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Preparation of Graphene-Copper Nanocomposite for Constructing Electrochemical Sensor for Paclitaxel Anti-Cancer Drug Detection in *Taxus Chinensis*

Yanjun Du[1,*], *Mengmeng Dou*[1], *Wang Ma*[2], *Xinjie Wang*[1], *Zhaosen Gu*[1] *and Xiaoming Deng*[1]

[1] The First Affiliated Hospital of Zhengzhou University, Traditional Chinese Medicine Department, No.1 Jianshe Eastern Road, Zhengzhou, Henan, China
[2] The First Affiliated Hospital of Zhengzhou University, Oncology Department, No.1 Jianshe Eastern Road, Zhengzhou, Henan, China
[*]E-mail: yanjundu_hn@qq.com

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In this work, a glassy carbon electrode modified with graphene and Au nanoparticles (GCE-graphene-EAu) was employed to electrochemically study paclitaxel which belonged to anticancer drug. Graphene and Au NPs were successfully electrodeposited on the surface of glassy carbon electrode in order to enhance the conductivity of electrode. An irreversible oxidation process was observed in the pH range of 3 to 10, which was diffusion-controlled. A linear relationship was observed between the anodic peak current and the concentration of paclitaxel ranging from 0.01 to 2 mM, where the limits of detection (LOD) and quantification (LOQ) were 0.005 and 0.004 mM, respectively. The designed approach was demonstrated to be efficient in the determination of paclitaxel in Chinese herb *Taxus chinensis*.

Keywords: *Taxus chinensis*; Paclitaxel; Sensor; Au nanoparticles; Graphene

1. INTRODUCTION

Paclitaxel (PAC), approved by Food and Drug Administration, is a promising drug for treating ovarian cancer in 1992, breast cancer in 1994 and Kaposi's sarcoma in 1997. In addition, paclitaxel exhibits significant efficiency for treating other cancers involving cancer of colon and lung, lymphoma and melanoma. Moreover, paclitaxel, known as the active agent, has been recognized as a promoter for the assembly of microtubule, which might be induced by its anti-tumour activity. During the past two decades, PAC has been considered as the most promising agent for cancer treatment by numerous

oncologists [1-7]. When employed in the cell treatment, PAC exhibits various disadvantages including cell division, chromosome segregation as well as mitotic spindle assembly. However, PAC can prevent the disassembly of microtubule polymer, which is different from other tubulin-targeting drugs like colchicine that inhibit the assembly of microtubule. Hence, the metaphase spindle configuration could not be obtained in chromosomes, where the progression of mitosis is blocked. Besides, the activation of mitotic checkpoint triggers apoptosis is prolonged as well as the reversion to G-phase of the cell cycle in the absence of cell division.

Drug analysis plays a critical role in controlling the quality of drugs, leading to the highly demand of developing an effective detection method with reliability, simplicity and sensitivity. So far, various approaches such as square wave voltammetry with Au electrode modified with the film of Cysteamine/DNA/SWNTs [8], liquid chromatography with anodic amperometric detection [9] and various HPLC approaches [10-15]. have been reported for the determination of PAC. However, all the above-mentioned approaches exhibit diverse problems, including the constructions of electrodes, the complicated separation processes and time-consuming extraction procedures.

In recent years, owing to the specific physicochemical characteristics such as outstanding thermal and electric conductivity, high surface area (2630 m^2/g of mono-layer graphene in theory) and strong mechanical strength, graphene has attracted intensive scientific and technological interest with its remarkably potential use in various applications including bioscience or biotechnologies as well as energy and electronics storage and conversion like solar and fuel cells, batteries and supercapacitor. The endow graphene and its relevant composites exhibit potential applications in the electrochemical biosensors due to the exciting properties including specific biocompatibility, massive production, easy functionalization and outstanding conductivity. Nevertheless, chemical groups are required to be modified onto the obtained hydrophobic graphene to enhance the applicability in prior to further usage. A variety of chemical reactions are involved in the synthesizing processes, leading to the requirement of complicated control of reaction conditions. The electrodeposition approach is a significantly facile and environment-friendly technique. The reduction of graphene oxide to graphene could be achieved in the dispersion during the process of electrodeposition, where the formed graphene could be deposited on the surface of the electrode directly.

Normally, the electrodeposition of Au NPs was performed to prepare the electrode modified with graphene-Au NPs by electrodeposition technique was employed for detecting biomolecules. Graphene, which is inert material, exhibits negligible influence on the bioactivity of biomolecules [16]. However, Au NPs was reported to display biocompatibility to biomolecules [17, 18]. Hence, the outstanding biocompatibility and affinity to the biomolecules were observed with such electrode [19, 20]. The conductivity of the electrode was improved remarkably by the electrodeposited layer of graphene and Au NPs, which was employed as the electron mediator.

Herein, a facile current voltammetric technique with low cost was proposed to determine PAC. The electrochemical oxidation of PAC was performed at the surface of electrode modified with graphene and Au NPs. Then, various parameters were optimized during the determination of PAC, resulting to the successful development of an electrically analytical approach which exhibited various characteristics of easy repairmen, fast response, renewal of paclitaxel, remarkable reproducibility as

well as low limit of detection. Furthermore, the designed approach was employed to determine PAC extracted from *Taxus chinensis*.

2. EXPERIMENTS

2.1. Materials

Graphite oxide was commercially available in XF Nano Inc. (Nanjing, China). Powder PAC was supplied by Reddy's laboratory (Hyderabad) and used as received. All the used chemicals were analytically pure. The electrochemical measurements were performed on a CHI760D electrochemical system (Chenhua Instrument, Shanghai, China) with three-electrode system. A glassy carbon electrode with a diameter of 3 mm (GCE) was used as working electrode, and saturated calomel electrode (SCE) and Pt foil were employed as reference electrode and auxiliary electrode, respectively. The morphology of the surface of the electrode was analyzed by scanning electron microscopy (JSM-6700F field emission scanning electron microscope, JEOL Ltd., Japan). Moreover, a Model CS501-SP thermostat (Huida Instrument, Chongqing, China), a Sigma 1-14 Microcentrifuge (Sigma, Germany), a Sigma 4K15 laboratory centrifuge and an AFS-9700 atomic fluorescence spectrophotometer (Kechuang Haiguang Instrument, Beijing, China) were employed for the assay. All the experiments were carried out at room temperature unless stated otherwise.

2.2. Sensor fabrication

In prior to the self-assembly of thiolated P1 on the electrode, graphene and Au was electrodeposited on the surface of GCE treated with H_2SO_4. In brief, the powder of graphite oxide was firstly added into PBS solution with the pH of 9.18 and then exfoliated through ultrasonication to obtain the colloidal dispersion of graphene oxide with a concentration of 1.0 mg/mL. Subsequently, the obtained dispersion was reduced by cyclic voltammetric reduction with N_2 bubbling under stirring at 4 ^oC between -1.5 and 0.5 V at 10 mV/s. Thereafter, Au was electrodeposited on the surface of GCE modified with graphene through chronoamperometry at 0.18 V in $HAuCl_4$ solution (1%, w/w) with perchloric acid (0.5 M). Then, P1 with a concentration of 1 mM was self-assembled on the GCE-graphene EAu. At last, 6-mercapto-1-hexanol was used to treat the electrode for 30 min.

2.3. Sample preparation

The viscous injection with the color of faint yellow can be achieved after the addition of colorless and clear taxeleon. Before the intravenous infusion, the injection was diluted through appropriate parenteral fluid as an actor of non-aqueous solution. The taxeleon solution with the concentration of 0.1 mM was obtained by putting exactly 2.84 ml of taxeleon into 200 ml of methanol, and the obtained solution was treated with sonication to guarantee complete dissolution. Partial solution was transported into the voltammetric cell, and the analysis was performed with same

condition which was used in the calibration. Pure PAC was acted as the description in the voltammograms record. Recycle experiments were conducted using the method with standard addition, to investigate the effect of excipients which was used in the forms of dosage and the precision of this method. In this investigation, the PAC of known concentration was added to the dosage of known amounts. The obtained mixture was examined like the analysis with pure PAC.

3. RESULTS AND DISCUSSION

Gold and graphene was successfully deposited on the surface of GCE by electronic method. As shown in classic CV of GO electrolysis (Figure 1A), conducting graphene was achieved on the GCE surface, which was proved by the increase of voltammetric current under consecutive scans of potentials. Meanwhile, as shown from the SEM image of GCE surface (Figure 1B), the graphene was successfully deposited as confirmed by the observed veli-like film coating. The further improvement of the microenvironment was essential to determine the target on the surface of electrode. An appropriate amount of electrodeposited Au would be advantageous to the graphene-Eau system [21]. EAu with excessive amounts would reduce the influence of graphene, which made the electron transformation more difficulty. Moreover, the response will decrease due to the unavailable enough active sites for electrochemical reaction. As can be seen from SEM image of graphene-EAu decorated GCE (Figure 1C), gold nanoparticles were highly dispersed on the surface of graphene.

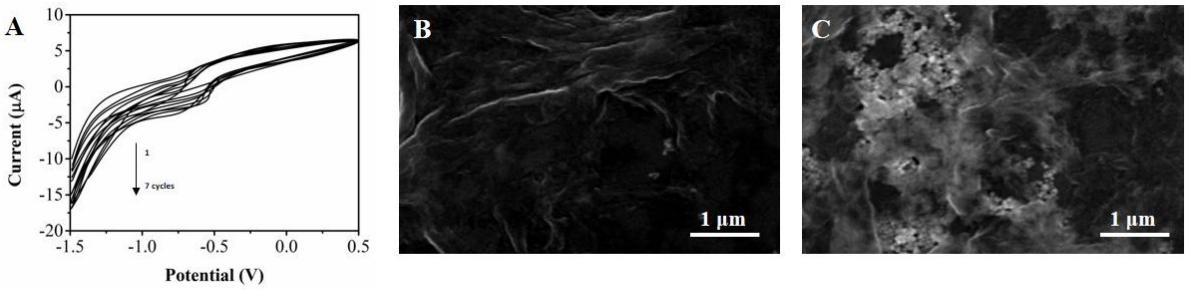

Figure 1. Characterized performance of the decorated surface of electrode: CV of graphene deposited by electronic method on the GCE under the GO reduction. Condition: 0.1M PBS; Scan rate: 10 mV/s (A); graphene decorated GCE SEM image (B); the graphene-EAu decorated GCE image of the SEM (D).

CV was employed to investigate the electrochemical performance of GCE-graphene-EAu for the determination of PAC. As shown in Figure 2, the excellent peak of anode at 1.187 V was exhibited, and the peak intensity was better in contrast to bare GCE. The electrode performance with PAC demonstrated irreversible from the results of the reverse scan, which showed the absence of reduction peak, indicating that, the electrode process of PAC is an irreversible one [22]. The first cycle record of the voltammograms was generally achieved. Well-established electro-oxidation process to PAC was achieved from the scans by comparing the GCE with and without graphene.

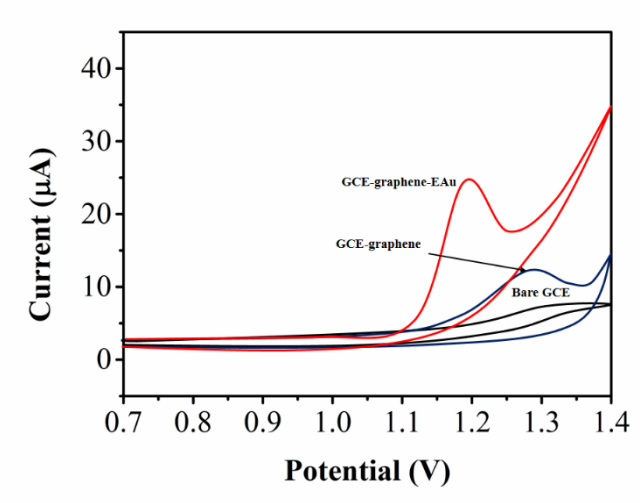

Figure 2. The CV comparison at 1 mM PAC among (GCE-graphene-Eau) in this system and GCE with/without graphene. Condition: electrolyte 0.1 M PBS; pH: 7; Scan rate: 50 mV/s.

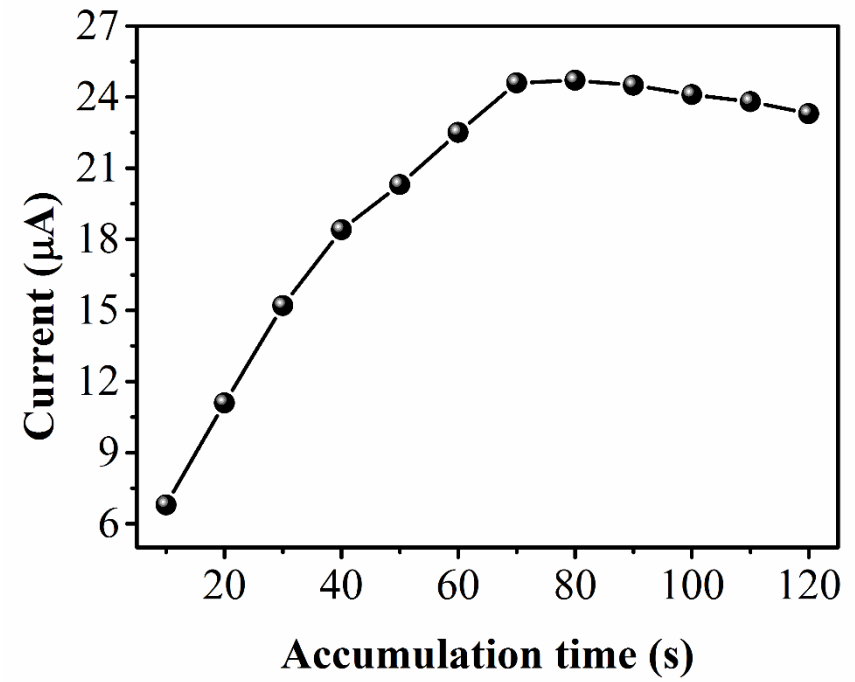

Figure 3. Influence of accumulating time to the peak current for 1 mM PAC oxidation. Condition: electrolyte 0.1 M PBS; pH: 7; Scan rate: 50 mV/s.

The potential and time of accumulation were investigated. Accumulation of analyte and improvement of sensitive determination in electrochemistry and analysis chemistry were conducted by the widely employment of open circuit. As illustrated from the effect of accumulating time (0-120 s) on the PAC oxidation in this system (Figure 3), the current increased with increasing accumulating time (0-70 s). The current showed no more increase when the time was higher than 70s, which meant that the time of 70s is optimal condition for next experiments.

The peak current in PAC showed no visible change by changing the accumulation potential. The current peak could not be determined by the accumulation potential, so the open circuit was used to perform the accumulation.

The medium pH could affect the electrode action. As illustrated in Figure 4A, CV was employed to investigate the electro-oxidation in the phosphate buffer with the pH ranging from 4 to 10 upon the 1 mM PAC, where the current and potential of peak were affected by the solution pH. As shown in Figure 4B, peak potential decreased corresponding to the increase of pH. This obeyed the following equation:

$$Ep(V) = 1.6406 - 0.0574\,pH \quad (1)$$

The slope in the equation is 57.4 mV/pH. The amounts of the hydrogen ions participating in the surface reaction of electrode were the same with the that of the transported electron, which was proved by the similarity between the experimental data and theoretical data (59 mV/pH) [23], suggests that the number of electrons transferred is equal to thatof the hydrogen ions taking part in the electrode reaction. In the proposed method, the electro-oxidation of PAC involvestwo electron and two proton transfer process. Literature survey reveals that C-7 hydroxyl group of PAC is easily oxidized than C-2 hydroxyl group [24, 25].

As shown in the Figure 4C, the relationship between I_{pa} and pH demonstrated volcano-like, where the peak was at pH 7.0. At pH 7, the best experimental data was achieved with highly sensitive detection and clear response, so this condition should be employed for further use.

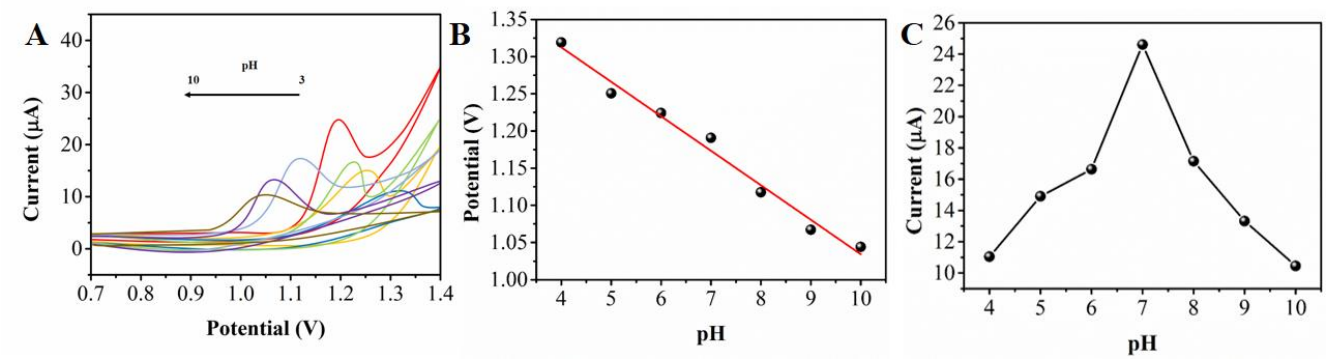

Figure 4. The pH effect to the shape of the peak of anode (A). A plot of I_{pa}(μA) *vs* PAC pH (B). The pH effect to the peak of potential of PAC (C). Condition: electrolyte 0.1 M PBS; Scan rate: 50 mV/s.

As shown in Figure 5A, linearly sweeping voltammetry was used to test the influence of scanning rate on electro-oxidation of PAC. In similarity with Figure 3B, these are typical current controlled by the diffusion, which is proved by the linearity between current and square root of the scan rate (0.05-0.425 V/s). As illustrated in Figure 5C, the relationship between log *v* and log I_{pa} was linear. The experimental slope (0.46) agreed well with theoretically data (0.5) in the absolutely diffusion controlled current, which in converse, current with diffusion controlled was present in this test. In Figure 5D, the peak of potential changed positively with the increase of the scanning rate, where relationship with linearity was obvious (0.05-0.425 V/s). This obeyed the following equation:

$$Ep(V)0.0259\log v(Vs-1)+1.2455 \quad (2)$$

If the electrode process is irreversible, according to Laviron formula, E_p is defined as the following equation:

$$Ep = E^0 + (\frac{2.303RT}{\alpha nF})\log(\frac{Rkk^0}{\alpha nF}) + (\frac{2.303RT}{\alpha nF})\log v \quad (3)$$

Here, α, k^0, n, v, E^0 are presented as transfer coefficient, standard heterogeneous rate constant of the reaction, number of transferred electrons, scan rate and is the standard redox potential, respectively. Other symbols are used as their usual meaning. Therefore, value of αn can be easily calculated as the slope of E_p vs. log v plot. In our case, the slope is obtained to be 0.0259. And thus, the value of αn is calculated to be 2.2149 (T = 298 K, R = 8.314 JK/mol, and F = 96480 C/mol). On the basis of Bard and Faulkner equation,

$$\alpha = \frac{47.7}{E_p - E_{p/2}} mV \quad (4)$$

where $E_{p/2}$ is the potential when the current is half peak value, the value of α is calculated equal to 0.9. Therefore, the number of transferred electrons (n) in the process of PAC electro-oxidation is calculated to be 2.37. In addition, E^0 is obtained from the intercept of E_p vs. v curve. Subsequently, when the value of E^0 is known, k^0 can be determined from the intercept of the previous plot. In our system, the intercept for E_p vs. log$v$ plot is 1.2237. E^0 and the k^0 are calculated to be 1.2087 and 1685, respectively. The number of transferred electrons for totally irreversible electron-transfer process was also calculated using the Randles–Sevcik equation which is defined as follow:

$$Ipa = (2.99\times10^5)n\alpha^{1/2}AC_0 * D_0^{1/2}v^{1/2} \quad (5)$$

where A is the electrode surface area, α is the charge transfer coefficient, D_o is the diffusion coefficient of the electroactive species, and C_o* is the bulk concentration of the solution. According to this equation, anodic peak current is presented as a linear function of square root of scan rate. Under experimental conditions, electro-active surface area of the GCE-graphene-EAu is to be 0.322 cm^2, diffusion coefficient of the electroactive species and concentration of the bulk solution are 6.11×10^{-5} cm^2/s and 5 mM, respectively.

Charge transfer coefficient α is calculated by using the following equation:

$$Ipa = 0.227nFAC^*k^0\exp\{(-\alpha F/RT)(E_p - E0')\} \quad (6)$$

Here, E_p and I_{pa} are presented as the peak potential and peak current, k^0, n, $E^{0'}$ are heterogeneous rate constant, number of transferred electrons, and formal redox potential. $E^{0'}$ value can be obtained from peak potential versus scan rate plot by extrapolating the straight line to v = 0. The value of α can be calculated as the slope of logI_p vs. (E_p-$E^{0\prime}$) curve. Under our experimental condition, slope is obtained to be 23.30, and the value of α is calculated to be 0.5879. Base on above calculation results, number of electrons is calculated equal to 2.39 which is equally the same with the calculation result in the previous irreversible electrode process.

As a precise electro-analytical technique with high sensitivity and low detection limits, differential pulse voltammetry (DPV) is widely employed to investigate the redox properties of chemicals at extremely small amounts. Therefore, DPV was used for electrochemical measurement of PAC in this work and results were shown in Figure 5. Phosphate buffer solution (pH = 7.0) was used

as the supporting electrolyte for the quantitative analysis of PAC. Peak current increased linearly with the increasing concentration of PAC ranging from 0.01 to 2 mM. However, when PAC concentration increases to a much higher level, linear relationship disappears due to the adsorption of PAC or its oxidation products on the electrode surface. The limit of detection (LOD) and limit of quantification (LOQ) are 0.005 mM and 0.004 mM, respectively. Table 1 shows the comparison of our proposed PAC electrochemical sensor with some previously reported sensor.

Table 1. Comparison of proposed PAC electrochemical sensor with other reports.

Method	LDR (μM)	LOD (μM)	Reference
Graphite pencil electrode	4-300	2	[22]
RP-HPLC	5-15	3	[12]
Micellar electrokinetic chromatography	0.1-500	0.02	[26]
HPLC	3-1000	1	[27]
HPLC	5-1000	3	[28]
Fluorescence and solid-state NMR spectroscopy	0.05-0.7	0.02	[29]
Graphene-copper	10-2000	5	This work

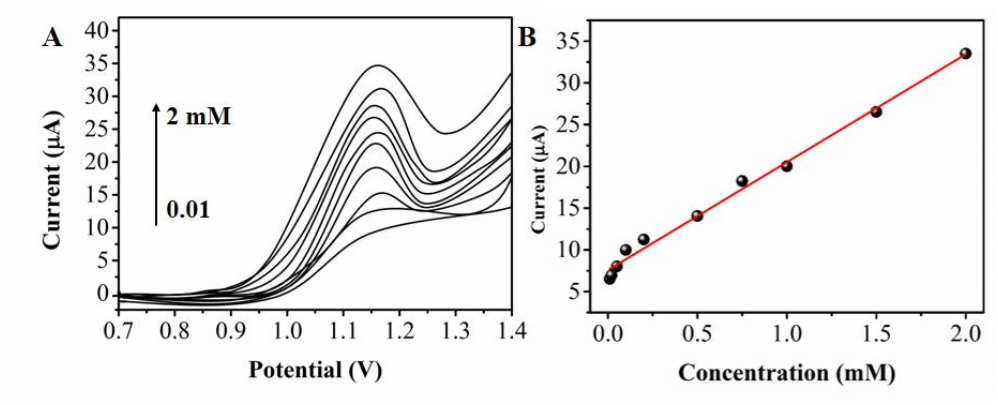

Figure 5. Differential pulse voltammograms results with different PAC concentrations under test conditions.

The proposed method mentioned above was applied to examine the effects of some common excipients used in pharmaceutical preparations. The tolerance limit is defined as the maximum concentration of the interfering substance, and error for determination of PAC is less than 5%. Each sample contained a fixed amount of PAC (0.1 mM) and excess amount of interfering substance, and the experimental results were listed in Table 2. It can be seen that the presence of even hundred-fold excess of interferents, such as citric acid, dextrose, glucose, gum acacia, lactose, starch, tartaric acid and sucrose did not interfere with the voltammetric signal of PAC, indicating that PAC can be assayed accurately using proposed method in the presence of interfering substance.

Table 2. Influence of interfering substances on the voltammetric responses under experimental conditions.

Excipients (1.0 mM) + PAC	Potential (V)	Current change (%)
PAC	1.187	0
Glucose + PAC	1.201	+2.54
Urea + PAC	1.197	-5.66
Ascorbic acid + PAC	1.187	+3.69
Citric acid + PAC	1.179	+5.14
Starch + PAC	1.182	-2.12

The proposed method above was also utilized to detect PAC in herb samples such as *Taxus chinensis*. The recoveries from urine were measured by injecting *Taxus chinensis* with known amount of PAC. Quantitative analysis were carried out by adding the standard PAC solution into the herb samples. The calibration graph are used for the determination of injected PAC in herb samples. The RSD values and detection results of four herb samples are listed in Table 3, where the detected recoveries are observed in the range from 98.57% to 103.13%.

Table 3. Determination of PAC in herb samples (n=3).

Sample	Added (mM)	Found (mM)	Recovery (%)	RSD (%)
1	0	0.11±0.005	—	—
2	0.05	0.165±0.012	103.13	4.54
3	0.1	0.207±0.030	98.57	3.54
4	0.2	0.314±0.027	101.29	6.23
5	0.5	0.612±0.044	100.16	5.12

4. CONCLUSION

In conclusion, a GCE-graphene-EAu was prepared using electro-deposition method, and subsequently utilized for electrochemical determination of PAC. The obtained PAC sensor shows a good linear analytical response in a wide PAC concentration ranging from 0.01 mM to 2 mM. The limit of detection (LOD) of this sensor is 5 μM. Furthermore, as-obtained sensor shows good selectivity of PAC detection, even in the presence of interfering molecules such as citric acid, dextrose and glucose at high concentrations. And thus, the proposed method can be further employed to analyze PAC in traditional Chinese medicine.

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