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A novel sensitive detection platform for antitumor herbal drug aloe-emodin based on the graphene modified electrode

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ABSTRACT

This paper has presented a novel strategy to carry out direct and sensitive determination of antitumor herbal drug aloe-emodin in complex matrices based on the graphene-Nafion modified glassy carbon (GN/GC) electrode. This proposed modified electrode showed good electrochemical response towards aloe-emodin (AE). Compared with the multiwall carbon nanotubes (MWCNTs) modified electrode, the GN/GC electrode has the advantages of higher sensitivity and lower cost. Under the optimized conditions, the calibration curve for AE concentration was linear in the range from 5 nmol/L to 1 μmol/L with the detection limit of 2 nmol/L. In addition, the practical analytical performance of the GN/GC electrode was examined by evaluating the selective detection of AE in natural aloe extracts and human urine samples with satisfied recovery. Therefore, the GN/GC electrode may hold great promise for fast, simple and sensitive detection and biomedical analysis of AE in complex matrices.

1. Introduction

Herbal medicines were one of the major resources for healthcare in early eras. Currently, they are gaining more attention from modern pharmaceutical institutes, as scientists become aware that herbal medicines are almost infinite resources for drug development [1]. Herbal medicines containing anthraquinones are being increasingly used for cosmetics, food and pharmaceuticals due to their wide therapeutic and pharmacological properties. Aloe-emodin (AE, Scheme 1) is a naturally occurring anthraquinone derivative present in the leaves and roots of a number of plants, such as aloe vera and rheum palmatum. Some studies have indicated that AE has a number of pharmacological effects, including antiviral, antimicrobial, and laxative activities [2,3]. In addition, AE has also been reported to exhibit an anticancer activity in several tumor cells, including lung squamous cell carcinoma [4], hepatoma cells [5], leukemia cells [6] and neuroectodermal cells [7]. However, the therapeutic action and toxicity of AE are still the subjects of considerable research. It prompts us to develop an analytical assay for the determination of AE in complex matrices such as biological fluids and tissues. Up to now, various techniques such as high performance liquid chromatography (HPLC) [8], synchronous fluorescence spectroscopy [9], gas chromatography/mass spectrometry [10], capillary electrophoresis [11] and micellar electrokinetic capillary chromatography [12] have been reported for the determination of AE. Compared with the above methods, the electrochemical methods have the advantages of celerity, simplicity, high sensitivity and low cost. Furthermore, they can unveil the messages about the reaction mechanism and the dynamics parameters of analytes. Moreover, some medicinal activity of AE is related to its redox activity. Therefore, in the investigation of AE, voltammetric method is very helpful in understanding the pharmacological effect and the antineoplastic mechanism.

Graphene, a two-dimensional carbon material, is a new member of the carbon family and has attracted great attention for both fundamental science and applied research [13]. One of the factors that make graphene so attractive is its low energy dynamics of electrons with atomic thickness [14]. Graphene was reported as a semiconductor with zero band gap and high carrier mobilities and concentrations and shows nearly ballistic transport at room temperature [15]. These unusually electronic properties make it ideal for designing nanoelectronic applications. Recently, graphene-based materials have been used as an advanced nanoelectrocatalyst for constructing electrochemical biosensors. Papakonstantinou and coworkers demonstrated that multilayer graphene nanoflake films showed excellent electrocatalytic activity for simultaneously determining dopamine (DA), ascorbic acid (AA), and uric acid (UA) [16]. Li and coworkers reported the graphene had fast electron-transfer (ET) kinetics and excellent electrocatalytic activity toward oxygen reduction and β-nicotinamide adenine dinucleotide (NADH) [17]. Niu and coworkers reported the use of the polyvinylpyrrolidone-protected graphene to fabricate a high-

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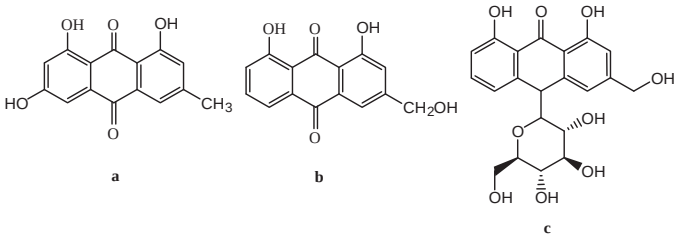


Scheme 1. Structures of (a) emodin, (b) aloe-emodin (AE) and (c) aloin.

performance glucose biosensor [18]. Dong and coworkers reported electrochemical reactivity of the four free bases of DNA at the chemically reduced graphene oxide modified glassy carbon electrode was greatly enhanced [19a]. However, it has not been reported to detect antitumor herbal drug using graphene modified electrode.

In this paper, we reported a novel strategy for sensitive determination of active antitumor drug (aloe-emodin, in this case) in medicine herbs based on the graphene-Nafion modified glassy carbon (GN/GC) electrode. The hybrid composite of graphene–Nafion was prepared and modified on the surface of glassy carbon (GC) electrode. The electrochemical behavior of AE was studied on the modified electrode. Compared with the multiwall carbon nanotubes (MWCNTs) modified electrode, the GN/GC electrode has the advantages of higher sensitivity and lower cost. Moreover, the GN/GC electrode could examine AE in real samples with good results.

2. Experimental

2.1. Apparatus and measurements

All electrochemical measurements were performed on an electrochemical workstation 660C (CH Instrument, China) with a conventional three-electrode system comprised of a platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference, and modified glassy carbon (GC) electrode diameter (3.0 mm) as working electrode at room temperature.

Atomic force microscopic (AFM) images were taken out using a Nanoscope III a multimode atomic force microscope (Veeco Instruments, USA) in tapping mode to simultaneously collect height and phase data. Raman spectra were recorded at ambient temperature on a Renishaw InVia Raman spectrometer with an excitation laser at 785 nm.

2.2. Reagents

Aloe-emodin, emodin and aloin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (China). They were dissolved in ethanol and stored in the dark. The multiwall carbon nanotubes (MWCNTs) were purchased from Shenzhen Nanotech Port Co., Ltd. (China), and then subjected to acid treatment as reported by Pan and coworkers [20]. Nafion (20 wt.%) was purchased from Sigma. All the phosphate buffered saline (PBS) buffer solutions contained 20 mM NaCl. The pH was adjusted with H_3PO_4 or NaOH. Other reagents were of analytical reagent grade and used without further purification. All solutions were prepared with MilliQ water (18 MΩ cm resistivity) from a Millipore system.

2.3. Synthesis of graphene

Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers method [21]. The reduction of GO to graphene was conducted according to the references [22]. Briefly, 6.2 mg GO was dispersed in 12 mL H_2O. Exfoliation of GO was achieved by the ultrasonication of the solution. The obtained homogeneous dispersion was mixed with 22 mL H_2O, 15 μL hydrazine solution and 80 μL 25 wt.% ammonia solution in a flask. After being vigorously shaken, the flask was put in a water bath (95 °C) for 1 h with consistently stirring.

Graphene dispersions prepared according to the above procedure were used for further characterization and film fabrication in this work.

2.4. Preparation of graphene–Nafion modified electrode, MWCNTs–Nafion modified electrode and Nafion modified electrode

Before modification, the GC electrode was successively polished to a mirror finish using 1, 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with water. After successive sonication in 1:1 nitric acid, acetone, and doubly distilled water, the electrode was rinsed with doubly distilled water. The cleaned GC electrode was dried with nitrogen steam for the next modification.

50 μL of 0.5 wt.% Nafion–alcohol solution was added into 150 μL of graphene sample solution with agitation to give a homogeneous solution (GN solution). The GN/GC electrode was prepared by casting 5 μL of GN solution (about 0.036 mg L^{-1}) on the GC electrode surface. The Nafion modified electrode and the MWCNTs-Nafion modified electrode were treated by the same way as the GN/GC electrode.

2.5. Sample solution preparation

Aloe extracts were prepared from the gel filet portions of leaves of Aloe barbadensis Miller, using methods as previously described with a slight modification [23]. In brief, the whole leaves (50 g) of Aloe vera were cut into thin pieces and broken up with a vortex mixer. The products were decanted into a round-bottomed flask containing 200 mL of ethanol. The mixtures were allowed to shake intensively for 5 h and then filtered. The filtrate was concentrated down to 10 mL via rotary evaporation and filtered through a 0.45 μm membrane prior to use.

Urine samples (obtained from laboratory personnel) were filtered through 0.45 μm membranes and then diluted with PBS buffer by 5-fold.

2.6. Analytical procedure

After 5 mL of pH 7.0 PBS (or urine samples) were placed in the electrochemical cell, the required volume of AE standard solution or aloe sample solution was added with a micropipette and deaerated with highly pure nitrogen for 3 min prior to measurements. After that, the electrodes were placed into the test solution and then the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were scanned after being adequately stirred for 180 s at open circuit. CV scanning was performed in the appropriate potential range, the sample interval of 0.001 V and the standing time of 2 s. DPV was performed in the appropriate potential range with pulse width of 0.05 V, pulse frequency of 0.05 s, pulse cycle of 0.2 s, pulse interval of 0.004 V and standing time of 2 s.

3. Results and discussion

3.1. Characterization of graphene

The structure and morphology of the resulting graphene were characterized using AFM. Fig. 1A indicated that the graphene sheets are almost single-layer. And the average thickness of single-layer graphene sheets is about 0.8 nm. This value is smaller than that of GO before the chemical reduction (~1.2 nm, Fig. 1B) and matches well with the reported apparent thickness of graphene [19a].

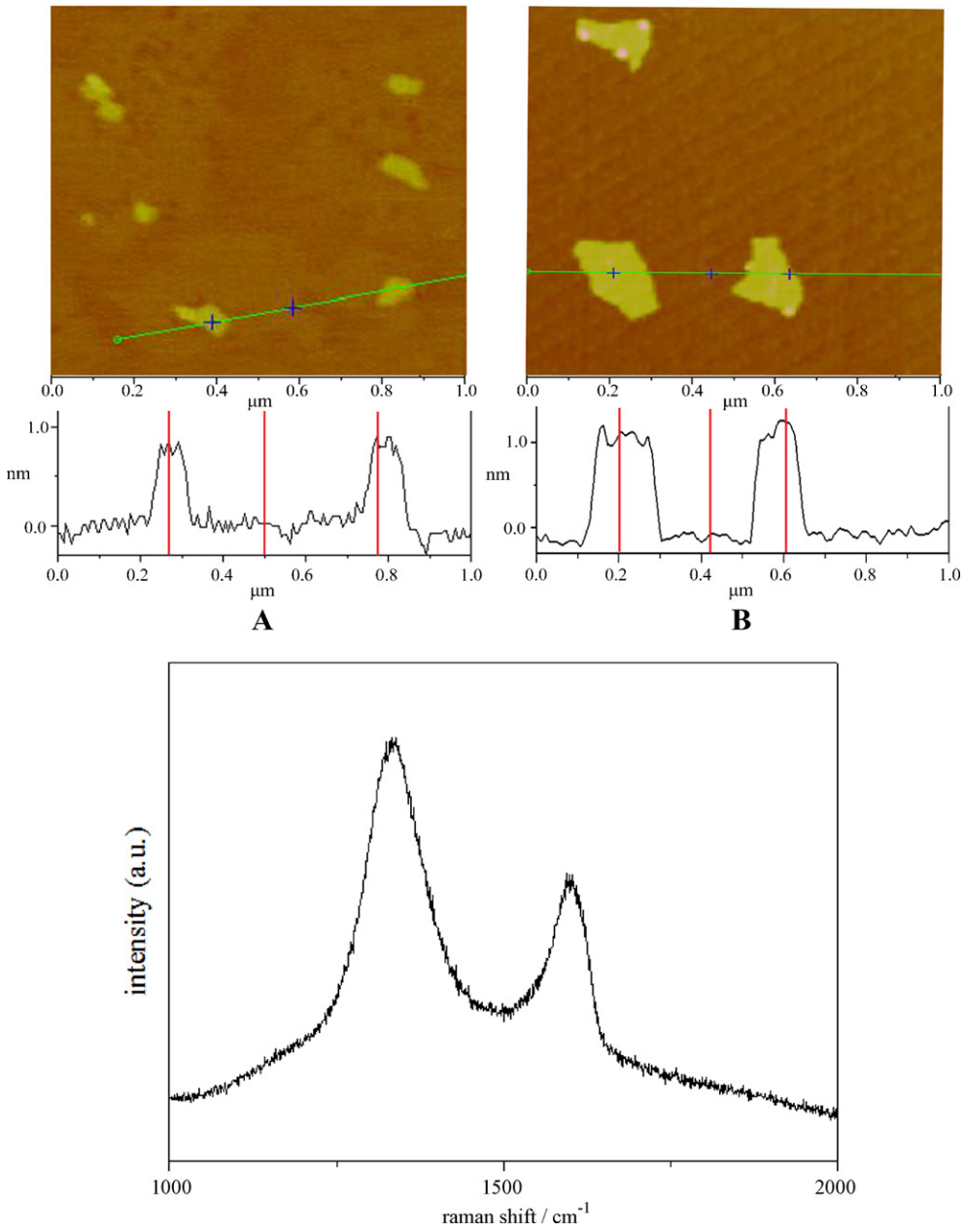


Fig. 1. (A) Tapping mode AFM images of graphene, (B) AFM images of GO on freshly cleaved mica substrates and (C) Raman spectra of graphene.

To obtain further information on the structure and topology of graphene, Raman spectroscopy was carried out. The Raman spectrum of the graphene in Fig. 1C displays a D band at 1337 cm^{-1} and G band at 1597 cm^{-1}. The D band arises from sp^3-hybridized carbon, and the peak represents the E2g zone center mode of the crystalline graphite [19b]. This means the relative intensity ratio of the D and G lines (I_D/I_G ratio) is proportional to the number of defect sites in graphite carbon. Raman spectra in Fig. 1C show the I_D/I_G ratio of graphene is about 1.64, indicating there are significant edge-plane-like defective sites existing on the surface of graphene [19b].

3.2. Electrochemical properties of GN/GC electrode

$Fe(CN)_6^{3-/4-}$ is close to an ideal quasi-reversible system on carbon electrodes. Fig. 2 shows the CV obtained for $Fe(CN)_6^{3-/4-}$ at GN/GC electrode, MWCNTs–Nafion modified electrode and Nafion modified electrode. The anodic and cathodic currents at the Nafion modified electrode were small and the peak-to-peak separation was 180 mV (curve c), demonstrating that the one-electron redox behavior of $Fe(CN)_6^{3-/4-}$ was blocked by Nafion film. The peak current of $Fe(CN)_6^{3-/4-}$ at the GN/GC electrode (curve a) was higher than that at the MWCNTs–Nafion modified electrode (curve b). Meanwhile, the peak-to-peak separation (ΔE) was 112 mV at GN/GC electrode, which was smaller than that at the MWCNTs–Nafion modified electrode (141 mV). The results indicated that graphene can increase the conductivity of the electrode and enhance the reversibility of the electron transfer process.

3.3. Electrochemical behavior of AE at GN/GC electrode

The CVs of AE in pH 7.0 PBS at different electrodes are indicated in Fig. 3. AE showed a sluggish and much small CV cathodic peak response at bare GC electrode. At the MWCNTs–Nafion modified

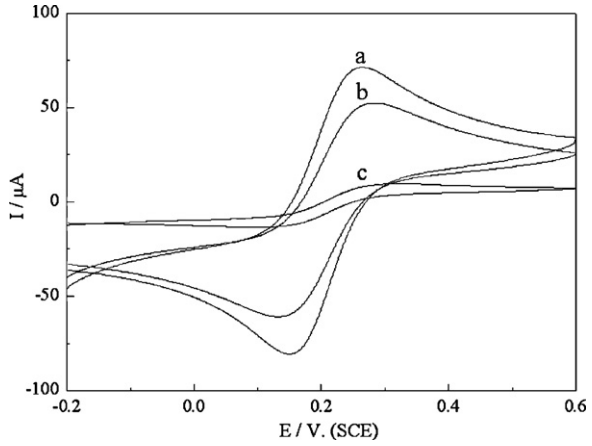


Fig. 2. CV of (a) the GN/GC electrode, (b) the MWCNTs–Nafion modified electrode and (c) the Nafion modified electrode in 5.0 mM $Fe(CN)_6^{3-/4-}$ in 0.5 M KCl. Scan rate, 0.1 V s^{-1}.

electrode, the anodic peak potential (E_{pa}) was −0.578 V and the cathodic peak potential (E_{pc}) was −0.618 with ΔE_p = 40 mV. However, a pair of symmetrical, Gaussian-shape peaks was observed at GN/GC electrode. The cathodic peak potential (E_{pc}) and the anodic peak potential (E_{pa}) appeared at −0.604 V and −0.589 V, respectively, ΔE_p = 15 mV, I_{pa}:$I_{pc} \approx 1$, which indicated that the reaction was a reversible electron transfer process. Obviously, the peak currents were higher and peak separation was smaller at GN/GC electrode than that of MWCNTs–Nafion modified electrode. It could be concluded that graphene had the better ability to promote electron-transfer reactions than MWCNTs due to the unique physicochemical properties of graphene (the single-sheet nature, high conductivity, large surface, etc.). Such significant current enhancement would allow developing a high sensitive electrochemical sensor for the determination of AE.

The effect of scan rates (ν) on the peak current of AE was studied. The peak current (I_{pa} and I_{pc}) increased with the scan rate, and was proportional to scan rate over the range of 10–100 mV s^{-1} (I_{pa} (μA) = 39.9ν − 117.4 (mV s^{-1}), R^2 = 0.998; I_{pc} (μA) = −40.1ν − 220.8 (mV s^{-1}), R^2 = 0.995). This meant that the electrochemical reaction was an adsorption-controlled process. In addition, with the increasing of υ, the peak potential almost did not vary with the scan rates,

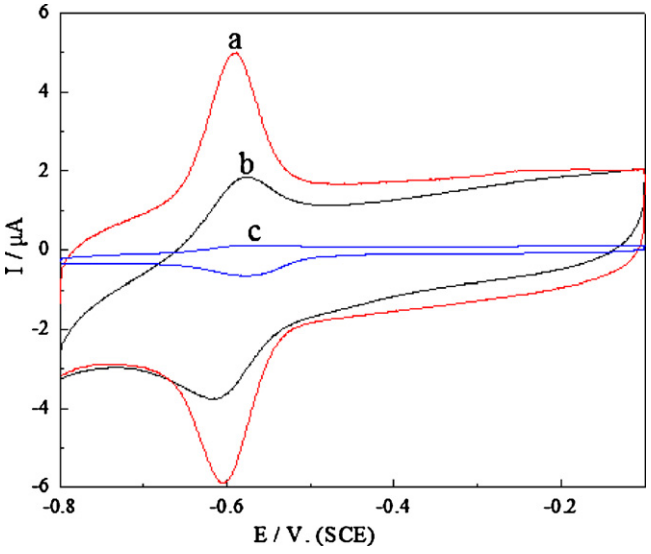


Fig. 3. CV of (a) the GN/GC electrode, (b) the MWCNTs–Nafion modified electrode and (c) the bare GC electrode in 1 μM AE in pH 7.0 PBS buffer, scan rate: 50 mV s^{-1}.

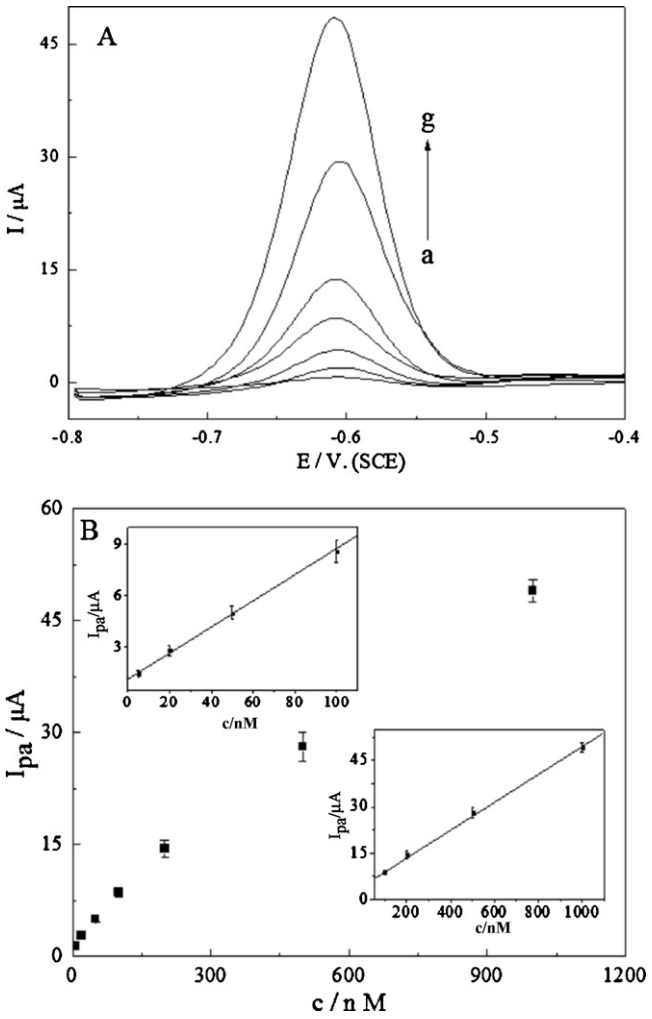


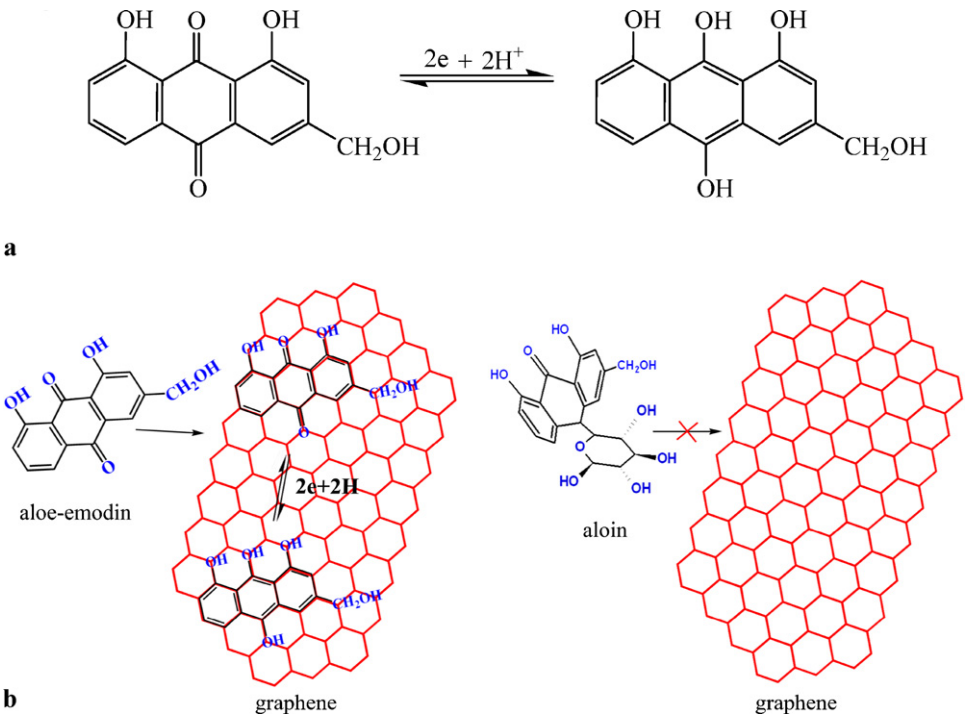
Fig. 4. (A) Background-subtracted DPV of AE at GN/GC electrode in pH 7.00 PBS. AE concentration (nM): (a) 5; (b) 20; (c) 50; (d) 100; (e) 200; (f) 500; (g) 1000. (B) Plots of I_{pa} versus C, showing two linear ranges. Error bars = ±relative standard deviation.

indicating that the electrochemical reaction was reversible. According to Laviron theory [24], the number of electrons involved in the reaction was calculated to be 2.

The redox process of anthraquinone derivatives was frequently related to proton participation, therefore the electrochemical behavior of AE at different pH values was studied. The CV curves indicated that the peak potential of AE at GN/GC electrode shifted toward negative direction with increasing of pH, which indicated that proton transfer was involved in the electrode reaction process. E^0 is the formal potential, the linear regression equation between E^0 and pH was E^0 = -52.02pH - 254.6, with a slope of 52.02 = 59m/n (n is the number of electrons transfer, m is the number of protons transfer), i.e. $m = n = 2$ (see above). Hence, two electrons and two protons were involved in this reaction. Therefore, electrode reaction mechanism of AE could be proposed in Scheme 2a.

3.4. Determination of AE

Since differential pulse voltammetry (DPV) has a much higher current sensitivity and better resolution than cyclic voltammetry, it was used in the determination of AE concentrations at GN/GC electrode and the estimation of the lower limit of detection. The I_{pa} of AE was measured in pH 7.0 PBS, and plotted against the bulk concentrations of AE after background subtraction. Statistical analysis of I_{pa} versus the concentrations of AE revealed two linear ranges (Fig. 4



Scheme 2. (a) Electrode reaction mechanism of AE. (b) A tentative mechanistic pathway proposed for the redox of AE and aloin at GN/GC electrode.

inset). The I_{pa} linearly increased with AE concentration from 5 to 100 nM with a calibration function of I_{pa} (μA) = 0.0745C + 1.1865, R^2 = 0.9986 and from 100 nM to 1000 nM with a calibration function of I_{pa} (μA) = 0.0444C + 5.0512, R^2 = 0.9980. The detection limit (defined as 3σ, where σ is the standard deviation of the blank) was 2.0×10^{-9} mol/L. This detection limit was better than the reported gas chromatography/mass spectrometry method [10] and capillary electrophoresis method [11]. In addition, it is comparable with that of electrochemical sensor based on liquid-type carbon paste electrode [25]. The relative standard deviation of the same electrode in 10 successive scans was 1.2% for 1 μM AE and 2.3% for 5 different electrodes, indicating that GN/GC electrode had an excellent reproducibility.

3.5. Separation of electrochemical responses to AE, aloin and emodin at GN/GC electrode

We used GN/GC electrode for the selective determination of AE in the presence of emodin and aloin. Emodin and aloin are the effective herbal components in aloe, which were traditionally used in China for treating various ailments. They have the similar molecular structures with AE (Scheme 1). Fig. 5 shows DPV for a mixture of 2 μM aloe-emodin, emodin and aloin at different electrodes. The oxidation peaks of AE and emodin became well resolved and were separated by about 324 mV at the GN/GC electrode (curve a), which enabled a highly selective and simultaneous determination of AE and emodin. Moreover, the peak current of AE and emodin at the GN/GC electrode was much higher than that at the MWCNTs–Nafion modified electrode (curve b) and the bare GC electrode (curve c). Furthermore, no oxidation peak of aloin was observed at GN/GC electrode. It may be due to the glycosyl group of aloin, which impeded the adsorption of aloin on graphene surface. A tentative mechanistic pathway proposed for the redox of AE and aloin at GN/GC electrode was illustrated in Scheme 2b. And we presumed that the enhancement of the molecule's hydrophilicity could also decrease its adsorption on graphene surface.

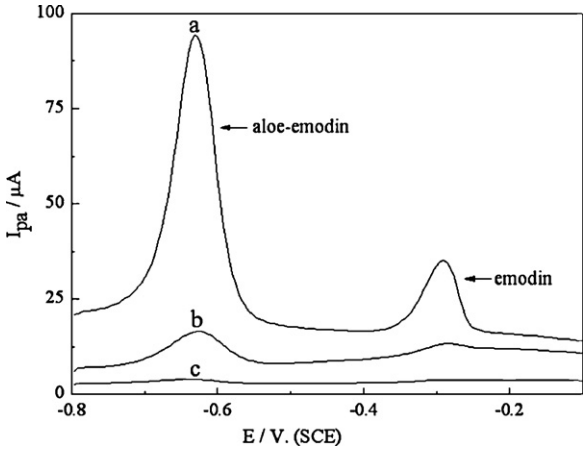


Fig. 5. Background-subtracted DPV of 2 μM aloe-emodin, emodin and aloin at (a) the GN/GC electrode, (b) the MWCNTs–Nafion modified electrode and (c) the bare GC electrode in 0.05 M PBS buffer (pH 7.0).

3.6. Interferences

To evaluate the interferences of some foreign species on the determination of AE, a systematic study was carried out. As indicated in Table 1, no interferences were observed for the following

Table 1
Influence of potential interferences on the response of 1 μM AE.

Interferences	Concentration	Signal change (I_{AE} = 100%)
Na^+	0.1 M	+2.56
K^+	0.1 M	−2.71
Starch	0.1 mg/mL	−2.63
Dextrin	0.1 mg/mL	−0.09
Vitamin A	1 mmol/L	−1.16
Ascorbic acid	1 mmol/L	−1.31
Uric acid	1 mmol/L	−2.52

Table 2
Determination of AE in the aloe vera extracts.

Aloe vera extracts				Spiked aloe vera extracts			
Detected by HPLC[2] (μM)	Detected by this method[a] (μM)	Recovery of this method (%)	R.S.D. (%)	AE added (μM)	AE found (μM)[a]	Average recovery (%)	R.S.D. (%)
20.8	21.5	103.4	1.9	0.5	0.509	101.8	2.1
32.5	33.2	102.2	2.3	0.8	0.810	101.3	1.8
46.2	46.9	101.5	2.4	1	0.989	98.9	2.6

[a] Average of five determinations.

Table 3
Determination of AE in the spiked urine samples.

Spiked urine samples			
AE added (μM)	AE found (μM)[a]	Average recovery (%)	R.S.D. (%)
0.5	0.504	100.8	2.3
0.6	0.596	99.3	1.9
0.7	0.709	101.3	2.7

[a] Average of five determinations.

compounds: Na^+, K^+, starch, dextrin, uric acid, ascorbic acid and Vitamin A, which commonly existed in the real samples, indicating that the proposed method had good selectivity for the determination of AE.

3.7. Analysis of AE in the aloe vera extracts and spiked urine samples

The GN/GC electrode was used to determine AE in the Aloe vera extracts. The results were shown in Table 2. Meanwhile, HPLC was also used to detect AE to testify the accuracy of the proposed method. The results obtained by the GN/GC electrode were in good agreement with the method of HPLC. Furthermore, in order to establish the suitability of the proposed method, known amounts of the standard AE were added into the analytical solution of aloe vera extracts, and the same analysis procedure was applied. The recoveries indicated that the accuracy of the proposed method was acceptable. From the experimental results, it was obvious that this novel GN/GC electrode had great potential for practical sample analysis.

The practical analytical application of this method was further studied by the measurement of AE in human urine. Lang reported that 20–30% of the dose was excreted in urine after oral administration of 4.5 mg/kg ^{14}C-aloe emodin (AE) to rats [26]. So detection of AE in urine is useful to investigate the pharmacokinetic properties of AE. The human urine samples obtained from laboratory personnel were determined by the method presented above. The results of the determination were listed in Table 3. AE was successfully detected in human urine and the recovery ranges from 99.3% to 101.3%. The results suggested this method can be used to evaluate the pharmacokinetic properties of AE.

4. Conclusions

In the present work, a platform for simple, rapid and sensitive determination of AE based on GN/GC electrode was proposed. A pair of well-defined redox peaks was obtained at the GN/GC electrode. From electrochemical results, we concluded that the graphene possessed higher electron transfer ability than MWCNTs. Furthermore, the GN/GC electrode can be used for selective detection of AE in the presence of several analogs. Finally, we also demonstrated the suitability of GN/GC electrode toward selective detection of AE in natural aloe extracts and human urine samples with good recovery. Therefore, the proposed modified electrode holds great promise for investigating the pharmacokinetic properties of AE in humans.

Acknowledgements

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