Voltammetric Determination of Bergenin with Graphene Modified Glassy Carbon Electrode

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This study utilized a sensitive electroanalytical method for the detection of bergenin in phosphate buffer solution (pH 5.0) with a graphene (GR) and chitosan (CS) nanocomposite modified glassy carbon electrode (GCE). Characterization of CS-GR/GCE was carried out by electrochemical impedance spectroscopy. Electrocatalytic oxidation of bergenin on CS-GR/GCE was greatly enhanced with the improvement of the anodic peak current, which allowed the development of a voltammetric sensor for bergenin determination. Under the optimized conditions with differential pulse voltammetry, the anodic peak responses enhanced linearly with bergenin concentration from 2.0×10^{-8} mol/L to 8.0×10^{-6} mol/L with the detection limit of 3.6 nmol/L (3σ). This electrochemical sensor showed high sensitivity and selectivity, excellent reproducibility and good stability. The analytical application of this sensor was successfully proved by the Tabellae Bergenini Compositae sample detection.

1. INTRODUCTION

Bergenin((2R,3S,4S,4aR,10bS)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a, 10b -tetrahydropyrano[3,2-c]isochromen-6(2H)-one, $C_{14}H_{16}O_9$) belongs to isocoumarins that is present rich in various plants such as *Bergenia purpurascens* [1]*, Peltophorum pterocarpum flowers* [2], *Maiiotus japonicus* [3] and *Vatica mangachapoi blanco* [4], which has lots of pharmacological effects, including anti-bacterial, anti-inflammatory, anti-narcotic, anti-litiasic, anti-arthritic, anti-malarial, anti-

tumor, anti-diabetic, immunomodulatory, hepatoprotective, anti-nociceptive properties [5]. Recently, it has been exhibited that bergenin attenuated inflammatory response in lipopolysaccharide-induced acute lung injury mice, lipopolysaccharide-induced mouse mastitis and in kidney injuries of diabetic rat [1]. Since bergenin is widely applied in the field of medicine, it is very important to establish sensitive method to determine bergenin. For example Li *et al.* designed a sensor based on poly(L-lysine) and graphene (GR) modified glassy carbon electrode (GCE) for bergenin analysis [6]. Chen *et al.* fabricated poly(4-(2-pyridylazo)-resorcinol) modified GCE for bergenin detection [7]. Zhu *et al.* studied the quenching behavior of bergenin to ECL of tris(2,2'-bipyridyl)-ruthenium(II)/tri-n-propylamine system and developed a new approach for bergenin detection by this ECL inhibition phenomenon [3].

GR is defined as a two-dimensional (2D) layer of carbon atoms with a theoretical thickness of 0.34 nm. It is sp^2 hybridized and possesses many remarkable properties, such as excellent electronic transport property, mechanical stiffness and elasticity [8]. The applications of GR have been reported due to their excellent electrochemical properties [9,10]. For example, Bo *et al.* prepared a DNA biosensor based on GR paste electrode modified with prussian blue and chitosan (CS) [11]. Priya *et al.* applied heparin and CS coated on reduced graphene oxide modified GCE (hep/CS-rGO/GCE) for detection of Pb^{2+} [12]. Li *et al.* used CS-GR nanocomposite modified electrode for detection of luteolin [13]. Kumar applied palladium modified GR nanocomposite modified GCE for detection of ethanol [14]. Liu *et al.* prepared a novel amperometric nitrite sensor based on immobilization of hemoglobin (Hb) on GR nanosheet modified electrode by CS-N,N-dimethylformamide hydrogel [15]. Shin *et al.* used flower-like palladium (Pd) nanoclusters decorated GR electrodes for ultrasensitive and flexible hydrogen gas sensing [16]. Zhang *et al.* applied the GR-based materials in biomedical domain [17]. Zhang *et al.* prepared graphene oxide/Pd nanocomposite modified GCE for detection of nitrite [18]. Our group investigated electrochemical behavior of Hb with Pd-GR modified electrode [19]. However, no reports about the application of CS-GR nanocomposite modified electrode for the investigation on bergenin electrochemistry had been reported.

This paper prepared a CS-GR nanocomposite modified GCE as the working electrode, which was used to study the electrochemical behavior of bergenin with electrochemical parameters calculated. Then electrochemical method for sensitive analysis of bergenin was established and further used to detection of Tabellae Bergenini Compositae sample with satisfactory results.

2. EXPERIMENTAL

2.1. Apparatus and reagent

Electrochemical measurements were performed on a CHI 660D electrochemical analyzer (Shanghai CH Instrument, China) with conventional three-electrode model. A modified GCE was used as the working electrode with a platinum (Pt) wire and saturated calomel electrode (SCE) as the counter electrode and the reference electrode, respectively.

Bergenin (Xi'an Yuquan Biotech. Ltd. Co., China), CS (Dalian Xindie Ltd. Co., China), Tabellae Bergenini Compositae (Yunnan Mingding Pharmaceutical Co., China) and GR (Taiyuan Tanmei Ltd. Co., China) were used as received. The supporting electrolyte was 0.04 mol/L phosphate buffer solution (PBS), which was deoxygenized before experiment. All the solutions were prepared with doubly distilled water and other chemicals used were of analytical reagents grade.

2.2. Fabrication of CS-GR/GCE

Prior to modification, GCE was successively polished to a mirror surface using 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with water. After successive sonication in a 1:1 (v/v) nitric acid solution and ethanol, GCE was rinsed with doubly distilled water and dried in room temperature.

GR (5 mg) and CS (10 mg) were dispersed into a 1.0 % acetic acid solution with a magnetic stirring to get the CS-GR mixture. Then CS-GR mixture was added on the surface of GCE and dried in the air to get CS-GR/GCE.

2.3. Electrochemical investigation

Electrochemical impedance spectroscopy (EIS) of different electrodes was performed in a 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol/L KCl with the frequency from 10^5 Hz to 0.1 Hz. The bergenin standard or sample solution was mixed with 0.04 mol/L PBS buffer (pH 5.0). Cyclic voltammetry was scanned in the range from 0 to 1.0 V at the scan rate of 0.1 V/s and differential pulse voltammetry was recorded from 0.1 V to 0.8 V with pulse amplitude as 50 mV, pulse width as 0.2 s and pulse period as 0.5 s.

3. RESULTS AND DISCUSSION

### 3.1. EIS of the modified electrodes

EIS is a commonly used method for the interfacial properties investigation and the electron transfer resistance (R_{et}) can reflect the interfacial resistance. This sensor assembly was characterized by EIS using GCE and CS-GR/GCE system with the Nyquist plots of different systems shown in Figure 1. The R_{et} value of GCE (curve a) equaled to 87.4 Ω and that of CS-GR/GCE (curve b) decreased to 44.4 Ω, which indicated that CS-GR nanocomposite exhibited good conductivity with the interfacial resistance decreased. GR has high conductivity with large surface area, therefore the conductivity was increased with the resistances decreased.
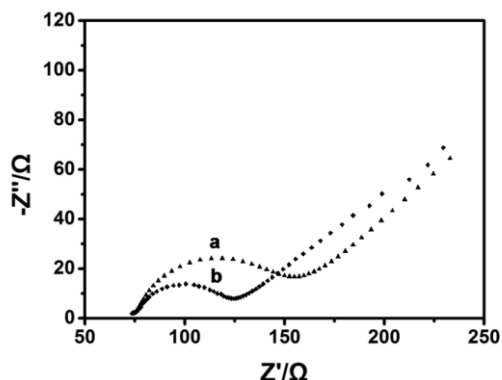
Figure 1. EIS of (a) GCE and (b) CS-GR/GCE in the presence of 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl solution with frequency range from 10^5 to 1.0 Hz.

3.2. Cyclic voltammetric behaviors of bergenin

Figure 2 depicts the typical cyclic voltammograms (CVs) of 10.0 μmol/L bergenin on CS-GR/GCE (curve a) and bare GCE (curve b) in 0.04 mol/L PBS (pH 5.0) at the scan rate of 100 mV/s. Both CS-GR/GCE and GCE showed a oxidation peak with the oxidation potentials (*Epa*) as 0.708 V and 0.729 V. The values of *Ipa* were found to be 12.8 μA and 5.18 μA respectively. The values of oxidation peak currents were about 2.47 times larger than that of GCE, which could be attributed to the presence of highly conductivity of GR. GR has many specific characteristics including low resistance, high conductivity and large surface area [20, 21], which could act as an effective mediator to promote the electrochemical reaction of bergenin. Therefore the GR modified on GCE surface was beneficial for the fastening of the electron transfer and acted as the electron bridges. Then the electrochemical analysis of bergenin was strengthened on CS-GR/GCE and it could be used to detect bergenin.
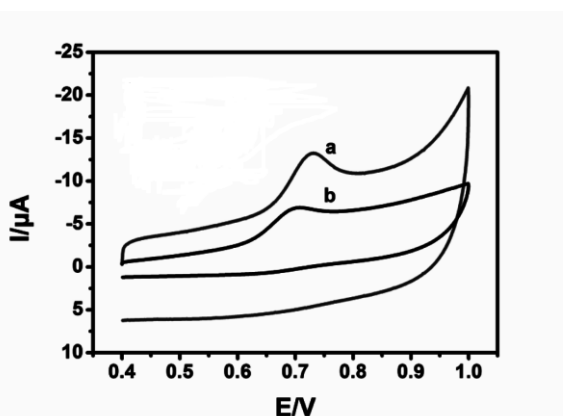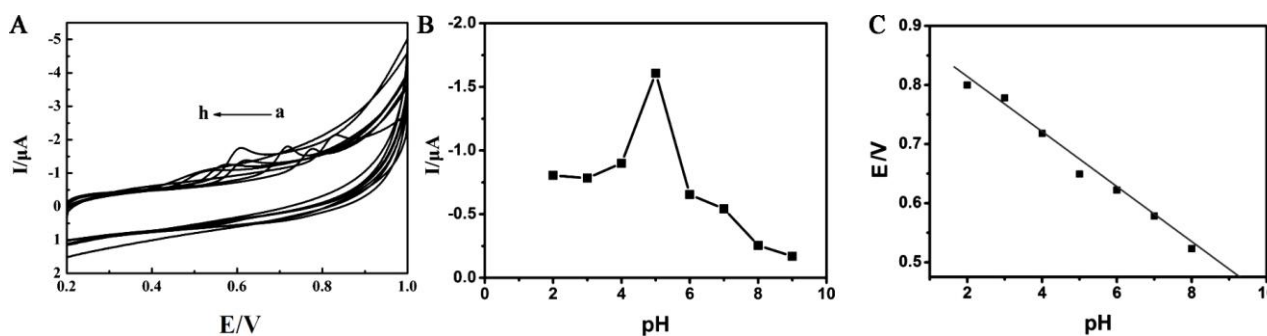

Figure 2. Cyclic voltammograms of 10.0 μmol/L bergenin on (a) CS-GR/GCE and (b) GCE in pH 5.0 PBS at the scan rate of 100 mV/s.

3.3. Electrochemical investigation

The effect of pH values to the electrochemical responses of 10.0 μmol/L bergenin on CS-GR/GCE was checked in the range from 2.0 to 9.0 with the curves shown in Figure 3A. It can be found

that the oxidation peaks appeared at different buffer pH, indicating that buffer pH influenced the electrochemical reaction. The peak currents increased with the increase of the buffer pH value until it reached 5.0 and then decreased when the pH value exceeded to 5.0. The relationship of the oxidation peak current with buffer pH was plotted with the results shown in Figure 3B and the biggest value appeared at pH 5.0, which could provide enough protons for the electrochemical reaction in the oxidation process of bergenin. Moreover, Figure 3C showed that the anodic peak potential changed linearly with pH values and the equation was $E^{0'}$(V) = -0.0466pH + 0.908 (γ=0.993). The slope value (-46.6 mV/pH) was approximately close to the theoretical value (-59 mV/pH), indicating that the same amounts of electrons and protons took part in the electrode reaction of bergenin.


Figure 3. (A) Cyclic voltammograms of 10.0 μmol/L bergenin on CS-GR/GCE with different buffer pH (from a to h: 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0) at the scan rate of 100 mV/s; (B) Relationship of I_{pa} against pH ; (C) Relationship of the oxidation potential (E_{pa}) against pH.

The influence of scan rates on the electrooxidation of 10.0 μmol/L bergenin in pH 5.0 was further examined at CS-GR/GCE. The superimposed cyclic voltammograms with the increase of scan rates from 10 mV/s to 400 mV/s was shown in Figure 4A. The oxidation peak currents increased linearly with the scan rates and the relationship of Ipa with v was obtained in Figure 4B. The linear regression equation was Ipa (μA) = -9.64v + 0.124 (V/s) (γ=0.999), which strongly suggested that the oxidation reaction of bergenin was an adsorption controlled electrode process. In other words, bergenin underwent a surface electrochemical reaction on CS-GR/GCE due to the large surface for adsorption.
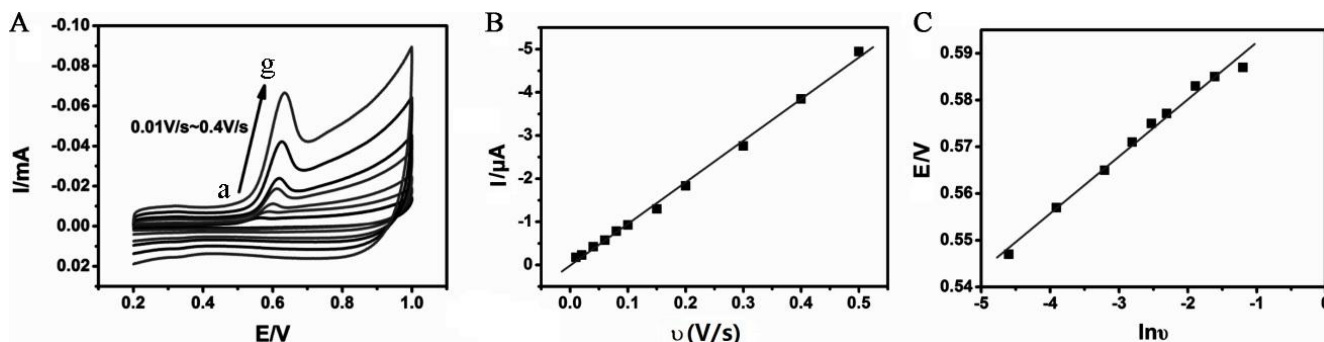
Figure 4. (A) Cyclic voltammograms of 10.0 μmol/L bergenin on CS-GR/GCE in pH 5.0 PBS at various scan rates (from a to g as 10, 40, 80,150, 200, 300, 400 mV/s); (B) Linear relationship of the oxidation peak currents versus scan rate (v); (C) Linear relationship of the oxidation peak potentials versus ln$v$.

The oxidation peak potential (*Epa*) shifted positively with the increase of scan rate and a linear relationship with the natural logarithm of scan rate (*lnυ*) was shown in Figure 4C with the regression equation as *Epa* (V) = 0.012ln*υ* (V/s) + 0.6843 (γ= 0.995). According to Laviron's equation [22], electrochemical parameters could be calculated with the electron transfer coefficient (*α*) as 0.5, the electrode reaction standard rate constant (*k_s*) as 1.6 s^{-1} and the electron transfer number (*n*) as 2. The results indicated that the electrode reaction of bergenin on CS-GR/GCE was an adsorption driven two-electron and two-proton process. A proposed electrode oxidation equation of bergenin was expressed in Scheme 1, which involved the oxidation mechanism of the phenolic hydroxy on benzene ring to carbonyl groups [7].
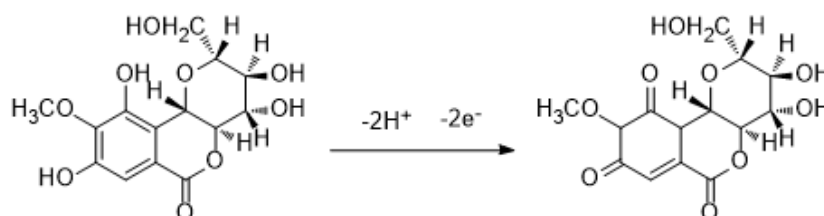
Scheme 1. Electrochemical oxidation equation of bergenin

3.4. Calibration curve

The effect of different concentration of bergenin on CS-GR/GCE was investigated by differential pulse voltammetry (DPV) with the typical curves shown in Figure. 5A. The oxidation peak currents increased with bergenin concentration from 0.02 μmol/L to 8.0 μmol/L and the linear regression equation was calculated as *Ipa*(μA)= 0.098 C (μmol/L) - 0.280 (γ=0.998). The detection limit of bergenin with CS-GR/GCE was calculated as 3.6 nmol/L based on the equation of $3S_0/S$, where 3 is the factor 99% confidential level, S_0 is the standard deviation of the blank measurements without bergenin (n= 9) and S is the slope value of the calibration curve.
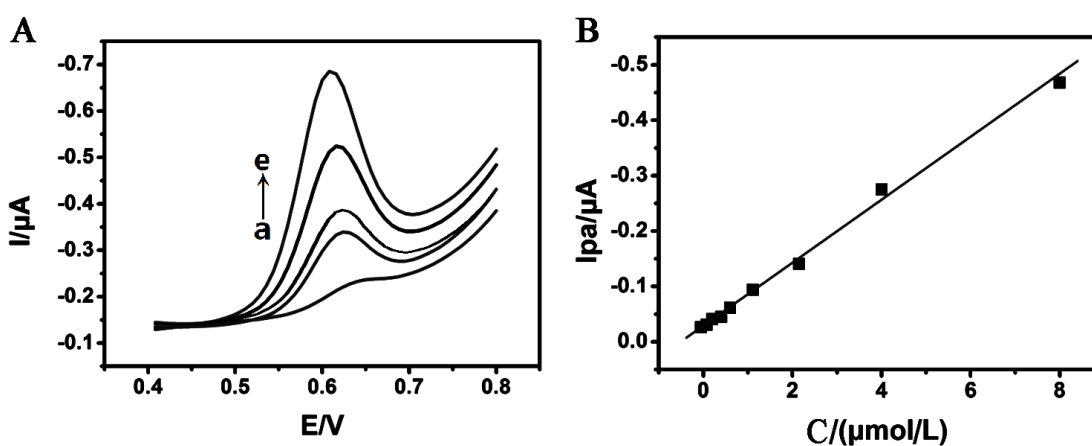
Figure 5 (A) DPV curves of different bergenin concentrations on CS-GR/GCE in 0.04 mol/L pH 5.0 PBS (from a to e: 0.2, 0.8, 2.0, 4.0, 8.0 μmol/L); (B) Calibration curve for 0.02~8.0 μmol/L bergenin.

A comparison of the analytical parameters of different modified electrodes for the electrochemical detection of bergenin was summarized in table 1. It can be seen that the proposed method has a wider detection range and lower detection limit for bergenin detection, which proved that CS-GR/GCE could be used for bergenin detection.

Table 1. Comparison of the analytical parameters for electroanalysis of bergenin.

Electrode	Linear range (mol/L)	Detection limit (mol/L)	Reference
Poly(L-lysine)/GCE	4.0×10^{-8} - 5.0×10^{-6}	1.0×10^{-8}	6
PAR/GCE	2.0×10^{-7} - 1.2×10^{-5}	2.0×10^{-8}	7
MWCNT/CPE	7.0×10^{-7} - 1.0×10^{-5}	2.0×10^{-8}	23
CS-GR/GCE	2.0×10^{-8} - 8.0×10^{-6}	3.6×10^{-9}	this work

GCE: Glassy carbon electrode, PAR: 4-(2-pyridylazo)-resorcinol, MWCNT: Multi-wall carbon nanotubes, CPE: Carbon paste electrode.

3.5. Interference

A study of the interference to the determination of 1.0 μmol/L bergenin in pH 5.0 PBS and the data were listed in table 2. These substances include amino acids and metal ions with the concentrations of 300.0 μmol/L, which was 300-fold lighter than that of bergenin. It was interesting to note that all the coexisting compounds did not affect electrochemical response of bergenin with the deviation below ±5％. This established the possibility of real sample analysis without any interference of these coexisting substances.

Table 2. Influence of coexisting compounds on the detection of 1.0 μmol/L bergenin (n=3)

Coexisting Substances	Concentration (μmol/L)	Relative error (%)	Coexisting substances	Concentration (μmol/L)	Relative error (%)
L-Alanine	300.0	3.16	$BaCl_2$	300.0	3.23
L-Cystine	300.0	3.65	NaCl	300.0	4.12
L-Lysine	300.0	3.41	$NiCl_2$	300.0	4.47
L-Threonine	300.0	2.53	$CdCl_2$	300.0	1.46
L-Arginine	300.0	2.08	$MnCl_2$	300.0	2.25

3.6. Reproducibility and stability

The reproducibility of the proposed bergenin sensor was evaluated by examining the DPV responses after continuous scanning in pH 5.0 PBS for 40 cycles, which gave nearly no decrease of the background response. It was also observed that CS-GR/GCE was stable at room temperature for 10 days and the response was about 90% of the initial value. These results demonstrated that CS-GR/GCE had good stability and reproducibility for electrochemical application.

3.7. Analytical application

To evaluate the practical analytical applications of CS-GR/GCE for the bergenin detection, Tabellae Bergenini Compositae purchased from Yunnan Mingding Pharmaceutical Co. (K130823) was detected. The procedure for preparing bergenin samples was as following procedure: fifteen pieces of Tabellae Bergenini Compositae were finely pulverized with the powder dissolved with ethanol. Then the mixture was sonicated for 30 min and refluxed for 120 mins, the turbid liquid was filtered carefully and the clear filtrate was diluted by pH 5.0 PBS to get the sample solution, which were detected by the experimental method with the content shown in table 3. The recovery test was checked by the addition of the standard bergenin solution in the sample solution, which was in the range from 98.8% to 103.2 %, indicating the real application of the proposed procedure for bergenin analysis.

Table 3. Determination of bergenin concentration in Tabellae Bergenini Compositae（n=3）

Samples	Detected (μmol/L)	Added (μmol/L)	Total (μmol/L)	Recovery (%)	RSD(%)
1	0.992	0.50	1.51	103.2	1.3
2	0.981	1.00	1.98	99.8	1.5
3	1.017	1.50	2.49	98.8	1.5

4. CONCLUSION

In summary, the present work had developed a new electrochemical senor based on CS-GR/GCE for bergenin detection, which had excellent electrochemical performance such as high sensitivity and selectivity, excellent reproducibility and good stability. Under the optimized conditions with differential pulse voltammetry, detection of bergenin in the linear concentration range from 2.0×10^{-8} to 8.0×10^{-6} mol/L was obtained with a detection limit of 3.6 nmol/L. Meanwhile, this modified electrode was further used for real sample tests by the successful detection of bergenin content in Tabellae Bergenini Compositae with the satisfactory results.

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