Res Chem Intermed (2015) 41:7151–7162
DOI 10.1007/s11164-014-1803-6

Electrochemical oxidation of catecholamines in the presence of aromatic amines: interplay between inter- and intramolecular nucleophilic addition

Lida Khalafi · Mohammad Rafiee · Maryam Norouznia · Yosef Abdossalami Asl

Received: 15 June 2014 / Accepted: 2 September 2014 / Published online: 26 September 2014

Abstract The reactions of electrochemically generated *o*-quinones from oxidation of catecholamines with aniline derivatives have been studied using cyclic voltammetry. There is a close and pH-dependent interplay between intra and intermolecular Michael addition reactions of side chain amine group of catecholamines and anilines toward the electrochemically generated *o*-quinone. The pH dependence of reactions has been studied and the condition for domination of each reaction was obtained. The observed homogeneous rate constants of reactions were estimated by digital simulation of cyclic voltammograms for each pathway. The reactivities and the rate constants of Michael addition of anilines depend on the electron-withdrawing character of their substituent, which have been studied quantitatively.

Keywords Catecholamine · Aniline derivatives · Diphenylamine · Half-wave potential

Introduction

Catecholamines (CAs) are known as the most significant family of biologically important compounds. They act as hormone and neurotransmitter in the central nervous system [1]. They contain a catechol nucleus and side chain ethylamine group. CAs undergo an oxidation reaction due to the intrinsic redox nature of their catechol group, which has been attracting wide attention in electrochemical studies [2–7]. Some of the most well-known catecholamines are dopamine (DA),

L. Khalafi (✉) · M. Norouznia
Department of Chemistry, College of Basic Science, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran
e-mail: l_khalafi@yahoo.com

M. Rafiee · Y. A. Asl
Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran

methyldopa (MD), and epinephrine (EP). Dopamine is one of the naturally occurring catecholamines and its hydrochloride salt is used in the treatment of acute congestive failure and renal failure Parkinson's. Parkinson's is a debilitating disease that is associated with deficiency of dopamine in the brain [8]. MD is a psychoactive drug used as a sympatholytic or antihypertensive that continues to have a role in otherwise difficult-to-treat hypertension and gestational hypertension [9]. Finally, EP or adrenaline is the most well known neurotransmitter and has many functions in the human body such as in regulating heart rate, blood vessels, and air passage diameters [10]. It is produced in some neurons of the central nervous system, and in the chromaffin cells of the adrenal medulla from the amino acids phenylalanine and tyrosine [11]. EP release is a crucial component of the fight-or-flight response of the sympathetic nervous system [12].

The main role of all these biologically important catecholamines in the nervous system is due to their unified skeleton (a benzene with two hydroxyl side groups and a side-chain ethylamine) whereas the presence of even simple substitution such as methyl changes their roles and functions considerably [13]. The biologically important roles of these CAs render them attractive in many areas of studies. Oxidation and derivatization of CAs are two applicable concepts from the synthetic and analytical aspect of view [14, 15]. Electrochemical methods have been used widely for the study and detection of the CAs either in vivo or in vitro and even the term of phsycoanalytical electrochemistry has been proposed for the electrochemical study of the neurotransmitters [16]. Electrochemical oxidation is also known as an efficient method for their derivatization and selective detection [17, 18]. The aim of this study is the voltammetric study of the above-mentioned catecholamines and investigation of the possibility of their derivatization and formation of some new diphenylamines in the presence of aniline derivatives by cyclic voltammetry.

Experimental

Apparatus: voltammetric experiments were performed using an Autolab model PGSTAT 101 potentiostat/galvanostat. In the typical voltammetric experiment a glassy carbon disc (2 mm in diameter) and a platinum wire were used as working and counter electrodes, respectively. The working electrode potentials were measured versus Ag/AgCl (all electrodes from AZAR electrode, Urmia, Iran). Alumina powder (0.3 μM) was used for mechanical polish of working electrode. This treatment was followed by ultrasonic cleaner for 30 s. The homogeneous rate constants were estimated by digital simulation of cyclic voltammograms (CVs) using the CVSIM simulation software and comparing the experimental and simulated CVs [19]. The fitting consists of finding a rate constant for which the differences between the digitally simulated and the experimental data reach to their minimum values [20].

Reagents: Dopamine, methyldopa, epinephrine, 3-nitroaniline, 4-methylaniline, *N*-methylaniline, 4-bromoaniline, and 4-nitroaniline were purchased from Fluka and were used without further purification. Sodium acetate, acetic acid, hydrochloric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate and phosphoric

acid were reagent-grade materials from E. Merck. All aqueous solutions were prepared with distilled water (Aquatron A8000 water purification system). The stock solutions of all catecholamines and aniline derivatives were prepared daily.

Results and discussion

The CVs of 1.0 mM solution of DA, MD, and EP in aqueous acetate buffer solution, pH = 5, are shown in Fig. 1. The entire voltammograms show one anodic peak (A_1) related to conversion of their catechol moiety to *o*-quinone at the electrode surface. The CVs of DA and MD (curves a and b) show one cathodic peak, C_1, as the counterpart of A_1 in negative going scan related to reduction of produced *o*-quinone. The CVs of DA and MD shows also C_2 reduction peak at more negative potential and its counterpart (A_2) in second consecutive cycle. The CV of EP (curve c) does not show the C_1 reduction peak, whereas the heights of its C_2/A_2 peaks are considerably more than those observed for DA and MD.

These voltammograms show that the electrochemically generated *o*-quinones from the oxidation of CAs undergo a chemical reaction in which the products of the reactions undergo electron transfer at more negative potentials than the parent molecules. This reaction is known as cyclization via nucleophilic addition of a side chain amine group to the *o*-quinone. The difference between A_1 and A_2 peak potentials strongly suggests the substitution of a resonance electron-donating amine group on the catechol ring, which have been reported and proven previously [2, 4]. The peak potentials of C_2 and its corresponding anodic peaks (A_2) agree well with the product of the electrochemical cyclization of CAs [4, 21]. The reaction mechanism is believed to be as noted below (Scheme 1).

All of the diagnostic criteria are indicative of an ECE mechanism. The ratio of C_1 over A_1 increases parallel to an increasing scan rate and reaches unity at very high scan rates. Also the height of normalized A_1 peak currents decreases at higher scan rates due to less chemical reaction and its following electron transfer [22]. The more cathodic to anodic (C_1/A_1) current peak ratio and nearby one (Fig. 1) for DA and MD can be attributed to the relative stability of their generated *o*-quinone at this condition. But unlike disappearing of C_1 peak in the CV of EP is indicative of the fact that its oxidized intermediate (*o*-quinone) is removed completely by the proposed chemical reaction. The higher reactivity of the side chain amine group of EP is related to the methyl group that enhances the nucleophilicity of the amine group. As is shown in Eq. (2) of Scheme 1, the addition of the amine group should be pH dependent. Therefore the voltammetric studies have been extended and the effect of pH has been studied on electrochemical behavior of all CAs. As shown in Fig. 2 for MD, increasing the ratio of A_1 over C_1 and the heights of A_2/C_2 redox peaks clearly demonstrates that the rate of the desired reaction increases parallel to an increase in solution pH. The rate and extent of the above-mentioned Michael addition is negligible at pHs lower than 5 and is enhanced drastically at pH values greater than 5 for MD.

The borderline pHs for the reactivity of DA and EP are 6 and 2.5, respectively, which confirm the lower and drastically higher reactivity of the oxidized form of

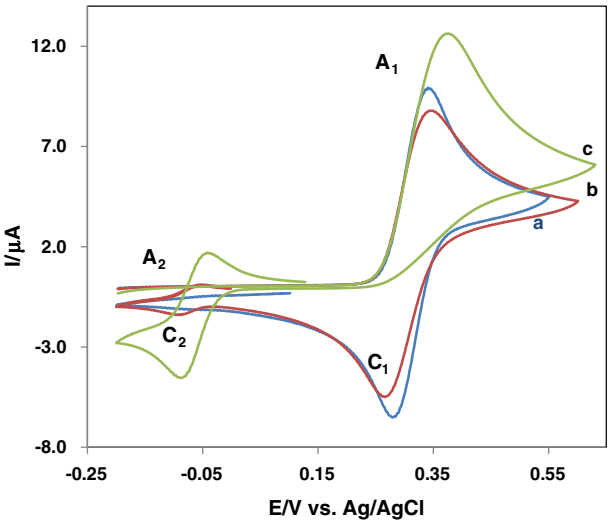
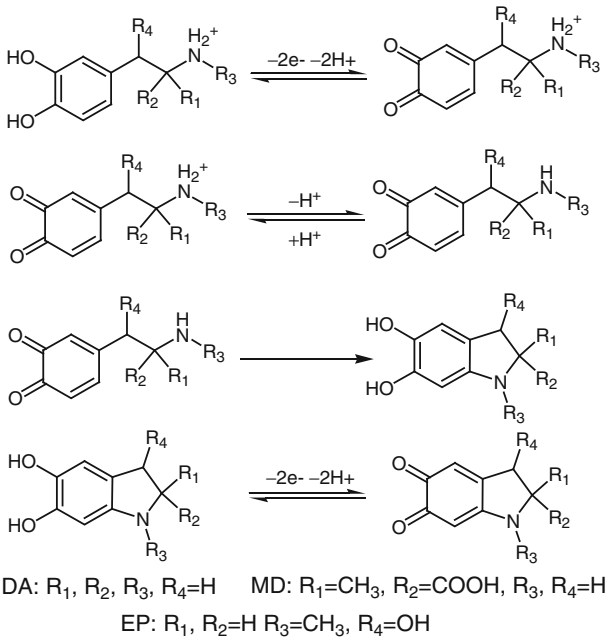


Fig. 1 Cyclic voltammograms of 1.0 mM DA (**a**), MD (**b**), and EP (**c**) at glassy carbon electrode in acetate buffer solution (pH = 5.0). Scan rate: 50 mVs^{-1}



Scheme 1 Proposed mechanism for electrochemical oxidation of CAs

these CAs, respectively. These results evidently demonstrate that the aliphatic side chain amine group of CAs undergoes nucleophilic addition in neutral and even mild acidic conditions despite their high basicity constants and considering the fact that

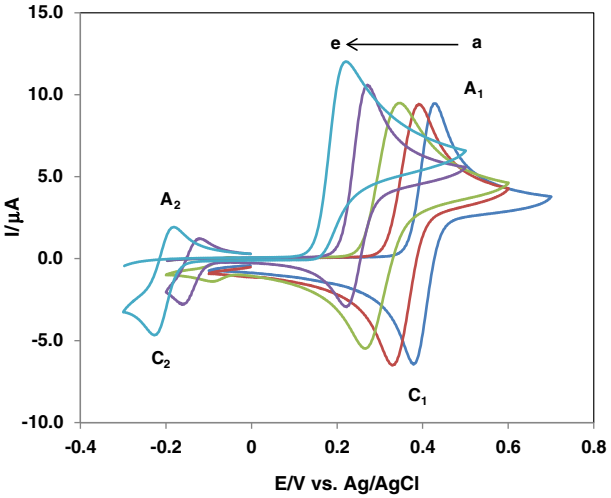


Fig. 2 Cyclic voltammograms of 1.0 mM MD at various pH values of 3.0 (**a**), 4.0 (**b**), 5.0 (**c**), 6.0 (**d**), and 7.0 (**e**). Scan rate, 50 mV s^{-1}

they are in their protonated forms [23]. The good extent of this chemical reaction is related to the coupled kinetic-equilibrium mechanism. In situ generation of reactive deprotonated form and shifting the fast acid–base equilibrium during the reaction is the most probable reason of their reactivities [22]. The same amine derivatives (aliphatic amines) in solution don't show any reactivity, intermolecular, toward *o*-quinone that produced from oxidation of catechol. Thus this good reactivity can be justified also considering the intramolecular character of the reaction for catecholamines.

Figure 3 shows the CVs of MD in the presence of 1.0 m of 3-nitraniline (3NA) at various pH values. In the presence of 3NA, one new cathodic peak (C_3) and its counterpart (A_3) appear in CVs. The half-wave potential of this new redox system, A_3/C_3, is more negative than A_1/C_1 and more positive than A_2/C_2.

The effect of CV scan rate as a useful parameter has been studied on the reaction and all of the diagnostic criteria are in good agreement with ECE mechanism in the presence of 3NA. An ECE mechanism is also observed in the presence of 3NA at the pH values that the side chain amine group of MD does not have any reactivity (curves a and b). The normalized CVs of MD in the presence of 3NA is shown in Fig. 4, which confirms the presence of a chemical reaction with electroactive product and its less extent at higher scan rates. Normalized CVs are obtained by dividing the currents by the square root of the scan rate [24].

The same mechanism is proposed for the nucleophilic addition of 3NA to electrochemically generated *o*-quinone from oxidation of MD and the same CAs (Scheme 2). Based on this proposed mechanism, the A_3/C_3 redox peaks are related to the electron transfer of the product of intermolecular 1,4 (Michael) addition of 3NA on *o*-benzoquinone at the electrode surface. 3NA is an aryl-amines with electron-withdrawing substituent that has a very weak basic character, $pK_b = 11.53$

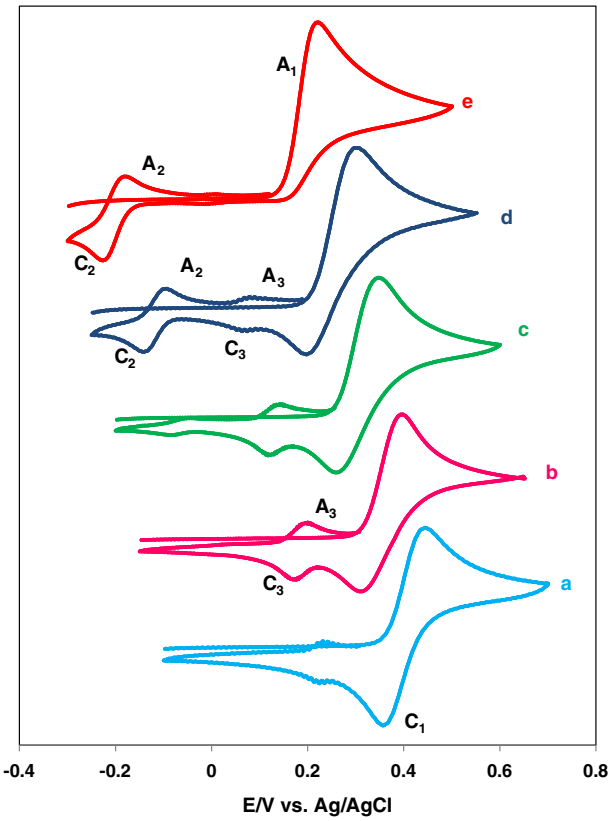


Fig. 3 Cyclic voltammograms of 1.0 mM methyldopa in the presence of 1.0 mM 3NA at various pH values of 3.0 (**a**), 4.0 (**b**), 5.0(**c**), 6.0 (**d**), and 7.0 (**e**). Scan rate, 100 mV s^{-1}

[25]. With a good estimation, more than 75 % of 3NA molecules are in deprotonated form at pH 3 and act as nucleophile at this mild acidic condition. The most probable reaction products are some diphenylamine derivatives, which have been reported earlier [2, 26].

At the pH values of 5 and 6, both inter and intramolecular reactions take place and their related redox peaks (A_2/C_2 and A_3/C_3) of products are observed. The A_3/C_3 redox peaks have the same half-wave potential as MD in the absence of 3NA. The CVs of MD at pH 7 and higher show two redox couple, A_1/C_1 and A_2/C_2, same as absence of 3NA. This is due to the predominance of the intramolecular over intermolecular Michael addition at these pH values. This interesting pH-dependent interplay between reactions is due to the difference of nucleophilicity and basicity of aromatic and aliphatic amines. The side chain amine group of CAs as an aliphatic amine is a stronger base and nucleophile than 3NA (as an aromatic amine). In acidic solution, the side chain amine group is fully protonated with good estimation and intramolecular reaction did not take place at all. Therefore, only the nucleophilic addition of 3NA take places and the peaks of its product are observed. In neutral and

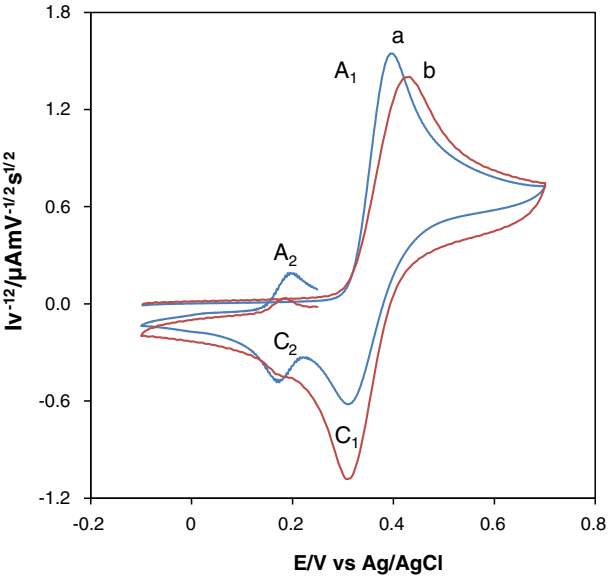
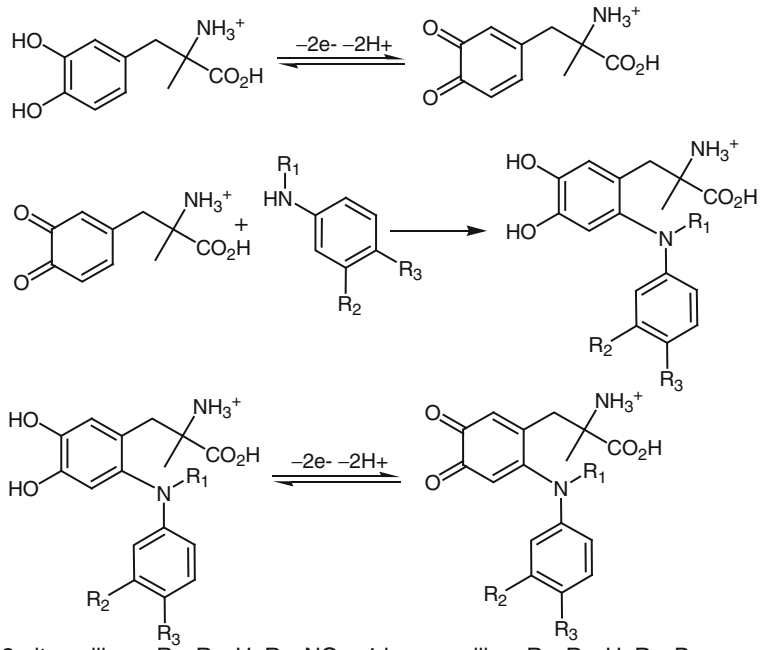


Fig. 4 Normalized cyclic voltammograms of 1.0 mM MD in acetate buffer solution (pH 7.0). Scan rates 50 (**a**) and 700 (**b**) mV s^{-1}, respectively

basic solutions that this side chain amine group is in reactive deprotonated form no competition was observed and the intramolecular reaction consumed all of the electrochemically produced *o*-quinone.

The rate of cyclization reaction increases more than the rate of addition of 3NA because the variation in the concentration of reactive form of side-chain amine is more significant than that of the 3NA [20]. Another interesting finding can be concluded by comparison of the half-wave potential of the products of inter and intramolecular reactions; the order of half-wave potential is $E_1^0 > E_3^0 > E_2^0$. The electron pair of aromatic 3NA is not free same as aliphatic amine chain of CAs to take part in resonance with catechol ring thus the E_s^0 of its products are more positive than the E^0 of cyclization products. Voltammetric behaviors of EP and DA in the presence of 3NA have been studied and the same trends were observed for their reactivity and E^0 of products. The CVs of all CAs at pH 5 are shown in Fig. 5.

Figure 5 shows that MD has an average reactivity toward both inter and intramolecular reaction. DA shows slightly less reactivity toward 3NA whereas for EP the intramolecular reaction is the dominant reaction overall. This confirms again the higher reactivity of EP in cyclization reaction due to its stronger nucleophilic character and appears even at less pH values. Voltammetric studies of all of the CAs above have been extended in the presence of 4-methylaniline, *N*-methylaniline, 4-bromoaniline, and 4-nitoaniline. Figure 6 shows the CVs of MD in the presence of the above-mentioned aromatic amines as nucleophile. All of the desired amines undergo nucleophilic addition with electrochemically generated *o*-quinone except 4-nitroaniline. Based on the above results, in an ECE mechanism, the ratio of A_1 to C_1 can be considered as the reactivity of nucleophiles toward *o*-quinone.



Scheme 2 Proposed mechanism for electrochemical oxidation of CAs in the presence of aniline derivatives

The order of anodic-to-cathodic peak current ratio suggests that the presence of an electron-donating group enhances the nucleophilicity of aniline derivatives to some extent and vice versa. The only exception to this order is *N*-methylaniline, with lower reactivity than expected, which can be explained by its higher steric hindrance. There is also an interesting order in the half-wave potential of products and the electron-donating or -withdrawing character of aniline derivatives. The presence of electron-donating groups on aniline rings increases their tendency for resonance of electron pair of nitrogen with catechol ring and thus shift the half-wave potential of products to more negative values and vice versa. There is also an exception for *N*-methylaniline; the presence of a methyl group in this derivative and its steric hindrance with the chain group of CAs in *ortho* position force it to the out of plane conformation that decrease the possibility of it resonance less than other first type aniline derivatives [27]. The exact same order was obtained for the reactivity of DA in the presence of the desired amines. In the case of EP not also the electron-withdrawing character of aniline substituents affect the half-wave potential of their product, but also influence their competitive interplay with the side chain amine group. Only the anilines with electron-donating groups undergo an acceptable competitive reaction with the amine group of EP as a better nucleophile than DA and MD. Figure 7 shows the CVs of EP in the presence of some selected

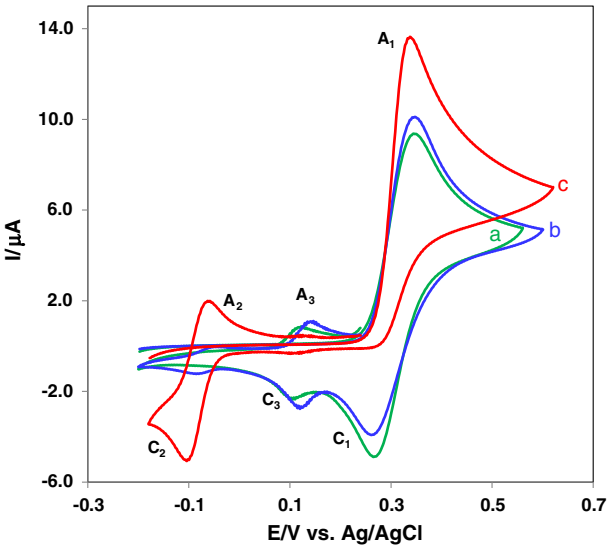


Fig. 5 The cyclic voltammograms of 1.0 mM solution of DA (**a**), MD (**b**), and EP (**c**) in the presence of 1.0 mM 3NA in acetate buffer solution (pH = 5.0). Scan rate, 50 mV s^{-1}

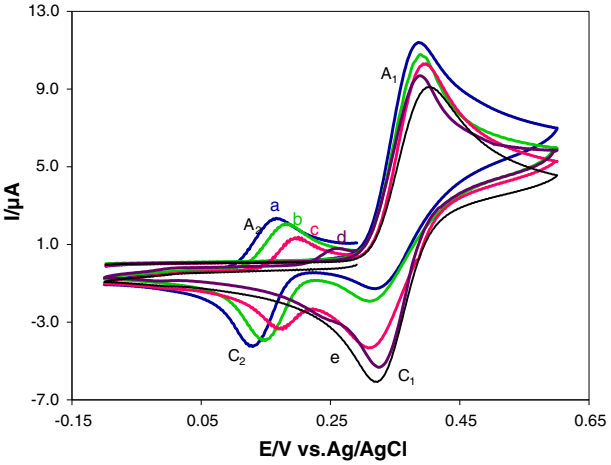


Fig. 6 The cyclic voltammograms of MD in the presence of 1 mM aniline (**a**), 4-bromoaniline (**b**), 3-nitroaniline (**c**), *N*-methylaniline (**d**), and 4-nitroaniline (**e**) in acetate buffer solution pH = 5. Scan rate, 100 mV s^{-1}

nucleophiles. It shows that the less extent of intermolecular reaction, for 3NA, cause to decrease in the height of A_3/C_3 peaks and increase the height of A_2/C_2 peaks related to its competitive intramolecular reaction.

Finally, in order to obtain more information and quantitative explanation, the rate constants of homogeneous coupled chemical reactions were obtained by digital simulation of CVs and comparison of the digitally simulated and experimental CVs.

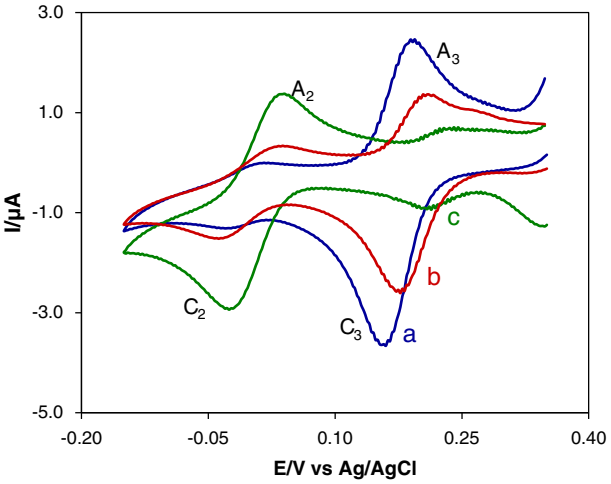


Fig. 7 The cyclic voltammograms of EP in the presence of 1 mM aniline (**a**), 4-bromoaniline (**b**), and 3-nitroaniline (**c**) in acetate buffer solution pH = 4. Scan rate, 100 mV s^{-1} the A_1/C_1 have been erased

Table 1 The observed homogeneous rate constant (k_{obs}) of Michael addition of aniline derivative and electro-generated *o*-quinone from oxidation of CAs and half-wave potential of products

	Methyldopa		Dopamine		Epinephrine	
	k^{*}_{obs}	$E^{**}_{1/2}$	k^{*}_{obs}	$E^{**}_{1/2}$	k^{*}_{obs}	$E^{**}_{1/2}$
4-methylaniline	290	0.142	289	0.138	317	0.142
Aniline	259	0.153	257	0.151	291	0.154
4-bromoaniline	227	0.174	205	0.171	231	0.175
3-nitroaniline	202	0.184	173	0.179	205	0.183
N-methylaniline	164	0.251	160	0.248	178	0.252

* Homogeneous rate constant at pH = 5 ($M^{-1}s^{-1}$)

** Half-wave potential of reaction product at pH = 5

The simulation was carried out assuming semi-infinite one-dimensional diffusion at a disk electrode geometry based on the proposed mechanism. E_{start}, E_{final}, scan rate, and electrode area were entered as experimental parameters. The formal potentials were obtained experimentally as the midpoint potential between the anodic and cathodic peaks (E_{mid}). The transfer coefficients (α) were assumed to be 0.5, and the heterogeneous rate constants (0.005 cm s^{-1}) for oxidation of CAs were estimated by use of experimental working curves [24, 28].

The rate constant of reactions (k_{obs}) were allowed to change through the fitting processes whereas all these parameters were kept constant. Good agreement between the simulated CVs and those obtained experimentally proves the accuracy of the proposed mechanism. The observed rate constant of coupling reaction of oxidized CAs with various aniline derivatives are presented in Table 1.

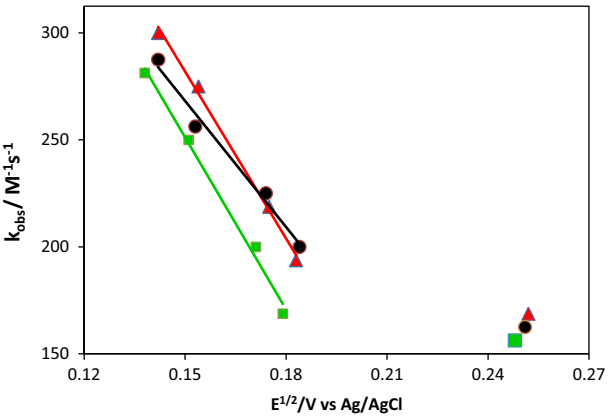


Fig. 8 Plot of observed rate constant versus half-wave potential of the products of aniline derivatives with EP (*filled triangle*), MD (*filled circle*), and DA (*filled square*)

Each reported rate constant is the average of five independent simulations at various scan rates and the relative standard deviations (RSD) of all reported values are <7 %. Moreover, the half-wave potentials of the reaction product of intermolecular reactions are listed in this table. As can be seen from the table, the order of rate constants is in good agreement with those obtained by diagnostic criteria of CVs; the anilines with electron-withdrawing substituent have smaller rate constants than aniline and 4-methylaniline. These consistent changes encouraged us to give a quantitative explanation for the relation of electron-withdrawing character and reactivity of products and/or aniline derivatives (Fig. 8).

It is interesting to note that in all CAs cases, the relevance of half-wave potential of products and electron-donating character of nucleophiles and their reactivity toward nucleophilic addition has a linear relation with good estimation. Therefore the half-wave potential of electroactive products can be used as determining parameter for the prediction of the reactivity of the electroinactive substrates.

Conclusions

Based on these results, the *o*-benzoquinones produced from the oxidation of catecholamines are attacked by their side chain amine groups or aniline derivatives in mild acidic solutions. There is a close interplay between inter- and intramolecular reactions in the presence of the aniline derivatives. The solution pH has an influential parameter that affects the superior of each nucleophilic addition toward *o*-quinones. It can be described taking into account of the difference of nucleophilicity and basicity of the side chain amine group as aliphatic amine and aniline derivatives as aromatic amines. The results show that the nucleophilicity and reactivity of epinephrine toward cyclization is substantially more than dopamine and methyldopa. This essay has argued that all of the desired aniline derivatives have a suitable reactivity toward *o*-quinone except for 4-nitroaniline, which is

expected due to its strong resonance electron-withdrawing character of nitro in *para* position of aniline. It is shown that the rate constant of Michael addition of anilines increases linearly by increasing the electron-withdrawing character of their substituent. There is also a significant correlation between half-wave potential of products and the nature of aniline substituents. Considering the fact that the reaction products are claimed to be diphenylamine derivatives, and they are known as antioxidants, the tuning of their half-wave potential and its electrochemical measurement may be interesting from the synthetic point of view.

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