Electrochemical Behaviour of Antioxidants: Part 2. Electrochemical Oxidation Mechanism of Quercetin at Glassy Carbon Electrode Modified with Multi-Wall Carbon Nanotubes; a Voltammetric and Chronocoulometric Study

Refat Abdel-Hamid*, Mostafa K. Rabia, Emad F. Newair
Department of Chemistry, Faculty of Science, University of Sohag, 82524, Sohag, Egypt

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Abstract

The electrochemical oxidation mechanism of quercetin at glassy carbon electrode modified with multi-wall carbon nanotubes in aqueous 0.2 M phosphate solutions with different pH values was studied using cyclic voltammetry and the double potential step chronocoulometric method. It was found that the oxidation proceeds in sequential steps, related to the five hydroxyl groups in the three aromatic rings. The oxidation mechanism was deduced by matching the experimental chronocoulometric charge ratio versus the logarithm of duration time plots to the theoretical working curves for different electrode mechanisms. It was concluded that the electrode reaction follows an "ECEC, first-order" mechanism. The proposed mechanism was confirmed by digital simulation using DigSim 3 software. The simulation was performed by comparing the simulated responses with the experimental ones. The homogeneous and heterogeneous kinetic parameters of the electrode reaction are estimated from the simulated data. Furthermore, it was found that, quercetin is strongly adsorbed on the electrode surface.

Keywords: Quercetin; Cyclic voltammetry; Chronocoulometry; Digital simulation.

Introduction

Flavonoids represent a large group of polyphenol secondary metabolites that are widely distributed in medicinal plants, fruits, teas and health beverages [2]. Quercetin, a derivative of benzo-γ-pyrone, is a bioflavonoid. Bioflavonoids are a large family of naturally occurring organic compounds widely distributed plants. Bioflavonoids are highly interesting because they may have a wide range of benefits to human health and have broad pharmacological activities including the prevention of cardiovascular diseases and different forms of cancer. Quercetin (3, 3’, 4’, 5, 7-pentahydroxyflavone) is predominant in vegetables and fruits. It was reported to be a potent hydroxyl antioxidant [3,4]. Quercetin electro-oxidation was studied on glassy carbon [5-13], platinum [14,15], polycrystalline platinum [16], multi-wall carbon nanotubes [17], paraffin-impregnated graphite disk modified with multi-wall carbon nanotubes [18], plastic formed
carbon \cite{19}, procaine and aminophenyl modified \cite{20} and a graphite-wax electrode \cite{21}. It was shown that, the electrooxidation of quercetin at a glassy carbon electrode involves an intermediate adsorption step \cite{7}. It was concluded that the initial stage of the process involves the oxidation of the two OH catechol groups in ring B on the quercetin, and is followed by oxidation of the remaining three hydroxyl groups, on the C and A rings. Gutiérrez et al \cite{17} reported that in the presence of carbon nanotubes the less positive wave (corresponding to the oxidation of the catechol groups) exhibit an increase in the oxidation and reduction currents but the peak potential separations are almost the same. The enhancement in the currents was attributed mainly to the increase of the electroactive area of the modified electrode surface.

Quercetin has five hydroxyl groups all of which are electroactive. It has been reported that its electro-oxidation mechanism is complex, pH-dependent and adsorption is involved. It was reported by Brett and Ghica \cite{13} earlier that the electrochemical oxidation of quercetin proceeds via two steps, each involving the transfer of one electron and one proton first to semi-quinone then to ortho-quinone. No electrochemical parameters were estimated. Furthermore, little attention has been paid to its electrochemical oxidation mechanism on carbon nanotubes (CNTs). However, in continuation of our work on electrochemical behaviour of antioxidants \cite{1}, a glassy carbon electrode modified with multi-wall carbon nanotubes was prepared and used to study the electrochemical behaviour of quercetin. The electrochemical oxidation mechanism is investigated, for a wide range of solution conditions, using cyclic voltammetry, double potential step chronoamperometry & chronocoulometry and digital simulation. The electrochemical oxidation mechanism of quercetin oxidation is proposed and discussed and their electrochemical parameters are estimated at different pH values. This may play a crucial role in understanding its antioxidant activity. The study is performed in aqueous phosphate solutions on modified electrode.

The chemical structure of quercetin

**Experimental**

*Materials and Methods*

All the electrochemical experiments were carried out using Model 273A potentiostat from EG & G Princeton Applied Research equipped with EG & G M 270
software. Glassy carbon/multi-walls carbon nanotubes modified electrode (GCE/MWCNTs) with a surface area of 0.0276 cm$^2$ was employed as a working electrode. 1.0 mg ml$^{-1}$ suspension of MWCNTs was obtained on dispersing five milligrams MWCNTs in 5.0 ml N,N-dimethylformamide (DMF). The mixed solution was ultrasonically agitated for 6 hs. The GCE/MWCNTs, modified electrode was prepared by dropping 1.0 ml of MWCNTs suspension (1.0 mg ml$^{-1}$) onto the clean surface of GCE. Then the solvent was evaporated overnight. The glassy carbon electrode was polished with 0.5 µm alumina powder on a polishing cloth. After rinsing the surface with deionized water, the polished GCE was rinsed in deionized water, then shaken while immersed in an ethanol/water mixture inside an ultrasonic vibrator for 5 min to remove traces of alumina powder from its surface and rinsed again with deionized water. Then, the surface was fully dried at atmospheric condition with a stream of purified nitrogen. A platinum electrode and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively. All the potentials were reported with respect to this reference electrode. All experiments were performed at 25°C.

The surface area of the electrode was determined from measurement the peak current of 1.0 mM potassium ferricyanide in 1.0 M KNO$_3$ system using the equation:

\[ i_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} \nu^{1/2} C \]

where $i_p$ is the peak current in amperes, $A$ is the area of the electrode in cm$^2$, $C$ is the concentration of the electroactive species in mM, $D$ is the diffusion coefficient in cm$^2$ s$^{-1}$, $n$ is the number of electrons transferred and $\nu$ is the sweep rate in Vs$^{-1}$.

All the chemical reagents used to prepare solutions were reagent grade from Merck and were used as received. The MWCNTs (purity >90%; carbon basis, D × L 110-170 nm × 5-9 µm) were obtained from Aldrich. All solutions were freshly prepared just before use with deionized water. The uncompensated resistance was corrected for by the potentiostat.

**Results and Discussion**

*Cyclic voltammetry*

The electrochemical behaviour of quercetin, Qu, at glassy carbon electrode modified with multi-walls carbon nanotubes is investigated in 0.2 M aqueous phosphate buffer solutions with different pH values. Figure 1 shows the cyclic voltammograms, cv, of 2.5 µM quercetin in buffer solution (pH 2.12) at a scan rate of 10 mV/s. The cv shows four anodic peaks associated with the oxidation centers present in the molecule occurring at the potentials of 0.47, 0.56, 0.84 and 1.13 volt versus SCE. The overall cyclic voltammogram profiles are similar to those previously reported.$^{[13]}$
The first anodic cv wave, located at \( \approx 0.47 \) volt, seems to be reversible. It is confirmed on reversing the potential scan just before the second wave, its cathodic counter-part is seen, c.f. figure 2. The peak separation, \( \Delta E_p = E_{pa} - E_{pc} \), is \( \approx 40 \) mV which agrees with the literature value and points to a reversible electrode reaction involving two electrons [22]. The somewhat large peak separation could point to a chemical contribution. On increasing the scan rate the anodic peak potential, \( E_{pa} \), is positively shifted and the cathodic peak potential, \( E_{pc} \), is negatively shifted. The relationship of peak potential to scan rate indicates that the electron-transfer process couples with a chemical reaction. The peak current relations of the anodic and cathodic waves with scan rate are constructed. It is found that the peak currents are directly proportional to the square root of scan rate \( \nu \) with a correlation coefficients of 0.985. This indicates that the oxidation process of Qu is diffusion-controlled. These findings point out to a diffusion-controlled electrode process involving a chemical reaction occurring after the electron transfer process [22].
Figure 2: Cyclic voltammograms of 2.5 µM Quercetin in 0.2 M phosphate buffer pH 2.12 at different scan rates (10, 15, 20, 25, 30, 40 mV/s), potential window is 0.30 to 0.53 V, just before the second cv wave.

The oxidation products at the first cv wave are reversibly reduced and could also be oxidized at higher potentials. The presence of the other waves can be attributed to the oxidation of other hydroxyl groups remaining in the products, such as the hydroxyl group attached position 5 on the A-ring, or even in another position of the existing structure.

On increasing the electrode potential, the oxidation products formed at the first peak is further oxidized, giving the second cv wave, located at ≈ 0.56 volt. The second cv wave is irreversible; it has no counterpart. Its peak potential, E_{pa}, is shifted anodically with increasing scan rate. The relatively low peak current of the wave is attributed to the hydrogen bond formed between the 3-hydroxyl group and the oxygen atom close to it at position 4 on ring C. It is related to oxidation of the 3-hydroxyl group at ring C \[^6\]. A linear least squares fit of log \(i_p\) versus log \(v\) is obtained with slope of 0.912 and correlation coefficient, \(r\), of 0.992. This reveals that, the reaction of quercetin along the second wave is mainly adsorption-controlled \[^{22}\].

On extending the potential window to more positive potential, a third oxidation cv wave is obtained at about 0.85 V. This wave is ascribed to the oxidation of the hydroxyl group attached on the 5-position of A-ring \[^{12}\]. A linear least squares log - log fit is obtained with slope of 0.865 and \(r\) of 0.996. This indicates that, the rate of oxidation of quercetin along this cv wave is controlled mainly by adsorption.

*Double potential step chronocoulometry*
Double potential step chronocoulometry has found considerable use in the investigation of the adsorption of electroactive species. The chronocoulograms of 2.5 µM quercetin in 0.2 M phosphate buffer solution (pH 2.12) are obtained at different duration times. For the chronocoulometric experiment, the electrode potential is stepped up from an initial value $E_i$ to a higher final value $E_f$. The potential is held at $E_f$ for a time $\tau$ after which it is stepped back to $E_i$ and maintained there for the same time interval. The charge that passes through the electrode during each of these time intervals is measured. It can be seen that the charge of the electrode potential from $E_i$ to a more positive value $E_f$ causes oxidation of both adsorbed and diffusing Qu. Upon reversing the potential step, the cathodic reaction proceeds, and the charge ratio, $Q(2\tau)_{\text{ox}}/Q(\tau)_{\text{red}}$ deviates from the expected theoretical value 0.586 \cite{23}. This is attributed to an irreversible consumption of the oxidized product generated in the forward step. The electrochemical responses are given by equations (1) and (2) for the forward and reverse steps.

\[ Q(t < \tau) = \frac{2nFAD^{1/2}C_{\text{bulk}}^{1/2}}{\pi^{1/2}} + Q_{dl} \]  
\[ Q(t > \tau) = \frac{2nFAD^{1/2}C_{\text{bulk}}^{1/2}}{\pi^{1/2}} \left[ t^{1/2} - (t - \tau)^{1/2} \right] \]  

where $A$ is the electrode area, $C_{\text{bulk}}$ the bulk concentration of analyte, $D$ the diffusion coefficient, $t$ the time, $Q_{dl}$ is the electrode-electrolyte double-layer charge and $n$ and $F$ have their usual meanings. When reactant adsorption occurs, the term $nFA\Gamma$ is added to equation (1) thus,

\[ Q(t < \tau) = \frac{2nFAD^{1/2}C_{\text{bulk}}^{1/2}}{\pi^{1/2}} + Q_{dl} + nF\Gamma \]  

where $\Gamma$ is the amount of the adsorbed species.

Thus, from equations (2) and (3), $Q_T(t) = Q(t) - Q(t > \tau)$ can be expressed by equation (4).

\[ Q_T(t) = \frac{2nFAD^{1/2}C_{\text{bulk}}^{1/2}}{\pi^{1/2}} \theta + Q_{dl} \]  

where $\theta = (t^{1/2} + (t - \tau)^{1/2} - t^{1/2})$.

The first term of the right-hand side of equation (1) represents the charge of species oxidation under diffusion-control. The second term is charge of the double-layer. This equation shows that a plot of $Q(t < \tau)$ against $t^{1/2}$ should be a straight line with an intercept equal to $(Q_{dl})$. It can be easily measured in a separate experiment for the supporting electrolyte alone. On plotting $Q(t < \tau)$ against $t^{1/2}$ for the first cv wave, a linear least squares fit gave a correlation coefficient of 0.999. This supports the conclusion that the first cv wave is diffusion-controlled. Moreover, the diffusion coefficient is determined to be $2.31 \times 10^{-6}$ cm$^2$ s$^{-1}$.  

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Figure 3 represents the plots of $Q(t<\tau)$ against $\theta$ and $Q(t<\tau)$ against $t^{1/2}$ for the second cv wave. Straight lines are obtained over the entire time range investigated. It is clear that the intercept according to equation (4) is always less than that obtained for the $Q(t<\tau)$ against $t^{1/2}$ plots. This further supports the view that Qu molecules adsorb on the working electrode. Furthermore, the amount of the adsorbate is estimated from the difference in the intercepts for the forward and reverse Anson plots (Eqs. (3) and (4)). The charge obtained for 2.5 µM quercetin is found to be 0.80 µC/cm$^2$. This value is equivalent to a surface excess $\Gamma_0$ of $1.002 \times 10^{-10}$ mol/cm$^2$.

![Anson Plots of $Q(t<\tau)$ vs. $t^{1/2}$ and $Q(t>\tau)$ vs. $\theta$ for 2.5 µM Qu second wave at pH 2.12](image)

Figure 3: Anson Plots of $Q(t<\tau)$ vs. $t^{1/2}$ and $Q(t>\tau)$ vs. $\theta$ for 2.5 µM Qu second wave at pH 2.12

Although cyclic voltammetry is used in quantitative measurements, the complex nature of the electrode mechanisms limits its use in quantitative investigations of coupled chemical reactions. In comparison the double potential step chronocoulometry method is more suitable for quantitative studies. The charge ratio $\frac{Q_b}{Q_r}$ [Equation (6)] is obtained from the ratio of equation (5) at $t = \tau$ and equation (2) at $t = 2\tau$.

$$Q(t<\tau) = \frac{2nFAD^{1/2}C_{bulk}}{\pi^{1/2}} t^{1/2} \quad (5)$$

$$Q_R = \frac{Q_b}{Q_r} = \frac{Q(\tau) - Q(2\tau)}{Q(\tau)} \quad (6)$$

The experiments are performed by varying the duration time, $\tau$, over a suitable range and measuring the charge ratio $Q_R$. The experimental results are compared with the theoretical response ratio for various mechanisms in an attempt to find a
satisfactory match. Chronocoulometric response ratios for 22 electrochemical mechanisms described by Hanafey et. al. are used for tracing the Qu oxidation mechanism \[24\]. The experimental $Q_R$ versus log $\tau$ plots match satisfactorily the theoretical working curves that had been calculated for the ECEC radical-radical dimer mechanism, the ECEC parent-radical dimer mechanism and the ECEC first-order different electrode mechanism. As figure 4 shows the best of these matches is that with the ECEC first-order mechanism.

**Figure 4**: Best fit of chronocoulometric charge ratio, $Q_R$ for experimental and theoretical working curves for the oxidation ECEC mechanisms of queretin.

**Digital simulation**

A scheme for the electrochemical oxidation of quercetin at pH 2.12 based on an ECEC first-order is proposed and tested by digital simulation, c.f. Scheme 1. Initially an electron is reversibly transfered to the quercetin molecule, species Qu, to form a radical-cation, $Qu^+$. This redox couple is assigned to the standard electrode potential $E^{\circ\prime}_1$ with a electron-transfer rate constant $k_1^{\circ\prime}$. The radical cation is next deprotonated in the first chemical step with a chemical rate constant $k_1^c$ to form the neutral radical species Qu. The neutral radical species (Qu) then undergoes further reversible electron-transfer step ($E^{\circ\prime}_2$) to form the monocation $Qu^+$ species with electron-transfer rate constant $k_2^{\circ\prime}$. The monocation $Qu^+$ will yield the final product QuO via a first-order deprotonation process with rate parameter $k_1^d$ following ECEC first-order mechanism. The steps in the mechanism are summarized as shown next in scheme 1.
To establish the theoretical working curves, digital simulation of the proposed mechanism is carried out. The experimental voltammograms are compared with the simulated ones calculated using the DigSim 3.03 software. This was done to confirm validity of the deduced ECEC oxidation electrode mechanism and to estimate the standard electrode potential, the kinetic parameters for the heterogeneous electron-transfers and their homogeneous associated chemical steps. For the diffusion coefficients, $D$, the default value of $2.26 \times 10^{-6}$ cm$^2$/s is used throughout. Different ECEC mechanisms including ECEC radical–radical dimmer; ECEC parent-radical dimmer, and ECEC first-order are tested. The transfer coefficient, $\alpha$, is assumed to be 0.5, and the formal potentials are obtained experimentally from the first cv wave observed in cyclic voltammetry. The procedure is performed to achieve the best fit between simulated and experimental cyclic voltammograms.

Figure 5 represents the matching of the simulated and experimental voltammograms for the different electrode ECEC mechanisms proposed for quercetin oxidation at pH 2.12. Good agreement between simulated and experimental voltammograms is obtained for the ECEC first-order mechanism. The kinetic parameters obtained for the best fit are given in Table 1. Thus, the simulation using the proposed ECEC first-order mechanism, c.f. Scheme 1, agrees well with the experimental data. This reveals good evidence for the mechanism given in scheme 1.

Table 1: Kinetic parameters for the electrochemical oxidation of 0.20 µM quercetin at pH 2.12 and scan rate of 20 mV s$^{-1}$.

<table>
<thead>
<tr>
<th>$E^0_1$/mV</th>
<th>$E^0_2$/mV</th>
<th>$k_0^\text{O}$/cm s$^{-1}$</th>
<th>$k_0^\text{d}$/cm s$^{-1}$</th>
<th>$K_1^\text{O}$/s$^{-1}$</th>
<th>$K_1^\text{d}$/s$^{-1}$</th>
</tr>
</thead>
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<tr>
<td>455</td>
<td>200</td>
<td>0.50</td>
<td>0.10</td>
<td>1.530</td>
<td>6.104</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$k_1^\text{O} \times 10^5$</td>
<td>$k_2^\text{O} \times 10^6$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$k_1^\text{d} \times 10^7$</td>
<td>$k_2^\text{d} \times 10^8$</td>
</tr>
</tbody>
</table>

Qu $\xrightarrow{k_e}$ Qu$^\text{+}$ + e$^{-}$

Qu$^\text{+}$ $\xrightarrow{k_1^\text{f}}$ Qu$^\text{+}$ + H$^\text{+}$

Qu$^\text{+}$ $\xrightarrow{k_2^\text{f}}$ QuO + H$^\text{+}$

Qu$^\text{+}$ $\xrightarrow{k_3^\text{f}}$ QuO + H$^\text{+}$

Scheme 1
Figure 5: Best fit for experimental and digital simulated data for the quercetin oxidation mechanisms, A) ECEC, first-order, B) ECEC, radical-radical dimer and C) ECEC, parent-radical dimer at pH 2.12 and scan rate of 20 mV/s.

Conclusion

The quercetin oxidation proceeds by a consecutive mechanism and is related mainly with the catechol groups in ring B and the three hydroxyl groups in rings A and C. The oxidation of the catechol moiety occurs first at low positive potentials and corresponds to a two-electron reaction and a two-proton reaction following the ECEC first-order electrode mechanism. The chemical steps, C, are deprotonation, since the peak potential is shifted to less positive values and the peak current decreases on increasing the pH of solution. Quercetin is strongly adsorbed on the electrode surface.
References