Electrochemical behavior of ascorbic acid and rutin on poly(L-arginine)-graphene oxide modified electrode

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The electrochemical behavior of ascorbic acid and rutin using a poly(L-arginine)-graphene oxide modified glassy carbon electrode (PLA-GO/GCE) has been studied by cyclic voltammetry and chronoamperometry. The modified electrode succeeds in distinguishing the electrochemical responses of ascorbic acid and rutin in a mixture solution due to its high electron transfer rate and good catalytic ability. The number of electrons involved in the reaction of ascorbic acid and rutin are calculated to be 1.994 and 1.968, respectively. The redox reaction of rutin is mainly an adsorption controlled process and the oxidation of ascorbic acid is a diffusion controlled process in pH 2.0 phosphate buffer. The diffusion coefficient of ascorbic acid is found to be $3.003 \times 10^{-6}$ cm$^2$ s$^{-1}$. In the case of rutin, the surface coverage concentration is $6.17 \times 10^{-9}$ mol cm$^{-2}$, and the electron transfer coefficient is 0.51, while the rate constant of electrode reaction is 0.967 s$^{-1}$. The PLA-GO/GCE electrode is of high sensitivity and selectivity, and has been successfully applied for simultaneous determination of ascorbic acid and rutin.

Keywords: Electrochemistry, Electrodes, Modified electrodes, Graphene, Graphene oxide, L-Arginine, Ascorbic acid, Rutin

As an increasingly important carbon-based material, graphene and graphene oxide (GO) have attracted considerable attention in recent years. Due to unique properties such as superior chemical stability, high specific surface area and excellent electronic conductivity, graphene is considered as a promising material in electronic devices. Some sensors combining graphene or graphene oxide with other materials such as amino acid, enzyme and nanoparticles have shown excellent usage in study of biomolecules and chemicals. Compared with graphene, GO is sticky and easier to adhere to substrates. Dong et al. obtained electrochemically reduced graphene oxide (ERGO) film on various conductive substrates. John et al. fabricated electrochemically reduced graphene oxide modified electrode with this method, and used it to simultaneously determine uric acid, xanthine, hypoxanthine and caffeine. Furthermore, the composite film of amino acid polymer and graphene oxide or graphene usually showed synergistic effect and had high electrocatalytic ability for some compounds.

Ascorbic acid (AA) and rutin usually coexist in foods and drugs, and play significant roles in human metabolism, diseases prevention and treatment. As a vital antioxidant and preservative, ascorbic acid can maintain the elasticity of veins, prevent cell agglutination and enhance the resistibility of capillary vessels. Rutin, a flavonoid glycoside, also called as vitamin P, is one of the naturally occurring bioactive compounds. Rutin has been widely used in the medicine since it can dilute the blood, reduce capillary permeability, lower blood pressure and treat easy bruising. Therefore, the investigation of ascorbic acid and rutin is important. It is necessary to study their relevant properties on detectors including electrochemical sensors.

In the present work, poly(L-arginine)-graphene oxide modified glassy carbon electrode was prepared and employed to study the electrochemical behavior of AA and rutin. A simultaneous detection method for AA and rutin was established and applied to analyze drug samples with satisfactory results.

Experimental

Electrochemical measurements were performed with a BAS 100B/W electrochemical workstation (BAS, USA). A classical three-electrode system, which consisted of an Ag/AgCl (saturated KCl) as reference electrode, a platinum wire as counter electrode, a bare GCE (3 mm in radius) or a modified electrode as working electrode was used for all electrochemical experiments. The morphology of the samples was observed using a scanning electron microscope from JSM-6610LV (JEOL, Japan). All pH measurements were made on a PHS-3C digital pH meter (Shanghai Leici Device Works, China).

Ascorbic acid and rutin were purchased from International Specialty Products (New Jersey, USA) and were used as received. Graphene oxide dispersion (2 mg mL$^{-1}$, dispersing agent: water; radius: 1~5 µm;
thickness: 0.8~1.2 nm; single layer ratio: 99%; purity: 99%) was obtained from Nanjing XFNANO Materials Technology Company, China. All other chemicals used were of analytical grade. Doubly distilled water was used throughout the experiments. Phosphate buffer solutions (PBS) were prepared with 0.1 mol L\(^{-1}\) H\(_3\)PO\(_4\)-Na\(_3\)PO\(_4\) and used as supporting electrolyte. All experiments were carried out at room temperature.

Before surface modification, the bare glassy carbon electrode (Φ = 3.0 mm) was polished with 0.05 µm alumina slurry on a polishing cloth and then the electrode was sonicated for 5 min in nitric acid, ethanol and doubly distilled water in sequence\(^\text{18}\). GO dispersion (2.0 µL) was cast on a bare GCE with a microsyringe, then dried under an infrared lamp. The graphene oxide modified glassy carbon electrode (GO/GCE) was fabricated until a colored film was formed. GO/GCE was cyclic scanned from 2.3 to −1.2 V at a scan rate of 0.10 V s\(^{-1}\) for 10 cycles in mixture containing 2.50×10\(^{-3}\) mol L\(^{-1}\) L-arginine, 8.00×10\(^{-2}\) mol L\(^{-1}\) HNO\(_3\) and 5.00×10\(^{-2}\) mol L\(^{-1}\) KNO\(_3\). After polymerization, the electrode was removed, rinsed thoroughly with doubly distilled water and air-dried to obtain the modified electrode PLA-GO/GCE.

**Results and discussion**

Figure 1 shows ten cycles of the cyclic voltammograms of L-arginine during polymerization process on GO/GCE and bare GCE, respectively. The cathodic current at negative potential increases sharply from a starting potential of −0.75 V, especially the first cycle in Fig. 1(a). However, the increase of current is not obvious in Fig. 1(b), indicating that GO contributes to the cathodic current. Compared with lower and higher negative potential during electrochemical polymerization, a potential of −1.2 V was enough to guarantee good catalytic activity of PLA-GO/GCE toward AA and rutin. Additionally, very high negative potential usually produced hydrogen bubbles on the surface of electrodes without further improving the response to AA and rutin. In fact, these bubbles not only influenced the electric current density, but also damaged the modified film. According to literature\(^\text{19}\), not all oxygen groups of GO can be electrochemically reduced at the potential of -1.2 V. In this case, it would be inappropriate to call the electrochemically reduced GO as the graphene, hence we still denoted this partially reduced graphene oxide as GO in this study.

Electrochemical impedance spectroscopy (EIS) of GO/GCE, GCE and PLA-GO/GCE is shown in Fig. 2; their corresponding resistances is 1698 Ω, 977.7 Ω and 283.2 Ω, respectively. The semicircle radius of GO/GCE is the largest, followed by that of bare GCE, while that of PLA-GO/GCE is almost a straight line. This indicates that PLA-GO/GCE has good electrical conductivity, and can also accelerate the rate of
electron transfer and lower the electron transfer kinetics at the electrode-liquid interface to some extent.

Figure 3 shows the SEM images of the surface of GCE, GO/GCE and PLA-GO/GCE. The bare GCE presented a smooth surface, while GO/GCE showed a crumpled and wrinkled structure. After L-arginine polymer was formed on the GO/GCE during electrochemical polymerization, PLA-GO/GCE exhibited sparse lines or bulges compared with GO/GCE. These images suggest that GO film and PLA-GO film had successfully modified the bare GCE.

The cyclic voltammograms of AA, rutin and their mixture at GO/GCE, GCE and PLA-GO/GCE are shown in Fig. 4. While there was no response at GO/GCE, bare GCE and PLA-GO/GCE responded to AA and rutin. Additionally, the peak current at PLA-GO/GCE is much higher than that at bare GCE. A serious anodic overpotential of AA occurs at bare GCE (Fig. 4a), disturbing the response of rutin in the mixture solution (Fig. 4c), which may be attributed to the slow electron transfer kinetics of bare GCE and the adsorption of the electroinactive oxidized product of AA. However, the overpotential is reduced and the peak current of AA is improved at PLA-GO/GCE, presumably due to the high electron transport rate and good catalytic activity of PLA-GO/GCE. In addition, a pair of sensitive redox peaks of rutin also occurs at PLA-GO/GCE (Fig. 4b), and since the ratio of the anodic peak current ($I_{pa}$) to the cathodic peak current ($I_{pc}$), is almost equal to unity, the redox process of rutin is reversible. Two anodic peaks at 0.273 V for AA and 0.605 V for rutin and a cathodic peak for rutin at 0.552 V were obtained at PLA-GO/GCE (Fig. 4c). Besides, the anodic peak between AA and rutin was separated by 0.332 V, implying that PLA-GO/GCE possessed good separating effect toward AA and rutin.

With an increase of scan rate, the anodic peak potential ($E_{pa}$) of both AA and rutin move to positive region, and the cathodic peak potential ($E_{pc}$) of rutin shifts to negative region (Supplementary data, Fig. S1). The relationships between scan rate, peak current and peak potential are expressed in Table 1.

The slope of $\log I$-$\log \nu$ for rutin is between 1.0 and 0.5 (closer to 1.0), while that for AA is approximate 0.5, suggesting that the redox of rutin is controlled by diffusion and adsorption together (mainly adsorption controlled process) and the oxidation of AA is a diffusion controlled process. From the slope of the
E-logv relationship\(^{21}\) \((2.303RT/anF)\) where \(n\) represents the number of electrons involved in the reaction, \(F\) is Faraday constant, \(R\) is gas constant, and \(\alpha\) is electron transfer coefficient, usually 0.5) the value of \(n\) for AA was calculated to be 1.994, i.e., the theoretical number of electrons involved should be 2.

From the chronoamperograms of AA and the corresponding plots of \(I\) versus \(t^{-1/2}\) (Supplementary data, Fig. S2), the diffusion coefficient \((D)\) of AA was determined by the equation\(^{22}\) \(i_d = (nFAD^{1/2}/C_0)/\sqrt{\pi t}\), where \(i_d\) is the limiting diffusion current \((A)\), \(A\) is the surface area of working electrode \((cm^2)\), \(C_0\) is the concentration of AA \((mol/cm^3)\), \(n\) is the number of electron transfer, \(F\) is the Faraday constant, and \(t\) is time \((s)\). The values of \(D\) \((cm^2 s^{-1})\) at GO/GCE, GCE and PLA-GO/GCE are \(2.628 \times 10^{-7}\), \(4.599 \times 10^{-7}\), and \(3.003 \times 10^{-6}\), respectively. PLA-GO/GCE gives the maximum diffusion coefficient, which indicates PLA-GO/GCE has a high sensitivity.

As shown in Table 1, the slopes of \(E_{pa}-\log v\) for anodic peaks and \(E_{pc}-\log v\) for cathodic peaks were 0.06103 and 0.05932, respectively. According to the Laviron’s theory\(^{23}\), the number of electron transferred \(n_a\) for rutin was calculated to be 1.968 (the electrons transferred is 2), and the electron transfer coefficient \(\alpha\) was calculated as 0.51 (close to the theoretical value of 0.5). This indicates that the electrode process is quasi-reversible in the scan rate range of 0.04-0.20 V s\(^{-1}\). Since the separation of the peak potentials \((AE_p = E_{pa} - E_{pc})\) was 0.109 V at a low scan rate of 0.20 V s\(^{-1}\), and \(n\Delta E_p > 0.2\) V, the rate constant of electrode reaction \(k_s\) was calculated as 0.967 s\(^{-1}\) by Eq. (1)\(^{24}\).

\[
\lg k_s = \alpha\lg(1-\alpha) + (1-\alpha)\alpha - \frac{RT}{n_F F} \frac{\alpha(1-\alpha) F n^2 D E_p}{2.3RT}\ldots(1)
\]

Using Eq. (2) according to literature\(^{25,26}\), and from the slope of the plots of anodic peak currents versus scan rate, the surface coverage concentration \(\Gamma\) of \(1.0 \times 10^{-5}\) mol L\(^{-1}\) rutin at PLA-GO/GCE in PBS \((pH\ 2.0)\) was estimated as \(6.17 \times 10^{-9}\) mol cm\(^{-2}\).

In most cases, the electrolyte pH is an important condition for electrochemical reaction. Figure 5 describes the cyclic voltammograms of AA and rutin. The peak currents of AA and rutin decrease with a rising value of pH. Both anodic and cathodic peak potentials shift in the negative direction with an increase of pH, suggesting that protons have participated in their electrode reactions. For rutin, according to Nernst equation, the approximate slope of 0.059V pH\(^{-1}\) implies the ratio of electrons-to-protons involved in the reactions was 1:1.

![Fig. 5—CVs of (a) 1.00x10^{-3} mol L^{-1} AA and (b) 2.00x10^{-5} mol L^{-1} rutin at different pH.](image-url)
Based on the results above and the literature, the possible mechanisms of AA and rutin were inferred as shown in Fig. 6.

A mixture of AA (5.00×10⁻⁴ mol L⁻¹) and rutin (1.00×10⁻⁵ mol L⁻¹) was studied in 30 parallel experiments. The relative standard deviations (RSD) of the anodic peak currents for AA and rutin were 3.3% and 4.2%, respectively, illustrating remarkable reproducibility. When the modified electrode was preserved in humid environment at room temperature for 20 days, the unchanged peak current and the similar shape of the original responses of AA and rutin indicate that PLA-GO/GCE had acceptable storage stability.

The modified electrode was tested for determination of AA and rutin. In a mixture (pH 2.0) consisting of appropriate concentrations of AA and rutin, PLA-GO/GCE was employed for cyclic voltammetry in a potential range of 0 −0.95 V at a scan rate of 0.12 V s⁻¹ with wait time of 10 min. The oxidation processes of AA and rutin was investigated when the concentration changed, and the linear regression equations and the linear ranges were obtained as follows,

\[
\begin{align*}
I_{(AA)} &= 5.796 + 2.658 \times 10^4 C (5.00 \times 10^{-6} - 5.00 \times 10^{-3} \text{ mol L}^{-1}); \\
I_{(rutin)} &= 0.1084 + 4.222 \times 10^6 C (3.00 \times 10^{-7} - 1.00 \times 10^{-6} \text{ mol L}^{-1}); \\
I_{(rutin)} &= 3.164 + 1.148 \times 10^6 C (1.00 \times 10^{-6} - 2.00 \times 10^{-5} \text{ mol L}^{-1}),
\end{align*}
\]

where the current (I) is in µA. The detection limits for AA and rutin were 3.0×10⁻⁶ mol L⁻¹ and 1.00×10⁻⁷ mol L⁻¹, respectively. Various foreign species such as K⁺, Na⁺, Ca²⁺, Mg²⁺, Ba²⁺, Zn²⁺, Al³⁺, Cl⁻, NO₃⁻, SO₄²⁻, C₂O₄²⁻, vitamin B₁, vitamin B₂, vitamin B₁₂, starch, L-threonine, L-serine, L-histidine (≥1.0 mg) and L-cysteine (0.6 mg), Cu²⁺ (0.6 mg), Ag⁺ (0.05 mg), and I⁻ (0.03 mg) were studied. No interference was observed in the determination of AA and rutin. This indicates that PLA-GO/GCE had good selectivity. In order to verify the reliability of PLA-GO/GCE, we applied it to the determination of AA and rutin in pharmaceutical tablet samples (Supplementary data, Table. S1). The results are in conformity with the recommended values of specification, suggesting that the established method could be efficiently used to determine AA and rutin in real samples.

In the present work, we have studied the SEM images showed that GO film and poly(L-arginine) film successfully covered the bare GCE and GO/GCE, respectively. GO/GCE and bare GCE failed to distinguish the voltammetric signals of AA and rutin, while, PLA-GO/GCE exhibited excellent performance including high distinguishing ability for AA and rutin, electrical conductivity and catalytic activity. Cyclic voltammetry and chronoamperometry investigations showed that two electrons of AA and rutin were involved in the electrochemical reactions at PLA-GO/GCE. The present modified electrode consists of inadequately electro-reduced GO and poly(L-arginine) film. GO is covered with poly(L-arginine) film, which reduces the loss of GO during use. Besides, several layers of poly(L-arginine) molecule are formed during cyclic scanning, and this composite film can provide considerable catalytic sites. This ensures a long life and good sensitivity of the electrode. This modified electrode displayed many desirable

![Fig. 6—The electrochemical reaction mechanism for (a) AA and (b) rutin.](image-url)
properties such as excellent stability, reproducibility, selectivity and sensitivity. PLA-GO/GCE is a promising sensor in electrochemical sensing and pharmaceutical analysis.

Supplementary data
Supplementary data associated with this article i.e., Figs S1 and S2 and Table S1, are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_54A(02)187-192_SupplData.pdf.

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