Development and Evaluation of a Portable Electrochemical Biosensor for Detecting Uric Acid in Urine

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In this work, we developed a highly sensitive electrochemical enzyme electrode that directly determines the uric acid (UA) concentration in urine. Results from cyclic voltammetry testing showed a well-defined oxidation peak at 0.35 V, but no reduction peak, indicating that an irreversible redox reaction was executed. The enzyme electrode's modification method was optimized as follows: 0.1% chitosan, 0.3% graphene oxide, 5 mmol L^{-1} uricase, and 25% glutaraldehyde. Under these optimal conditions, there was a good linear relationship between the redox peak current and UA concentration in the range of 0.1-2 mmol L^{-1} ($I=4.1661\ C_{UA} + 2.0445$; $R^2=0.9995$); and the detection limit was 0.023 mmol L^{-1} (S/N = 3.3). The modified electrode showed high repeatability (RSD = 3.34%), good stability (12 days), and strong anti-interference capability. Therefore, this electrode can quickly and accurately determine the UA concentration in urine. This study is highly significant for monitoring UA levels in gout populations and preventing kidney damage caused by gout.

1. INTRODUCTION

In the human body, uric acid (UA) is the final metabolic product of guanidine derivatives. The UA concentration and its changes can reflect the states of metabolism, immunity, and other physiological functions [1]. In healthy people, the body regulates the concentration of UA in urine. However, when the regulatory mechanism fails to function, the concentration of UA in the urine rises, which can lead to some serious diseases. A typical example is gout, a condition in which the UA crystals are deposited between the joints, causing painful joint swelling. If left untreated, the disease progresses to where the UA concentration in urine oversaturates, resulting in urate being deposited in renal tubules or interstitium, ultimately causing severe kidney damage [2]. Therefore, rapid, sensitive, and accurate detection of UA levels in human urine is highly significant for diagnosis and prevention of kidney related diseases.

To date, UA detection methods primarily include spectroscopy, high-performance liquid chromatography, enzymatic analysis, electrochemiluminescence, and electrophoresis. However, these methods require cumbersome sample pretreatment, expensive instruments, and complicated and expensive analytical processing, making them difficult to apply in the home [3-6]. In recent years, the electrochemical method has attracted extensive attention [7, 8]. This method is characterized by low cost, rapidity, high sensitivity, and simple operational procedures; and can be further developed into a small portable sensor. Thus, people with gout can have consistent, in-home UA monitoring, which provides families, communities, and the patient the convenience of reliable, home-based healthcare [9-11]. However, most traditional electrodes (e.g., glassy carbon, gold, and platinum electrodes, etc.) cannot distinguish the UA oxidation signal due to interference from other components in the urine (e.g., ascorbic acid (AA)) [12,13].

In this study, we manufactured an electrochemical biosensor using Graphene Oxide (GO), Prussian Blue (PB) electronic media, and Chitosan (CS) polymer to modify the electrode and optimize the enzyme electrode's preparation conditions. Following appropriate modification, the electrode's anti-interference capability was improved as compared with the traditional UA electrode [14-17]. Moreover, the electrochemical signal index was accurately converted to a UA physiological index and shown to be repeatable. Thus, this work provides a new, simple, and cost-effective technique for detecting UA in urine [Figure 1].
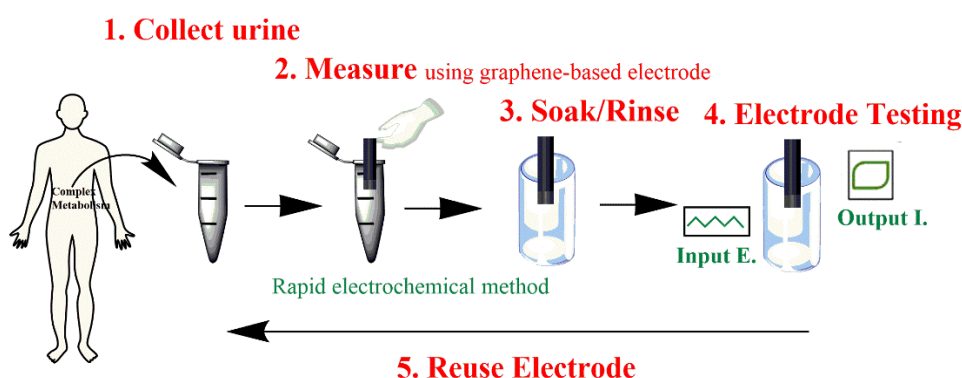
Figure 1. Electrode measurements offer the potential for simple and rapid measurement of UA in human urine

2. MATERIALS AND METHODS

2.1 Reagents

Uricase from *Candida sp.* was purchased from Sigma-Aldrich; Graphene oxide from Nanjing Ji Cang Nano Technology Co., Ltd; UA from Shyuanye Co., Ltd; and Glutaraldehyde and Chitosan from Aladdin Co., Ltd. Other materials were purchased from Sinopharm Chemical Reagent Co., Ltd. Phosphate-buffered saline (PBS, 0.1 mol L^{-1} NaH_2PO_4, and 0.1 mol L^{-1} Na_2HPO_4) was used in this study. Most of the solutions in the experiments were prepared with ultrapure water.

2.2 Apparatus

All electrochemical assay measurements were carried out with an electrochemical workstation (CHI760D, Shanghai CH Instruments Co., China). The three-electrode system was composed of a glassy carbon electrode (GCE) (diameter: 3 mm), a platinum wire electrode, and a Ag/AgCl electrode (saturated KCl), which served as the working, counter, and reference electrodes, respectively. The scanning electron microscope (SEM) images were acquired with a SUPRA™ 55 Thermal Field SEM.

2.3 Methods

2.3.1 Preparation of the UOx/CS-PB-GO/GCE electrode

Prior to use, a GCE was polished with 0.5 and 0.05 μm alumina powder to obtain a mirror-like surface, then ultrasonically rinsed with distilled water and ethanol, respectively. 10 μL of GO (0.3%) was sprinkled onto the electrode working surface and air dried. This electrode was denoted as GO/GCE. Next, the GO/GCE was immersed in a PB solution, and PB was deposited on the surface of the GO/GCE electrode by cyclic voltammetry (10 cycles). The electrode surface was then cleaned and 10 μL CS solution (0.1%) was dropped on. After drying, the electrode was denoted as CS-PB-GO/GCE. The working face of CS-PB-GO/GCE was soaked in glutaraldehyde for 1 h, cleaned, and dried; then immersed in uricase for 1 h. The resulting electrode was denoted as UOx/CS-PB- GO/GCE.

2.3.2 Determination of the electrode surface activity

The electrochemical impedance spectroscopy (EIS) spectra of GO/GCE and GCE were recorded using the AC impedance method in a 1×10^{-3} mol L^{-1} $K_3Fe(CN)_6$ solution. The bare GCE electrode and GO modified GO/GCE electrode were detected. Surface electron transfers were in the frequency range of 2×10^6-100 Hz. Cyclic voltammetry was used to compare the UOx/CS-PB-GO/GCE electrode with modified GO and the UOx/CS-PB/GCE electrode with unmodified GO. The electrochemical behavior of GCE was assessed in a 1×10^{-3} mol L^{-1} UA substrate solution.

2.3.3 Optimization of electrode modification conditions

The modification conditions for electrode preparation were optimized by testing under the following conditions: 1) 0.1% CS solution, 25% glutaraldehyde solution, and 3 mmol L^{-1} uricase, the electrodes were modified with different GO concentrations (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%); 2) 0.3% GO, 25% glutaraldehyde solution, and 3 mmol L^{-1} uricase, the electrodes were modified with different CS concentrations (0.06%, 0.08%, 0.1%, 0.12%, and 0.14%); 3) 0.3% GO, 0.1% CS solution, and 3 mmol L^{-1} uricase, the electrodes were modified with different glutaraldehyde solutions concentrations (15%, 20%, 25%, 30%, and 35%); 4) 0.3% GO, 0.1% CS solution, and 25% glutaraldehyde solution, the electrodes were modified with different concentrations of uricase (3 mmol L^{-1}, 4 mmol L^{-1}, 5 mmol L^{-1}, 6 mmol L^{-1}, and 7 mmol L^{-1}). Three parallel experiments were conducted. See Table 1 for experimental conditions.

Table 1. Experimental conditions for electrode modification optimization

	CS solution (%)	Glutaraldehyde (%)	GO (%)	Uricase (mmol L^{-1})
Experiment 1	0.1	25	0.1, 0.2, 0.3, 0.4, and 0.5	3
Experiment 2	0.06, 0.08, 0.1, 0.12, and 0.14	25	0.3	3
Experiment 3	0.1	15, 20, 25, 30, and 35	0.3	3
Experiment 4	0.1	25	0.3	3, 4, 5, 6, and 7

2.3.4 Characterization of the electrode morphology

Enzyme electrodes were prepared according to 2.3.3 optimized electrode modification conditions. The working faces of PB-CS-GO/GCE and UOx/PB-CS- GO/GCE electrodes were separately made on the GCE microscope electrode, and characterized by SEM.

2.3.5 Determination of the electrode properties

The UOx/PB-CS-GO/GCE electrodes were prepared according to 2.3.3 optimized electrode modification conditions. A standard UA solution with a concentration range of 0.1-2 mmol L^{-1} was prepared. The response current intensity of the UOx/PB-CS-GO/GCE electrode was measured, and the electrode's linear range was analyzed. The UA solutions' current was repeatedly measured at concentrations of 0.1 mmol L^{-1}, 0.2 mmol L^{-1}, 0.4 mmol L^{-1}, 0.6 mmol L^{-1} and 1.0 mmol L^{-1} to analyze the repeatability of the electrode preparation method. An enzyme electrode, stored at 4 ℃, was used daily to determine the concentration of a 0.4 mmol L^{-1} UA substrate solution, and analyze the stability of the enzyme electrode. Furthermore, the enzyme electrode's cyclic voltammetry curve in different pH UA solutions (0.8 mmol L^{-1}) was measured, and the influence of pH on the electrode performance was analyzed.

The recovery of the UOx/PB-CS-GO/GCE electrode was analyzed by detecting the UA in spiked human urine samples, which were provided by local hospitals. Different concentrations of UA were added to the urine samples using the standard addition method for testing recovery. No other pretreatment process was performed.

*2.3.6 Determination of anti-interference performance of the electrode*

The peak potential and current intensity of oxalic acid, AA, salicylic acid, citric acid, glucose, and dopamine solutions were measured using the UOx/ PB-CS-GO /GCE electrode to detect the influence of interfering substances on UA detection. All interfering substances were added in concentrations of 50

mmol L^{-1}. It has been reported that the peak potential for AA is close to that of UA [18, 19] and the peak current is often partially coincident, thus affecting the determination of UA concentration. In this study, AA and UA were added simultaneously, and cyclic voltammetry was performed to observe whether the peak currents of AA and UA overlapped.

3. RESULTS AND DISCUSSION

3.1 *Analysis of electrode surface activity*

GO provides a high specific surface area that increases the electrode's effective working space and provides more binding sites for electrochemical deposition of the mediator material [20-22]. The diameter of the EIS map semicircle represents the charge transfer resistance (Rct) at the interface between the electrode and the electrolyte. Figure 2-I shows that the semi-circular diameter of the EIS spectrum for the GO/GCE electrode is significantly reduced when compared to that of the GCE electrode. Thus, it can be inferred that the Rct was significantly reduced as well. These results demonstrate that the addition of GO on the electrode's surface increases the surface charge transfer, thereby improving the electrode's working efficiency [23-24]. In addition, Figure 2-II shows that the UOx/PB-CS/GCE modified electrode without GO has no response to UA detection. This indicates that GO plays an important role in modification of the enzyme electrode.
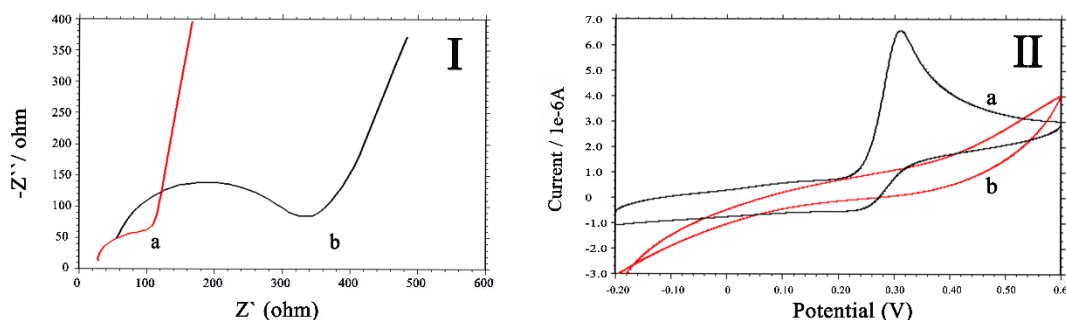

Figure 2. I: A. C. Impedance of (a) GO/GCE and (b) GCE; II: Cyclic voltammograms of (a) UOx/CS-PB-GO/GCE and (b) UOx/CS-PB/GCE. All measurements were carried out in 0.1 mol L^{-1} PBS (pH 7.4).

### 3.2 *Optimization of electrode modification conditions*

Figure 3 depicts the optimal concentration for CS, glutaraldehyde, GO, and UA oxidase, using current intensity as a measure of electron transport. Note that all four chemical constituents demonstrated a similar trend consisting of three distinct parts: 1) increasing concentration correlates with increasing current intensity; 2) the current intensity maximum was reached; and 3) after the current intensity maximum is exceeded, it decreases as the concentration increases. For CS, the current intensity reached a maximum when the concentration was 0.1%. This indicates that a CS concentration exceeding 0.1% adversely affects electron transfer ability and electrode response strength. The current intensity reached a maximum for glutaraldehyde and uricase at concentrations of 25% and 5 mmol L^{-1}, respectively, indicating

that these concentrations provide the best cross-linking effect. Finally, the optimum modified concentration of GO was 0.3%; as excessively high concentrations of GO will result in reduced sensitivity of the electrode. Therefore, the optimal modification conditions for the enzyme electrode were 0.1% CS, 25% glutaraldehyde, 5 mmol L^{-1} uricase, and 0.3% GO.
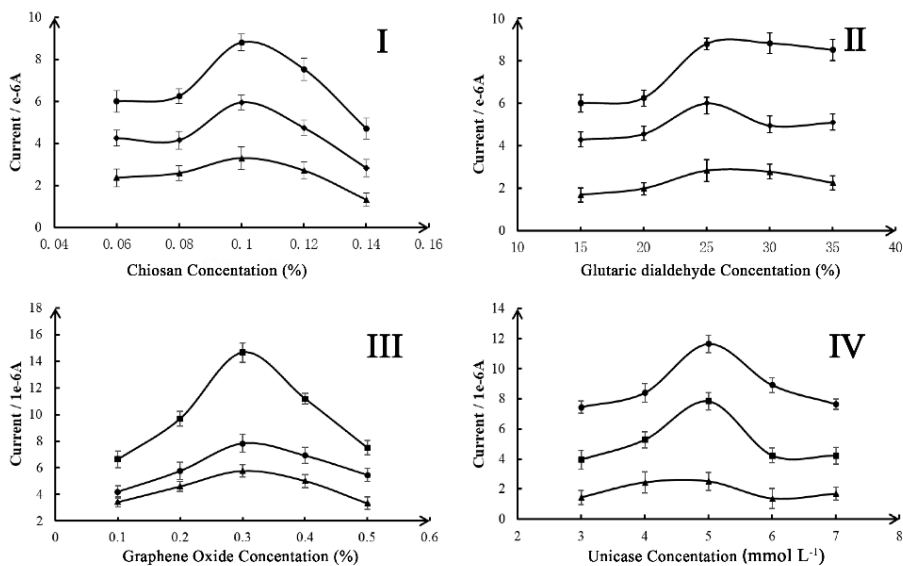
Figure 3. Optimization of the UOx/CS-PB-GO/GCE electrode modification parameters (I: Chitosan; II: Glutaric dialdehyde; III: Graphene oxide; IV: Uricase)

3.3 Characterization of the electrode morphology
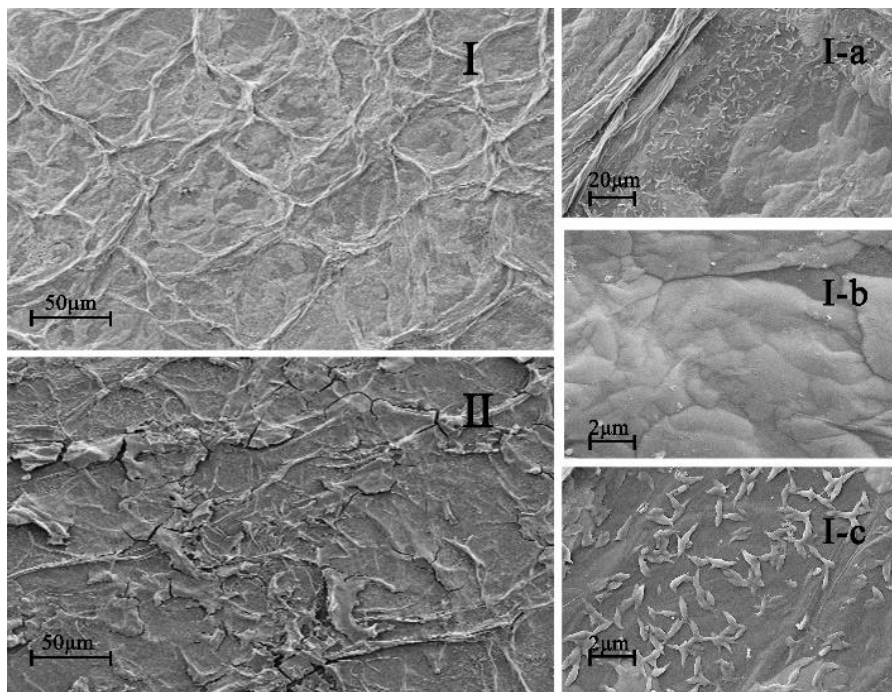
Figure 4. SEM images of the electrode (I: CS-PB-GO/GCE and II: UOx/CS-PB-GO/GCE）

The SEM images of UOx/PB-CS-GO/GCE are displayed in Figure 4. As shown in Figure 4-I, GO and CS combined to form a stable thin film structure (4-I-a, 4-I-b) by electrostatic interaction. Figure 4-II displays the CS-enzyme unit uniformly distributed on the electrode's surface. The SEM photo depicts CS molecules being uniformly adsorbed on the modified electrode's surface and subsequently forming a stable film. The enzyme molecules cross-linked with CS via glutaraldehyde, and formed a stable, sufficiently large, three-dimensional structure. The space is advantageous for improving the enzyme electrode's working performance. As shown in Figures 4-I-a and 4-I-c, PB is evenly distributed across the electrode surface. PB is an excellent electrochemical material for hydrogen peroxide detection [25-27], which has a strong capacity toward electron transfer. The uniform distribution of PB is beneficial for enhancing the response signal [28].

3.4 *Effects of electrode properties on the electrochemistry*

In order to further optimize the UOx/CS-PB-GO/GCE electrode's performance, the linear range, repeatability, stability, influence of pH on the electrode, and the electrode's anti-interference capability were studied.

Figure 5 demonstrates that the uricase electrode's peak current gradually increased as the substrate concentration increased. The correlation coefficient between the redox peak current and its concentration is 0.9995, indicating a good linear fit. According to the 3 σ rule, when the signal-to-noise ratio is 3.3 (S/N=3.3), the UA detection limit is 0.023 mmol L^{-1}.Thus, the UA electrode has high sensitivity; and because the UA concentration in human urine is ~ 1.79 mmol L^{-1} [29], can be used for direct detection of UA in human urine.
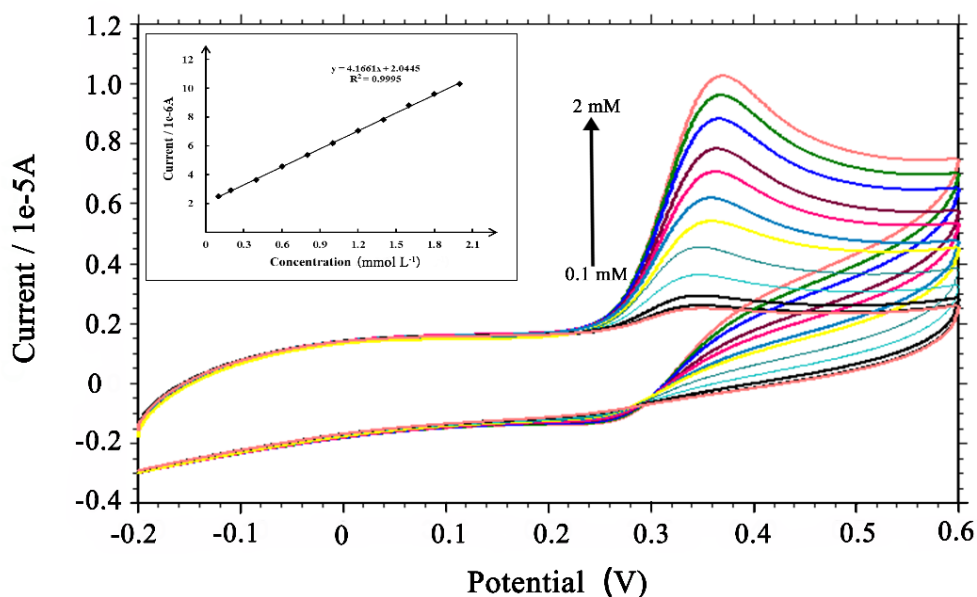
Figure 5. The linear ranges of the UOx/CS-PB-GO/GCE electrodes. Measurements were carried out in 0.1 mol L^{-1} PBS (pH 7.4).

The electrode repeatability test results are depicted in Figure 6-I. The relative standard deviation (RSD) of the current response values were all below 5%, with an average of 3.34%. These values indicate that the experimentally prepared UOx/CS-PB-GO/GCE electrode has good reproducibility. The UA enzyme electrode's stability is shown in Figure 6-II. Throughout the course of a 14 day test, the current intensity did not show significant fluctuations before the 12th day, indicating that the electrode remained stable for 12 days. Figure 6-III shows that pH did affect the electron transfer response, in that the current increases as the pH gradually increases. Because the current was affected in an alkali environment and UA can't dissolve in an acidic environment, pH=7 was selected as the electrode's working pH.

Human urine samples were selected as the real samples for investigating the reliability of the proposed method. No other pretreatment process was performed. The results demonstrated that the human urine concentration was 1.75 mmol L^{-1}. The standard addition method was used for testing recovery and showed that the recovery rates of UA spiked samples were 95%-103%, with an average recovery rate of 98.33% [Table 2]. These results clearly indicate the applicability and reliability of the proposed method.
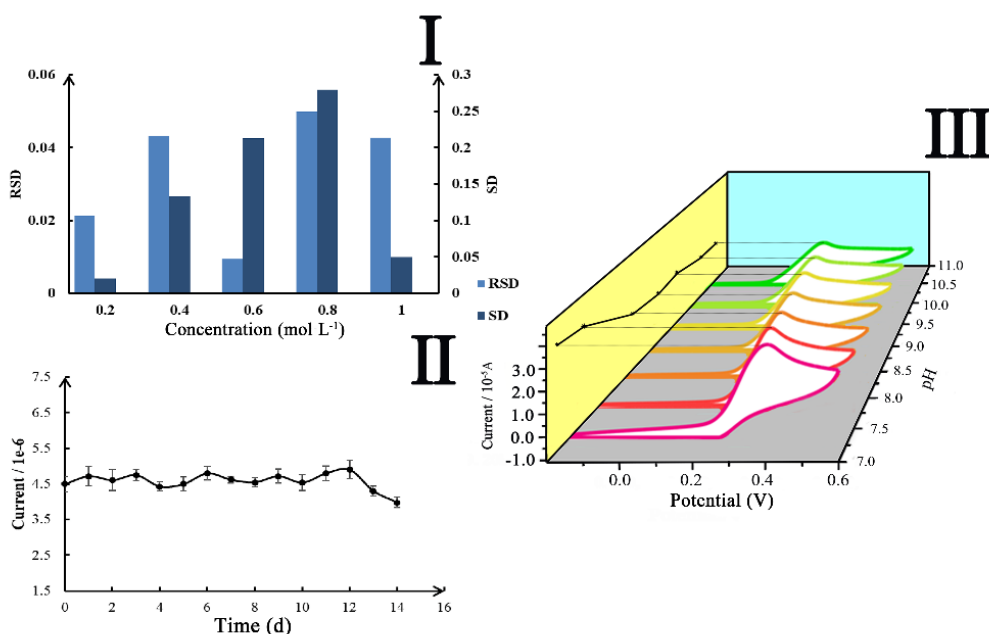
Figure 6. The working properties of the UOx/CS-PB-GO/GCE electrodes (I: electrode repeatability; II: electrode stability; and III: influence of pH on electrode performance. Measurements were carried out in 0.1 mol L^{-1} PBS.)

Table 2. Recovery results of UA at different concentrations spiked into human urine sample.

| Urine found（mmol L^{-1}）	Added（mmol L^{-1}）	Total found（mmol L^{-1}）	Recovery (%)	Average Recovery (%)
	0.1	1.845	95	
1.75	0.1	1.847	97	98.33
	0.1	1.853	103	

3.5 Analysis of anti-interference performance of the electrode

Urine's primary constituents consist of oxalic acid, AA, salicylic acid, citric acid, glucose, and dopamine. The influence of urine's primary components on UA oxidation response current were also investigated [30, 31] by preparing a solution for each constituent and testing their electrochemical response currents (and that of UA) using cyclic voltammetry. As shown in Table 3, the electrochemical sensor has no response current to salicylic acid, citric acid, and glucose. The response current to oxalic acid and dopamine was weak, but peak potentials are visible at 0.599 V and 0.082 V, respectively. Since the peak potential of UA was 0.351 V, oxalic acid, dopamine, salicylic acid, citric acid, and glucose have no effect on the determination of UA.

However, the UOx/PB-CS-GO/GCE modified electrode has a strong response to AA. Therefore, a cyclic voltammetric scan of the AA and UA mixture was performed using a UOx/PB-CS-GO/GCE modified electrode. The results are shown in Figure 7. AA and UA oxidation peaks are clearly distinguishable, thus AA did not affect UA detection. These results demonstrate that the UOx/PB-CS-GO/GCE electrode has good anti-interference capability.

Table 3. Anti-interference experiment of the UOx/PB-CS-GO/GCE electrode

Interference	Concentration (mmol L^{-1})	Potential (V)	Current ($1e^{-6}$A)
Uric Acid	1	0.351	6.177
Oxalic acid	50	0.599	1.926
Ascorbic acid	50	0.198	31.12
Citric acid	50	——	——
Glucose	50	——	——
Salicylic acid	50	——	——
dopamine	50	0.082	-2.290
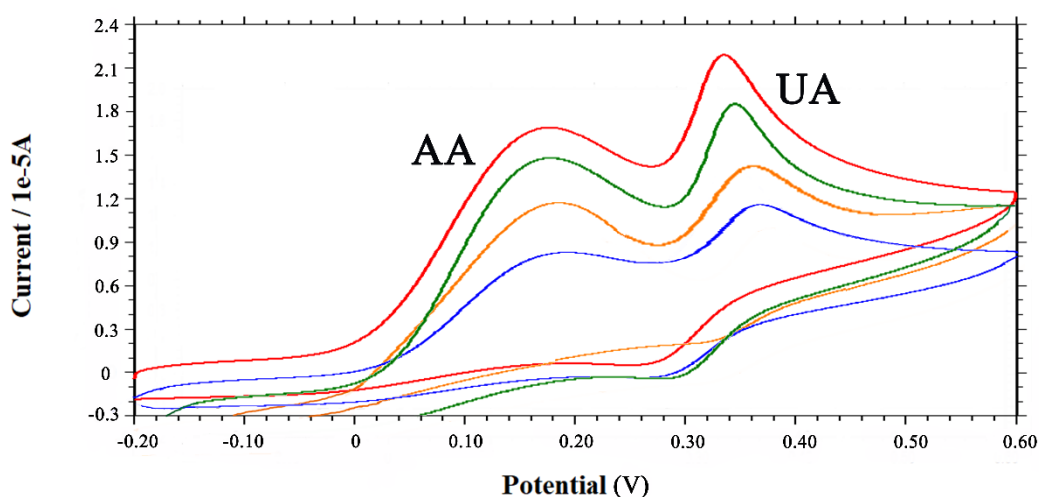
Figure 7. CV curves of the AA/UA mixture on the UOx/PB-CS-GO/GCE electrode. Measurements were carried out in 0.1 mol L^{-1} PBS (pH 7.4).

4. CONCLUSION

In this study, we prepared an electrode for rapidly detecting the UA concentration in urine, using PB-CS-GO as the stable skeleton. PB was deposited to increase electron transfer efficiency; chitosan was used to promote cross-linking; and the modification conditions of the electrode were optimized. Compared with the traditional electrode, the UOx/PB-CS-GO/GCE electrode has high repeatability (RSD 3.34%) and good stability (12 days), which can quickly and accurately determine the UA concentration in urine. In addition, the electrodes have good anti-interference capability. Of particular note is its good selectivity for ascorbic acid, which has the strongest interference potential. Finally, the electrode's measurement range (0.1-2 mmol L^{-1}) in this study includes the concentration range of UA in human urine (approximately 1.79 mmol L^{-1}). Therefore, the UOx/PB-CS-GO/GCE electrode provides a new technical approach for detecting UA in human urine quickly, accurately, and conveniently.

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