

A New Method for Puerarin Determination Based on Poly (Alizarin Red S/Graphene) Modified Electrode

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Received: 22 August 2016 / Accepted: 13 October 2016 / Published: 10 November 2016

A poly(alizarin red S/graphene) (PARS-GR) composite membrane modified glassy carbon electrode was constructed by a facial one step electrodeposition method. The surface morphology and electrochemical characteristic of the modified electrode were investigated carefully by the assistance of scanning electron microscopy and cyclic voltammetry, respectively. The electrochemical behavior of puerarin was investigated by cyclic voltammetry and a oxidation peak appeared at +0.61 V (vs. SCE) in pH 6.5 phosphate buffer solution. The PAR-GR/GCE displayed satisfactory electrochemical properties including higher conductivity and lower electron flowing resistance. The oxidation process of puerarin on PAR-GR/GCE surface is calculated to be a one-electron and one-proton transfer process. Under the optimized experimental conditions, the oxidation peak current showed good linearity with puerarin concentration under the range of $1.0 \times 10^{-7} \sim 7.5 \times 10^{-4}$ mol/L accompanied by a detection limit of 3.4×10^{-8} mol/L (3σ). The modified electrode was successfully utilized to examine the puerarin samples with satisfactory results.

Keywords: Puerarin, Alizarin red S, Graphene, Chemical modified electrode

1. INTRODUCTION

It is well-known that puerarin (7, 4'-dihydroxyisoflavone-8 β -glucopyranoside) is the main isoflavone constituent of the plant Radix Puerarine, which endowed the name of GeGen in traditional Chinese medicine[1]. Owing to excellent antioxidative and antithrombotic effects, puerarin has been widely applied in clinical treatment for non-alcoholic fatty liver disease and alcohol-induced adipogenesis [2-6]. Recently, Wei et al. reviewed the new progress on the pharmacological studies of puerarin [7]. Various researchers have demonstrated its good neuroprotective effect, which has been utilized in clinically as an effective ingredient for the treatment of Alzheimer's disease [8], Parkinson's disease [9], cerebral ischemia [10] and other cell lines [11-13]. Nowadays, several sampling techniques

such as HPLC [14], capillary zone electrophoresis [15] and liquid chromatography tandem mass spectrometry [16] have been explored for puerarin analysis in different matrix. For example, Yan et al established a simultaneous determination for puerarin and other six constituents in rat plasma on a liquid chromatography-electrospray ionization-tandem mass spectrometry [17]. However something unsatisfactory exists, too. Some of them suffered from the drawbacks including time-consuming, toxic organic solvents, high-cost instruments or low precision and sensitivity and so on. For comparison with the established methods above, electroanalytical techniques obtained more and more attentions in the field of quantitative analysis with advantages of low cost, ease to operation, fast and sensitive analysis and few sample preparation. Some electroanalytical approaches have been reported for puerarin determination based on different modified electrodes, such as single-walled carbon nanotube/poly Evans blue membrane [18] and CdTe quantum dots-decorated poly (diallyldimethylammonium chloride)-functionalized graphene nanocomposite [19].

In order to improve analytical performance of the working electrode, the combination of various materials have obtained wider interests for the modification of electrode surface. Graphene, the thinnest carbon material, possesses excellent merits of large specific surface area, good physical and chemical stability, excellent mechanical, and high electrical and thermal properties [20-22]. Therefore, graphene is an ideal carbon material used in the domain of chemical modification electrode [23-25]. Zhou et al reported a DNA sensor based on reduced graphene oxide modified glassy carbon electrode [26]. Alizarin red S (ARS) is a common member of dihydrodiketoanthracene family, which has been widely used as a coordination reagent for metal species analysis by spectrophotometric method [27]. Owing to good electrochemical behaviours, ARS has been studied as an electrochemical probe for large biomolecules determination [28, 29]. Furthermore, ARS has been explored as a modifier material to get modified electrodes with higher properties by electropolymerization, and its possible mechanism of the electropolymerization was also demonstrated [30, 31]. The benzoquinonyl and hydroxyl groups in the molecule endowed the polymeric product (PARS) act as a receptor for protons, which could facilitate the transfer rate of electron between electrode and reagent [32].

In this work, a PARS-GR composite membrane modified glassy carbon electrode (PARS-GR/GCE) was constructed by electrochemical co-deposition of ARS and GO via cyclic voltammetry technique. Because of the synergistic effect of PMB and GR, resulting the formed nanocomposite combined the merits of PARS and GR, including large specific surface area, high catalytic activity and unique electrochemical properties. The modified electrode displayed good catalytic ability, and a new electrochemical approach for puerarin determination was established.

2. EXPERIMENTAL

2.1 Apparatus and Reagents

Electrochemical measurements were all carried out by an electrochemical workstation (EC 550, Wuhan Gaossunion Technology Co., Ltd., China) with a traditional three-electrode system consisting of a modified GCE as the working electrode, Pt wire as the auxiliary electrode, and a saturated calomel

electrode (SCE) as the reference electrode. Scanning electron microscopy (SEM) was conducted with a JSM-7500F scanning electron microscope (Japan Electron Company, Japan).

Puerarin (Zhejiang Fangge Pharmaceutical Co. Let., China) , alizarin red S (ARS, Tianjin Kemiou Chemical Reagent Co., Ltd., China) were used directly. 0.2 mol/L phosphate buffer saline (PBS) with different pH values was applied as supporting electrolyte. Deionized water were used for all experiments and other reagents were all of analytical grade and used as purchased.

2.2 Fabrication of PARS-GR/GCE

First, the bare GCE should be awarded a common precondition to get a mirror-like surface, including polish and wash. Then the PARS-GR/GCE was prepared as follows. Second, The GCE was applied cyclic voltammetry from -1.4 V to 1.8 V at 100 mV/s for 30 cycles in a homogeneously dispersed solution containing 10.0 mmol/L alizarin red and 1.0 mg/mL GO to get PARS-GR/GCE. The obtained PARS-GR/GCE was washed with deionized water and preserved in a refrigerator at 4 °C when was out of use.

3. RESULTS AND DISCUSSION

3.1 Characterizations of PARS-GR composite film

As showed in Figure 1, the SEM images illustrated the morphologies of different modified electrodes. It can be seen that sheet-like graphene can be observed on GR/GCE (Figure 1A). PARS film on GCE surface has a fine branch-like structure (Figure 1B). On PARS-GR/GCE (Figure 1C), a uniform and loose surface was got, which can be ascribed to the integration of GR nanosheet with the polymer. Under the selected conditions, ARS was electropolymerized to form a polymer as well as GO was electrochemically reduced to GR. Thus PARS-GR composite was simultaneously formed on GCE surface, which resulted in the increasement of electrode surface area and charge transfer rate.

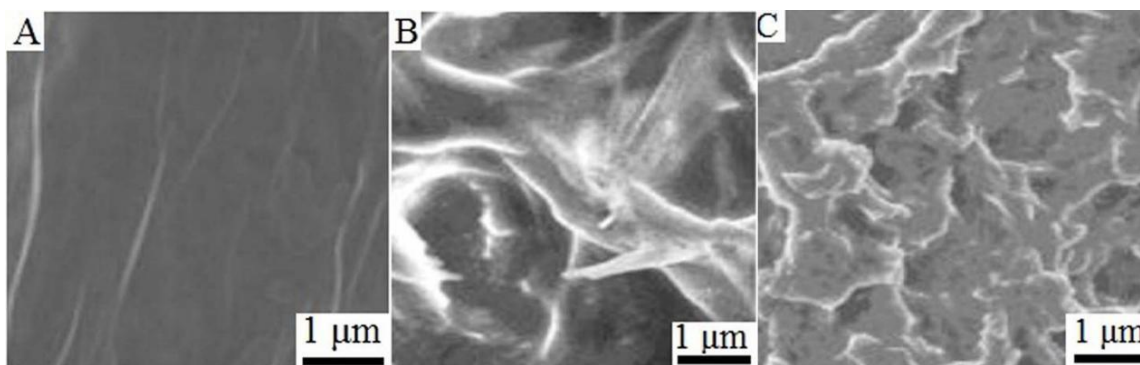


Figure 1. SEM images of GR/GCE (A), PARS/GCE (B) and PARS-GR/GCE (C).

Electrochemical impedance spectroscopy (EIS) is an effective technique to investigate the interface properties. Herein it was utilized to characterize various modified electrodes with the results shown in Figure 2. At bare GCE the electron-transfer resistance (R_{et}) was obtained as 1940 Ω (curve c), which was larger than that of on GR/GCE (310 Ω , curve d), demonstrating that the graphene film on the GCE surface greatly enhanced the conductivity and the electron transfer rate. However, the R_{et} of PARS/GCE was increased to 2590 Ω (curve a), which could be ascribed to the electrostatic repulsion force between the negatively charged $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and PARS film[33]. While on PARS-GR/GCE, the R_{et} was reduced to 1130 Ω (curve b), which confirmed the successful modification of PARS-GR composite on GCE. During the electrodeposition process, ARS and GO could be coelectropolymerized together, resulting the formation of GR-doped electropolymer, which could accelerate electron transfer rate.

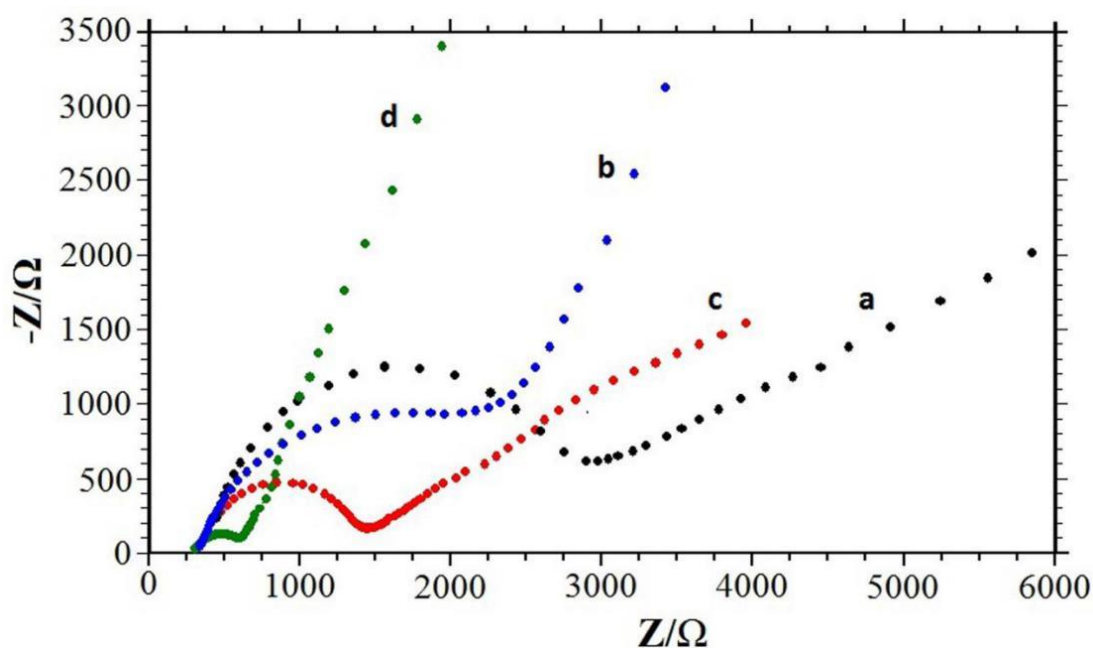


Figure 2. EIS curves of PARS/GCE (a), bare GCE (b), PARS-GR/GCE (c) and GR/GCE (d) in pH 6.5 PBS containing 5.0×10^{-3} mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 mol/L KCl solution.

The influences of the electropolymerized conditions on electrochemical properties of PARS-GR/GCE was optimized. The scan cycles and the scan rate can effect the thickness of the polymer membrane and the response of the modified electrode. Therefore, the two factors were studied by single factor experiment within the range from 5 to 30 cycles and from 10 to 100 mV/s in a 1.0 mmol/L $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution by cyclic voltammetry, with the values of redox peak currents and the peak-to-peak separation as the indicator. The results demonstrated that the optimized cycle number was 20 and scan rate was 50 mV/s.

3.2 Electrochemical behaviors of puerarin

Cyclic voltammetry was applied to investigate the electrochemical behaviors of 1.0×10^{-5} mol/L puerarin in 0.1 mol/L pH 6.5 PBS with various modified electrodes as working electrodes with results shown in Figure 3. It was obvious that no visible peak appeared on GCE (curve a), indicating the reduction of puerarin was difficult to realized. While on PARS/GCE (curve b), the weak oxidation was observed at 0.62 V (vs. SCE). In contrast, a clear oxidation peak of puerarin was obtained on GR/GCE (curve c) and PARS-GR/GCE (curve d) at the potential of 0.61 V (vs. SCE), which was a typical character of irreversible electrode reaction. While on PARS-GR/GCE the oxidation peak current (I_p) was enhanced obviously with the value of $8.68 \mu\text{A}$, which was 1.6 and 1.4 times larger than those on PARS/GCE and GR/GCE, respectively. The results showed that the PARS-GR film on GCE was favorable for the electron transfer rate, which could be attributed to the synergic effect of PAES and GR. According to Ref. [31], the possible mechanism of the electropolymerization of AR was shown in scheme 1, the electro-reactions occurring at the modified electrodes may be accompanied by the exchange of protons with the solution, which was beneficial to the realization of puerarin redox on PARS-GR/GCE. Thus PARS-GR/GCE was applied as the working electrode in the following studies.

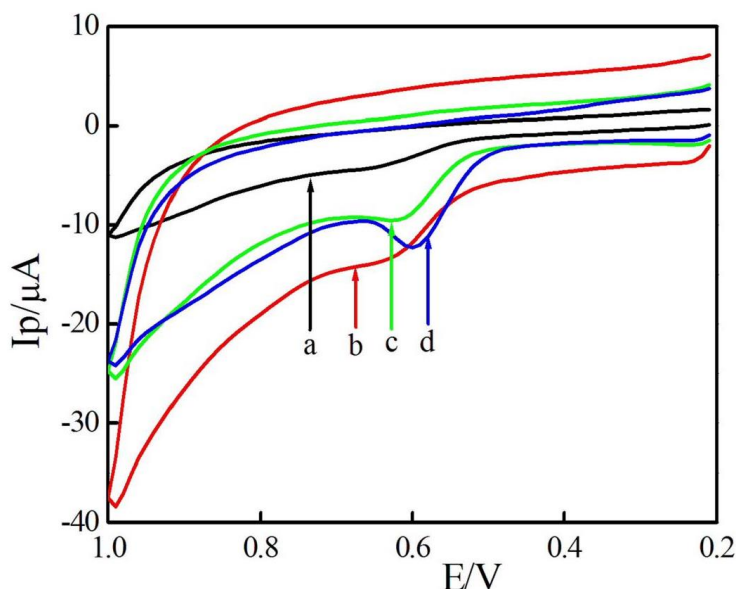


Figure 3. Electrochemical behaviors of 1.0×10^{-5} mol/L puerarin on different electrodes. Curves a-d: bare GCE, PARS/GCE, GR/GCE and PARS-GR/GCE.

3.4 Effect of buffer pH

The correlation of pH with electrochemical response of 1.0×10^{-5} mol/L puerarin on PARS-GR/GCE was studied in PBS solution within the pH range from 5.0 to 8.0. The results was shown in Figure 4. It can be seen that the oxidation peak potential shifted negatively while the pH value increased. The pH dependence on E_p was in linearity with linear regression equation as $E_p(\text{V})=0.897-0.0418 \text{ pH}$. The slope of the equation between E_p and pH was 41.8 mV pH^{-1} , which was approximate

to the theoretical value of 59.0 mV pH^{-1} , indicating the same number of electrons and protons involved in the electrode reaction [34]. In addition, the peak current of puerarin reached its maximum at pH 6.5. So pH 6.5 was selected as the optimal acidity for all following experiments.

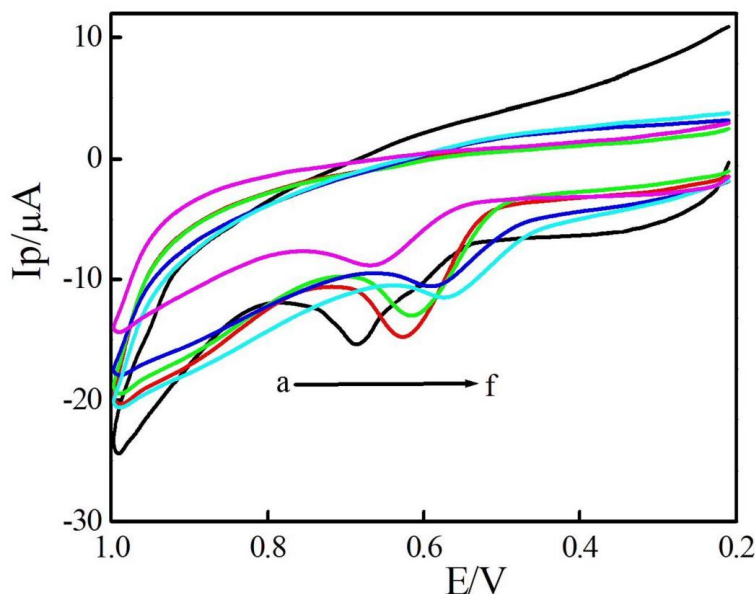


Figure 4. Cyclic voltammograms of 1.0×10^{-5} mol/L puerarin on PARS-GR/GCE with different buffer pH (from a to f are 5.0, 6.0, 6.5, 7.0, 7.5, 8.0) at the scan rate of 100 mV/s.

3.4 Effect of scan rate

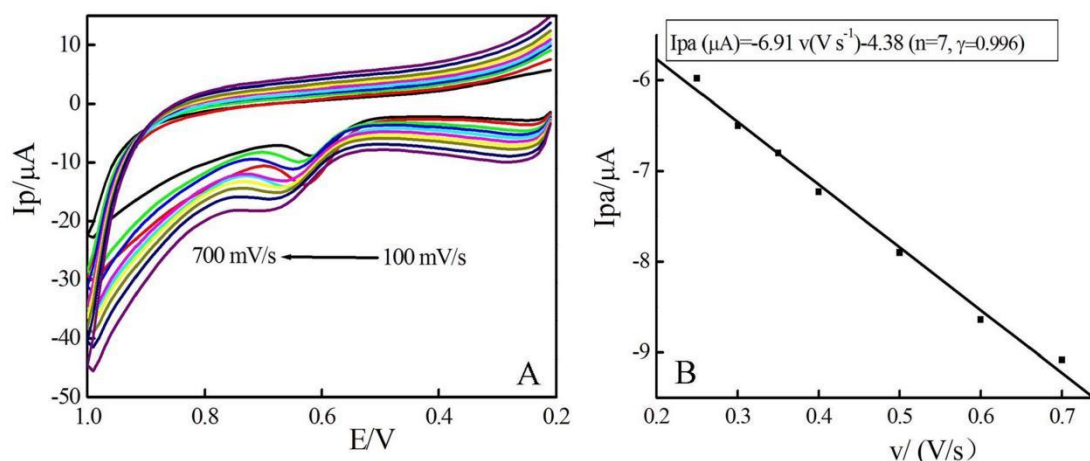


Figure 5. (A) Cyclic voltammograms of 1.0×10^{-5} mol/L puerarin on PARS-GR/GCE with with different scan rates (a-j: 100, 150, 200, 250, 300, 350, 400, 500, 600, 700 mV/s) in pH 6.5 PBS; . (B) Linear relationship of the peak current I_p with the scan rate (ν) in the range from 200 to 700 mV/s.

The influence of scan rate on electrochemical response of puerarin at ARS-GR/GCE was studied by cyclic voltammetry over the range of 30~700 mV/s. As seen from Figure 5A, with the scan rate increasing, the oxidation peak currents grown with the positive movement of redox potentials. A good linear relationship was plotted between I_{pa} and scan rate (ν) with the result shown in insert of

Figure 5B, indicating that the electrode reaction was an adsorption-controlled process. Therefore, an adsorption and enrichment process of puerarin on PARS-GR/GCE surface occurred before the electrode reaction, consequently an increased oxidation current response was obtained.

The relationship between E_p and ν could be presented by the following equation:

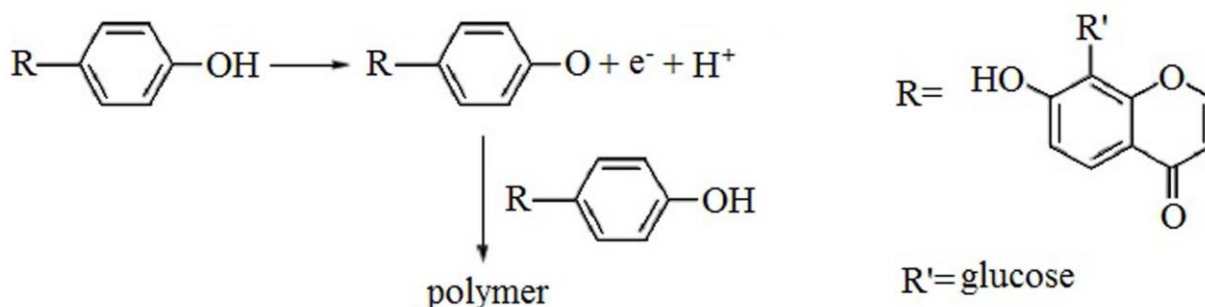
$$E_p(\text{V}) = 0.717 + 0.0536 \ln \nu \quad (\text{V/s}) \quad (1)$$

According to the Laviron's equation below [35].

$$E_p = E_p^0 + \frac{RT}{(1-\alpha)nF} \ln \nu \quad (2)$$

where E^0 is formal potential, α is transfer coefficient of the oxidation of puerarin, n is the number of electrons transferred in electrode reaction. Other symbols have their common meanings.

Based on the Eqs. (1) and (2), the value of $(1-\alpha)n$ was calculated as 0.48. For a totally irreversible and adsorption-controlled electrode process, the value of α is normally presumed as 0.5. Therefore, n was obtained as 0.96, which is almost equal to 1.0, indicating the oxidation process of puerarin is a one-electron and one-proton transfer process. This result was in agreement with the previous work and a possible electrochemical oxidation mechanism of puerarin on the modified electrode was proposed as follows (Scheme 1) [36].



Scheme 1. The possible oxidation mechanism of puerarin.

3.5 Calibration curve

With the purpose of enhancing the sensitivity, differential pulse voltammetry (DPV) was applied to examine puerarin under the optimized experimental parameters. The results indicated that I_{pa} value increased gradually with puerarin concentration, obeying a linear regression equation of $I_{pa} (\mu\text{A}) = 0.3227 C (\text{mol/L}) + 1.1284$ ($n=11$, $\gamma=0.996$) over the range of $1.0 \times 10^{-7} \sim 7.5 \times 10^{-4} \text{ mol/L}$. The detection limit was obtained as $3.4 \times 10^{-8} \text{ mol/L}$ (3σ). The comparison of this new explored method to some other reported electrochemical methods for puerarin analysis was listed in Table 1. Among of them, CdTe-PDDA-Gr/GCE showed the lowest detection limit, which may be ascribed to the high conductivity and electrocatalytic activity of CdTe nanoparticles toward puerarin. However it necessitated more complicated electrode construction process. Compared to other methods, this as-proposed approach

showed the advantages of wider determination range and lower detection limit, which was suitable for micro-analysis.

Table 1. Comparison of different modified electrodes for puerarin detection

Electrode	Linear range ($\mu\text{mol/L}$)	Detection Limit ($\mu\text{mol/L}$)	Reference
Dropping mercury electrode	0.4-6.0	0.05	[37]
GCE	0.1-57.6	0.1	[38]
MCNT-PE	0.8-20.0	0.36	[36]
CeO ₂ /MWCNTs/GCE	0.04 - 6.0	0.008	[18]
CdTe-PDDA-GR/GCE	0.001-0.1	0.0006	[19]
PAR-GR/GCE	0.1-750.0	0.034	This work

*MCNT:multi-wall carbon nano-tubes; PE: paste electrode; PDDA: poly (diallyldimethylammonium chloride).

The relative standard deviation of 9 consecutive measurements of 2.0×10^{-5} mol/L puerarin was got as 1.47 %, suggesting that PARS-GR/GCE displayed a good reproducibility for electrochemical determination of puerarin in its linear range. The stability of PARS-GR/GCE was also studied by examining the current response to 2.0×10^{-5} mol/L puerarin. After continuous 30 potential cycles, the current response remained 97.8 % of the initial value. PARS-GR/GCE gave a response decrease of 5.56 % after a storage of 30 days in 4 °C refrigerator. The results obtained above showed a good reproducibility and stability of PARS-GR/GCE.

3.6 Interference studies

Table 2. Influence of coexisting substance on the determination of 1.0×10^{-5} mol/L puerarin

Coexisting substance	Concentration (mol/L)	Relative error (%)
K ⁺	1.0×10^{-3}	1.27
Na ⁺	1.0×10^{-3}	1.78
Ca ²⁺	1.0×10^{-3}	2.67
Mg ²⁺	1.0×10^{-3}	3.12
Zn ²⁺	1.0×10^{-3}	2.98
Ca ²⁺	1.0×10^{-3}	2.87
Cu ²⁺	1.0×10^{-3}	-2.36
citric acid	1.0×10^{-4}	1.80
urea	1.0×10^{-4}	3.45
glutamic acid	1.0×10^{-4}	2.48
ascorbic acid	1.0×10^{-4}	2.25
glucose	1.0×10^{-4}	1.07

The potential influences of common interfering substance to 1.0×10^{-5} mol/L puerarin were investigated. From the results listed in Table 2, it can be seen that no obvious influences appeared on the puerarin determination, indicating a high anti-influence ability of the proposed method.

3.7 Analytical applications

Four artificial puerarin samples were prepared and further used to evaluate the practical application of the as-explored method by a standard addition method. As shown in Table 3, puerarin could be determined satisfactorily with a recovery range of 98.4 %~104.0 % and the relative standard deviation was up to 2.50 %, demonstrating the promising application of PARS-GR/GCE to puerarin detection.

Table 3. Determination of puerarin in artificial samples (n=5)

Sample	Specified (mol/L)	Added (mol/L)	Detected (mol/L)	RSD (%)	Recovery (%)
1	1.0×10^{-5}	5.0×10^{-6}	1.52×10^{-5}	2.12	104.0
2	1.0×10^{-5}	1.5×10^{-5}	2.52×10^{-5}	1.76	101.3
3	1.0×10^{-5}	2.0×10^{-5}	2.98×10^{-5}	2.25	99.0
4	1.0×10^{-5}	2.5×10^{-5}	3.46×10^{-5}	2.50	98.4

4. CONCLUSIONS

A new kind working electrode of PARS-GR/GCE was constructed successfully by electrochemical co-deposition of ARS and GO on the surface of GCE. Owing to the synergistic effect, PARS-GR/GCE showed good electrocatalytic activity to puerarin with enhanced electrochemical responses. Consequently a novel electrochemical method was explored for puerarin analysis with a wider linear range of $6.0 \times 10^{-6} \sim 7.5 \times 10^{-4}$ mol/L and a detection limit of 2.1×10^{-6} mol/L, which also displayed high sensitivity, good stability and anti-influence ability.

ACKNOWLEDGMENTS

This work received financial support from the project of Shandong Province Higher Educational Science and Technology Program, China (No. J14LD59), the Natural Scientific Foundation of Shandong Province, China (ZR2014CM046, ZR2015CL019) and Collaborative Innovation Center of Chinese Medicine Antivirus in Shandong University of Traditional Chinese Medicine (XTCX2014B01-07).

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