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Electrochemical Determination of Quercetin in Hawthorn and Onion Using a poly (L-lysine)/graphene Film Electrode

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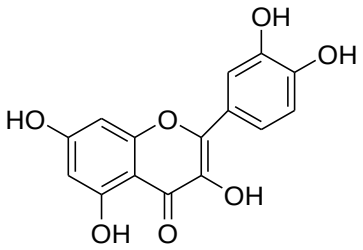
A novel voltammetric method was developed for the fast determination of quercetin using apoly (L-lysine)/grapheneglassy carbon electrode (PLYS/GR/GCE). Using this modified electrode as the working electrode, cyclic voltammetry (CV) was performed on quercetin in pH 6.0 phosphate-buffered saline (PBS). A high electrocatalytic activity was shown for the oxidation of quercetin at the PLYS/GR/GCE in this work. The oxidation of quercetin at the PLYS/GR/GCE was significantly higher than that obtained at a bare electrode. Under the optimal experimental conditions, there was a linear relationship between the oxidation peak current and the concentration of quercetin in the range of 8.1×10^{-7}~1.9×10^{-5} mol·L^{-1} with a detection limit of 2.4×10^{-9} mol·L^{-1}. The method showed good reproducibility and sensitivity for the determination of quercetin. Moreover, this method was successfully applied to the determination of quercetin in hawthorn and red onion, which indicated that the PLYS/GR/GCE has promising novel applications in the detection of quercetin in real samples.

Keywords: Poly (L-lysine); Graphene; Quercetin; Electrochemical determination

1. INTRODUCTION

Quercetin [2-(3, 4-dihydroxyphenyl)-3, 5 ,7-trihydroxy-4H-chromen-4-one] is a flavonol and is widely distributed in fruits, vegetables and grains. The quercetin molecular structure is shown in Scheme 1. Quercetin has anticancer, anti-allergic, anti-inflammatory and antiviral activities[1-3] and thus has attracted considerable interest in recent years. This molecule is an ingredient in supplements, beverages and foods. Additionally, it is a Chinese herbal medicine[4-7]. Therefore, it is very important to develop some simple, economic and efficient methods for quercetin determination in crude drugs or plants.



Scheme 1. Molecular structure of quercetin

Some methods have been used for the analysis of quercetin, such as HPLC-UV[8], solid-phase extraction[9], and capillary electrophoresis[10]. These techniques often have a high sensitivity and efficiency, but on the other hand, they also have disadvantages, such as complicated operation procedures, time-consuming sample pretreatment, high reagent consumption, and high cost. Electrochemical methods can overcome these shortcomings because they have a higher sensitivity, lower costs, the capability for in-field detection and reduced interference by non-electroactive substances. Quercetin exhibits electroactivity because the molecule contains phenolic hydroxyl groups. Hence, electrochemical methods are useful for the analysis of this electroactive compound. Some electrochemical detection techniques have been described for the quantification of quercetin, and several materials have been used to fabricate modified electrodes, including carbon nanotubes[4, 11-13], metal microparticles[14, 15], graphene nanosheets[16] and others[17, 18]. It can be seen that carbon has been widely used as an electrode material because it exhibits many advantages, including extraordinary mechanical, electrical and chemical properties. Among these forms of carbon materials, graphene (GR) has attracted considerable attention since its discovery by Geim and Novoselov in 2004[19] because of its unique properties. The electrochemical behavior of graphene, a novel form of carbon, has been intensively studied [20-22]. Poly (L-lysine) (PLYS) has also attracted considerable attention due to its good biocompatibility and flexible structural framework. Numerous novel applications of PLYS have been investigated, including in electrochemical biosensors [23, 24]. In particular, PLYS combined with carbon materials has been widely used as modified electrodes [25, 26]. From these above reports, it can be seen that this polymer sensor can not only enhance the sensitivity of the electrode but also improve the adhesion of the carbon material to the electrode surface. Here, we report a novel electrochemical analytical method for detecting quercetin using a PLYS/GR/GCE. Although the application of the conductive polymer PLYS combined with graphene has been examined in electrochemical studies [24, 25, 27-30], to the best of my knowledge, this method of quercetin determination has not yet been reported.

In this study, we describe an electrochemical sensor based on poly(L-lysine)/graphene for the analysis of quercetin. The results showed that the PLYS/GR/GCE had good sensitivity, selectivity and stability for quercetin detection. The present method was rapid, specific and highly selective and was successfully applied to the determination of quercetin in hawthorn and red onion, which indicated that the PLYS/GR/GCE has promise for novel applications in the detection of quercetin in real samples.

2. EXPERIMENTAL

2.1 Apparatus and reagents

A CHI660E electrochemical analyzer (Shanghai Chenhua Instrument Co., Ltd., Shanghai, China) with a conventional three-electrode cell was used for the electrochemical measurements. The working electrode consisted of a bare GCE or modified GCE. The reference and counter electrodes were a Ag/AgCl electrode and platinum (Pt) wire, respectively. Ultrasonic cleaning was performed in a KQ-100 ultrasonic cleaner (Kunshan, China), and the pH of the solution was adjusted using a pHS-3B pH meter (module PHS-3B, Shanghai, China). An SYZ-550 quartz sub-boiling high-purified water distiller (Jiangsu, China) and an MT infrared lamp (Guangzhou, China) were used. Scanning electron microscopy (SEM) measurements were taken with a Sirion 200 field emission SEM (FEI, America).

L-lysine was provided by Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China. Quercetin was purchased from the National Institutes for Food and Drug Control. Graphene was made in house according to the synthetic method reported in our previous report[21]. Graphene (0.5 mg) was dispersed in 1.0 mL of double-distilled water under ultrasonication until a homogeneous solution was formed. The buffer solution was prepared by mixing stock solutions of 0.20 $mol \cdot L^{-1}$ sodium phosphate dibasic (Na_2HPO_4) and 0.10 $mol \cdot L^{-1}$ citric acid. All other chemicals in this study were of analytical grade and used directly without further purification. Double-distilled water was used in all experiments.

2.2 PLYS/GR/GCE preparation

Prior to modification, a GCE was first polished carefully with gold sand paper (2000-Grit) and 0.5 mm alumina slurry and then sequentially washed with 50% nitric acid, absolute alcohol and deionized water in an ultrasonic cleaner. The cleaned GCE was dried thoroughly for the next modification step. First, 5 μL of a graphene suspension was cast on the pretreated GCE surface using a microinjector and then dried under infrared light to obtain the GR/GCE. The PLYS/GR/GCE was prepared by electrodepositing 2.0×10^{-4} $mol \cdot L^{-1}$ L-lysine by cyclic scanning on the GR/GCE surface between 0.3-2.1 V at 80 $mV \cdot s^{-1}$ for five cycles in pH 6.0 PBS.

2.3 Analytical measurements

Quercetin and PBS (pH 6.0) were added into the electrochemical cell. The voltammetric detection of quercetin was performed by cyclic voltammetry (CV) between -0.3 V and 1.4 V at 120 $mV \cdot s^{-1}$. After each scan, the electrodes were placed in a blank solution to eliminate the peaks for subsequent use.

3. RESULTS AND DISCUSSION

3.1 Optimum preparation conditions of the modified GCE

The results showed that the amount of graphene and the polymerization conditions of lysine have an important influence on the performance of the PLYS/GR/GCE. The effect of the amount of graphene and polymerization conditions of lysine on the peak current of quercetin by CV investigated. It was found that 5 μL was the most appropriate amount of GR at the optimal concentration (0.3 mg·mL^{-1}) used for the GR/GCE preparation. On the other hand, the modified electrode prepared in PBS (pH=6.0) with 2.0×10^{-4} mol·L^{-1} L-lysine in a potential range from -0.3 V to 2.1 V with a scan rate of 80 mV·s^{-1} had a better electrocatalytic effect in the determination of quercetin.

Further, two different electrodes, GR/GCE (a) and PLYS/GR/GCE (b), were characterized using SEM, as shown in Figure 1. As shown in this image, lysine was successfully polymerized on the surface of the graphene-modified electrode to form a rod-like structure with a large surface area.

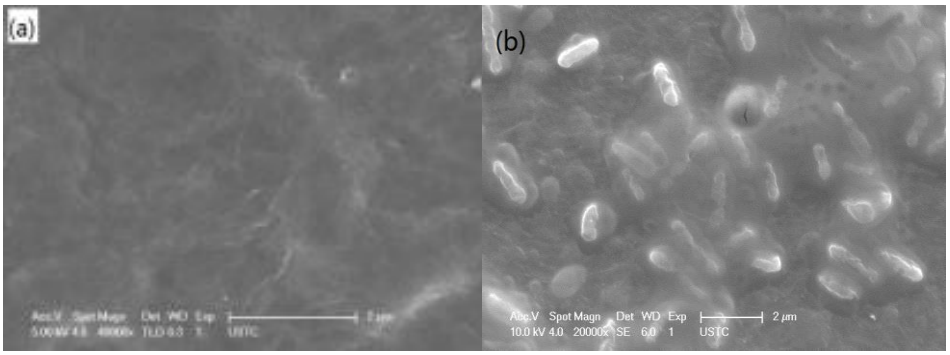


Figure 1. The SEM of GR/GCE(a) and PLYS/GR/GCE(b).

3.2 Electrochemical behavior of quercetin at various electrodes

We investigated the electrochemical behavior of quercetin by CV at various electrodes. The results are shown in Figure 2. Figure 2 (a) depicts the cyclic voltammetric analysis curve on the PLYS/GR/GCE in the absence of quercetin. The CVs of quercetin were investigated at the bare GCE (b), PLYS/GCE (c), GR/GCE (d) and PLYS/GR/GCE. The corresponding oxidation peak currents were 6.84 μA, 13.68 μA, 20.30 μA and 30.46 μA, respectively. The results indicated that oxidation of quercetin was significantly improved on the PLYS/GR/GCE. As can also be seen, the oxidation peak potential was negatively shifted and the oxidation peak current of quercetin on the PLYS/GR/GCE exhibited the highest current. The results indicated that the PLYS/GR/GCE has a good electrocatalytic effect on quercetin. The effect was probably because graphene has good conductivity and a large specific surface area, and the formation of three-dimensional rod-like structures by lysine increased the surface area of the electrode. On the other hand, the formation of hydroxyl hydrogen bonds between the nitrogen atom of lysine and the hydroxyl groups in quercetin weakened the bonds of hydrogen and oxygen in the quercetin molecule to make the oxidation of

quercetin easier, and thus, the oxidation peak current of quercetin significantly improved on the PLYS/GR/GCE [24, 25, 27-30].

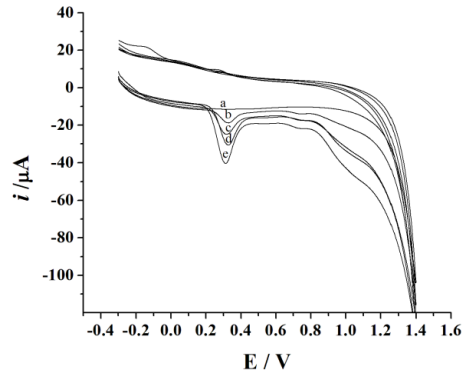


Figure 2. Cyclic voltammograms of PLYS/GR/ GCE in blank solution(a), quercetin (8.0×10^{-6} mol·L^{-1}) at the bare glass carbon electrode (b), PLYS/GCE (c), GR/GCE(d), PLYS/GR/GCE (e).

3.3 Optimum conditions for the determination of quercetin

3.3.1 Influence of pH

The influence of the pH on the peak currents of quercetin was investigated. Figure 3 shows that the CVs of quercetin at the PLYS/GR/GCE differed at pH values ranging from 3.0 to 7.0. The results showed that the highest oxidative peak of quercetin was observed at pH 5.0 (Figure 3A). The oxidation peak potentials for quercetin became more negative as the pH increased from 3.0 to 7.0, indicating that the oxidation of quercetin at the PLYS/GR/GCE was a proton-involved reaction. There was a linear relationship between the potential and pH value, which was found to be Epa(V)=0.67−0.063pH with r=0.9992 (Figure 3B). The slope of 0.063 was close to 0.059, which indicated that the electron transfer number was equal to the proton transfer number, which is in accordance with these reports [31-33].

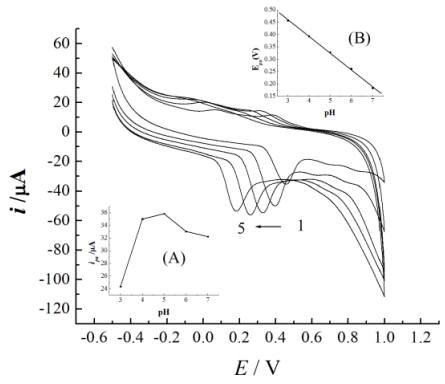


Figure 3. CVs of 2.0×10^{-5} mol·L^{-1} quercetin at a PLYS/GR/GCE in PBS of various pH values. Scan rate: 80 mV·s^{-1}. Each of the letters from 1 to 5 corresponds to a pH of 3.0, 4.0, 5.0, 6.0 and 7.0, respectively. Inset（A）is the plot of the peak potential of quercetin versus pH value of PBS, Inset（B）is the plot of the oxidation peak current of quercetin versus pH value of PBS.

3.3.2 Influence of scan rate

The influence of the scan rate on quercetin oxidation was investigated in a rather wide range of 40 to 360 mV·s^{-1}. The oxidation peak current versus the square root of the scan rate had a good linear relationship, and the linear regression equation was i_{pa}(A)=1.09×10^{-5}+1.28×10^{-7}[v (mV·s^{-1})]$^{1/2}$ with r=0.9992 (Inset of Figure 4). The oxidation peak current increased proportionally with the square root of the scan rate, which suggested that the oxidation of quercetin was a diffusion-controlled process [34]. It was found that the peak potential shifted positively and that the oxidation peak current values increased gradually when the scan rate was increased (Figure 4). The scan rate was 120 mV·s^{-1} in our experiment because the shape of the peak and the sensitivity were good.

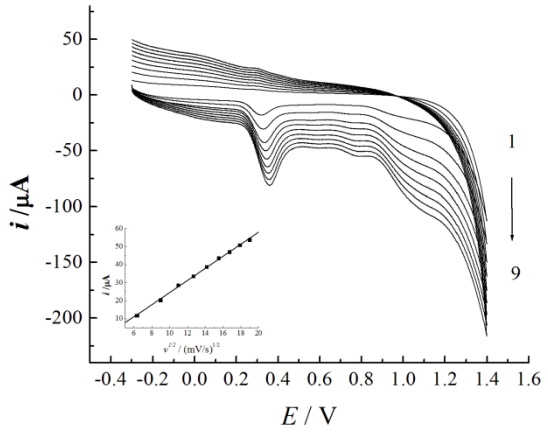


Figure 4. CVs of 2.0×10^{-5} mol·L^{-1} quercetin at PLYS/GR/GCE. Each of the numbers from 1 to 9 corresponds to a scan rate of 40, 80, 120, 160, 200, 240, 280, 320 and 360 mV·s^{-1}, respectively. Inset is the plot of oxidation peak current of quercetin versus the square root of scan rate.

3.3.3 Effect of quercetin concentration

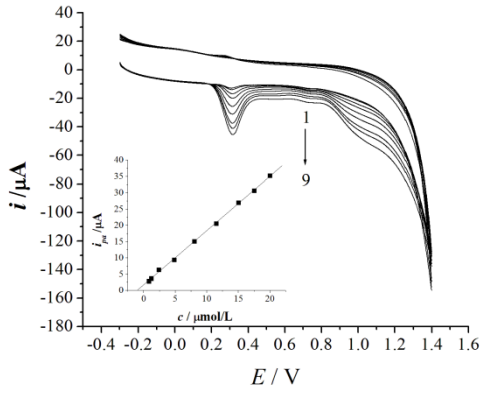


Figure 5. CVs of various concentrations of quercetin at PLYS/GR/GCE. Each of the numbers from 1 to 9 corresponds to a concentration of 8.1×10^{-7}, 1.2×10^{-6}, 2.4×10^{-6}, 4.8×10^{-6}, 8.0×10^{-6}, 1.1×10^{-5}, 1.5×10^{-5}, 1.75×10^{-5} and 1.9×10^{-5} mol·L^{-1}, respectively. Inset is the plot of oxidation peak current versus the concentration of quercetin.

Under the optimal experimental conditions, the oxidation peak current densities increased when the concentration of quercetin increased (Figure 5). As seen from the illustration of Figure 5, the oxidation peak current value was proportional to its concentration from 8.1×10^{-7} to 1.9×10^{-5}mol·L^{-1}, and the regression equation could be expressed by the equation: i_{pa}(A)= $1.72\times10^{-6}+1.67c$ (mol·L^{-1}), where r=0.9997. The detection limit was 2.4×10^{-9} mol·L^{-1}.

3.4 Reproducibility and Stability

The reproducibility and stability of the PLYS/GR/GCE were also investigated. The reproducibility of the PLYS/GR/GCE was studied by measuring the response to a solution of quercetin six times, and the relative standard deviation (RSD) was 2.4%. The stability of the PLYS/GR/GCE was also studied for 5, 10 and 20 days. The results clearly showed that the quercetin oxidation peak currents were 98.2%, 97.0% and 96.5% of the original curve, respectively, indicating that the proposed electrode was very stable.

3.5 Interferences

The interferences of some metal ions andcommon organic compounds in quercetin determination were studied. The results showed that 100-fold higher concentrations of glucose, sucrose, K^+, Mg^{2+}, Ca^{2+}, $A1^{3+}$, Cl^-, NO_3^- and SO_4^{2-}, 50-fold higher concentrations of Ca^{2+}, Cu^{2+}, Zn^{2+}, $A1^{3+}$ and rutin, and a 10-fold higher concentration of Fe^{3+} had no influence on the signals of quercetin, with deviations below 5%.

3.6 Determination of Real Samples

First, 10 grams each of hawthorn and red onion skin was carefully washed with water, dried in drier at 80-90°C and then ground to fine powders. Next, 1 gram of each fine powder was transferred to a Soxhlet extractor and extracted with ethanol. The filtrate was concentrated by vacuum distillation and diluted to 50 mL with methanol in a volumetric flask.

Table 1. Determination results of quercetin in hawthorn and red onion sample (n=6)

Sample	Quercetin added ($\times10^{-6}$mol/L)	Average found ($\times10^{-6}$mol/L)	Recovery (%)	RSD (%)
hawthorn	-	0.86 [a], 0.89 [b]	-	3.4 [a], 3.9 [b]
	1.00	1.82 [a], 1.86 [b]	96.0 [a], 97.0 [b]	3.2 [a], 2.6 [b]
onion	-	4.37 [a], 4.21 [b]		2.2 [a], 2.6 [b]
	5.00	9.24 [a], 9.03 [b]	97.4 [a], 96.4 [b]	3.0 [a], 2.8 [b]

A:The results obtained by using the proposed method. B: The results obtained by HPLC method.

3.7 Comparison of the proposed method with those reported in the literature

Table 2 shows the comparison between the analytical performance of the proposed method and some previous electrochemical methods [35-41] for the determination of quercetin. It can be seen that the proposed method also was rapid, specific and highly selective and was successfully applied to the determination of quercetin.

Table 2. Comparison of different electrodes for quercetin determination

Sensors	Linear range (μmol/L)	Detection limit (μmol/L)	References
Activated silica gel / Carbon paste electrode	0.0165–0.3309	12	35
Pt-PDA@SiO_2 /GCE	0.05–0.383	16	36
Graphene quantum dot/Gold nanoparticle /GCE	0.01–6.0	0.002	37
Co_3O_4 /GCE	0.50–330	100	38,39
MIP/GO/GCE	0.6–15	48	40
SPE │MrGO-MIP	0.02–250.0	0.013	41
PLYS/GR/GCE	0.81-19	0.0024	This work

It can be seen clearly that this proposed method was successfully applied to the determination of quercetin in pretreatment samples. The analysis results obtained using this proposed method were compared with those obtained by the HPLC method. Table 1 shows the measurement results obtained by the proposed method (Table 1a) and the HPLC method (Table 1b). Compared with several modified electrodes[35-41], the proposed modified electrode was better or comparable to these previous reported methods. It also showed a wide LDR and a low LOD [35-36, 38-41]. These maybe attributed to the synergistic effects resulted from the combination of L-lysine and graphene those particles.

From these above reports, it can be seen that this polymer sensor could not only enhance the sensitivity of the electrode but also improve the adhesion of the carbon material to the electrode surface. It exploited new ways in food determination. According to Table 1 and Table 2, the good recovery and precision, and the corresponding results from the HPLC method and other electrochemical sensor reported previously[35-41], this proposed method was suitable for the determination of quercetin in real samples.

4. CONCLUSIONS

In summary, a novel and highly enhanced voltammetric sensing method based on poly (L-lysine)/graphene at a glassy carbon electrode was established for the analysis of quercetin. The results showed that the PLYS/GR/GCE had good sensitivity, selectivity and stability in quercetin detection. The present method was rapid, specific and highly selective and was successfully applied to the determination of quercetin in hawthorn and red onion, which indicated that the PLYS/GR/GCE has promising novel applications in the detection of quercetin in real samples.

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