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Glassy carbon electrodes modified with gelatin functionalized reduced graphene oxide nanosheet for determination of gallic acid

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Abstract. A simple approach for the preparation of gelatin functionalized reduced graphene oxide nanosheet (Gel-RGONS) by chemical reduction of graphene oxide (GO) using gelatin as both reducing agent and stabilizing agent in an aqueous solution was developed. The morphology and structure of the Gel-RGONS were examined by X-ray diffraction, transmission electron microscopy, ultraviolet–visible spectroscopy and Raman spectroscopy. Gelatin acted as a functionalizing reagent to guarantee good dispersibility and stability of the r in distilled water. Moreover, a new electrochemical sensor was developed based on Gel-RGONS modified glassy carbon electrode (Gel-RGONS/GCE). Gel-r exhibits excellent electrocatalytic activity to gallic acid (GA) oxidation. The experimental conditions such as pH, adsorption time and scan rate were optimized for the determination of GA. Under optimum conditions, the sensor responded linearly to GA in the concentration of 1.0×10^{-6} to 1.1×10^{-4} M with detection limit of 4.7×10^{-7} M at 3σ using linear sweep voltammetry (LSV). The method has been successfully applied to the determination of GA in sample of black tea.

Keywords. Reduced graphene oxide nanosheet; gelatin; gallic acid; electrocatalysis.

1. Introduction

Graphene has many novel properties, such as high surface area, excellent electrical conductivity and electron mobility at room temperature, and unique thermal and mechanical properties.[1] These characteristics drive the dreams of applying graphene in various areas, such as nanoelectronic devices,[2] transparent conductors,[3] sensors,[4] capacitors[5] and nanocomposite materials.[6] At present, great efforts have been made for the preparation of graphene oxide nanosheet (RGONS), such as micromechanical exfoliation of graphite,[7] chemical vapour deposition,[8] epitaxial growth on electrically insulating surfaces[9] and solution-based reduction of exfoliated graphene oxide (EGO).[10] Among these methods, the chemical reduction of EGO is the most commonly used approach because it has advantages of low-cost and bulk-scale production. However, the RGONS is hydrophobic and tends to form agglomerates or even re-graphitized to graphite due to the strong π–π stacking and van der Waals interactions which may limit its further biological applications.[11] Therefore, the prevention for aggregation by functionalizing RGONS is of vital importance because most of its unique properties are only associated with individual sheets.

Gelatin is a linear polypeptide that consists of different amounts of 18 amino acids which is generally prepared by the partial hydrolysis of collagen, the chief protein component

in skin, bones and white connective tissues.[12] Gelatin backbone has abundant amino side chain, which could be oxidized to nitrite; therefore, it could be naturally employed as reductants due to its mild reductive ability in the synthesis of nanomaterials.[13] Furthermore, the non-polar amino acid chain of the gelatin could immobilize on the surface of graphene through hydrophobic–hydrophobic interactions, which leads to the formation of stable dispersion of graphene.[14]

Gallic acid ($pK_a = 4.40$), GA, is one of the main natural phenolic components encountered in green tea, black tea, humid substances and several other plants.[15] The rapid and accurate detection of gallic acid is of great interest to analytical chemistry because it has not only strong anti-mutagenic, anti-carcinogenic and antioxidant activities but also potential health effects which have been found in recent times.[16] GA has a slight toxicity at low concentrations,[15] and hence its accumulation in the environment should be avoided, especially in waters where it presents a very low biodegradability.

Various methods have been applied to the determination of GA, such as flow injection analysis,[17,18] resonance light scattering,[19] thin-layer chromatography,[20] reversed phase high-pressure liquid chromatography[21] and electrochemical method.[22–26] Unfortunately spectroscopic techniques are time-consuming and laborious; chromatographic techniques are slow and expensive and the complicated instruments are also required.[16] Direct electrochemical oxidation of GA can

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provide a fast model for analysis of GA in real samples. Electrochemical reaction of GA happens on the surfaces of electrodes, so the choice of modified electrodes is very important.

A simple and environmentally friendly synthetic route for the preparation of gelatin functionalized graphene nanosheet was reported by using graphene oxide (GO) as a precursor, in which gelatin acted as not only a reducing reagent but also a functionalization reagent to guarantee good dispersibility and stability of the RGONS in distilled water. Moreover, the electrochemical sensor based on RGONS/GCE was developed to investigate the voltammetric behaviour of the GA and develop its simple, rapid and direct detection method at low concentration levels. As an example of application, the gallic acid content in black tea samples was determined with satisfactory results.

2. Experimental

2.1 *Chemicals and apparatus*

Graphite flakes were purchased from Asbury Graphite Mills, Inc. (Asbury, NJ). Chloridric acid (HCl, 37.5%), sulphuric acid (H_2SO_4, 98%), hydrogen peroxide (H_2O_2, 30%) and phosphoric acid (H_3PO_4, 60%) were obtained from Fluka Chemicals (Germany). Gelatin was obtained from Beijing Aoboxing Universeen Bio-Tech Co. Ltd. (Beijing, China). Potassium permanganate was purchased from Tianjin Jiang-tian Chemical Reagent Co. Ltd. in China.

Electrochemical measurements were performed with an Autolab Potentiostat/Galvanostat (Netherlands) coupled with a Pentium IV personal computer. The three-electrode system consists of a Gel-RGONS/GCE as working electrode, Ag|AgCl|KCl$_{3M}$ as the reference electrode, and a platinum wire as an auxiliary electrode (Metrohm) were used in all voltammetric experiments. The crystallization and purity of the synthesized samples were characterized by X-ray diffraction (XRD). UV–vis absorbance spectroscopy was performed using a spectrophotometer (Shimadzu, Kyoto, Japan). LEO-Libra 120 microscope was employed for Gel-RGONS transmission electron microscopy (TEM) images. Raman spectra were conducted on a Renishaw Raman microscope (Britain).

2.2 *Synthesis of Gel-RGONS by gelatin*

GO was prepared based on modified Hummers' method as described elsewhere.[27] In a typical procedure, about 20 mg of GO was loaded in a 250 ml round bottom flask and 100 ml of distilled water was then added, yielding an inhomogeneous yellowish-brown dispersion. The gelatin was prepared separate by adding 2 g of gelatin crystal in 100 ml of distilled water using hot plate stirrer for maintaining the temperature of 80°C for 30 min to complete dissolution. After that, the pale yellow coloured gelatin solution was added dropwise to yellowish-brown coloured GO dispersion at 80°C using same hot plate stirrer for complete addition. This mixture was stirred for 20 h at 95°C to form blackish gel. Finally, the resulting blackish gel was mixed with small amount of hot water and it was centrifuged at 7000 rpm for 30 min followed by washed with hot water three times to remove excess gelatin.

2.3 *Fabrication of Gel-RGONS /GCE*

Prior to modification, the bare GCE was polished on chamois leather with 0.05 μm alumina powder. Then it was thoroughly sonicated in deionized water and absolute ethanol, respectively. Briefly, 1 mg of synthesized r was dispersed in 5 ml water with the aid of ultrasonic agitation. The cleaned GCE was coated by casting 5 μl of the Gel-r solution and dried at 70°C in an oven air to remove the solvent.

3. Results and discussion

3.1 *Characterization of Gel-RGONS*

XRD is an effective method to investigate the interlayer changes and the crystalline properties of the synthesized material, which can monitor the synthetic process and the extent of reaction simultaneously. Figure 1 displays XRD pattern of the GO compared with that of Gel-RGONS obtained from gelatin reduction of GO. GO (pattern a) had a peak centred at $2\theta = 10.0°$ corresponding to (001) reflection. After the functionalization with gelatin, no obvious peak was observed at 10.0°, indicating the GO was reduced completely. The Gel-RGONS sample (pattern b) shows a broad peak at approximately $2\theta = 21°$ corresponding to (002) reflection of RGONS.

TEM image (figure 2) also illustrated the morphology of the Gel-RGONS, which exhibited a general view of the RGONS and clearly illustrated the flake-like shapes of graphene on the plane of which some corrugations and scrolling were also observed. Moreover, the appearance of separate and stable graphene sheets in the TEM image indicates that the Gel-RGONS possessed high soluble dispersity.

Raman spectroscopy is a sensitive and powerful nondestructive tool that can provide a quick and facile structural and quality characterization of the produced material. Thus,
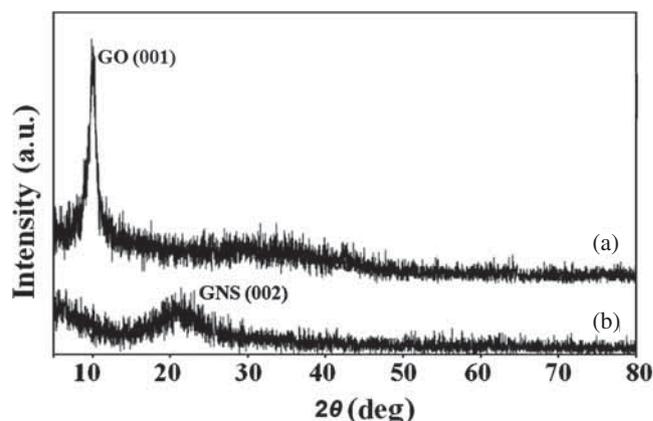

Figure 1. XRD patterns of (**a**) GO and (**b**) Gel-RGONS.

it is widely used for the study of the ordered and disordered crystal structures of graphene.[8] Figure 3 shows the Raman spectra of GO (pattern a) and Gel-RGONS (pattern b). Two strong Raman peaks are observed at around 1360 and 1600 cm^{-1}, which originated from the disordered carbon atoms (D-band) and the crystalline graphitic carbon atoms (G-band) of graphene layer, respectively. The G-band is usually assigned to the E$_{2g}$ phonon of C sp^2 atoms, while the D band is a breathing mode of κ-point phonons of the A$_{1g}$ symmetry. A prominent D-band is an indication of disorder in the Raman of the GO, originating from defects associated with vacancies, grain boundaries and amorphous carbon species. The G-band value for GO is 1593.7 cm^{-1} while for Gel-RGONS is 1601.1 cm^{-1}. The downshift of 7.4 cm^{-1} for Gel-RGONS compared with GO can be attributed to the reduction of GO to form RGONS or the attachment of the gelatin on the RGONS surface.[28,29] The peak area ratio of the D-band to the G-band (I_D/I_G) can give an indication of the surface functionalization degree for graphene surface. The calculated area ratios are 0.94 for GO and 1.32 for Gel-RGONS. The increment of I_D/I_G ratio for Gel-RGONS
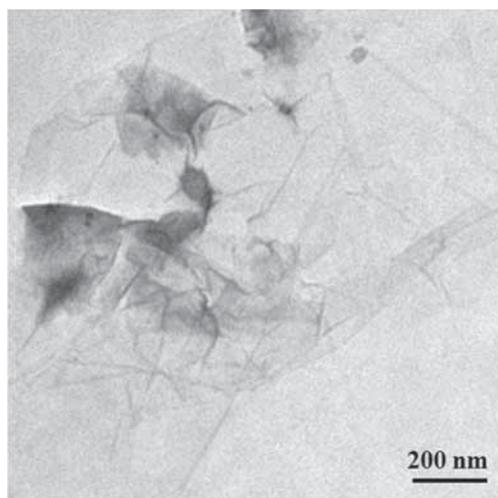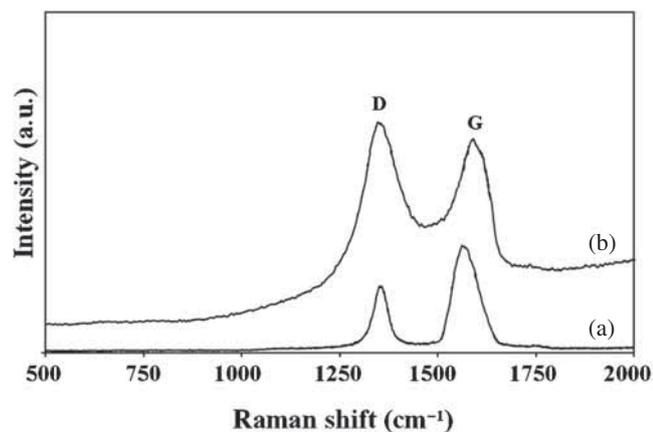
can be ascribed to the presence of unrepaired defects that remained after the removal of large amounts of oxygen-containing functional groups. Considering the relationship of I_D/I_G with the extent of π-conjugation and concentration of defects on the G-band, the present route allows the modification of the electronic or optical properties of the G-band readily.

3.2 *Electrochemical behaviour of GA at Gel-RGONS/GCE*

We have examined the electrocatalytic activity of Gel-RGONS film deposited on GCE towards GA electrooxidation in buffer solution (pH 2.00) using cyclic voltammetry (figure 4). Curves a, b and c show that two anodic peak currents are detectable. Peak I was attributed to the formation of the semiquinone radical, followed by its oxidation to the quinone form (peak Π), but the resolution of peak Π is poor. A comparison of the cyclic voltammograms of GA at bare GCE (curve a), RGONS/GCE (curve b), and Gel-RGONS/GCE (curve c) demonstrates the oxidation peak potential of GA at Gel-RGONS/GCE occurs at a potential less positive than at a bare GCE and RGONS/GCE. The obtained clear voltammetric signal with higher oxidation current for GA at Gel-RGONS/GCE electrode was attributed that the oxidation of GA was catalysed by Gel-RGONS.

It is well known that the electrochemical behaviour of GA is dependent on the pH value of the aqueous solution.[22-26] The effect of pH value on the electrooxidation of GA at the surface of Gel-RGONS/GCE was investigated through the use of different 0.1 M phosphate buffer solutions (pH 2.00–6.00). The current response of GA at Gel-RGONS/GCE decreases with the increase in the pH of solution. The protonation of GA decreases with the increase in pH, thus, the static attraction interaction between GA and Gel-RGONS decreases. So the anodic peak current decreases with the increase in pH. Therefore, pH 2.00 was chosen as optimum pH and further studies were performed at pH = 2.00.
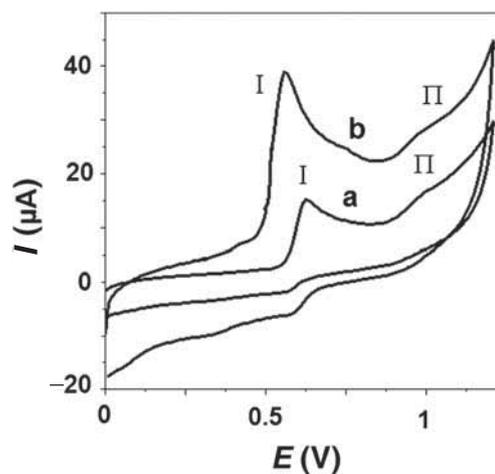

Figure 2. TEM image of Gel-RGONS.

Figure 3. Raman patterns of (**a**) GO and (**b**) Gel-RGONS.

Figure 4. Cyclic voltammograms of 0.1 mM GA at (**a**) GCE, (**b**) RGONS/GCE and (**c**) Gel-RGONS/GCE in 0.1 M PBS solution (pH 2.0) at scan rate 50 mV s^{-1}.

Figure 5 shows the dependence of the anodic peak potential (E_{pa}, peak I) for GA (0.03 mM) as a function of the buffer pH. The values of peak potential for GA shifted to more negative potentials with the increase in solution pH, indicating that protons are directly involved in the rate determination step of the oxidation reaction. The peak potential of GA also shows linear relativity to the pH with the slope of −54 mV/pH. This value of slope are close to that given by the Nernstian equation, indicating that an equal number of electrons and protons are involved in the electrochemical process of GA according to the reaction as shown in scheme 1. One proton and one electron were transferred in each process, with no peaks on the reverse scan, indicating an irreversible
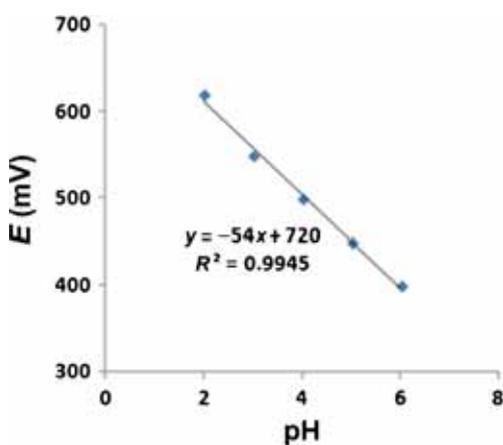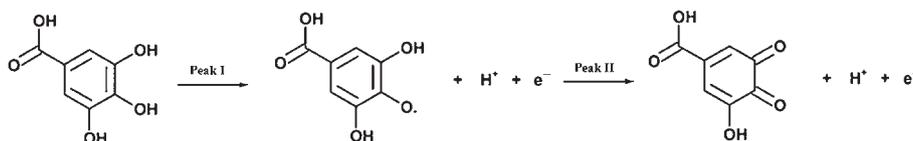
process, which corresponds to the direct electrochemical oxidation of the gallic acid.[22,23] The oxidation mechanism of GA had been confirmed in acidic solutions via two electrons and two protons transfer in the voltammetric experiments.[30]

Figure 6A shows the cyclic voltammograms of the Gel-RGONS/GCE in 0.1 M PBS (pH 2.00) in the presence of 0.03 mM GA at various scan rates. It was observed that the values of E_{pa} was shifted slightly to the positive direction with the increase in scan rate. The anodic peak current is linearly proportional to scan rate (figure 6B), suggesting that the electrode process was controlled by adsorption step.

Effect of accumulation time on anodic peak current was investigated at the different time ranges of 5–60 s. On increasing time, the peak current increases to a maximum value at 10 s and scales off at longer accumulation time. This indicates that, GA on the modified electrode surface is rapidly adsorbed. Further the increase of accumulation time did not increase the response of GA on the electrode. This is due to a complete coverage of electrode surface and the peak current remains almost scale off. For practical purposes, a 10 s accumulation time is sufficient for analytical procedure.

In real samples, some co-existing electroactive species might affect the sensor response, for example, ascorbic acid (AA), uric acid (UA), acetaminophen (AP), cysteine (Cys), glutathione (Glu), tryptophan (Trp), tyrosine (Tyr) and methionine (Met). The selectivity and anti-interference advantages demonstrated by comparing the responses of relevant electroactive species (0.1 mM) and GA (0.1 mM) on a modified electrode. The results showed that Cys, Glu, Trp and Tyr compounds have interference to detection of GA.

Figure 5. The dependence of the anodic peak potential (E_{pa}, peak I) for GA as a function of the buffer pH.

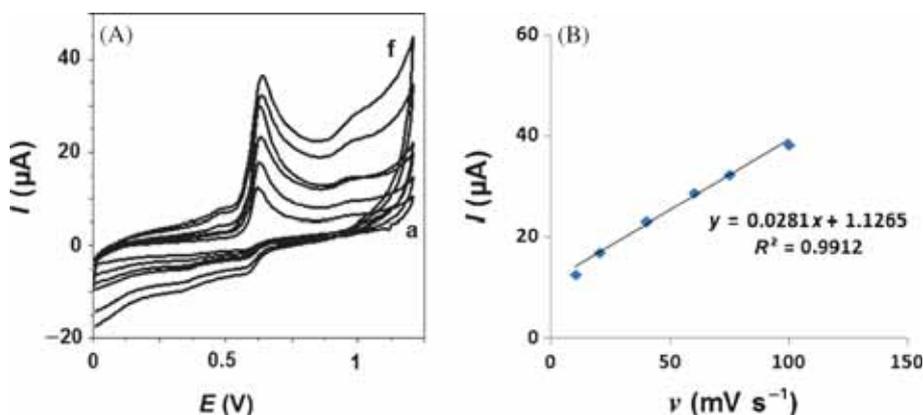**Scheme 1.** Pathway proposed of gallic acid oxidation.

Figure 6. (**A**) Cyclic voltammograms of 0.03 mM GA at Gel-RGONS/GCE in 0.1 M PBS solution (pH 2.00) and 0.1 M KCl at different scan rates. The scan rates are: (a) 10, (b) 20, (c) 40, (d) 60, (e) 75 and (f) 100 mV s^{-1}, respectively. (**B**) The plot of cathodic and anodic peak currents *vs.* scan rates (v).
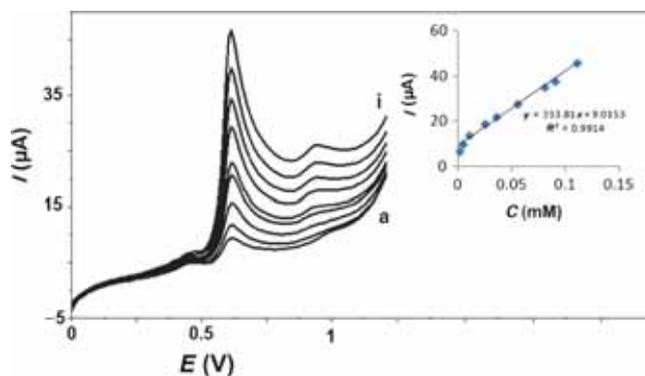
Figure 7. Cyclic voltammograms of Gel-RGONS/GCE in the presence of (a) 0.001, (b) 0.004, (c) 0.010, (d) 0.025, (e) 0.035, (f) 0.055, (g) 0.080, (h) 0.090 and (i) 0.12 mM of GA in 0.1 M PBS solution (pH 2.00) and 0.1 M KCl at scan rate 20 mV s^{-1}. (Right) Plot of anodic peak current *vs.* GA concentrations.

3.3 *Analytical application*

The linear sweep voltammetry (LSV) response of the GA sensor is investigated by successively adding various concentrations of GA into 0.1 M PBS solution (pH 2.00). Figure 7 illustrates LSVs and the calibration plot of anodic peak current *vs.* GA concentrations. The dependence of current response with respect to concentration of GA was linear from 1 to 110 μM with an detection limit of 0.47 μM at Gel-RGONS/GCE, a high sensitivity of 331.06 μA mM^{-1} and an correlation coefficient of 0.9914 (figure 6, inset). The observed linear range and detection limit and sensitivity of GA were compared with the reported papers.[22–26]

We also investigated the concentration of GA in black tea as real sample using the proposed method. The sample was diluted with PBS (pH 2.00) before the detection. The concentration of GA was calculated to be about 2.1 μM. The given value corresponds to the mean value of three determinations. The results show that the Gel-RGONS/GCE electrochemical sensor can be used successfully to give a good estimate of the GA content in foods.

4. Conclusion

A green and facile reduction route of GO based on gelatin to produce Gel-RGONS, in which the reducing agent gelatin plays an important role as a capping agent in stabilizing graphene simultaneously was demonstrated. The obtained Gel-RGONS showed excellent dispersibility and stability in distilled water and various physiological solutions. Moreover, Gel-RGONS was applied to directly modify GCE to obtain a new electrochemical sensor, which was used to investigate GA and was applied to the detection of GA with detection limit of 4.7×10^{-7} M at 3σ using LSV. The method has been successfully applied to the determination of gallic acid in sample of black tea. The Gel-RGONS showed excellent catalytic activity, indicating that the present method may be used for real sample applications.

Acknowledgement

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