Electrochemical Characterization of Glassy Carbon Electrode and Boron-doped Electrode for Determination of Quercetin

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ABSTRACT

The present paper studied the process of electro-oxidation of quercetin with a glassy carbon electrode and boron-doped diamond electrode, in 0.05 M phosphate buffer, in 20% ethanol solution. Quercetin electrochemical oxidation was an irreversible reaction at the glassy carbon electrode. The oxidation mechanism proceeds in sequential steps, related to the catechol and resorcinol groups. The oxidation is pH dependent. The quercetin quantitative analysis at the glassy carbon electrode was detected by amperometry in phosphate buffer (pH 6.0) with potential 0.6 V. The results obtained show high sensitivity, excellent dynamic range, and a low detection limit of 0.0005-0.412 mM, 0.36-1.60 mM, and 0.2 μM (S/N = 3), respectively.

Keywords: Quercetin, Glassy carbon electrode, Boron-doped diamond, Cyclic voltammetry, Amperometry

INTRODUCTION

Quercetin is one of the most abundant natural flavonoid compounds (3, 3′, 4′, 5, 7-pentahydroxyflavone) present in plants in substantial amounts. The structure is shown in Scheme 1, which occurs naturally in plant extract. It has anti-oxidant, antibacterial, anti-inflammatory, and antitumor properties and prevents cancer, heart and age related diseases. It also acts as an anti-mutagen which accelerates cell oxidative damage and also protects human colonocyte DNA from oxidative attack in vitro. Therefore, the determination of quercetin is very important in human health. There are so many techniques used in quercetin determination, such as HPLC-UV (Careri, Corradini, Elviri, Nicoletti, & Zagnoni, 2003; Jiang, Zhou, & Li, 2011; Zielinska, Nagels, & Piskula, 2008), spectrophotometry (Arvand & Anvari, 2013), capillary electrophoresis (Castro Lopez, Lopez Vilarino, Gonzalez Rodriguez, & Barral Losada, 2011; Chen, Zhang, & Ye, 2000; Wang et al., 2007), and liquid chromatography with mass spectrometry (Ma et al., 2013). These techniques are expensive with less sensitivity; hence, an alternative, low-cost and highly sensitive technique is desire. Although several electrodes such as glassy carbon electrode (Brett & Ghica, 2003), platinum electrode (Andreescu, Andreescu, & Sadik, 2003), and multi-walled carbon nanotubes paste electrode (Xiao, Zhao, & Zeng, 2007), were widely used in voltammetric determination of quercetin, glassy carbon electrode and

boron doped diamond electrode have been proposed for the electrochemical studies of quercetin.

In this study, we used glassy carbon comparison with boron doped diamond as the working electrode for the determination of quercetin. The study demonstrated that cyclic voltammetry and amperometry provided a simple and rapid way of identifying quercetin. In addition, the developed amperometry showed a high sensitivity for determination. The reaction of quercetin utilizing glassy carbon and boron-doped diamond was characterized by cyclic voltammetry.

METHODOLOGY

Chemicals

Quercetin (HPLC grade, >98%) and sodium phosphate dibasic dodecahydrate were obtained from Sigma. The stock solution of quercetin (10 mM) was prepared by dissolving into ethanol. Phosphoric acid was purchased from MERCK. Sodium phosphate monobasic dehydrate was purchased Fluka. All other reagents were analytical grade. Ultrapure water (Simplicity® ultrapure water (Type 1), Millipore, France) was used throughout.

Apparatus

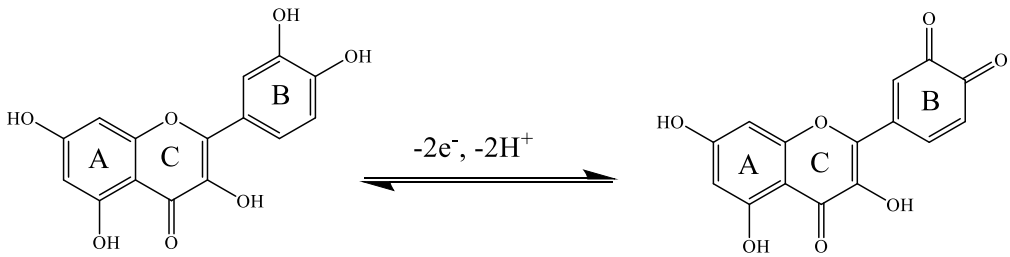
Electrochemical measurements were performed on a DY2011 Potentiostat (Digi-Ivy, Inc., Austin, USA) at room temperature in a conventional three-electrode electrochemical system, which was used for cyclic voltammetric and amperometric experiments. An Ag/AgCl reference electrode (CH Instruments, Austin, TX), and a stainless steel counter electrode were used. Boron-doped diamond (supplied by Professor Yasuaki Einaga's laboratory (Yano, Popa, Tryk, Hashimoto, & Fujishima, 1999)) and a glassy carbon disk electrode (3-mm diameter, CH Instruments Austin, TX) were used as working electrodes, respectively. The boron-doped diamond electrode was pressed against a smooth ground joint at the bottom of the cell, and isolated by an O-ring (3-mm diameter). Ohmic contact was made by placing the backside of the Si substrate onto a brass plate. The pH values of phosphate buffer solution were measured with a pH meter (Mettler Toledo MP220, Mettler Toledo, Switzerland). Magnetic stirring was employed during the amperometric experiment.

Electrochemical measurement

The glassy carbon electrode was polished to a mirror-like surface with 0.05mm alumina slurries and then rinsed with deionized water prior to use. Electrochemical measurements were performed at room temperature. The electrochemical behavior of quercetin was investigated in a 0.05 M phosphate buffer solution (pH 3-7), dissolved 20% (v/v) ethanol, by cyclic voltammetry, at glassy carbon and boron-doped diamond electrodes. The potential range was varied from -0.4 to +1.2 V at scan rate of 100 mVs^{-1} for cyclic voltammetry analysis. The best electrode and the highest current of pH phosphate buffer solution was used for quantitative analysis by amperometry. Hydrodynamic amperometric measurements were performed while stirring in 2.0 mL of the phosphate buffer (pH 6.0). The optimum anodic potential was applied (0.1, 0.2, 0.3,….,0.9 V, respectively) to the glassy carbon working electrode. After the background current reached steady state, quercetin was added, and the corresponding current responses were recorded as a function of time.

RESULTS AND DISCUSSIONS

The electrochemical behavior of 10 mM quercetin, in 20% ethanol and phosphate buffer supporting electrolyte (pH 3-7), at scan rate of 0.1 V s^{-1} , was investigated by cyclic voltammetry, at glassy carbon and boron-doped diamond electrodes. The effect of pH value over the range of pH 3.0 to 7.0 was investigated as in Figure 1 and Figure 2, respectively. They also show that, when the solution pH increases, the peak shifts in the negative direction, at a rate of about 64 mV pH^{-1} for glassy carbon electrode, and 47 mV pH^{-1} for boron-doped diamond electrode. These results were shown Figure 1(f) and Figure 2(f), the slops of the equation are approximately close to the theoretical value of 58.5 mV pH^{-1}, indicating that the numbers of electrons and protons transferred are equal in the electrochemical reactions (Manokaran, Muruganantham, Muthukrishnaraj, & Balasubramanian, 2015; Yao, Zhang, Wang, Xu, & Wen, 2014; Zare, Namazian, & Nasirizadeh, 2005).



Quercetin

Scheme 1. The reaction mechanism of electro-oxidation of quercetin.

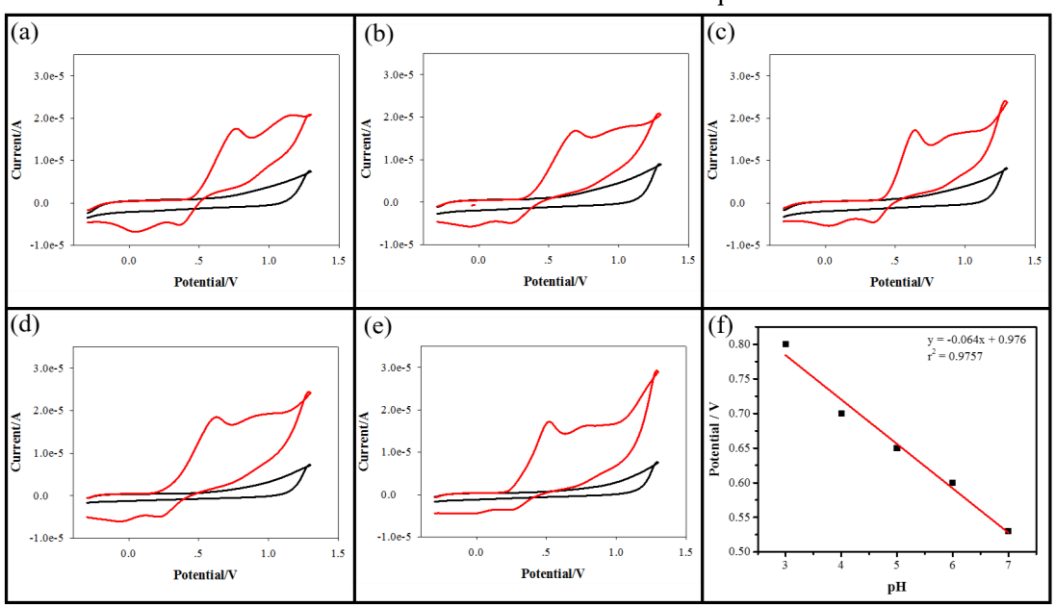


Figure 1. Cyclic voltammograms of 10 mM quercetin in 0.1 M phosphate buffer (a) pH 3.0 (b) pH 4.0 (c) pH 5.0 (d) pH 6.0 and (e) pH 7.0, using glassy carbon as the working electrode. Scan rate: 0.1 V s^{-1}. (f) The graph shows the relationship between the anodic potential of quercetin and the pH of phosphate buffer.

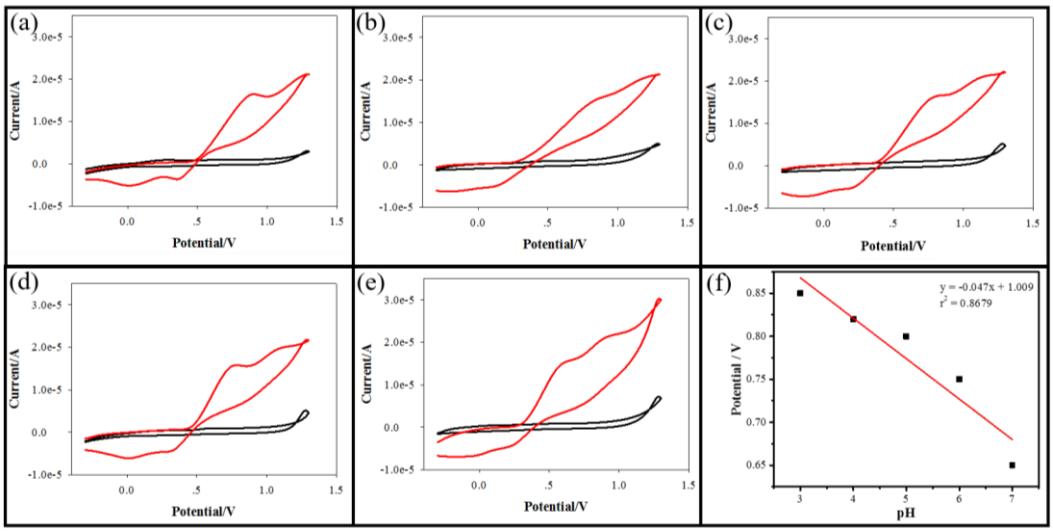


Figure 2. Cyclic voltammograms of 10 mM quercetin in 0.1 M phosphate buffer (a) pH 3.0 (b) pH 4.0 (c) pH 5.0 (d) pH 6.0 and (e) pH 7.0 using boron-doped diamond as the working electrode. Scan rate: 0.1 V s^{-1}. (f) The graph shows the relationship between the anodic potential of quercetin and the pH of the phosphate buffer.

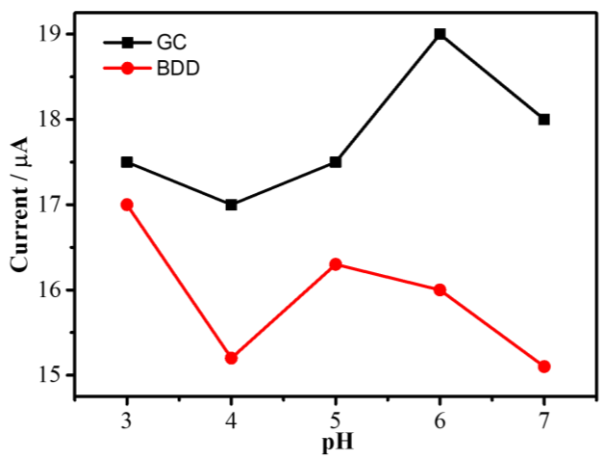


Figure 3. The graph shows the relationship between the anodic current of 10 mM quercetin (from cyclic voltammograms of Figure 1 and 2) and the pH of the phosphate buffer.

The effect of electrodes as shown in Figure 1 and Figure 2 demonstrated that the glassy carbon electrode showed higher current and lower oxidation potential than the boron-doped diamond electrode. Thus, the glassy carbon electrode was employed in the experiments. The optimum pH value of the phosphate buffer electrolyte for quercetin determination at the glassy carbon, over the range of pH 2.0-7.0, was studied by cyclic voltammetry and the resultant analysis shown in Figure 3. The highest anodic peak currents of quercetin at the glassy carbon electrode was pH 6.0. Therefore, pH

6.0 was chosen for the following experiments as the best pH condition according to the work of Manokaran (Manokaran et al., 2015).

To obtain the optimal potential for amperometric detection under stirred conditions, the hydrodynamic behavior of quercetin was studied. Figure 4 shows a amperogram *I–E* curve obtained at the glassy carbon electrode, for 100 μL addition of 10 mM quercetin, in 0.05 M phosphate buffer (pH 6.0) as the electrolyte solution. Each data potential represents the average of three additions. The maximum current was 0.6 V. Hence, this potential was set for quantitative amperometric potential detection in next experiment.

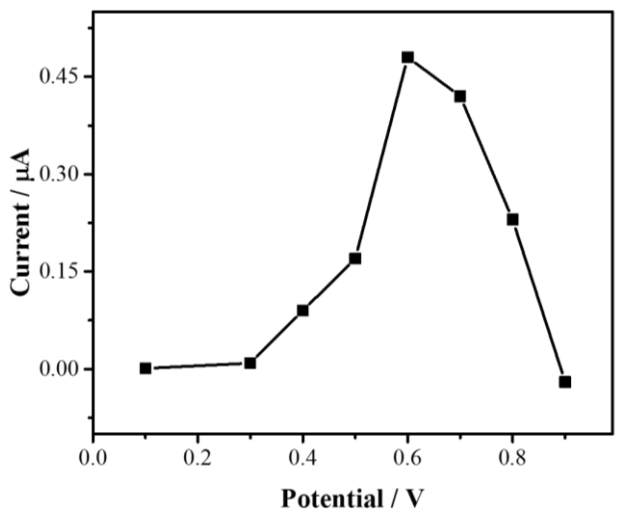


Figure 4. The optimum potential graph of 0.48 mM quercetin analysis at various potentials from 0.1 -1.0 V by the glassy carbon electrode with amperometry.

The voltammetric results described above show the feasibility of using the glassy carbon electrode for amperometric detection of quercetin. Amperometry under stirred conditions was performed to evaluate the potential of the glassy carbon electrode for determination of quercetin. According to the voltammetric results, the optimum electrode potential was selected at 0.6 V which is the same as the oxidation peak of quercetin at pH 6.0 (Figure 1). Figure 5A shows the amperogram results recorded at the glassy carbon electrode, at successive additions of quercetin, concentration range of 0.5 μM – 18 mM. The resulting responses of each concentration were plotted in Figure 5B. Under these conditions, the response of the glassy carbon electrode was linear over the concentration range of 0.0005–0.412 mM (0.15 – 125 mg L^{-1}) and 0.36-1.60 mM (109 – 486 mg L^{-1}) with a slope of 6.62 μA mM^{-1} and 2.77 μA mM^{-1} and a correlation coefficient of 0.9946 and 0.9868, respectively. The linearity is better than Jin's results (Jin, Kwon, Park, Kim, & Jung, 2008). The detection limit was 0.2 μM (61 μg L^{-1}) based on three signal-to-noise ratio, which was better than Muti's work (Muti, Gencdag, Nacak, & Aslan, 2013).

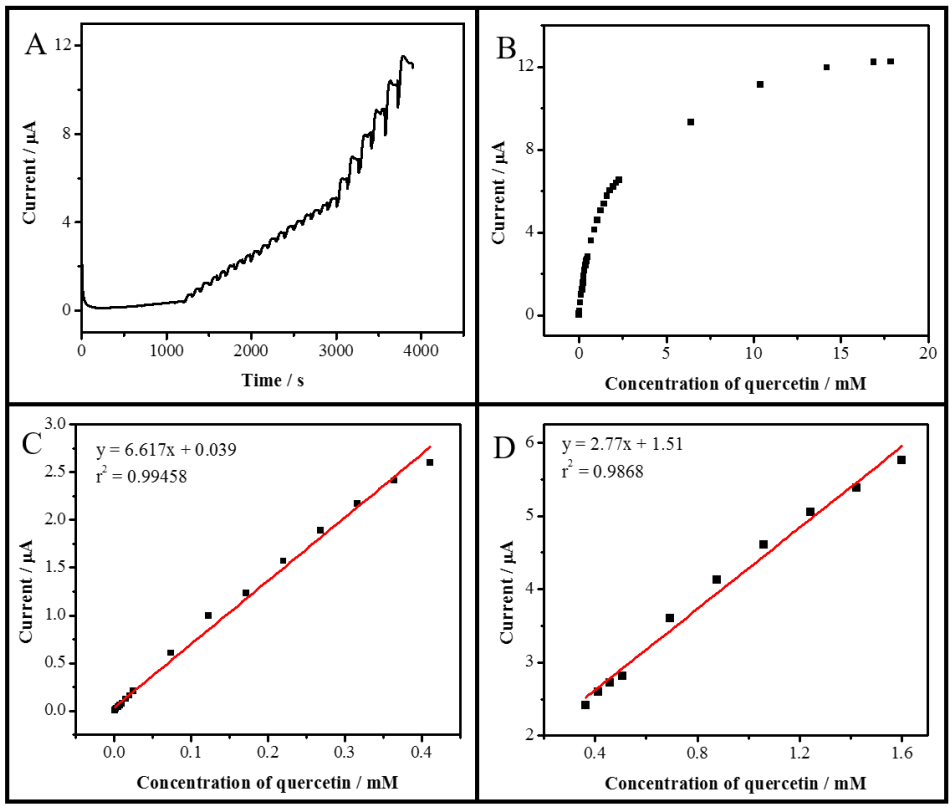


Figure 5. (A) Current–time response of the glassy carbon electrode in stirring phosphate buffer (pH 6.0) for the successive addition of quercetin at 0.6 V. (B) Linear response of steady state current with quercetin for the glassy carbon electrode. (C) The first linearity graph at concentration 0.0005 – 0.412 mM and (D) the second linearity graph at concentration 0.36 – 1.60 mM.

CONCLUSION

The results obtained in these studies show that the electrochemical behavior of quercetin is strongly dependent upon the solution pH. Throughout the whole pH range, the process of electro-oxidation of quercetin follow on the numbers of electrons and protons transferred are equal in the electrochemical reactions mechanism. The proposed protocol demonstrates the successful application of the glassy carbon electrode for the determination of quercetin. Quercetin can be accumulated at the glassy carbon electrode and exhibits a sensitivity anodic peak at about 0.6 V in a 0.05 M phosphate buffer solution of pH 6.0. Under optimized conditions, the anodic peak current is linear to the quercetin concentration in the ranges of 0.0005–0.412 mM and 0.36-1.60 mM, respectively at lower 10 %RSD (n=3) and with excellent sensitivity (6.617 µA/mM), and limit of detection (0.2 µM). In the further, this simple method, for the determination of quercetin, will apply to real samples of plants and human bodies.

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