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Voltammetric study of the oxidation of quercetin and catechin in the presence of cyanide ion

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Abstract The reaction of electrochemically generated *o*-benzoquinones from oxidation of quercetin and catechin as Michael acceptors with cyanide ion as nucleophile has been studied using cyclic voltammetry. The reaction mechanism is believed to be EC; including oxidation of catechol moiety of these antioxidants followed by Michael addition of cyanide ion. The observed homogeneous rate constants (k_{obs}) for reactions were estimated by comparing the experimental voltammetric responses with the digitally simulated results based on the proposed mechanism. The effects of pH and nucleophile concentration on voltammetric behavior and the rate constants of chemical reactions were also described.

Keywords Catechin · Quercetin · *o*-Quinone · Cyanide ion · Digital simulation

Introduction

An antioxidant is defined as a molecule capable of slowing or preventing the oxidation of other molecules [1], and flavonoids are the most widely used group of antioxidants. The electrochemical oxidation of flavonoids is of great interest because of their action as antioxidants with the ability to scavenge radicals by electron transfer processes [2]. Also, many other biologically important processes involve redox reactions, and electrochemical methods compose a collection of

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extremely useful tools for the analysis of their reactions. Among the characteristics of electrochemical methods, in situ generation of reactive intermediates and inversion in polarity by transfer of electrons at the electrode–solution interface have found considerable attention in various areas of chemistry [3]. Molecular electrochemistry studies the mechanistic events at or near an electrode on a molecular level and voltammetry is convenient and also the most frequently used technique for this purpose [4].

Quercetin and catechin as natural flavonoids were selected for this study. They have several biological activities including radical scavenging, anti-inflammatory, antimutagenic, and anti-cancer activities [5]. Quercetin is a frequent component of major dietary constituents, such as onions and apples [6]. Research shows that quercetin influences cellular mechanisms in vitro and, in animal studies, there is evidence from human population studies that quercetin may, in a very limited fashion, reduce the risk of certain cancers [7]. Catechin is extracted from plants and is present in natural foods and drinks such as green tea [8]. The application of catechin as a phenolic standard in quantization of reducing equivalents in food and biological fluids and its oxidation mechanism are of particular interest [9]. The interest in the quantification of catechin is consistent with its importance as being potentially beneficial to human health. The evidence of the presence of catechin in foodstuffs can be related to epidemiological studies that take into account the relationships between catechin intake and diseases [10].

Quercetin and catechin can be chemically or electrochemically oxidized and their electrochemical behaviors have been studied in some papers [11]. Both quercetin and catechin have a catechol skeleton in their structures and electrochemical experiments show that the first oxidation of these compounds is related to the oxidation of their catechol group. Oxidation of catechol derivatives occurs at very low positive potentials, and is a reversible reaction. The product of oxidation generates *o*-quinone, a highly reactive species, which undergoes various chemical reactions. It can be attacked by the side chain group of parent molecules or suitable nucleophiles via a Michael addition reaction [12, 13].

The aim of this work is the electrochemical oxidation of catechin and quercetin in the presence of cyanide ion and then the estimation of the rate constants of reactions by digital simulation. Cyanide is acutely toxic to mammals by all routes of administration, with a very steep and rate-dependent dose–response curve that involves inactivation of cytochrome oxidase and inhibition of cellular respiration and consequent cellular anoxia. In humans and animals, cardiovascular, respiratory, and central nervous systems are primarily affected [14].

Experimental

Apparatus

Cyclic voltammetry was performed using a Behpajoh Model BHP 2061-C potentiostat/galvanostat. In the voltammetry experiments, 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 (KCl 3.0 M). All electrodes were from Azar Electrode (Ourumieh, Iran).

Reagents and solutions

All of the experiments were performed with analytical reagent-grade chemicals purchased from Merck. These chemicals were used without further purification. The stock solutions of the catechin and sodium cyanide were prepared fresh daily by dissolving the compounds in distilled water. The stock solution of quercetin was prepared daily by dissolving it in distilled water/acetonitrile (60/40) solution. Samples were prepared by taking the appropriate aliquots from the stock solutions followed by dilution with buffer solutions. The 0.15 M buffered solutions were prepared based on Kolthoff tables [15]. The homogeneous rate constants were estimated by analyzing the cyclic voltammetric responses using the simulation CVSIM software [16].

Results and discussion

Voltammetric study

Figure 1, curve a, shows the cyclic voltammograms of 1.0 mM quercetin in the absence of cyanide ion at pH 6.0 in the mixture of acetonitrile/water, 20/80, solution. The cyclic voltammograms of quercetin shows one anodic (A_1) and corresponding cathodic peak (C_1) with peak current ratio nearly but less than unity,

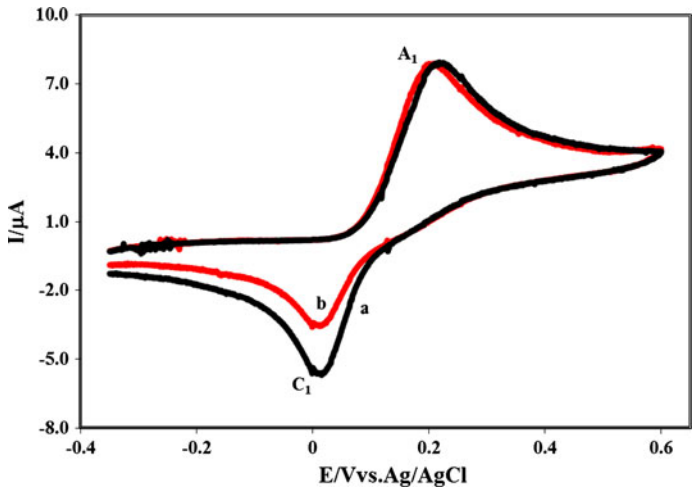


Fig. 1 Cyclic voltammograms of 1.0 mM quercetin: *a* in the absence, *b* in the presence of 1.0 mM of cyanide ion, at glassy carbon electrode, in aqueous/acetonitrile solution containing phosphate buffer (pH = 6.0, c = 0.15 M), *Scan rate* 100 mV s^{-1}

within two-electron process. The electrode response of quercetin can be related to the transformation of its catechol group to *o*-quinone and vice versa.

Figure 1, curve b, shows a cyclic voltammogram of 1.0 mM quercetin in the presence of 5.0 mM cyanide ion in the same solution. In this condition, the most important difference between the voltammograms in the presence and absence of cyanide ion is decreasing magnitude of cathodic peak (C_1) currents. As mentioned above, the C_1 peak is due to reduction of *o*-quinone and its currents are proportional to the quantity of it. A decrease in the C_1 peak currents is indicative of this fact that the oxidized intermediate formed at the surface of electrode removed by a chemical reaction.

The time scale of a voltammetric experiment is determined by the scan rate. It is an important parameter in the study of homogeneous reactions coupled with electrode reactions [17]. Figure 2 shows the effect of the potential scan rate on voltammograms of quercetin in the presence of cyanide ion at pH 6.0. It is seen that when the scan rate is decreased, the C_1–A_1 current peak ratio decreases. This is reflected the low extent of chemical reaction at the period of recording the cyclic voltammograms at high scan rates. The inset of Fig. 2 shows the peak current ratio (I_{pC}/I_{pA}) versus scan rate for a mixture of quercetin and cyanide ion.

Also the voltammetric studies were performed at various concentrations of cyanide ion (Fig. 3). By increasing the concentration of cyanide ion, the height of the cathodic peaks and their ratio over the A_1 peak is decreased.

Such behaviors are good criterions for EC mechanism consists of an electron transfer reaction (*E*) followed by a chemical reaction (*C*) [17]. According to these results, electrochemical oxidation of quercetin causes the formation of related

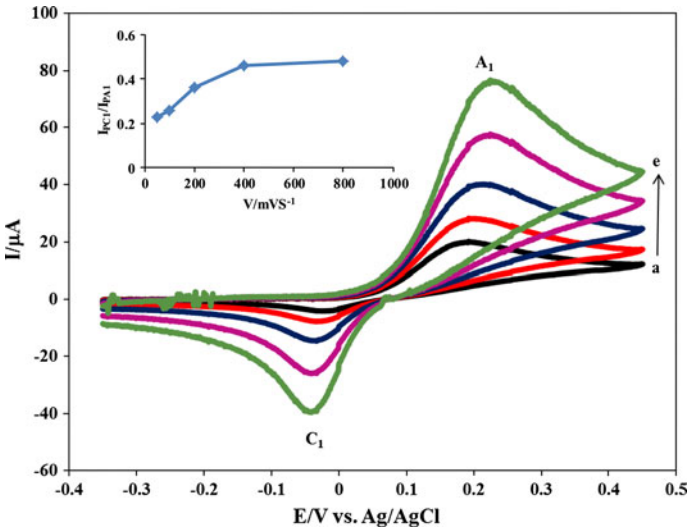


Fig. 2 Cyclic voltammograms of 1.0 mM quercetin in the presence of 5.0 mM cyanide ion at a glassy carbon electrode in phosphate buffer solution (pH = 6.0, c = 0.15 M). Scan rates from *a* to *e* are 50, 100, 200, 400, and 800 mV s^{-1}, respectively. *Inset* variation of cathodic to anodic peak current ratios versus scan rate

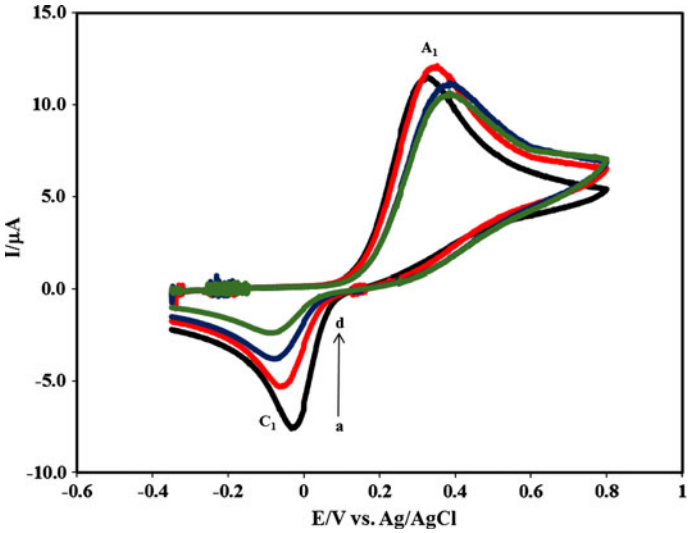
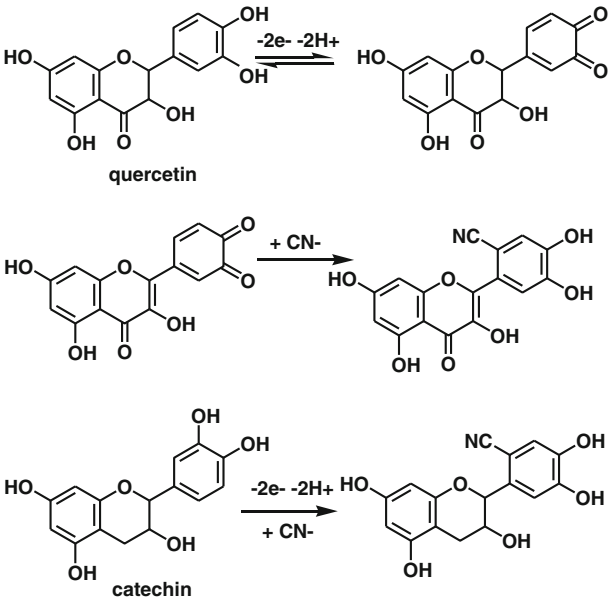


Fig. 3 Cyclic voltammograms of 1.0 mM quercetin in the presence of different concentration cyanide ion, pH = 6.0, Concentrations from *a* to *d* are 1.0, 2.0, 4.0, and 8.0 mM; *Scan rate* 100 mV s^{-1}

o-quinone that undergoes Michael addition reaction with cyanide ion. It is faster than other secondary reactions and leads to the related nitrile derivatives. The oxidation of the product is more difficult than the oxidation of the parent molecules



Scheme 1 Proposed mechanism for electrochemical oxidation of quercetin and catechin in the presence of cyanide ion

and was prevented by virtue of the presence of the electron-withdrawing nitrile group on catechol ring [18]. The proposed reaction pathway is shown in Scheme 1.

Because of the dependence of the quercetin oxidation pathway and the acid base equilibrium of cyanide ion on pH, the voltammetric studies were performed at various pHs (Fig. 4).

In basic solutions, the height of quercetin reduction peak decreases due to some considerable side reactions. They consist of the intramolecular reaction of hydroxy groups and coupling of anionic forms of quercetin with *o*-quinones, but the peak current ratio near unity at neutral and acidic solutions can be considered as relative stability of the *o*-quinone [12]. Therefore, the effect of pH has been studied at pHs lower than 7.0. At the desired solutions and presence of cyanide ion, the peak current ratio is less than unity and decreases with increasing pH. The plot of the current peak ratio versus solution pH is shown in the inset of Fig. 4. Cyanide ion is a weak base and variations of reaction rates with pH are due to protonation of cyanide ion and variation of its percentage [15, 22]. The electrochemical study of catechin has been studied in aqueous solution using the method described for quercetin. The results of the investigation of catechin in the presence of cyanide ion are the same as those obtained for quercetin.

Kinetic evaluation

The schemes for the electrochemical oxidation of quercetin and catechin in the presence of cyanide ion were proposed and tested by diagnostic criteria of cyclic voltammograms. One of the successful methods for obtaining kinetic parameters of the coupled homogeneous reaction is digital simulation [19]. The simulation was

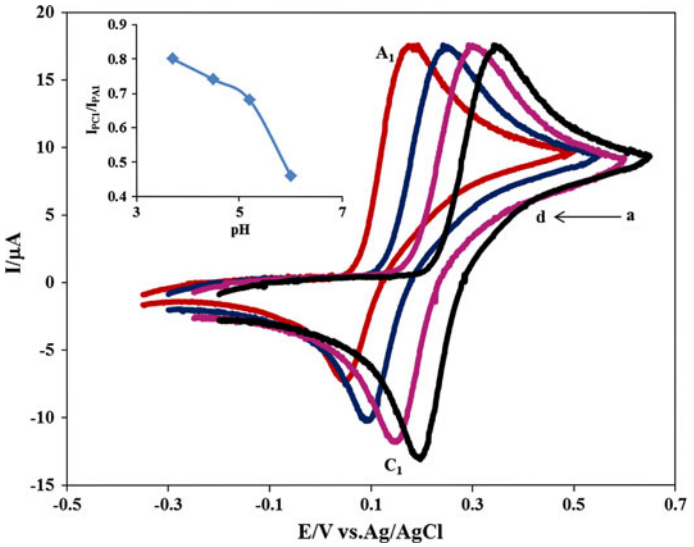


Fig. 4 Cyclic voltammograms of 1.0 mM quercetin in the presence of 5.0 mM cyanide ion, at glassy carbon electrode, at various pH values *a* 3.7, *b* 4.5, *c* 5.2 and *d* 6.0; *Scan rate* 200 mV s^{-1}

carried out based on proposed EC mechanism and assuming semi-infinite one-dimensional diffusion on a planar electrode. The experimental parameters entered for digital simulation consist of the following: starting potential (E_{start}), switching potential (E_{switch}), scan rate *(v)*, half wave potential ($E_{1/2}$), and analytical concentration of species. The formal potentials were obtained experimentally as midpoint potential between the anodic and cathodic peaks (*E*mid). The transfer coefficients (α) and heterogeneous rate constants for oxidation of quercetin and catechin were estimated by experimental working curves [20, 21]. All of these parameters were kept constant and the observed rate constant of chemical reaction k_{obs} was allowed to change during the fitting processes. The fitting consists of finding a rate constant for which the differences between the digitally simulated and the experimental data reach to its minimum [22]. The rate constants of the reaction of produced quinones from oxidation of quercetin and catechin with cyanide ion were estimated for various pHs, cyanide ion concentrations and scan rates. As is shown in Fig. 5, there are good agreements between the simulated voltammograms with those obtained experimentally.

The observed rate constant of coupling reaction of oxidized quercetin with cyanide ion at various pHs are presented in Table 1. Based on these results, the rate of reaction enhances at high pH values, which are related to increasing the cyanide ion percentages.

Also, the observed rate constants of reactions were obtained by digital simulation at various concentrations of cyanide ion. Figure 6 shows the plots of k_{obs} as a function of cyanide ion concentration.

The observed rate constants (k_{obs}) increase linearly as a function of cyanide ion concentrations, which is expected based on voltammetric results. Each point is the average of five independent simulations at various scan rates. Since $k_{obs} = k\,[CN^-]$, where k is the rate constant of reaction, therefore, the value of rate constant can be obtained from the slope of the obtained plot in Fig. 6. The values of the obtained rate constants (k) at pH 6.0 are 37.6 and 34.4 $M^{-1}s^{-1}$ for catechin and quercetin, respectively. The standard deviations were obtained for four independent scan rates for each concentration of cyanide ion. The standard deviations are 9.3% for catechin and 7.4% for quercetin.

Conclusions

The results of this work show that quercetin and catechin are oxidized to their respective *o*-quinones derivative at very mild conditions. Electrochemically generated *o*-quinones are quiet reactive toward the nucleophilic attack of cyanide ion. The Michael addition reaction of this nucleophile with *o*-quinones leads to the formation the nitrile derivatives of parent flavonoids. Based on voltammetric and simulation results, the chemical reaction, which leads to the formation of corresponding nitrile derivatives of flavonoids, has considerable homogeneous rate constants. This is a good example of in situ generation of reactive species by electrochemical methods and kinetic study of coupled homogeneous reaction by the analysis of voltammetric responses.

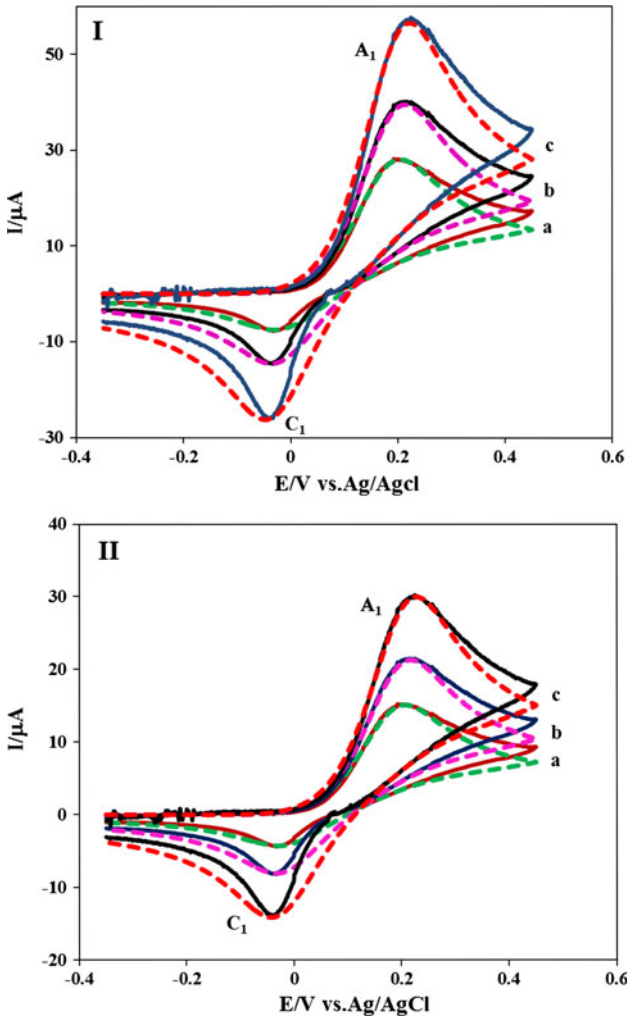


Fig. 5 Simulated (*doted*) and experimental (*line*) cyclic voltammograms of quercetin (I) and catechin (II) at various scan rates *a* 100, *b* 200, and *c* 400 mV s^{-1}

Table 1 Homogeneous rate constants (k_{obs}) for quercetin at various pHs

pH	3.7	4.5	5.2	6.0
k_{obs} (s^{-1})[a]	0.002	0.004	0.014	0.054

[a] The observed rate constants in the presence of 5.0 mM cyanide ion and scan rate of 200 mV s^{-1}

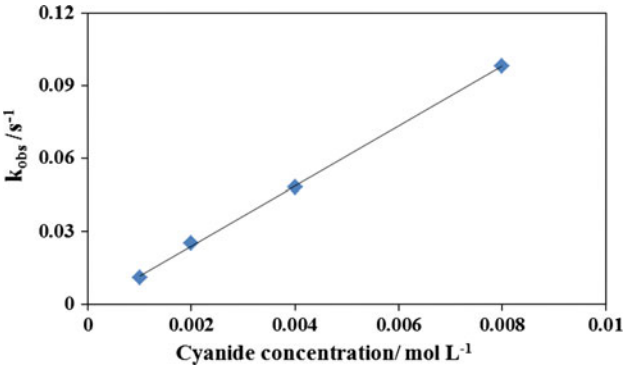


Fig. 6 The plot of observed rate constant (k_{obs}) versus cyanide ion concentration for Michael addition reaction of oxidized quercetin at pH 5.2

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