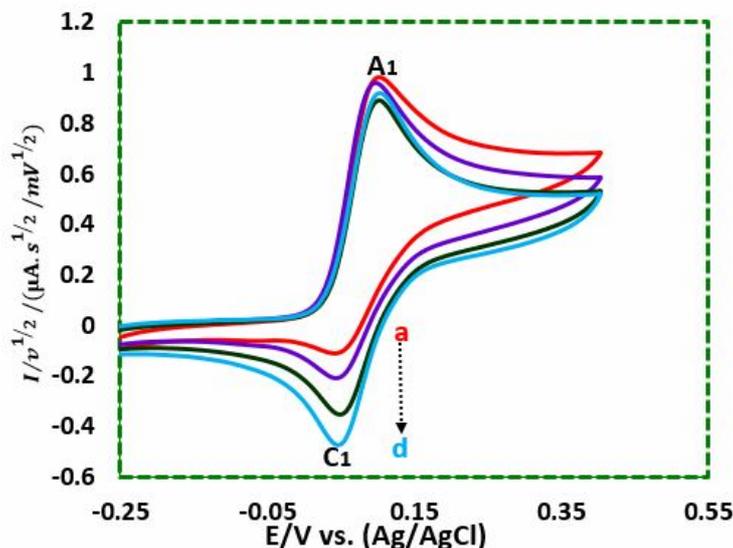


Fig. 6. Normalized voltammograms of CA (1 mM) in the presence of 4A-DMU (1 mM) at the same conditions as Fig. 5 at different scan rates. Scan rates from a-d are 5, 10, 25 and 50 mV s^{-1} . Temperature: 25 ± 1 $^{\circ}\text{C}$.

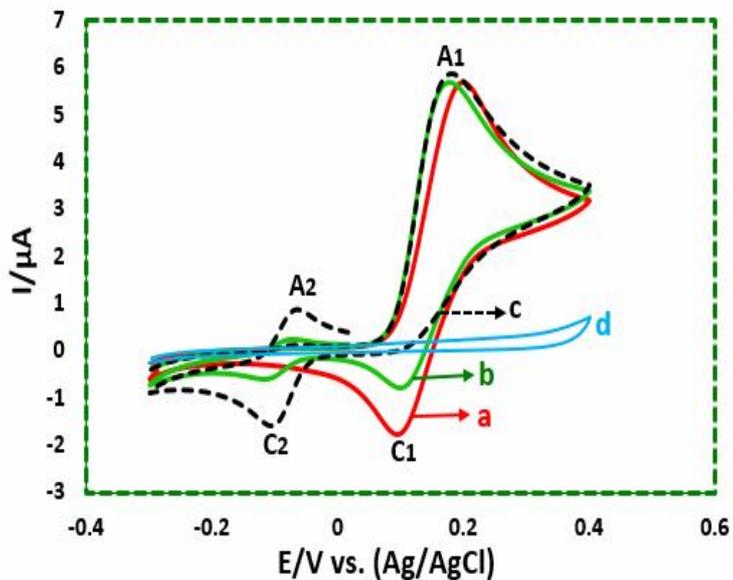


Fig. 7. Cyclic voltammograms of CA (1.0 mM): (a) in the absence of *p*-TI, (b) in the presence of 1.0 mM *p*-TI, and (c) in the presence of 3.0 mM of *p*-TI. (d) CV of 1.0 mM of *p*-TI. Scan rate: 25 mV s^{-1} . Other conditions are the same as in Fig. 5.

vanish of cathodic peak of CA_{OX} to CA indicates the chemical reaction of CA_{OX} and *p*-TI (as nucleophile) at the electrode surface. In addition, a new cathodic peak appeared

in negative potentials, and in the second cycle, the counterpart of C_2 peak was formed in $E = -0.084$ V vs. Ag/AgCl. This new redox couple (A_2/C_2) is related to the

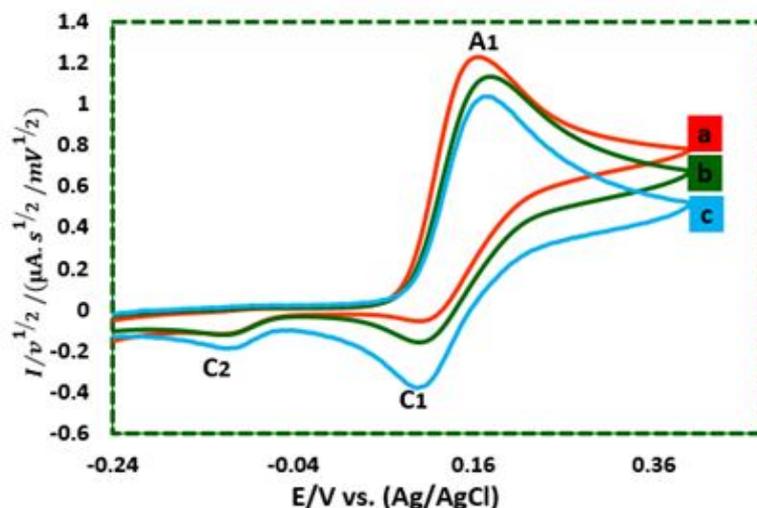


Fig. 8. Normalized CVs of 1.0 mM CA in the presence of 1.0 mM *p*-TI at various potential scan rates (v). The potential scan rates of a-c are 10, 25 and 100 mV s^{-1} . Working electrode: a glassy carbon electrode. Solvent: in 0.15 M sodium acetate aqueous solution/ethanol (80:20, v/v) mixture. Temperature: 25 ± 1 $^{\circ}\text{C}$.

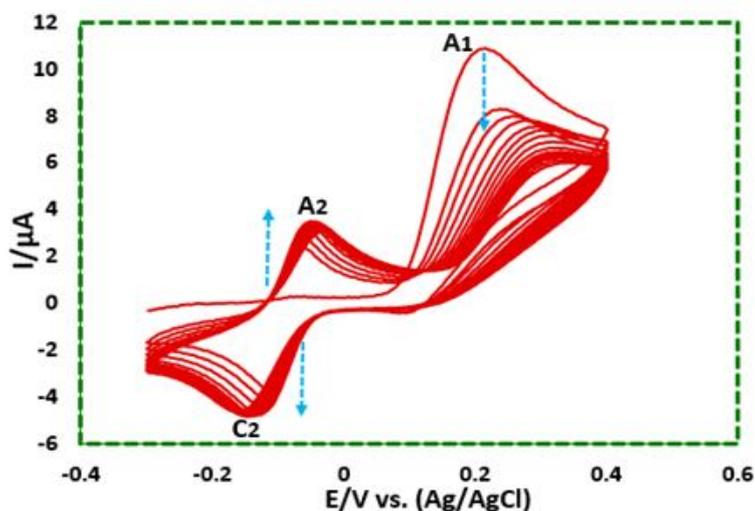
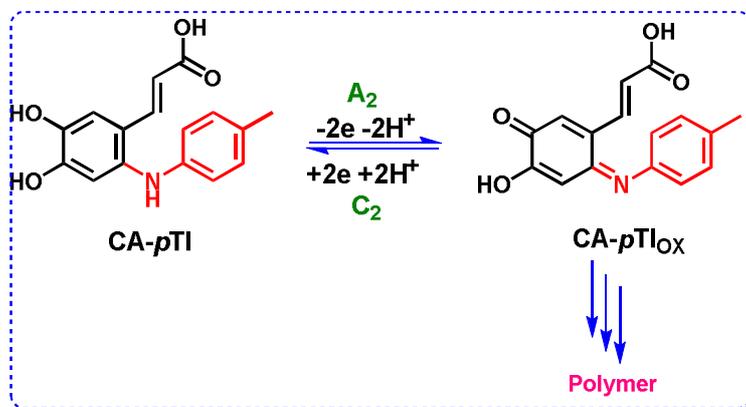


Fig. 9. Repetitive cyclic voltammograms (15 cycles) recorded in a 1.0 mM solution of CA in the presence of *p*-TI (3.0 mM). Temperature: 25 ± 1 $^{\circ}\text{C}$. Scan rate 50 mV s^{-1} .

oxidation and reduction of the products formed (*CA-pTI* /*CA-pTI_{ox}*) from the reaction of *CA_{ox}* and *p*-TI. In this potential window, there is no any anodic and cathodic peak for *p*-TI (Fig. 7d). To prove the ECE mechanism for this study, normalized cyclic voltammograms of CA in the

presence of *p*-TI at different scan rates indicated that the anodic current (I_{pA1}) is decreased in a higher scan rate (Fig. 8).

To obtain more electrochemical data, repetitive cyclic voltammograms (15 cycles) of CA in the presence of *p*-TI



Scheme 3. Electrochemical oxidation and polymerization of CA in the presence of *p*-TI

were obtained at the same conditions as before. As the results show, the height of I_{pA1} is decreased in parallel with the increase of I_{pA2}/I_{pC2} (Fig. 9). Based on these results, we proposed electrochemical polymerization of CA-*p*TI_{OX} at the glassy carbon electrode (Scheme 3).

CONCLUSIONS

In this study, the electrochemical behavior of CA in the presence of aromatic amines (4A-DMU, *p*-TI and SA) has been performed *via* cyclic voltammetry at a glassy carbon electrode. This approach can provide good information for C-N bond formation in green conditions. The results show that the mechanism may be EC or ECE in in 0.15 M sodium acetate aqueous solution/ethanol (80:20, v/v) mixture solution. In this media (ECE mechanism), the aromatic amines are deprotonated and their nucleophile features are remarkable. CA oxidized to CA_{OX} forms in the first scan and in the presence of aromatic amines, the Michael-type addition reaction has occurred in solution. In the second cycle, a new anodic peak appeared in negative potentials that contributed to oxidation of adduct formed at the electrode surface. In the CA-*p*TI case, the electrochemical polymerization was illustrated with an increase in the number of scans. As a consequence, all observations for the ECE mechanism may not be observed in the time scale of cyclic voltammetry, but at the time scale of coulometry, the second oxidation reaction can proceed, resulting in production of quinone imine.

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