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ORIGINAL PAPER

Voltammetric behavior and the determination of quercetin at a flowerlike Co_3O_4 nanoparticles modified glassy carbon electrode

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Abstract Flowerlike Co_3O_4 nanoparticles were used as a modifier on the glassy carbon electrode to fabricate a quercetin (Qu) sensor. The morphology and crystallinity of the prepared Co_3O_4 material were investigated by scanning electron microscopy and X-ray diffraction. Electrochemical behavior of Qu at the sensor was studied by cyclic voltammetry and semi-derivative voltammetry. Results suggested that the modified electrode exhibited a strong electrocatalytic activity toward the redox of Qu. The electron transfer coefficient (α), the number of electron transfer (n), and the diffusion coefficient (D) of Qu at the sensor were calculated. Under the optimum conditions, the catalytic peak currents of Qu were linearly dependent on the concentrations of Qu in the range from 5.0×10^{-7} to 3.3×10^{-4} M, with a detection limit of 1.0×10^{-7} M. This proposed method was successfully applied to determine the quercetin concentration in Ginkgo leaf tablet and human urine samples.

Keywords Flowerlike · Co_3O_4 nanoparticles · Quercetin · Sensor

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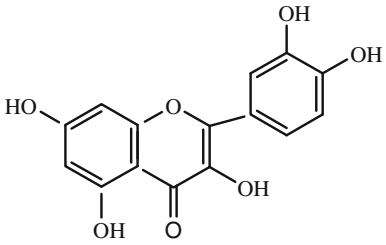
1 Introduction

Flavonoids are a group of polyphenolic compounds and widely distributed in plant [1]. Quercetin (Qu) is one of the most common flavonoids, which has the general structure of a 15-carbon skeleton, consisting of two aromatic rings linked by an oxygen containing heterocycle (Scheme 1). Quercetin has been shown to act as a scavenger of various oxidizing species, such as superoxide anion, hydroxyl radical, and peroxyl radicals [2, 3]. As a result of these biological activities, its pharmacological functions have been widely developed including anti-bacterial, anti-inflammatory, anti-tumor, anti-virus, harmostat, etc. [4–9]. Currently, over 130 preparations containing Qu or other flavonoids have been registered as therapeutic medicine worldwide [10]. Therefore, it is necessary to develop simple, economic, and sensitive methods for Qu determination in pharmaceutical drugs and plants. Various analytical methods have been reported for the determination of Qu. Liquid chromatograph [11–14], UV–Vis spectrophotometry [15, 16], and mass spectrometry (MS) [17, 18] have been used to determine the presence of Qu and other flavones in plants. These techniques provide high sensitivity for the assay; yet, they also have disadvantages, as complexity of operation, reagent-consuming, and high cost. Since Qu contains phenolic hydroxyl groups which exhibit electroactivity at oxidation potential, electrochemical methods are preferable with their advantages of rapid response, low cost, and simple use.

Among the electrochemical methods for the detection of natural products, the design and fabrication of electrodes modified by carbon nanotubes and metal nanoparticles have been much focused on. Xu and Kim [19] used carbon nanotubes-modified glassy carbon (GC) electrode for selective determination of Qu in the presence of interfering



Scheme 1 The molecular structure of Qu

species such as ascorbic acid, uric acid, glucose, and catechol in large excess. Yang et al. [20] studied the electrochemical properties of catechin at single-wall carbon nanotubes-modified GC electrode. Gutiérrez et al. [21] utilized GC electrodes modified with multiwall carbon nanotubes dispersed in polyethylenimine and polyacrylic acid for sensitive detection of Qu. Franzoi et al. [22] investigated the feasibility of amperometric detection of Qu at a biosensor through immobilizing Laccase in a modified β-cyclodextrin matrix containing Ag nanoparticles in ionic liquid. Lim et al. [23] reported an amperometric biosensor for detection of glucose based on electrodeposited glucose oxidase enzymes and palladium nanoparticle onto a Nafion-solubilized carbon nanotube film. Yogeswaran et al. [24] developed an amperometric biosensor immobilized with nano platinum and nano gold on a carbon nanotubes film. Recently some articles have reported that the addition of oxide is efficient to improve catalytic activity of the modified electrode. Cobalt oxide nanoparticles are known to be highly reactive, and they have been utilized in processes such as energy storage systems [25], electrochromic thin films [26], anode material [27], and heterogeneous catalysis [28]. Moreover, Co_3O_4 and other cobalt-based oxides continue to attract significant interest, primarily due to their excellent electrocatalytic activity toward various compounds, peroxide and oxygen. Jia et al. [29] synthesized vertically aligned Co_3O_4 by heating Co foil on a hot plate and described its electrocatalytic activity to H_2O_2; Casella [30] anodically deposited cobalt hydroxide film on a GC substrate and checked the electrochemical activity of the cysteine; Ozer et al. [31] deposited cobalt hydroxide thin film by sol–gel processes and characterized the electrochromic behavior of the cobalt hydroxide thin film.

In this study, we reported the construction of a Qu sensor based on the modification of flowerlike Co_3O_4 nanoparticles (FCo-Np) onto a GC electrode. The modified electrode was signed as FCo-Np/GC electrode. The amperometric and kinetic characteristics of the sensor and its application for Qu analysis were investigated in detail by electrochemical methods. The results demonstrated that the modified electrode has excellent sensing performance toward Qu, including high sensitivity and selectivity. Also the modified electrode can be used for Qu determination in drug and urine samples, without a previous treatment.

2 Experimental

2.1 Reagents and solution

All aqueous solutions were prepared with doubly distilled and deionized water. Britton–Robinson (B–R) buffer solutions of different pH were prepared by mixing four stock solutions of 0.04 M H_3PO_4, HAc, H_3BO_3, and 0.2 M NaOH. B–R buffer solutions with various pH values were used as supporting electrolytes. Qu was purchased from Sigma and a 5.0×10^{-4} M stock solution was dissolved in 5% (v/v) ethanol and prepared in B–R buffer solution at pH 6.8. Other solutions were prepared from the stock solution by appropriated dilutions with the same buffer solution. All other chemicals were of analytical grade. High pure nitrogen was used for deaeration. The compound Ginkgo leaf tablets were purchased from Shanghai Third Chinese Medicine Factory (090812, 091103), Yangtze River Pharmaceutical Co., Ltd. (09032901, 08070302), and Tianjin Feiying Pharmaceutical Co., Ltd. (20091005), respectively, with the specified amount of 10 mg per tablet.

2.2 Apparatus

All electrochemical experiments including cyclic voltammetry (CV) and semi-derivative voltammetry (SDV) were carried out with a CHI660A electrochemical workstation (Shanghai Chenhua Co., China) connected to a Pentium 200 MHz PC. A conventional three-electrode system was used for all electrochemical experiments, which consisted of a working electrode, a platinum wire auxiliary electrode, and a saturated calomel reference electrode (SCE). All potentials reported are versus SCE. A SCS-1200 ultrasonic apparatus (Qingchao Electronic Apparatus Company, Shanghai, China) was applied in the ultrasonic experiment. The morphology of the prepared FCo-Np sample was characterized by scanning electron microscopy (SEM). These images were taken using a JSM6700F scanning electron microscope (JEOL, Japan), using an accelerating voltage of 20 kV. The crystallinity of the prepared FCo-Np material was analyzed via X-ray diffraction (XRD) on a Philips X Pert' PRO SUPER instrument (Philips, Holland) based on CuKα radiation source ($\lambda = 1.5418$ Å) scanned from 10° to 80° (2θ) with a step size of 0.02°.

2.3 Fabrication of FCo-Np/GC electrode

The FCo-Np/GC electrode was fabricated as follows: first, 1.0 mg FCo-Np were dispersed into 4.0 mL doubly distilled water by 10 min ultrasonic agitation to give a homogeneous nano-Co_3O_4 suspension. FCo-Np were obtained via the sequential process of a hydrothermal reaction and heat treatment by using $Co(CH_3COO)_2{\cdot}4H_2O$ and $Co(NH_2)_2$ as reagents [32]. The GC electrode (3 mm in diameter) was polished successively with 1.0, 0.3, and 0.05 μm aluminum oxide powder on chamois leather. Then it was rinsed with doubly distilled water and sonicated in ethanol and doubly distilled water for 5 min. The electrode was immediately dried with high purified nitrogen gas. After cleaning, A 2.5 μL FCo-Np suspension was placed onto the cleaning GC electrode surface, and the solvent was allowed to evaporate under an infrared lamp. The prepared FCo-Np/GC electrode was rinsed twice with doubly distilled water, and then pretreated in 0.2 M phosphate buffer solution by scanning the electrode repeatedly between −0.5 and 0.5 V until the shape of the cyclic curves no longer changed. This FCo-Np/GC electrode was stored in air at room temperature.

2.4 Sample preparation and application

One of the samples was the compound Ginkgo leaf tablet. Ten pieces of compound Ginkgo leaf tablets were carefully washed with water to remove their sugar-coating and then dried at room temperature. The tablets were carefully ground to a fine powder and thoroughly mixed. Sample solutions were prepared by weighing a certain amount of the powder and dissolving it in ethanol with the aid of ultrasonic agitation and then filtering it through filter paper. The filtrate was diluted 50 times with pH 6.8 B–R buffer solution before the measurement.

Another sample was urine taken from one atherosclerotic patient 4 h after his administration of two Yangtze River Ginkgo leaf tablets. This urine sample was directly analyzed by the modified electrode just after being filtered through 0.45-μm polypropylene acrodisc syringe filter.

The three-electrode system was immersed in a 10-mL cell containing proper amounts of Qu sample and B–R buffer solution (pH 6.8). The cyclic voltammograms were recorded in the potential range from −0.2 to 0.6 V with the scan rate of 0.1 V s^{-1}. A standard addition method was adopted to determine the Qu contents in samples.

A high-performance liquid chromatographic (HPLC) method for the determination of Qu available in the Pharmacopoeia of the People's Republic of China [33] was used to compare the obtained analytical results with the proposed modified electrode.

3 Results and discussion

3.1 Characterization of the FCo-Np

Figure 1a shows the SEM image of the resultant Co_3O_4 nanoparticles. It can be seen that the products are flowerlike structures composed of Co_3O_4 rods. Each of the rods has one end outside and another end bound to other rods. The diameters of these flowerlike structures are about 5–6 μm. From the insert graph with high magnification, it is clear that the rods consisted of very tiny Co_3O_4 particles. The structures of Co_3O_4 were further investigated by XRD analysis. In the 2θ range of 10°–80°, the typical peaks (111), (220), (311), (222), (400), (422), (511), and (440) can be indexed as pure cubic phase of Co_3O_4 spinel (JCPDS card 42-1467) (Fig. 1b). No other peaks for impurities were detected. According to the Scherer and

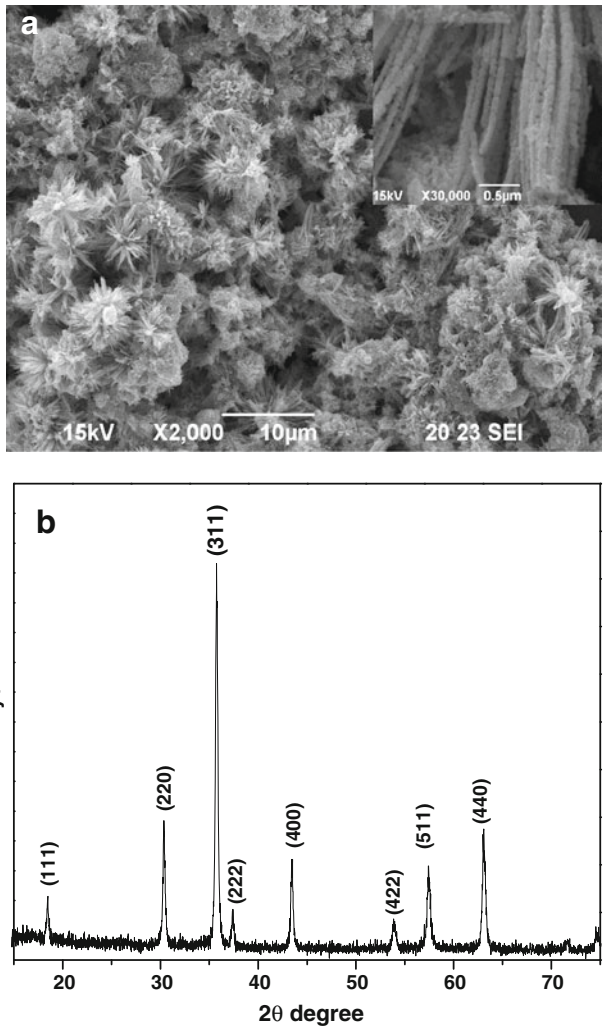


Fig. 1 **a** SEM images of the FCo-Np, where the *inset* shows the magnified image of Co_3O_4 nanorods. **b** XRD pattern of Co_3O_4 nanoparticles

Bragg formula, calculations based on the strongest 311 diffraction peak reveal the average crystal size of Co_3O_4 was about 25 nm. The results indicated that the Co_3O_4 rods consisted of 25 nm sized crystals, which also confirms the SEM results.

3.2 Voltammetric behavior of the FCo-Np/GC electrode

Preliminary cyclic voltammetric experiments were performed to study the electrochemical behavior of the FCo-Np/GC electrode. As shown in Fig. 2, the voltammograms recorded in the B–R buffer solution reveal a set of peaks at about −0.165 V in the forward scan and at −0.081 V in the reverse scan of the modified electrode, respectively. The redox couple can be attributed to the conversion between Co_3O_4/CoOOH species. These values are consistent with the previous reports [30, 34].

Figure 3 showed the cyclic voltammograms of Qu on bare GC (curve a) and FCo-Np/GC electrode (curve b) with the scan rate as 0.1 V s^{-1}. On GC electrode, a pair of sluggish redox peaks appeared with the anodic peak potential (E_{pa}) as 0.095 V and the cathodic peak potential (E_{pc}) as −0.144 V. The anodic (I_{pa}) and cathodic (I_{pc}) peak current was got as 2.4 and −4.3 μA, respectively. The ratio of I_{pa}/I_{pc} was calculated as 0.56 and the peak-to-peak separation (ΔE_p) was got as 0.239 V. The results indicated the electrode reaction was a quasi-reversible process. While on the FCo-Np/GC electrode, the reversibility of Qu was significantly improved with the redox peak current increased greatly. From curve b it can be seen that E_{pa} was negatively shifted to −0.019 V and E_{pc} was positively shifted to −0.120 V. The peak-to-peak separation was got as 0.101 V. The peak currents were about 5 times larger

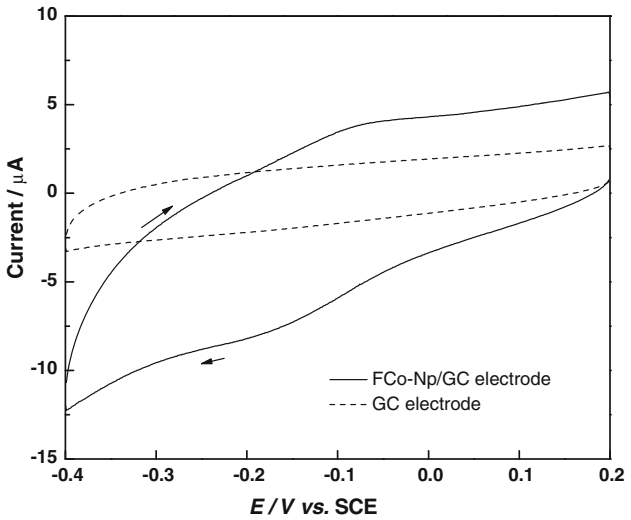


Fig. 2 CVs of bare GC and FCo-Np/GC electrode in B–R buffer solution (pH 6.8). Scan rate: 0.1 V s^{-1}

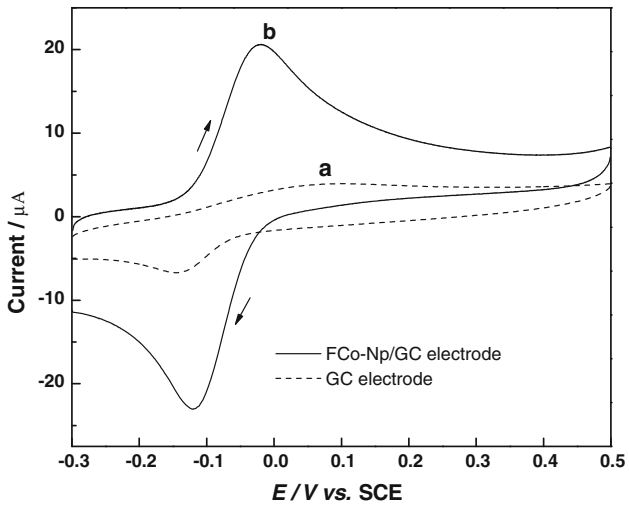


Fig. 3 CVs of bare GC (*a*) and FCo-Np/GC electrode (*b*) in pH 6.8 B–R buffer solution in the presence of 2×10^{-5} M Qu, respectively. Scan rate: 0.1 V s^{-1}

than that on the bare GC electrode with the ratio of I_{pa}/I_{pc} as 0.91. It is well known that the increase of the redox peak current and the decrease of the overpotential are typically indicative of an electrocatalytic reaction. The improvement of the reversibility and sensitivity might be caused by the enlargement of effective electrocatalytic active surfaces on the modified electrode. So nano-Co_3O_4 material plays an important role in improving the electrochemical performance of the modified electrode.

3.3 Influence of potential scan rates

To investigate the reaction mechanism, scan rate-dependent experiments were carried out for Qu electrocatalytic reaction at FCo-Np/GC electrode, the result is shown in Fig. 4. The redox peak current was proportional to the square root of scan rate in the range 20–400 mV s^{-1} (I_{pa} (μA) = 4.2713 + 45.09 $v^{1/2}$ (V s^{-1}), $r = 0.9987$; I_{pc} (μA) = −1.4698 − 53.08 $v^{1/2}$ (V s^{-1}), $r = 0.9997$), indicating diffusion-controlled kinetics.

The relationship of peak potential with scan rate was further constructed. As shown in Fig. 5, with the increase of scan rate the E_{pa} was positively shifted and the E_{pc} was negatively shifted, indicating that the electro-transfer rate was not very fast and the electrochemical reaction gradually became less reversible. At higher scan rates, the peak potential and log$v$ showed a linear relationship. The regression equations were $E_{pa} = -9.81 \times 10^{-4} + 0.0303 \log v$ ($r = 0.994$) and $E_{pc} = -0.133 - 0.0306 \log v$ ($r = 0.996$) (E_p: V, v: V s^{-1}). According to the Laviron's equations [35]:

$$E_{pc} = E^{\circ\prime} - 2.3RT \log v/\alpha nF \quad (1)$$

$$E_{pa} = E^{\circ\prime} + 2.3RT \log v/(1-\alpha) nF \quad (2)$$

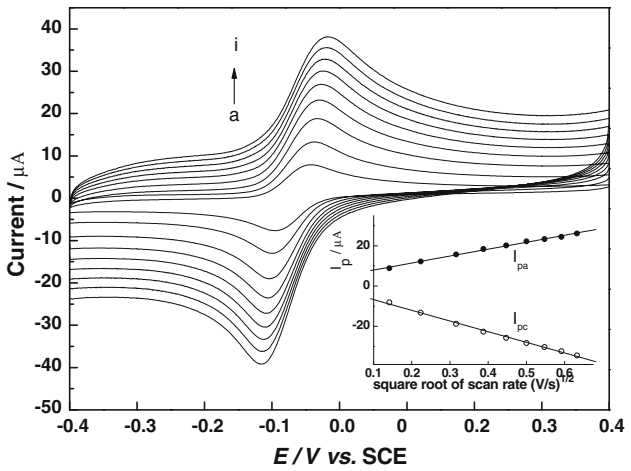


Fig. 4 CVs of 2×10^{-5} M Qu on the modified electrode at different scan rates (from *a* to *i*): 0.02, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 V s^{-1} in pH 6.8 B–R buffer solution. The *inset* is the dependence of redox peak current on the square root of potential scan rate

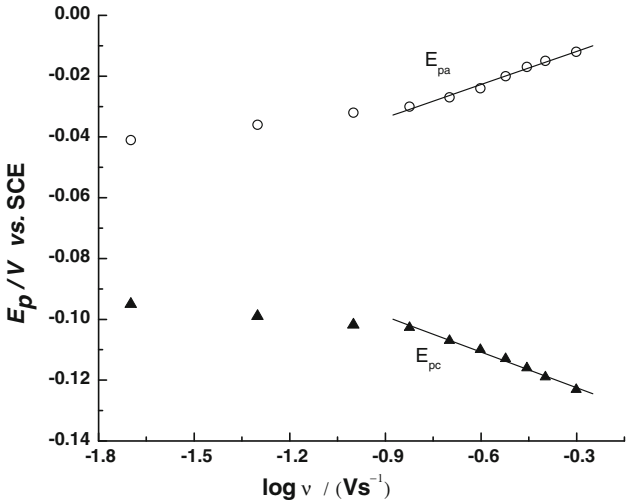


Fig. 5 The relationship between redox peak potential and logarithm of potential scan rate

where α is the charge transfer coefficient, n is the number of electron transfer, v (V s^{-1}) is the scan rate, $E^{o\prime}$ is the formal potential, and F (C mol^{-1}) is the Faraday's constant.

The values of α and n were calculated to be 0.497 and 1.8, respectively, suggesting that two electrons were involved in the oxidation process. This was in according with that reported in the literature [36].

3.4 Influence of supporting electrolyte and pH

The type of supporting electrolytes played a key role in the voltammetric response of Qu. The current responses of 2×10^{-5} M Qu were investigated in different supporting

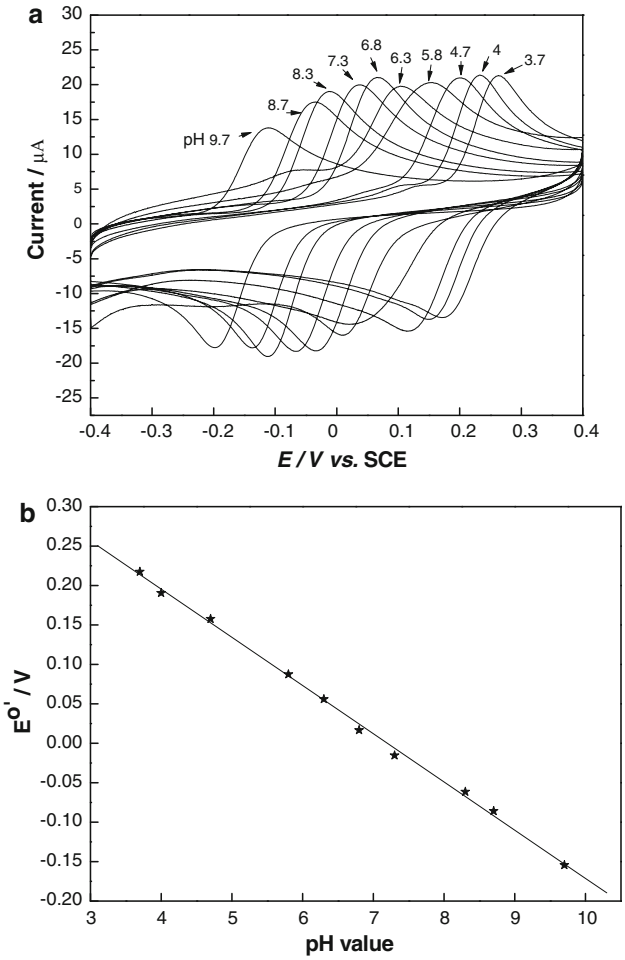
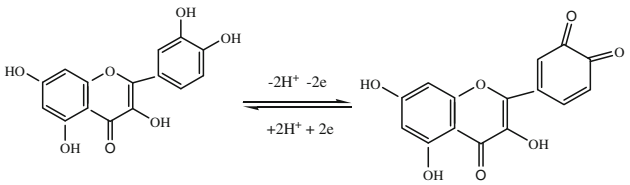


Fig. 6 **a** Influence of buffer solution pH on CVs of 2.0×10^{-5} M Qu at the FCo-Np/GC electrode. Solution pH: 3.7, 4.0, 4.7, 5.8, 6.3, 6.8, 7.3, 8.3, 8.7, and 9.7 (from *right* to *left*). **b** Relationship of formal potential $E^{o\prime}$ with pH value. Scan rate: 0.1 V s^{-1}

electrolytes such as NaAc–HAc, sodium citrate–HCl, tartaric acid–sodium tartrate, and B–R buffer solution. Results indicate that a higher peak current and better peak shape could be obtained in a B–R buffer solution. Therefore, the B–R buffer solution was adopted.

The effect of pH of B–R buffer solution, which ranged from 3.7 to 9.7, on the electrochemical response of 2×10^{-5} M Qu was observed. As can be seen in Fig. 6a, when the solution pH is changed from 3.7 to 6.8, the peak currents stay nearly unchanged, but when the pH exceeds 6.8, the peak currents begin to decrease with the further increase in pH value. Therefore, pH 6.8 was chosen for the determination of Qu. The effect of buffer pH on the formal potential ($E^{o\prime}$) was investigated. As shown in Fig. 6b, when the pH changes from 3.7 to 9.7, the formal potentials shift to a negative direction. There is a linear relationship between the $E^{o\prime}$ and the pH value, and the regression equation is $E^{o\prime} = 0.440 - 0.061$ pH ($n = 10$, $r = 0.9990$). The slope is 0.061 V pH^{-1}, which was close to the

theoretical value of 0.059 V pH^{-1} at 25 °C. According to the equation: $-0.061x/n = -0.059$, where n is the electron transfer number and x is the number of hydrogen ions participating in the reaction, the undertaking of electrons was accompanied by an equal number of hydrogen ions. According to the above results, the electrochemical reaction of Qu on the modified electrode was a two-electron two-proton process and the electrode reaction equation was expressed as follows:



3.5 Chronocoulometric response

Since the electrode process was diffusion-controlled, the diffusion coefficient could be calculated by a chroncoulometric experiment. According to the equation given by Anson [37]:

$$Q = 2nFAD^{1/2}Ct^{1/2}/\pi^{1/2} + Q_{dl} + Q_{ads}$$

where n is the number of electron transferred, F (C mol^{-1}) is the Faraday constant, A (cm^2) is the area of the electrode, D (cm^2 s^{-1}) is the diffusion coefficient of species, C (mM) is the bulk concentration of species, t(s) is the potential pulse width, Q_{dl} (C) is the double layer charge, and Q_{ads} (C) is the faradic component due to the oxidation of adsorbed species. The parameter D can be calculated from the slope Q versus $t^{1/2}$ plot if the values of A, n, and C are known.

The relationship between Q and $t^{1/2}$ was constructed with a good linear regression equation as Q (μC) = 10.24 $t^{1/2}$ +10.32 ($n = 6$, $r = 0.998$). From the slope the diffusion coefficient of Qu was calculated as 5.2×10^{-5} cm^2 s^{-1}.

3.6 Calibration curve, reproducibility, and stability

In order to examine the practical feasibility of the modified electrode, the calibration curve, stability, and reproducibility were tested. SDV curves of ten different concentrations of Qu were studied under the optimum conditions mentioned above.

It was found that the anodic peak currents were linear to Qu concentrations over the range 5.0×10^{-7}–3.3×10^{-4} M (Fig. 7). The linear regression equation was $I_{pa} = 1.721 + 0.618\ C$ (I_{pa}: μA, C: μM, $r = 0.9992$), and the detection limit was found to be 1.0×10^{-7} M on the signal-to-noise ratio of 3.

The reproducibility of the biosensor was estimated by comparing the redox peak current of 2×10^{-5} M Qu. The relative standard deviation was 2.1% with ten determinations, revealing that this modified electrode had good reproducibility. The storage stability of the biosensor in air at room temperature was evaluated by measuring the current response with 2×10^{-5} M Qu by every day use. The response of the modified electrode decreased to 95% after 10 days, whereas 92% of the original response retained after 30 days. This implies that the biosensor is considerably stable. The electrode-to-electrode reproducibility of the modified electrodes was examined on six modified electrodes constructed individually; the RSD of the six average peak currents of 2×10^{-5} M Qu was calculated to be 2.3%.

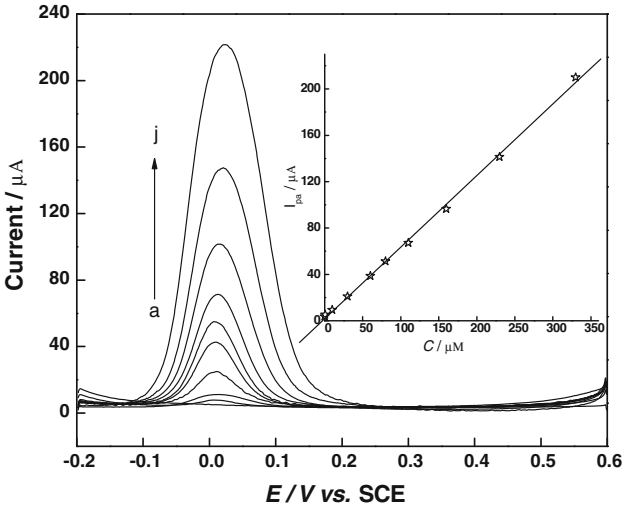


Fig. 7 SDV curves of different concentrations of Qu in pH 6.8 B–R buffer solution at FCo-Np/GC electrode: (*a*) 0; (*b*) 0.5; (*c*) 10; (*d*) 30; (*e*) 60; (*f*) 80; (*g*) 110; (*h*) 160; (*i*) 230; and (*j*) 330 × 10^{-6} M. *Inset* curve of I_{pa} versus Qu concentration

3.7 Interference study

Some organic compounds and inorganic ions were tested to check their levels of interference in Qu determination. The results suggest that 50 times concentration of lactic acid, urea, glucose, starch, sucrose, 30 times of rutin, phenothiazine, five times of dopamine, and three times for epinephrine have no influence on the signals of Qu with deviation below 5%. Otherwise, some metal ions such as 100 times concentration of Na^+, K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, and Zn^{2+} have no influence on the determination of Qu.

Ascorbic Acid (AA) is the main coexisting substance in compound Qu table, so the electrochemical response of Qu in the presence of AA on the FCo-Np/GC electrode was studied. The typical cyclic voltammograms of

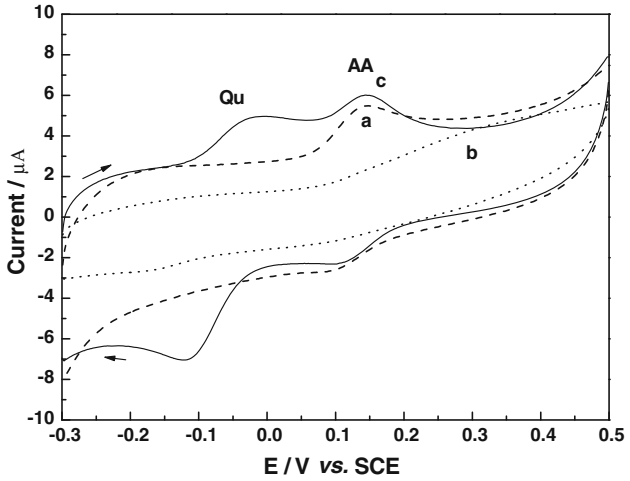


Fig. 8 CVs of 1.0×10^{-5} M AA on the FCo-Np/GC electrode (*a*); the mixed solution of 1.0×10^{-5} M AA and 5.0×10^{-6} M Qu on GC (*b*); and FCo-Np/GC electrode (*c*) with scan rate as 0.1 V s^{-1}

AA on FCo-Np/GC electrode was shown in Fig 8 (curve a), and the oxidation peak appeared at 0.143 V. Curve b was the cyclic voltammograms of AA and Qu mixture solution on the GC electrode. A rather flat electrochemical response was obtained, which showed that the electrochemical signals could not be distinguished due to the oxidation of AA and Qu almost at the same potential. While on the FCo-Np/GC electrode, two separated oxidation peaks appeared on the cyclic voltammograms with the potential at 0.145 and −0.087 V, which was attributed to that of AA and Qu, respectively (curve c). The oxidation peak potential separation was 0.230 V for AA and Qu detection, which was large enough for simultaneously determination of AA and Qu in the mixed solution.

3.8 Samples determination

This proposed method was applied to the determination of Qu in the compound Ginkgo leaf tablet. Table 1 showed the measurement results, and the recovery values of five independent experiments varied from 97.1 to 103.7%. The method was also applied to the determination of Qu in urine samples. The measurement results were shown in Table 2, and the recovery values varied from 96.0 to 103.5%. The results of the analysis using the modified electrode were compared with those obtained using the standard HPLC method [33]. According to Table 1 and Table 2, the good recovery and precision, and the accordant results with standard HPLC method, indicate that the present method is suitable for determination of Qu in drug and urine samples.

4 Conclusion

A sensor was constructed by modification of a GC electrode with FCo-Np. The FCo-Np have shown an excellent electrocatalytic activity to the redox of Qu. An electrochemical sensor for Qu detection was developed, and experimental parameters were optimized. Moreover, the sensor can be applied to the determination of Qu in Ginkgo leaf tablet and human urine samples. This study was expected to be useful for providing a sensitive and stable sensor for Qu detection.

Table 1 Determination of Qu in tablet[a] samples by SDV

Samples	Detected[b] (mg/tablet)	Added (mg L^{-1})	Found[b] (mg L^{-1})	Recovery (%)	HPLC[b] (mg/tablet)
090812	10.1 ± 0.3	1.08	1.12 ± 0.03	103.7	9.9 ± 0.4
091103	9.7 ± 0.4	2.44	2.37 ± 0.06	97.1	9.8 ± 0.2
09032901	10.3 ± 0.4	2.96	2.89 ± 0.05	97.6	10.2 ± 0.1
08070302	9.8 ± 0.3	3.29	3.24 ± 0.08	98.5	9.9 ± 0.4
20091005	10.4 ± 0.2	4.67	4.84 ± 0.10	103.6	10.2 ± 0.3

[a] Lable amount: 10 mg Qu per tablet

[b] Mean value ± standard deviation ($n = 5$)

Table 2 Determination of Qu in urine samples and recovery study

Samples	Added (mg L^{-1})	Found[a] (mg L^{-1})	Recovery (%)	HPLC[a] (mg L^{-1})
1	0	0.45 ± 0.01	–	0.49 ± 0.04
2	0.50	0.93 ± 0.03	96.0	1.00 ± 0.03
3	1.00	1.48 ± 0.04	103.0	1.54 ± 0.03
4	1.50	1.92 ± 0.03	98.0	1.99 ± 0.04
5	2.00	2.52 ± 0.02	103.5	2.47 ± 0.03

[a] Mean value ± standard deviation ($n = 5$)

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