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Electrooxidation of 4-Methylcatechol in the Presence of Azide Ion and Its Kinetic Study by Digital Simulation

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The electrooxidation of 4-methylcate-chol has been studied in the presence of azide ion (N_3^-) as a nucleophile in aqueous solution using cyclic voltammetry and differential pulse voltammetry. Multicyclic voltammetry of 4-methylcatechol indicates one anodic peak and corresponding cathodic peak without any polymerization. Cyclic voltammogram of 4-methylcatechol in the presence of azide ion shows one enhanced anodic peak and decreased corresponding cathodic peak in acidic solution. However, in the neutral solution, cyclic voltammograms show one anodic and two cathodic peaks. Effects of some parameters such as scan rate and azide ion concentration on the shape of cyclic voltammograms were investigated. Also, the cyclic voltammograms of 4-methylcatechol in the presence of azide ion in acidic and neutral solutions were digitally simulated based on EC′ and ECE mechanisms, respectively. The observed catalytic rate constant (k_{cat}) for catalytic reaction and homogeneous rate constant (k_m) for Michael addition reaction were estimated by comparing the experimental cyclic voltammetric responses with the digitally simulated ones.

Key Words: Electrooxidation, Azide, 4-Methylcatechol, Cyclic voltammetry, Digital simulation.

INTRODUCTION

Catechols are widespread in the environment, especially as constituents of edible plants. A number of these catechols may undergo oxidative metabolism to electrophile *o*-quinones by oxidative enzymes such as cytochrome P450 and peroxidases[1]. *o*-Quinones are cytotoxic molecules that have deleterious effect not only on the foreign organisms but also on the self-matter[2]. In addition, they are electrophiles[3-16] as well as redox active compounds

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and as a result, they are ultimate cytotoxins formed from oxidative metabolism of numerous aromatic compounds[17]. Among catechols, 4-alkylcatechols have special properties. *o*-Quinones formed from oxidation of parent 4-alkylcatechols can be converted to their tautomeric *p*-quinone methides form[18]. The conversion of 4-alkylcatechols to *p*-quinone methides may contribute to the cytotoxic and/or genotoxic activity of 4-alkylcatechols. These species are much more electrophile than their *o*-quinone tautomers. 4-Methylcatechol is a representative of 4-alkylcatechols and also a model for biological molecules with biologic therapeutic effects[19-21]. It has been shown that 4-methylcatechol converts to related quinone methide during enzymatic oxidation[18]. So with the aim of investigation of electrooxidation of 4-alkylcatechols in the presence of nucleophiles, this present work is carried out.

On the other hand, azide is often used as an inhibitor to detect active site metal ions of enzymes such as tyrosinases and laccases[22,23]. It is believed that some of observed inhibitation is due to reaction of enzymatically generated quinones with azide to form azido-catechols[24]. Also, azidoquinones constitute a remarkably versatile of synthetically useful reagents[25].

To our best knowledge, electrochemical oxidation of 4-methylcatechol in the presence of azide has not been reported in the literature, Therefore, due to the importance of this reaction in view of mechanistic, biological aspects and synthetic applications, the anodic oxidation of 4-methylcatechol in the presence of azide ion is investigated.

EXPERIMENTAL

All reagents were obtained from Fluka and used without further purification. All solutions and subsequent dilutions were prepared using double-distilled water. All experiments were carried out in phosphate buffer (0.1 M) with different values of pH. The voltammetric experiments were performed using an Autolab potentiostat & galvanostat (Netherlands) coupled with a Pentium IV personal computer with a standard three electrode configuration. Glassy carbon disk (Metrohm, diameter 2 mm) served as working electrode, a platinum wire electrode provided the counter electrode with a Ag|AgCl|KCl 3M reference electrode completing the cell assembly. All potentials are quoted *versus* Ag|AgCl|KCl 3M. The glassy carbon electrode was polished between each set of experiments with aluminum oxide powder on a polishing cloth. All experiments were conducted at 25 ± 0.1 ºC using of water thermostat circulator (polystat CC1, Huber, Germany). The homogeneous rate constants were estimated by analyzing the cyclic voltammetric responses using a commercial digital simulation software (DigiElch 3.0).

RESULTS AND DISCUSSION

Electrochemical behaviour of 4-methylcatechol: The literature show that catechol could be polymerized *via* oxidation[9]. By considering the possibility of competition of polymerization reaction with nucleophilic reaction, the possibility of polymerization of 4-methylcatechol is investigated. Therefore, subsequent cyclic voltammograms of 1 mM 4-methylcatechol in 0.1 M phosphate buffer (pH=7) at scan rate of 50 mV s^{-1} were recorded. As shown in Fig. 1, at the first scan, there is only one anodic peak (A_0) attributed to the oxidation of 4-methylcatechol to related *o*-benzoquinone and one corresponding cathodic peak (C_0) related to the reduction of 4-methyl-*o*-benzoquinone with half-wave potential of about 0.135 V (**Scheme-I**, eqn. 1). The peak separation potential, ΔE_p (= E_{pa}- E_{pc}) is about 53 mV, indicating a quasi-reversible behaviour of a two electron-two proton process. At subsequent scans, cyclic voltammograms show decrease of their peaks height (A_0 and C_0) without appearance of new peaks. Also, the cyclic voltammetry and differential pulse voltammetry of this electrode after rinsing with distilled water in the pure buffer solution show no peaks. These results show that 4-methylcatechol could not go under electropolymerization in this condition.

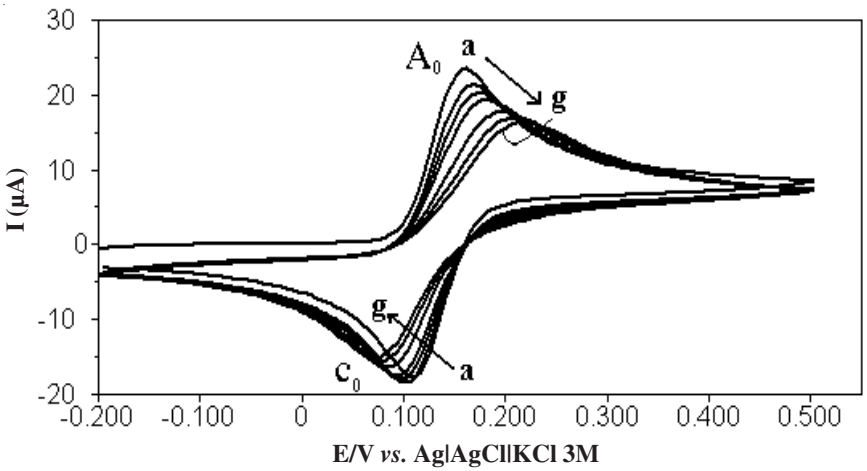
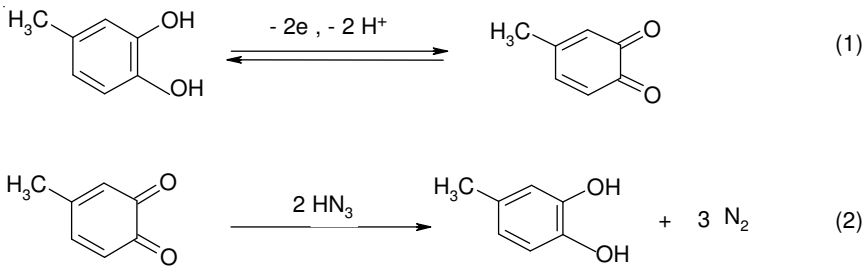


Fig. 1. Subsequent cyclic voltammograms of aqueous solution containing of 1 mM 4-methylcatechol and 0.1 M phosphate buffer (pH 7) at scan rate of 50 mV s^{-1} at the surface of GC electrode. (a to g) show the 1st, 2nd, 3rd, 5th, 10th, 15th and 20th subsequent cycles, respectively

Influence of pH: The influence of pH on the electrochemical behaviour of 4-methylcatechol and azide ion is assessed separately and as mixture by examining the electrode response in solution buffered between pH 2.0 and



Scheme-I

9.0. As can be seen in Fig. 2, azide can be oxidized irreversibly with peak potential of 1.16 V (pH 3). With pH increasing, its peak potential is firstly shifted to less positive potential but remains unchanged in pH higher than 5. These results confirm that azide exists as protonated form in low pH named hydrazoic acid (HN_3). By plotting E_{pa} as a function of pH, acidic dissociation constant (pK_a) of hydrazoic acid obtained 4.78. This value is in agreement with data in the literature[26]. On the other hand, recording of cyclic voltammograms of 1 mM 4-methylcatechol show that the peak potential position of the redox couple of 4-methylcatechol is pH dependent. Some typical cyclic voltammograms presenting the oxidation of 1 mM 4-methylcatechol in the absence and presence of 1 mM azide at different pHs are compared (Fig. 3). In acidic solution, 4-methylcatechol gave a well developed reversible wave. With increasing pH values from 2 to 7, the anodic and cathodic peaks are shifted to the less positive potentials whereas anodic and cathodic peaks heights does not change considerably. These observations can be demonstrated by considering oxidation reaction of 4-methylcatechol to 4-methyl-*o*-benzoquionone (eqn. 1 in **Schemes I** and **II**). This equation shows that increasing the pH, facilitates the 4-methylcatechol oxidation and consequently decreases its oxidation potential. Also, because of all pH values from 2 to 7 are sufficiently below the pK_{a1} of 4-methylcatechol (> 9.4), the electroactive species that effectively are being oxidized are the same and consequently anodic peak current (A_0) does not change considerably. In addition, comparison of oxidation potentials of 4-methylcatechol and azide shows that in the oxidation potential of 4-methylcatechol, azide cannot be electrochemically oxidized, consequently can act as a nucleophile.

Electrochemical behaviour of 4-methylcatechol in presence of azide ion: 4-Methylcatechol oxidation produces the corresponding *o*-benzoquinone which undergoes frequently nucleophilic attack. The nucleophile usually reacts by a Michael 1,4-addition mechanism to form a substituted *o*-benzoquinone. If the substituent is such that the potential for the oxidation of product is higher, the product cannot be oxidized at the working potential

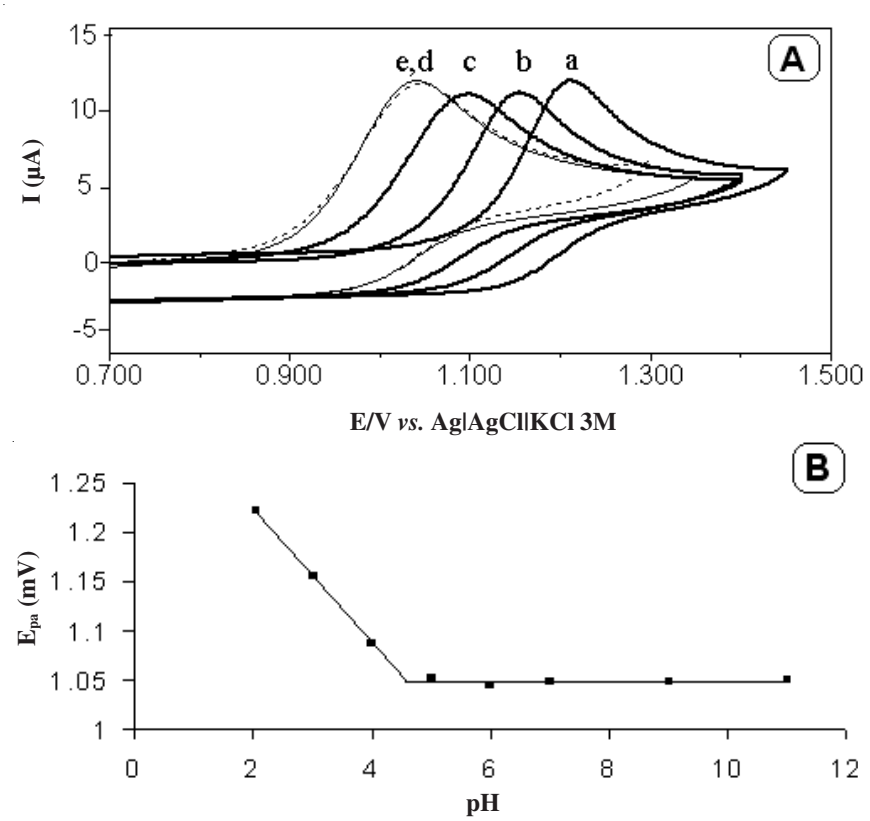


Fig. 2. A) Cyclic voltammograms of 1 mM azide ion in 0.1 M phosphate buffer solution in scan rate of 50 mV s^{-1} and different pH at the surface of GC. The pH of cyclic voltammograms (a to e) is 2, 3, 4, 5 and 9, respectively. B) Plot of Epa *versus* pH for azide ion

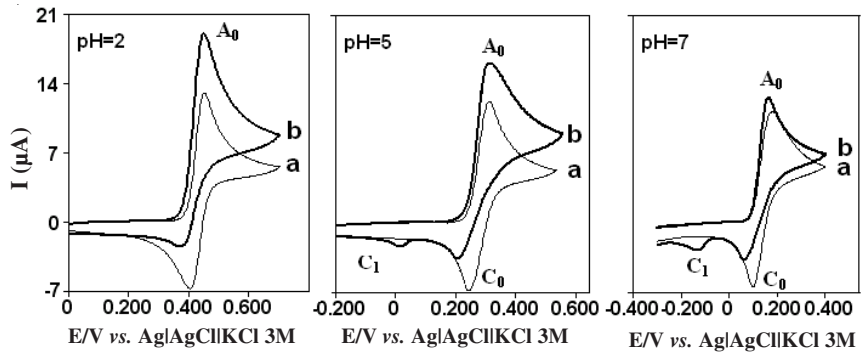
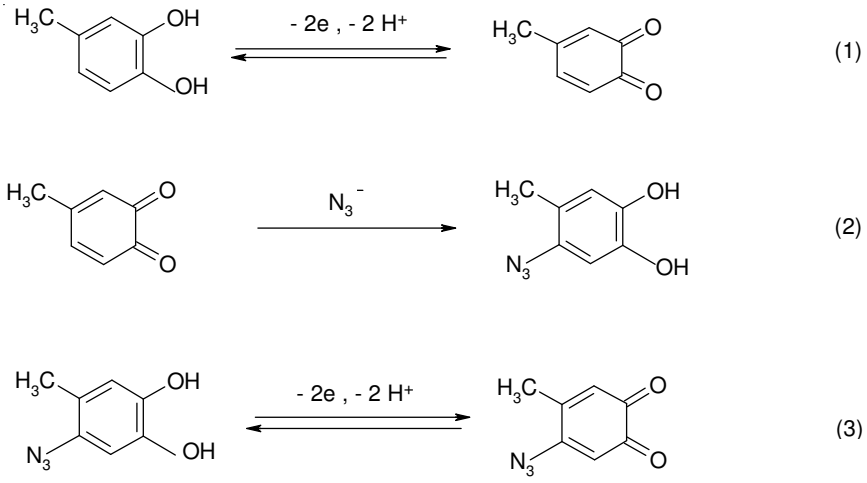


Fig. 3. Cyclic voltammograms of 1 mM 4-methylcatechol in the absence (a) and the presence of 1 mM azide (b) in 0.1 M phosphate buffer with different pHs at scan rate of 10 mV s^{-1}. All pHs have the same current scale

(oxidation potential of 4-methylcatechol). Thus, the reaction will be complete after the first oxidation-addition process. However, if the substituent is such that the potential for the oxidation of product is lower than that of 4-methylcatechol, the product can be oxidized again and further addition reaction may be occur.

First cycle of cyclic voltammograms of 1 mM 4-methylcatechol in the absence and presence of 1 mM azide at different pHs were compared in Fig. 3. As can be seen, in more acidic solution (*e.g.*, pH= 2), there is an increase in anodic peak current (A_0) against a decrease in current of cathodic peak (C_0) without appearance of any new peak. This observation allow us to propose a homogeneous catalytic pathway for the electrochemical oxidation of 4-methylcatechol in the presence of azide ion in acidic solution (**Scheme-I**). Also, controlled potential coulometry (at potential of 0.6 V) is used to determine the number of consumed electrons per molecule of 4-methylcatechol. Value of 4.8 e^- per molecule of 4-methylcatechol was obtained for controlled potential coulometry of a solution containing 1 mM 4-methylcatechol, 1 mM azide ion and 0.1 M phosphate buffer at pH = 2. Catalytic pathway (**Scheme-I**) is responsible for increasing the number of consumed electrons from 2 to 4.8 e^- per molecule of 4-methylcatechol and consequently, considerable enhancement in height of A_0 peak of 4-methylcatechol in the presence of azide ion at pH = 2. This type of reaction, previously reported by Couladouros *et al.*[27] for the reaction of hydrazoic acid with naphtoquinone. But at higher pH (*e.g.* pH 5), one anodic peak (A_0) and two cathodic peaks (C_0 and C_1) appear. Appearance of this new cathodic peak (C_1) can be attributed to the formation of an adduct *via* 1,4-Michael addition reaction of azide ion to electrochemically generated 4-methyl-*o*-benzoquinone in the reaction layer (**Scheme-II**). Anodic peak current (A_0) of 4-methylcatechol in the presence of azide is larger than that of in the absence of azide. It seems that at this pH, azide reacts with 4-methylcatechol based on two parallel mechanisms including catalytic mechanism and 1,4-Michael addition (ECE) mechanism. Because of oxidation potential of adduct is lower than that of 4-methylcatechol, the formed adduct can be oxidized in the potential of 4-methylcatechol oxidation and consequently, the height of A_0 peak increases in the presence of azide ion. Also, it is well-known that catalytic reaction increases the A_0 peak current. Thus, both of these mechanisms are responsible for increasing the anodic peak current (A_0). Cyclic voltammogram of 4-methylcatechol in the presence of azide ion at pH 7 shows one anodic peak and two cathodic peaks. The anodic peak current (A_0) of 4-methylcatechol in the presence of azide ion is larger than that that 4-methtylcatechol in the absence of azide. As stated above, the formed adduct is oxidized at the oxidation potential of 4-methylcatechol and increases the A_0 peak current.



Scheme-II

The comparison of cyclic voltammograms of 4-methylcatechol in the presence of azide ion shows that with changing pH from 5 to 7, the anodic peak height (A_0) decreases whereas new cathodic peak (C_1) grows. As stated above, new cathodic peak (C_1) is attributed to the reduction of Michael addition adduct. The growth of C_1 peak height with changing pH from 5 to 7 can be associated to the concentration of Michael reaction adduct, as demonstrated below. With plotting the composition of HN_3 (α values) as a function of pH, we have: pH 5: 35.5 % of HN_3 and 64.5 % of N_3^-; pH 7: 0.5 % of HN_3 and 99.5 % of N_3^-. Thus, concentration of azide ion (that can act as a nucleophile) at pH 7 is more than that of pH 5, consequently Michael addition reaction of azide ion with 4-methyl-*o*-benzoquinone at pH 7 occurs in larger scale and produces adduct with larger quantity. The decrease of anodic peak current (A_0) of 4-methylcatechol in the presence of azide with changing pH from 5 to 7 indicates that at pH 7, in contrary to pH 5, azide can react with 4-methyl-*o*-benzoquinoe only *via* Michael addition mechanism and consequently has smaller A_0 peak current. It must be noted that the thermodynamic driving force of catalytic reaction depends on the difference between the $E^{\circ\prime}$ of mediator and $E^{\circ\prime}$ of analyte. The larger difference value ($\Delta E^{\circ\prime}$) means that thermodynamic driving force of catalytic reaction is more and consequently catalytic reaction is more favoured[28-30]. Thus, we estimated the difference between the $E^{\circ\prime}$ of 4-methylcatechol (taken as $E'm$ evaluated from CVs) and the $E^{\circ\prime}$ of azide at pHs 2 and 7[31-33]. The values 3.11 and 2.28 V were obtained for pHs 2 and 7, respectively. These data show that $\Delta E^{\circ\prime}$ value at pH 2 is more than that of pH 7 and consequently the driving force of catalytic reaction at pH 2 is more. Thus, at pH 2 catalytic reaction can be occur.

At pHs higher than 7, hydroxide attacks to electrogenerated 4-methyl-*o*-benzoquinone and hydroxylation of 4-methylcatechol occurs. Thus, the pH = 7 is a convenient pH for Michael addition reaction between the oxidized 4-methylcatechol and azide.

The effect of subsequent cycles on the voltammetric signal of a solution containing 1 mM 4-methylcatechol and 1 mM azide ion were shown in Fig. 4. The results show that, parallel to the decrease in current of peak A_0 and shift of its potential in a positive direction, new anodic peak A_1 and its cathodic counterpart (C_1) appear at less positive potentials and grow. Growth of A_1 and C_1 peaks with increasing cycles number is an evidence of adsorption of adduct at the surface of electrode. Thus, these new peaks are related to electro-oxidation and electro-reduction of azide-catechol adduct. The positive shift of the A_0 peak in the presence of azide ion in the subsequent cycles is probably due to the formation of a thin film of product at the surface of electrode, inhibiting to a certain extent of the performance of the electrode process.

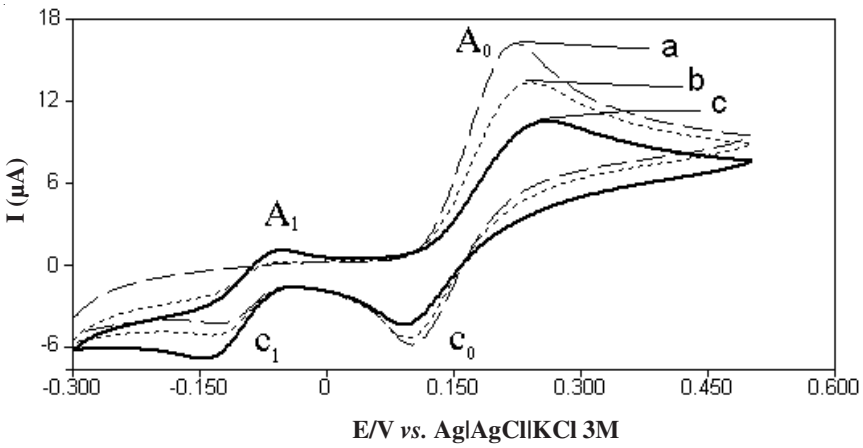


Fig. 4. Cyclic voltammograms of 1 mM 4-methylcatechol in the presence of 1 mM azide ion at the different subsequent cycles. a) 1st, b) 10th and c) 15th. Scan rate is 50 mV s^{-1} and pH = 7

The effect of scan rate on the electrochemical behaviour of 4-methylcatechol in the presence of azide ion also was studied. Fig. 5A shows typical cyclic voltammograms obtained for 1 mM 4-methylcatechol in the presence of 1 mM azide ion at various scan rate As can be seen, only in scan rates up to 50 mV s^{-1}, new cathodic peak (C_1) exists and at higher scan rates begins to disappear. On the other hand, the value of current function, I_p (A_0) /$v^{1/2}$ was found to be decrease exponentially with increasing scan rate (Fig. 5B), which is characteristics of an ECE mechanism[3].

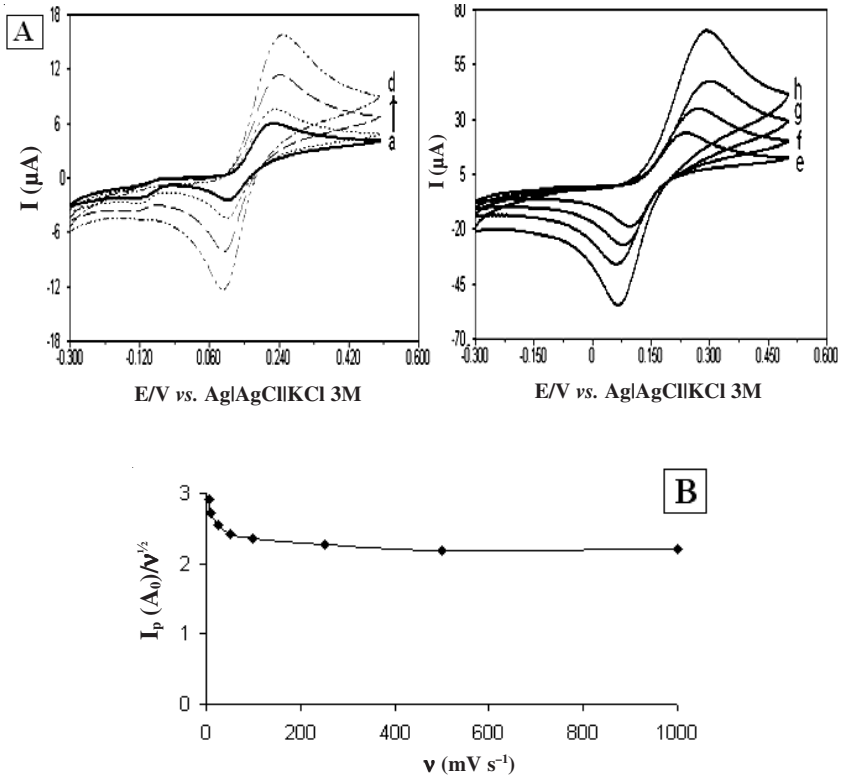


Fig. 5. A) Second cylce of cyclic voltammograms of solution containing 1 mM 4-methylcatechol, 1 mM azide ion and 0.1 M phosphate buffer (pH 7) at various potential scan rates : a to h are 5, 10, 25, 50, 100, 250, 500 and 1000 mV s^{-1}, respectively. B) Variation of peak current function (I_p (A_0) / $v^{1/2}$) *versus* scan rate

Effect of azide ion concentration on electrochemical behaviour of 4-methylcatechol was studied with cyclic voltammetry and differential pulse voltammetry. Cyclic voltammograms of 4-methylcatechol in the presence of different concentrations of azide ion from 0 to 13 mM is shown in Fig. 6A. As can be seen with increasing of azide ion concentration, height of C_0 is decreased and in contrast, C_1 is increased. Also, differential pulse voltammograms 4-methylcatechol in the presence of azide ion show two completely separated peak C_0 and C_1 which could be attributed to the reduction of electrogenerated 4-methyl *o*-benzoquinone and oxidized form of 4-methylcatechol-azide adduct, respectively. The height of peak of attributed to the reduction of electrogenerated 4-methyl *o*-benzoquinone (C_0) decreased while the height of peak attributed to reduction of oxidized form of 4-methylcatechol-azide adduct (C_1) enhanced with increasing azide ion concentration.

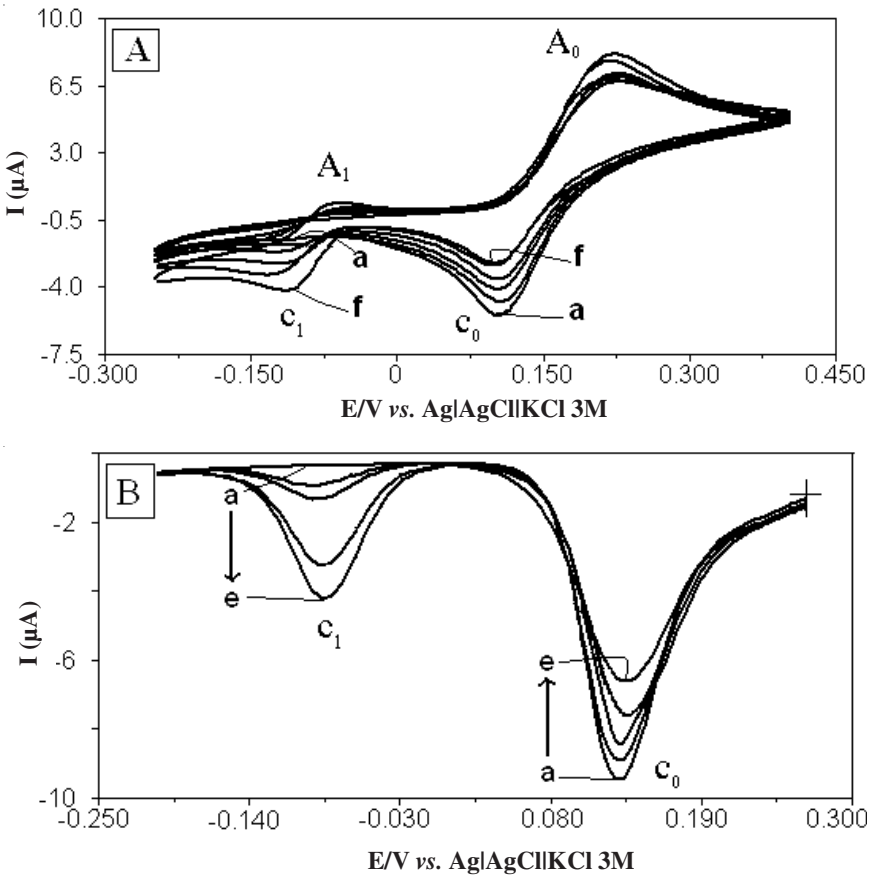


Fig. 6. A) Second cycle of cyclic voltammograms of 1 mM 4-methylcatechol in the presence of different concentration of azide ion in pH = 7 at scan rate of 10 mV s^{-1}. a) 1 mM, b) 2 mM, c) 4 mM, d) 8 mM and e) 13 mM respectively. B) Typical differential pulse voltammograms of related to A). Initial oxidation potential 0.5 V, modulation time 0.05 s, interval time 0.5 s, step potential 0.005 V and modulation amplitude 0.025 V

Kinetic evaluations: Two mechanisms were proposed for the electrochemical oxidation of 4-methylcatechol in the presence of azide in acidic and neutral solution and were confirmed by digital simulation. Simulations were performed based on EC′ and ECE mechanisms for acidic and neutral solutions, respectively. In acidic solution (*e.g.* pH = 2), based on an EC′ mechanism, the observed catalytic reaction rate constant (k_{cat}) was estimated by comparison of the simulated cyclic voltammograms with experimental ones (first cycle) in different scan rates (Fig. 7A). The transfer coefficients (α) were assumed to be 0.5 and the formal potentials were obtained experimentally as midpoint potential between the anodic and cathodic peaks

observed in cyclic voltammetry. The heterogeneous rate constant is estimated by use of an experimental working curve[34]. The estimated value is 0.0007 cm s^{-1} is used for simulation of cyclic voltammograms. The calculated catalytic reaction rate constant (k_{cat}) is 19.3 M^{-1} s^{-1} ($RSD^{n=5}$ = 8.7 %), where RSD is the relative standard deviation and n is the number of individual catalytic reaction rate constants obtained in different scan rates. But, for neutral solution (pH = 7), simulation was performed based on an ECE mechanism. Fig. 7B shows the typical cyclic voltammograms of 4-methylcatechol in the presence of azide ion and corresponding simulated cyclic voltammograms in neutral solution (pH = 7) at different scan rates. The homogeneous rate constant (k_m) of Michael addition reaction has been estimated by the same method which was used for the evaluation of catalytic reaction rate constant (k_{cat}). The calculated homogeneous rate constant [for Michael addition of azide ion to 4-methylcatechol in pH = 7 (k_m)] is 2.1 M^{-1} s^{-1} ($RSD^{n=5}$ = 5.8 %). Since this work has performed in aqueous media with high ionic strength (phosphate buffer 0.1 M), the effect of the uncompensated resistance on obtained results is negligible. So, the homogeneous rate constants have calculated without correction of uncompensated resistance.

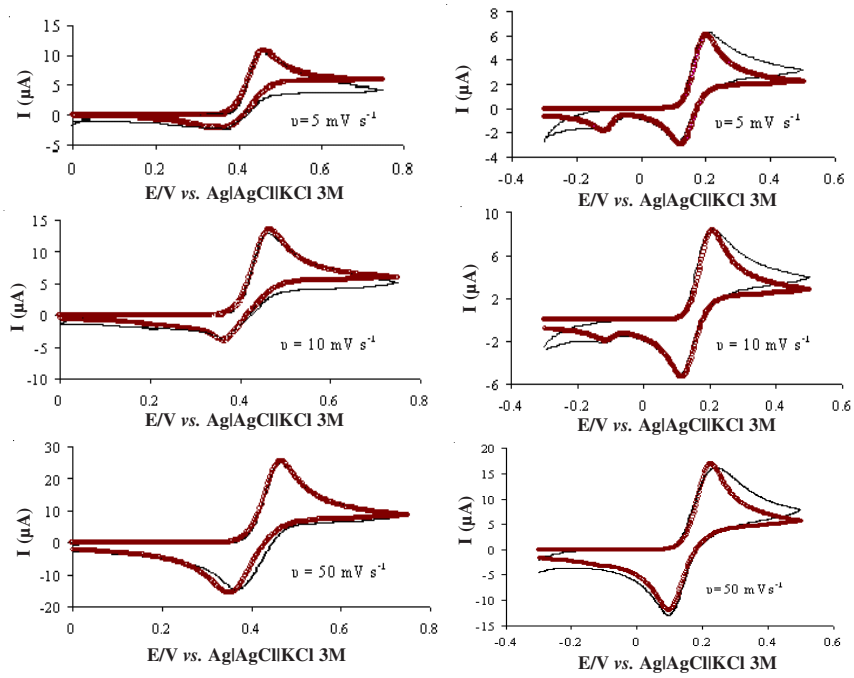


Fig. 7. A) Cyclic voltammograms of 1 mM 4-methylcatechol in the presence 1 mM azide ion in aqueous solution containing 0.1 M phosphate buffer with pH = 2 at various scan rates with corresponding simulated cyclic voltammograms. B) as (A) with pH = 7. experimental: solid line and simulated: circle

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