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An electrocatalytic oxidation and voltammetric method using a chemically reduced graphene oxide film for the determination of caffeic acid

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ABSTRACT

The present work describes the characterization of a chemically reduced graphene oxide (CRGO) modified glassy carbon electrode (GCE) for electrochemical investigation of caffeic acid (CA). Cyclic voltammetry (CV), differential pulse voltammetry (DPV), amperometry, and electrochemical impedance spectroscopy (EIS) techniques were used to characterize the properties of the electrode. There was an obvious enhancement of the current response and a decreased over potential for the oxidation of CA. The interfacial electron transfer rate of CA was studied by EIS. Under optimal conditions, the CRGO displayed a linear response range of 1×10^{-8} to 8×10^{-4} M and the detection limit was 2×10^{-9} M (S/N = 3), with a sensitivity of 192.21 μA mM^{-1} cm^{-2} at an applied potential of +0.2 V (vs. Ag/AgCl reference), which suggests that the CRGO is a promising sensing materials for the electrochemical investigation of CA. The results showed the good sensitivity, selectivity and high reproducibility of the CRGO modified electrode. Moreover, this modified electrode was further applied to investigate the CA in real samples of wine with satisfactory results.

1. Introduction

Polyphenolic compounds are secondary metabolites in plants and are present in plant-derived foods including berries, apples, citrus fruit, cocoa, and grapes; vegetables such as onions, olives, tomatoes, broccoli, lettuce, and soybeans; grains and cereals; green and black teas, propolis, and red and white wines [1,2]. There are a variety of naturally occurring phenolic acids such as caffeic and gallic acids and the well-known pharmacological, medicinal and biochemical properties of phenolics have been extensively studied [3]. They have antioxidant properties that prevent auto-oxidation via the inhibition of radical formation. Thus, a diet rich in fruits and vegetables that contain these compounds plays a protective role against many human diseases such as cancers, cardiovascular disease, immunoregulation diseases, asthma and allergic reactions [4–7].

In recent decades, caffeic acid (CA) has been found to be naturally present in higher plants in various forms such as glycosides, esters and the free form. The best described property of CA is as an antioxidant but it can also act as a carcinogenic inhibitor [8,9]. For example, the caffeic acid phenethyl esters that occur in honeybee hive products have both anticarcinogenic and immunomodulatory properties [10–12]. Several studies have focused on caffeic acid activity against HIV integrase and it has been found to completely block HIV replication with moderate anti-HIV activity in cell cultures. Derivatives of caffeic acid are abundant in wine. The phenolic compounds of caffeic acid are the main contributors to color stability and protection against oxidation in the wine [4,13].

In recent years, various methods have been developed for caffeic acid (CA) sensors. Among these methods, it has been found that liquid and gas chromatography, spectrophotometry [4] and capillary electrophoresis [14] have the facility for the determination of phenolic acids in food samples and plant materials. However, these methods have the disadvantages of being time consuming, necessitating expensive instruments, and have poor selectivity and sensitivity. Since CA is an electroactive compound, electrochemical techniques for detection have also received

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considerable interest, because they are easy to use, more selective, less expensive, faster, and have good selectivity for biological analysis. However, there has been little reported in the literature on the study of the mechanism of caffeic acid oxidation using the electrochemical measurements [15,16]. Numerous studies have focused on the quantitative analysis of caffeic acid in different matrices [6,5]. In 2004, Sousa et al. published an interesting report on the use of glassy carbon electrodes with an activated GCE surface for investigation of the electrochemical oxidation of CA in natural products such as orange juice, without interference from the ascorbic acid [17]. Santos et al. [18] fabricated a poly(glutamic acid) (PG) film modified electrode that could be used for the electrochemical determination of CA [18]. Fernandes et al. [19] developed a biosensor based on green beans which was constructed in order to determine the caffeic acid content in white wine by using square-wave voltammetry [19]. Glassy polymeric electrodes modified with poly(caffeic acid) films have also been used for the electrochemical oxidation of CA [6]. Tyszczuk et al. [20] studied lead modified GCE(PbFE-GCE) and investigated its usefulness for the electrochemical determination of CA using square-wave voltammograms [20]. In recent years, there have been few reports on the electrochemical determination of CA using electrochemical biosensors fabricated based on immobilized tyrosinase, laccase or peroxidase [21]. To the best of our knowledge a chemically reduced graphene oxide modified electrochemical sensor has not yet been produced for the determination of CA using the amperometric method.

Graphene, which can be described as a one-atom-thick planar sheet comprising a sp^2-bonded carbon atoms bonded together in a hexagonal lattice, has been the focus of a wide range of research in recent years [22,23]. Furthermore, graphene is less expensive compared to other materials from the carbon family, due to its unique properties such as large surface area, extraordinary electronic transport property, high electrocatalytic activity, good mechanical strength, high thermal conductivity and high mobility of charge carriers [24,25]. In addition, over the last few years, several graphene preparation procedures have been developed, such as the micromechanical exfoliation of graphite, chemical vapor deposition, and solution-based chemical reduction of graphene oxide (GO) [26,27]. Owing to their low-cost and applicability for bulk-scale production, methods involving the chemical reduction of GO are the most attractive. In the most successful cases, the chemical reduction of GO has been conducted using hydrazine or hydrazine hydrate as the reducing agent. Moreover, previous reports have demonstrated that graphene has the ability to accelerate electron transfer allowing for the design of novel electrochemical sensors and biosensors. Graphene based modified electrodes have attracted enormous interest in the past few years and have been successfully applied for the study of some biological and organic molecules, including DNA [28,29], glucose oxidase [30], hydrogen peroxide [31], dopamine [32], methanol fuel [33] Nitrite [34] and nitrobenzene [35].

In this present work, a caffeic acid (CA) sensor was developed by coating chemically reduced graphene oxide (CRGO) onto a glassy carbon electrode (GCE) by the drop casting method.

2. Experimental procedure

2.1. Materials

The graphite (powder, <20 μm), caffeic acid, Na_2HPO_4 and NaH_2PO_4, *N*,*N*-dimethyl formamide (DMF) and the other chemicals were all purchased from the Sigma Chemical Company. The buffer solution (0.05 M) was prepared from Na_2HPO_4 and NaH_2PO_4 to be applied as the supporting electrolyte. The pH values were adjusted with 0.1 M HCl or NaOH solutions. The water used in all experiments was deionized and further purified using a Milli-Q system (Millipore Corporation).

2.2. Apparatus

All electrochemical measurements were performed with a CHI 400A electrochemical workstation, using a three-electrode test cell. A conventional three-electrode system was used with a modified glassy carbon (GC) electrode (5 mm in diameter) as the working electrode, an Ag/AgCl/1 M KCl reference electrode (Biometra, Germany) and a Pt-wire counter electrode. All measurements were carried under ambient conditions. X-ray photoelectron spectroscopy (XPS) was recorded using a PHI 5000 Versa Probe equipped with an Al Kalpha. UV–visible absorption spectroscopic measurements were performed with a Hitachi U-3300 spectrophotometer. Scanning electron microscopy (SEM) (Hitachi S-3000 H) and energy-dispersive X-ray spectroscopy (EDX, HORIBA EMAX X-ACT Model 51-ADD0009) were employed to characterize the morphology. Electrochemical impedance spectroscopy (EIS) was performed in a frequency range from 100 MHz to 100 kHz (ZAHNER, from Kroanch, Germany).

2.3. Fabrication of the sensors

Graphene oxide was prepared using graphite according to the modified Hummer's method [34,36] whereby 1 g of graphite (graphite powder, <20 μm, Aldrich) was suspended in 2.5 g of $K_2S_2O_8$ and 46 mL H_2SO_4 and then stirred in a round bottom flask at 0 °C for 15 min. Next, 2.5 g of P_2O_5 was added into the mixture over 15 min in order to avoid a temperature spike and the mixture was left to be stirred vigorously for 6 h at 20 °C. On completion, the mixture was then poured and diluted with 1 L of water and filtered. Afterward, a 6 g portion of $KMnO_4$ and 1 g of $NaNO_3$ in 31.2 mL of water were added gradually while being stirred. The temperature of the mixture was controlled to below 20 °C. The reaction occurred as the mixture was stirred at 35 °C for 2 h, after which 500 mL of distilled water was added slowly to keep the temperature below 50 °C. After that further reaction was allowed to proceed for 2 h, and then 250 mL of water and 6 mL of H_2O_2 (30 weights %) were added. The color of the mixture changed to brilliant yellow. Finally, the solid suspension was washed with a 2 M HCl solution and then washed 3–4 times with ethanol and dried in a vacuum at 60 °C overnight. The graphite oxide slurry was then dried in a vacuum oven at 60 °C for 48 h before use. Afterward, a sample of CRGO (0.5 g mg mL^{-1}) was prepared by dispersion in DMF with the aid of ultrasonication for 30 min. The CRGO sample was prepared by mixing 20 mg of graphite oxide with 40 mL of 50 mM aq. $NaBH_4$ for 1 h. Thereafter, the mixture was ultrasonicated for 1 h. The CRGO dispersion was then washed continually with water and dried at 60 °C for 48 h in a vacuum oven. Prior to modification, the GCE (3 mm in diameter) was first polished with 0.05 μm alumina slurry using a Buehler polishing kit. After successive sonication processes in ethanol and double distilled water, the sample was dried. Then, about 5 μL of the CRGO suspension was cast onto the pretreated GCE surface and dried for 2 h at 40 °C. Finally, the modified GCE was immersed in 0.05 M PBS (pH 7) to remove the loosely adsorbed CRGO to be used for the electrochemical studies Fig. 1.

3. Results and discussion

3.1. Characterization of CRGO

The morphology of the CRGO was characterized by SEM. Fig. 2(A) shows the structure of the CRGO which consisted of crumpling,