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Sensitive Electrochemical Determination Uric Acid at Pt Nanoparticles Decorated Graphene Composites in the Presence of Dopamine and Ascorbic Acid

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In this paper, the composites of Pt nanoparticles and graphene (PtNPs/GR) were prepared by one-step electrochemical reduction technique, and then were used as modified materials for electrochemical determination of uric acid (UA) in the presence of dopamine (DA) and ascorbic acid (AA). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods were employed to study their electrocatalytic activity toward the oxidation of UA in the presence of DA and AA. It showed PtNPs/GR modified electrode displayed good electrocatalytic activity toward UA, which can be ascribed to the synergistic effect between the high conductivity and large surface area of GR and the high catalytic activity of PtNPs. The anodic peak current was linear to the concentration of UA in the range from 0.5 μM to 180 μM, and the detection limits was 0.22 μM (S/N = 3). Moreover, the prepared electrode displayed highly selectivity, good stability and reproducibility.

Keywords: Uric acid; Pt nanoparticles; Graphene; Electrochemical determination

1. INTRODUCTION

Uric acid (UA) is an end-product of purine metabolism and present in urine and in blood serum [1,2]. Generally, the normal concentration of UA in the healthy human serum is in the range of 0.24 – 0.52 mM, while that in urine is 1.49 – 4.46 mM [3]. Abnormal levels of UA in blood serum and the urine cause a series of diseases such as hyperuricemia, arthritis, gout and renal insufficiency [4-6]. In addition, previous reports have shown that once the concentration of UA exceeds a certain value, the insulin signaling will be inhibited directly [7]. Many methods have been employed to detect UA concentration, including liquid chromatography [8], chemometric-assisted spectrophotometry [9], chemiluminescence [10] and electrochemical methods [11-13]. Due to the fact that UA is an

electroactive biological molecules, electrochemical methods based on modified electrodes are preferred to the conventional methods, because they have many advantages, such as simple instrumentation, high sensitive, cost-effective and available to *in-situ* monitoring for its determination [6, 14,15]. However, the voltammetric responses are plagued by interference from DA and AA due to their very close electrochemical redox potentials. Especially the amount of AA in biological fluids is very large [16,17]. Therefore, quantitative detection of UA in the presence of DA and UA is practically significant.

Electrodes modified with graphene [18], carbon nanotube [19], polymer film [20] and metal nanoparticles (Pt, Au, etc.) [21,22] can simultaneously detect UA, DA and AA. Especially, PtNPs have attracted great attentions because of their high electrocatalytic ability, excellent conductivity and biocompatibility [23,24]. What's more, the immobilization of PtNPs on some nano-supporting materials, such as conducting polymer, GR and other carbon materials, can obtain high sensitivity and selectivity for the simultaneous detection of UA, DA and AA [25-27]. It is worth noting that, among these supporting materials, GR has received tremendous attention because of its high electrical conductivity, large surface area, rapid electron transfer and outstanding mechanical properties [28,29]. For example, Feng et al. reported that PtNPs modified reduced graphene oxide sheets (RGO) were synthesized by *in-situ* chemical reduction method, and the nanocomposite of PtNPs/RGO were applied for the simultaneous detection of DA and UA in the presence of AA [30]. However, the above-mentioned chemical reduction procedures are usually time-consuming and some complicated instruments are always needed [31]. While, electrochemical method is rapid, relatively simple approach to achieve the PtNPs decorated GR composites modified electrode.

In this work, PtNPs/GR modified glassy carbon electrode (PtNPs/GR/GCE) was constructed by a simple effective electrochemical method, and the modified electrode was used for detecting UA in the presence of DA and AA. PtNPs/GR/GCE enhanced the electrochemical oxidation of UA compared with bare GCE and GR/GCE, which was ascribed to the synergistic effect between PtNPs and GR. Thus, a highly selective and sensitive electrochemical method for UA determination was developed based on PtNPs/GR/GCE.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Uric acid (UA), dopamine (DA), L-(+)-ascorbic acid (AA) and H_2PtCl_6 were purchased from Sigma-Aldrich. Graphene oxide (GO) was purchased from Nanjing XianFeng Nano Material Technology Co. Ltd. Phosphate buffer was prepared from stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, which were purchased from Sinopharm chemical reagent Co. Ltd. All other reagents were of analytical grade, and double distilled water was used throughout the experiment.

2.2 Apparatus

Electrochemical measurements including cyclic voltammetric (CV) and differential pulse voltammetry (DPV) were carried out on a CHI660D electrochemical workstation (Shanghai, China). A

conventional three electrodes cell contained a modified glassy carbon electrode (GCE) (Φ = 3 mm) as working electrode, a saturated calomel reference electrode (SCE) as reference electrode, and a platinum wire as auxiliary electrode. All experiments were carried out at room temperature and all potentials were measured versus the SCE. DPV was carried out with the parameters of an increment potential of 0.004 V, a pulse amplitude of 0.05 V, a pulse width of 0.2 s, a sample width of 0.02 s, a pulse period of 0.5 s and a quiet time of 20 s.

2.3. Preparation of the modified electrodes

Prior to modification, GCE was mechanically polished with chamois leather containing 0.05 μm Al_2O_3, and then it was ultrasonically cleaned with doubly distilled water, absolute ethanol and doubly distilled water each for 5 min, respectively.

5 mg GO was dissolved in double distilled water and ultrasonicated for 3 h to obtain a stable suspensions (0.5 mg mL^{-1}). 5 μL the obtained GO suspension was dropped onto GCE and then dried in air. Then the electrochemical co-reduction of H_2PtCl_6 and GO was performed by using chronoamperometry in 0.1 M phosphate buffer (pH 5.0) containing 1 mM H_2PtCl_6 at a potential of -1.0 V for 60 s to produce PtNPs/GR/GCE. For the comparison, GR/GCE was fabricated using a similar method without H_2PtCl_6 solution.

3. RESULTS AND DISCUSSION

3.1 Cyclic voltammetric behaviors of UA at various electrodes

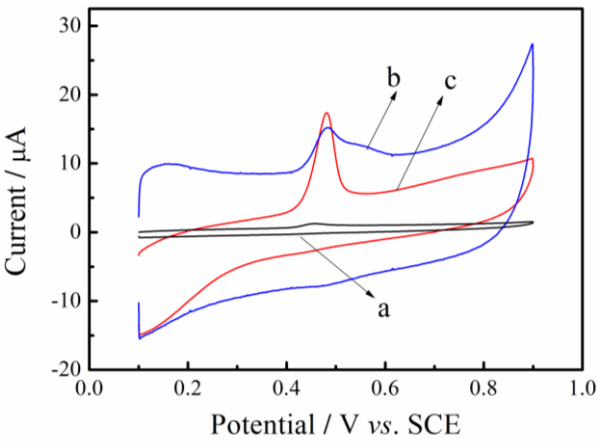

Figure 1. Cyclic voltammograms of 200 μM UA in 0.1 M phosphate buffer (pH 5.0) at bare GCE (a), GR/GCE (b) and PtNPs/GR/GCE (c) at the scan rate of 50 mV s^{-1}.

The electrochemical behavior of UA on bare GCE (a), GR/GCE (b) and PtNPs/GR/GCE (c) was investigated by CV in 0.1 M phosphate buffer (pH 5.0) containing 200 μM UA (Figure 1). From curve (a), it can be seen that the electrochemical response of UA was very poor at bare GCE. At

GR/GCE (b), the electrochemical response was larger than that at bare GCE, which was attributed to the excellent catalytic effect of GR. The highest peak current toward the oxidation of UA can be observed (curve c) at PtNPs/GR/GCE, which can be ascribed to the synergistic effect of GR and metal NPs.

3.2 Determination of UA in the presence of DA and AA

DPV is a sensitive technique, which has been usually undertaken for electrochemical detection [32,33], and it also has been applied for the electrochemical determination of UA in the presence of DA and AA. As shown in Figure 2, the oxidation peaks of UA, DA and AA on bare GCE (a) were seriously merged with only one broad peak, indicating the low selectivity and sensitivity for simultaneous detection of UA, DA and AA.

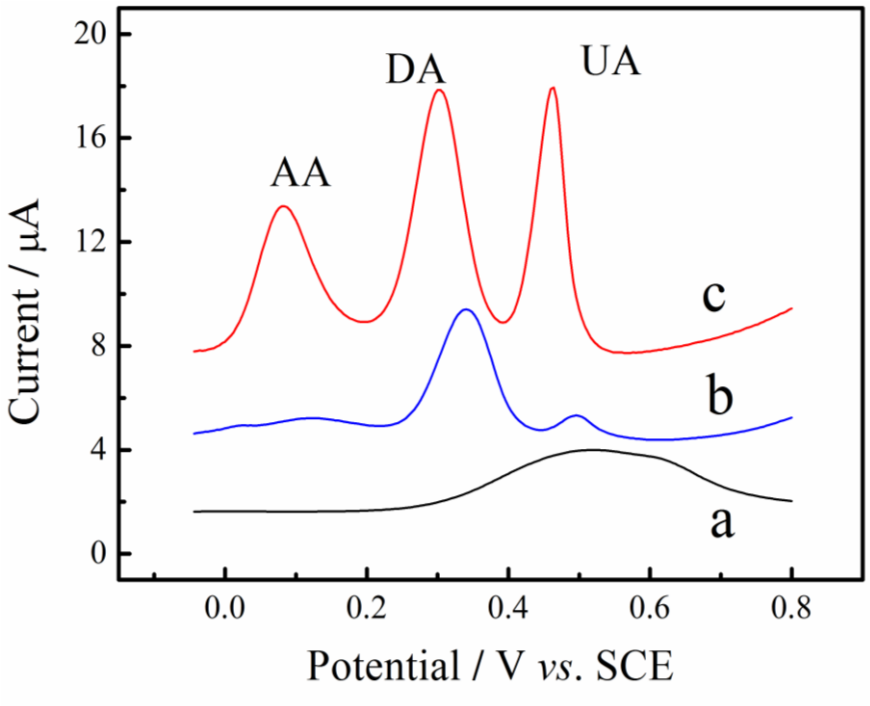

Figure 2. DPV curves at bare GCE (a), GR/GCE (b) and PtNPs/GR/GCE (c) in 0.1 M phosphate buffer (pH 5.0) containing the mixture of 150 μM UA, 150 μM DA and 1 mM AA.

In contrast, the peak currents of DA and UA at GR/GCE (b) increased, which was mainly attributed to the π–π interaction between the aromatic nucleus structure and GR. However, the three species still cannot be well-distinguished from each other at GR/GCE. On PtNPs/GR/GCE (c), three obvious oxidation peaks with larger peak separations and peak currents corresponding to the oxidation of AA, DA and UA appeared respectively, demonstrating that PtNPs/GR/GCE could be used to construct a sensitive electrochemical sensor for the determination of UA in the presence of DA and AA with much higher electrocatalytic activity and selectivity.

3.3 Optimization of the experimental conditions

3.3.1 Influence of pH Values

The peak potentials and the peak currents are closely related to the pH of buffer solution. Figure 3A showed the effect of different pH on the response of 100 μM UA. It was found that the anodic peak was perfect in pH 5.0.Meanwhile, the maximum current of anodic peak appeared in pH 5.0. Thus, pH 5.0 was selected as the optimal pH for detection in the following experiments. It also can be seen the anodic peak moved to the negative direction along with the pH changed from 4.0 to 8.0 (shown in Figure 3B). The anodic peak potential was proportional to pH with the linear regression equations E_{pa} (V) = – 0.066 pH + 0.8024 (R^2 = 0.9964). The slope of the equation was approximately close to the theoretical value of 58.5 mV pH^{-1}, which demonstrated that the electrochemical reaction involved equal numbers of proton-transfer and electron-transfer [34].

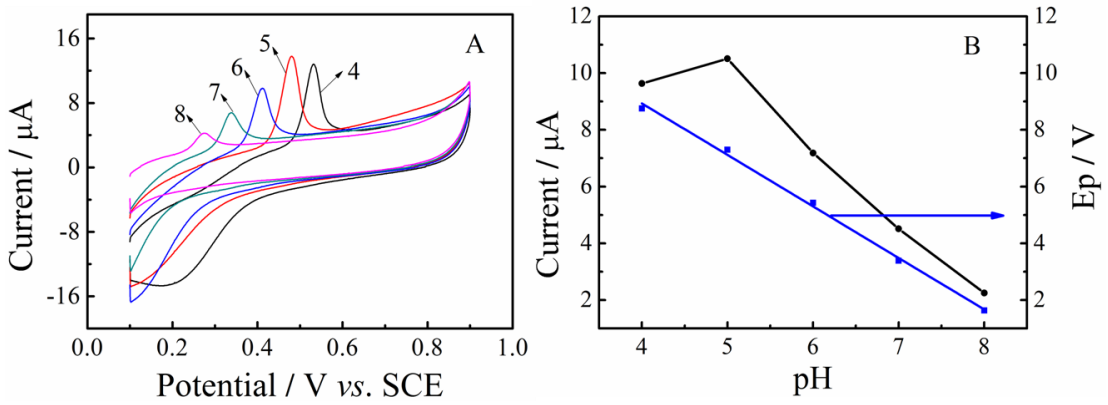

Figure 3. (A) CV of 100 μM UA at different pH values (4.0, 5.0, 6.0, 7.0 and 8.0) in phosphate buffer at PtNPs/GR/GCE. (B) The influences of pH on the oxidative peak current of 100 μM UA.

3.3.2 Influence of scan rate

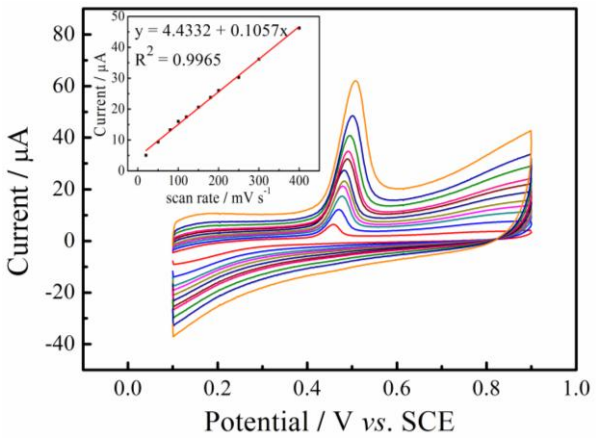

Figure 4. CVs of 200 μM UA on PtNPs/GR/GCE at different scan rates in 0.1 M phosphate buffer (pH 5.0) (from the inner to the outer are 20, 50, 80, 100, 120, 150, 180, 200, 250, 300 and 400 mV s^{-1}). Insert: the plot of the peak currents versus scan rate.

The influence of scan rates on the oxidation peak of 200 μM UA at PtNPs/GR/GCE was also examined by CV. As shown in Figure 4, it can be seen that the oxidation peak currents increased with the increase of potential scan rate. Moreover, the oxidation peak currents (I_{pa}) was proportional to the scan rate *v* (shown in the inset), and the liner regression equation was expressed as I_{pa} (μA) = 0.1057*v* (mV s^{-1}) + 4.4332 (R^2 = 0.9965). It indicated that the electrochemical behavior of UA at PtNPs/GR/GCE was an adsorption-controlled electrode process.

3.4 Electrochemical determination of UA

Because the method of DPV has a better resolution and higher sensitivity than CV, it was carried out to determine the concentration of UA at PtNPs/GR/GCE. Figure 5A shows the DPV responses of different UA concentrations at PtNPs/GR/GCE. It can be observed that the oxidation peak currents increased linearly with the increase of the concentration of UA from 0.1 μM to 40 μM and 40 μM to 200 μM (Figure 5B). The linear regression equations were I_{pa} = 0.0531 *c* + 1.1029 (R^2 = 0.9969) and I_{pa} = 0.0756 *c* + 0.0805 (R^2 = 0.9971), respectively. The limit of detection (LOD) was calculated to be 0.026 μM according to the following equation using International Union of Pure and Applied Chemistry (IUPAC) definitions [35]:

LOD = 3.3*s*/*m*

where s is the standard deviation of the blank response and m is the slope of the calibration curve.

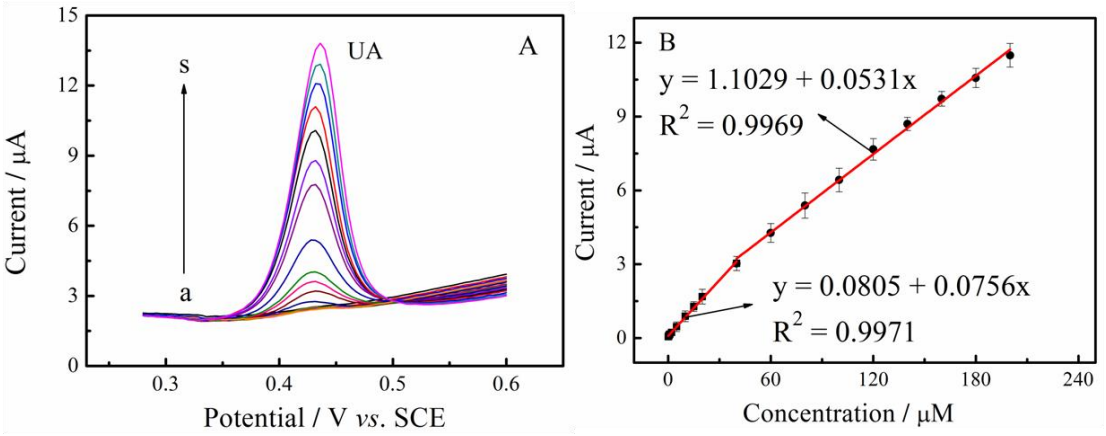

Figure 5. (A) DPV of PtNPs/GR/GCE in different concentrations of UA solutions (0.1, 0.3, 0.5, 0.7, 1, 2, 5, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 μM); (B) Plot of the oxidation peak current against the concentration of UA.

To evaluate the applicability of this modified electrode, the DPV measurements were recorded at various concentrations of UA in the presence of constant concentrations of DA and AA (25 μM and 1 mM, respectively). As can be seen in Figure 6A, the electrochemical oxidation peak currents of UA increased with increasing of its concentrations. The two linear regression equations for the detection of

UA were also obtained which are calculated as I_{pa} = 0.2671 *c* + 0.3819 (0.5–20 μM, R^2 = 0.9954) and I_{pa} = 0.0766 *c* + 4.5259 (20–180 μM, R^2 = 0.9954) with the detection limit of 0.22 μM (Figure 6B). The PtNPs/GR/GCE presented a wide linear range for UA determination and offered a lower detection limit in relative with those of other modified electrodes in literature [31, 36-40] (Table 1), indicating that the composites of PtNPs/GR was good sensing substrate for the fabrication of electrochemical sensors for detecting UA in the presence of DA and AA.

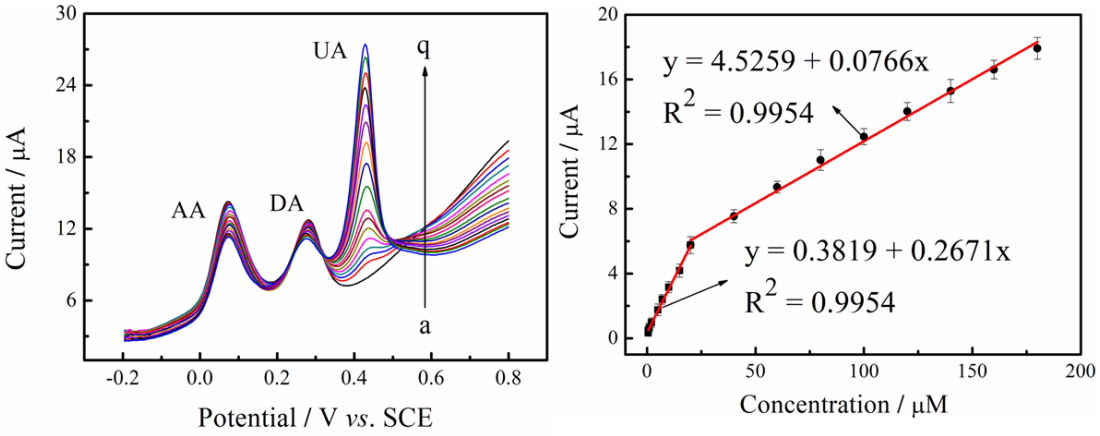

Figure 6. (A) DPV of PtNPs/GR/GCE in different concentrations of UA (0, 0.5, 1, 2, 5, 7, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160 and 180 μM) in the presence of 25 μM DA and 1 mM AA, respectively. (B) Plot of the oxidation peak current against the concentration of UA.

Table 1. Comparison of the proposed sensor for detecting UA in the presence of DA and AA at PtNPs/GR/GCE with others.

Type of electrode	Linear range (μM)	Detection limit (μM)	Refs.
Au/RGO/GCE	8.8–53	1.8	[31]
[a]OMC/Nafion/GCE	5.0–80	4.0	[36]
ERGO/GCE	0.5–60	0.5	[37]
Chitosan/graphene/GCE	2.0–45	2.0	[38]
[b]MWCNT/PEDOT/GCE	10–250	10	[39]
Fe_3O_4/r-GO/GCE	4.0–20 and 20–212	0.5	[40]
PtNPs/GR/GCE	0.5–20 and 20–180	0.22	This work

[a]OMC/Nafion: Ordered mesoporous carbon/Nafion;

[b]MWCNT/PEDOT: MWCNT/poly(3.4-ethylendioxythiophene).

3.5 Reproducibility, stability and selectivity of PtNPs/GR/GCE

The reproducibility of the modified electrode was examined for detecting UA (100 μM) at five electrodes employing a DPV measurement to present the electrochemical signals. The relative standard

deviation (RSD) of the measurements was about 5.68%, indicating the sensor displayed a good reproducibility. In addition, in order to test the repeatability of PtNPs/GR/GCE, 100 μM UA was detected successively 15 times with the same electrode of PtNPs/GR/GCE. The value of RSD was 4.32%, indicating the PtNPs/GR/GCE had a good repeatability.

The storage stability of PtNPs/GR/GCE was examined by the DPV response to 100 μM UA in 0.1 M phosphate buffer (pH 5.0). After each measurement, PtNPs/GR/GCE was washed with phosphate buffer and stored in a refrigerator at 4 °C. Losses of 10.2% in the current response of the PtNPs/GR/GCE for UA was observed at the 15 days, and the results showed an acceptable stability.

Moreover, to study the selectivity of PtNPs/GR/GCE, interfering substances of tyrosine, citric acid, glucose and possible interferential ions (such as K^+, Na^+, Al^{3+}, Mg^{2+} and Ca^{2+}) were tested in this study and the results were shown in table 2. It was found that the RSD value observed in the DPV responses of UA in the presence of these foreign substances was lower than ± 5%. The results indicated a good selectivity of the fabricated sensor.

Table 2. The influences of some possible interferential ions and biological substances on the peak currents of UA 0.1 M phosphate buffer (pH 5.0) at the PtNPs/GR/GCE.

Interferents	Signal change (%)
K^+	2.18
Na^+	3.22
Al^{3+}	-2.15
Mg^{2+}	3.64
Ca^{2+}	-3.27
tyrosine	4.56
citric acid	-2.32
glucose	4.61

3.6. Determination of UA in human urine samples

The application of this method in real sample analysis was also studied by direct analysis of UA in human urine samples. In order to fit into the linear range of UA, all the urine samples were diluted 500 times with 0.1 M phosphate buffer (pH 5.0). Standard addition method was employed. The recovery determined by spiking the samples with a measured amount of standard UA was found to be between 96% and 105.6 %. Also, the results were compared with those obtained by spectrophotometric method, and were shown in Table 3. The UA values measured by our method were in good agreement with those by spectrophotometric method, which demonstrated the feasibility of the present method.

Table 3. Determination of UA in urine samples.

Sample	UA content (g=L) by electrochemical method	UA content (g=L) by spectrophotometric method
1	1.36	1.35
2	3.25	3.27
3	5.15	5.13

4. CONCLUSIONS

PtNPs/GR/GCE was prepared by a simple one-step electrochemical co-reduction of H_2PtCl_6 and GO using chronoamperometry. PtNPs/GR modified electrode was successfully fabricated for the selective and quantitative detection of UA in the presence of DA and a very high concentration of AA. And its electrochemical properties were discussed in detail. PtNPs/GR/GCE showed excellent catalytic activity for the electrochemical oxidation reaction of UA. A low detection limit of 0.22 μM was obtained because of the synergistic effect of metal NPs and GR. What's more, the modified electrode presented excellent stability, reproducibility and high selectivity.

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