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Electrochemical Sensor Based on Glass Carbon Electrode Modified With Graphene Quantum Dots (GQDs) for Detection of Uric Acid

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Uric acid (UA) is a decomposition product of purine nucleotide metabolism in the human body, and is a normal component of urine. Most UA dissolves in the blood, passes through the kidneys and leaves the body in urine. High levels of UA in the blood can lead to hyperuricemia that cause the formation of UA crystals, and result in permanent damage of bone, joint and tissue, and kidney and heart disease. The most typical and common related diseases are that the crystals cause gout in the joints and form kidney stones in the kidneys. In this work, graphene quantum dots (GQDs) was successfully synthesized from citric acid as reaction substrate by pyrolysis method and was used to modify glass carbon electrode (GCE) to prepare an electrochemical sensor for the detection of UA. The electrochemical property of GCE modified with GQDs was characterized by differential pulse voltammetry, and cyclic voltammetry. The electrochemical response of the modified GCE for detecting UA showed a good linear relation in the ranges of 10~1000 μmol/L in phosphate buffer saline (pH=6.5). The as-prepared sensor has the advantages of good selectivity, high sensitivity and acceptable precision for the detection of UA, and can be used to detect UA in serum. The developed electrochemical sensor can be considered as a valuable tool for the analysis of UA in human serum, and has the potential to be developed for detecting UA outside the classical diagnostic laboratory. In addition, the electrochemical sensing technology involved in this study can be applied to the detection of UA, which lays a foundation for the further development of portable UA detector and expands the application of electrochemical sensing technology in laboratory medicine.

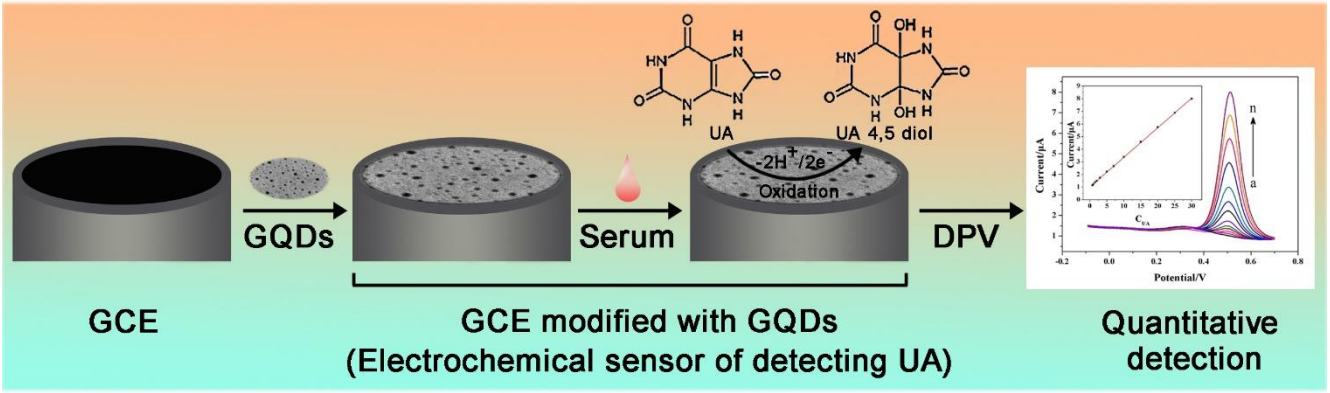
Keywords: Uric acid, Graphene quantum dots, Glass carbon electrode, Nanomaterials, Electrochemical sensor

1. INTRODUCTION

Uric acid (UA) which is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$, is an end product of purine metabolism in humans and great apes, and is a normal component of urine [1]. UA forms ions and salts known as urates and acid urates, such as ammonium acid urate, and is a very significant biological molecule in body fluids [2]. Because of its low solubility in aqueous solution (about 60 mg/L), UA may accumulate in human body [3]. Normal UA levels in serum are 2.6-5.7 mg/dl (155-339 μmol/L) in premenopausal women and 3.5-7.0 mg/dl (208-416 μmol/L) in men and postmenopausal women [1]. However, excessive amount of UA in blood can cause several diseases, such as gout, hyperuricemia and Lesch–Nyan syndrome [4, 5]. Thus, the level of UA in the blood and urine acts as a clinical diagnosis index for the aforementioned diseases. Therefore, the detection of UA is of great significance to prevent related diseases and reduce associated complications in clinical. At present, there are several analytical methods, such as fluorometry [6], high performance liquid chromatography (HPLC) [7], chemiluminescence [8], capillary electrophoresis (CE) [9] and colorimetry [10], which are used for the detection of UA. Nevertheless, most of these methods have the of procedures laborintensive and time-consuming, and the requirements of costly instrumentation. Hence, the development of an efficient detecting method for detecting UA is highly desirable in clinical.

The nanomaterials with unique properties are used for the clinical medicine, especially carbon nanomaterials, such as carbon nanotube [11], graphene oxide [12] and carbon dots [13]. Although these carbon nanomaterials possess excellent electronic transfer characteristics which are extensively used by researchers, the preparation process is tedious and complicated. However, Graphene quantum dots (GQDs), a new member of carbon nanomaterials family, draw more and more attention [14] the preparation process of which is handy, green and time-saving. In this work, we synthesized the GQDs by one-step process.

In recent years, electrochemical technologies get more and more attention and are applied to detect a variety of materials, such as carbohydrate antigen [15-18], carcinoembryonic antigen [19-22], tumor necrosis factor [23], alpha fetoprotein [24], dopamine [25], and glucagon-like peptide-1 [26]. What's more, more and more nanomaterials unite with electrochemical technologies to structure electrochemical sensor. In this study, GQDs were applied to modify GCE to build an electrochemical sensor for the detection of UA, as shown in Scheme 1. The obtained sensor successfully determined the UA, showed satisfactory sensitivity, selectivity and reproducibility and is better than the previous reports about the detection of UA. The as-prepared electrochemical sensor in the paper has several remarkable merits, such as simple fabrication process, ideal selectivity, speedy response and miniaturized potential. And the sensor could be utilized to detect UA levels of the human in phosphate buffered saline (pH=6.5).



Scheme 1. Schematic diagram showing preparation process of electrochemical sensor and detection of uric acid.

2. MATERIALS AND METHODS

2.1. Reagents and apparatus

Citric acid monohydrate (CA, $C_6H_8O_7•H_2O$), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4•2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4•12H_2O$), potassium chloride (KCl), sodium chloride (NaCl), L-ascorbic acid (AA), D(+)-glucose monohydrate ($C_6H_{12}O_6•H_2O$) and uric acid (UA, $C_5H_4N_4O_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). (±)-Epinephrine hydrochloride, glutathione (reduced), calcium chloride dihydrate ($CaCl_2•2H_2O$), magnesium chloride hexahydrate ($MgCl_2•6H_2O$), zinc sulfate heptahydrate ($ZnSO_4•7H_2O$) and iron (III) chloride ($FeCl_3$) were purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai China). Ultrapure water (18.2 MΩ•cm) purified from Millipore water purification system was used throughout the experiments in this work. All the reagents used in this work were analytically grade reagents and were not purified during all the experiments.

After precisely weighing 0.7801 g of $NaH_2PO_4•2H_2O$ and 1.7907 g of $Na_2HPO_4•12H_2O$, these two substrances were dissolved in an appropriate amount of ultrapure water. The liquid was then transferred to a 100 mL volumetric flask, followed by adding ultrapure water to the scale mark and shaking well. The pH values were adjusted to 6.5 with HCl or NaOH solution. The 0.1 mol/L phosphate buffer solution (PBS, pH=6.5) was obtained and used for the detection of UA.

Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were measured at CHI 660C electrochemical workstation (Shanghai ChenHua Instruments Co., China). The conventional three-electrode system composed of the modified GCE as working electrode, Ag/AgCl electrode (containing saturated KCl solution) as reference electrode and platinum wire as auxiliary electrode was used for the measurements of DPV and CV in 0.1 mol/L PBS (10 mL, pH=6.5). Transmission electron microscopy (TEM) images were obtained with a JEOL JEM-1400 Microscope. Fourier transform infrared spectroscopy (FTIR) spectrum was collected on Nicolet Avatar 360 spectrometer. The related experimental parameters of DPV and CV were given, according to the concrete experiment. All the experiments were carried out at room temperature.

2.2. Preparation of graphene quantum dots

In this work, CA was pyrolyzed and carbonized at a high temperature to synthesize graphene quantum dots (GQDs). The specific synthsis process was as follows. A 2 g of CA was accurately weighed and heated at 200 ℃ for 30 min in a 50 mL beaker. It was evident that the solid CA crystals changed into a colorless viscous liquid, and then into a light yellow viscous liquid with continuous stirring. The viscous liquid continued to deepen in color until it became a dark brown viscous liquid, indicating that GQDs was sucessfully synthesized. After cooling to room temerature, 10 mL of NaOH solution (10 mg/mL) was added to dissolve the dark brown solid to obtain GQDs solution. The pH of the GQDs solution was adjusted to 7.5 with HCl to make GQDs stable. The obtained GQDs solution with pH 7.5 was stored at 4 ℃ with keeping out of light, when not to use.

2.3. Preparation of electrochemical sensor (GCE modified with GQDs) for detecting UA

Before the modification was performed, the glass carbon electrode (GCE) was polished successively with 0.3 and 0.05 μm aluminum oxide on a wet microcloth pad, and then properly sonicated for 5 min in HNO_3 solution (volume ratio of HNO_3 to H_2O was 1:1), ethanol (95%) and deionized water, respectively. After the polished GCE was blow-dried with nitrogen, 5 μL of the above GQDs solution was dropped onto the surface of the GCE and dried at room temperature (25 ℃) for 24 h. The surface of GCE would form a homogeneous GQDs film and was named as GQDs modified GCE.

3. RESULTS

3.1. Characterizations of GQDs by TEM

The particle size of GQDs prepared by the pyrolysis method were characterized by TEM, as shown in Figure 1.

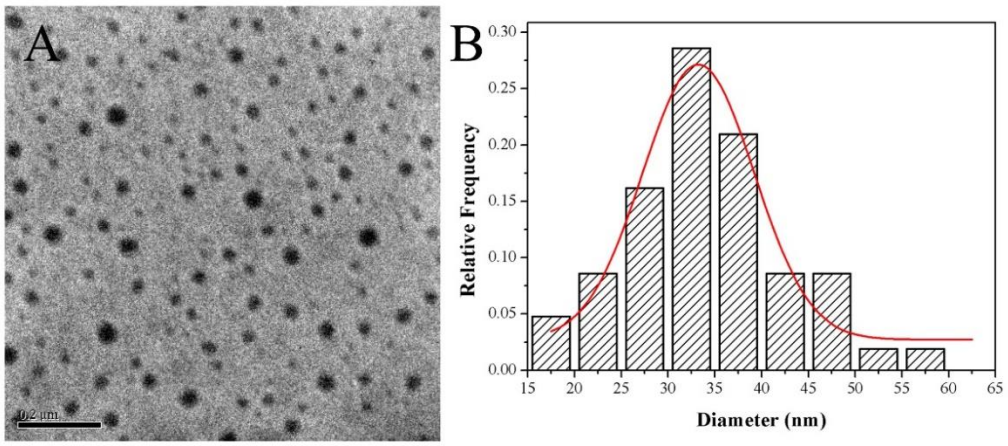


Figure 1. (A) TEM image of GQDs, and (B) Histogram of the particle size distribution of GQDs.

The TEM image (Figure 1A) showed that the GQDs were uniformly distributed, and the histogram of particle size distribution (Figure 1B) clearly showed that the diameters of the GQDs were mainly in range of 25~40 nm. The results indicated the as-prepared GQDs was uniform and evenly distributed, which would lay a good foundation for their subsequent application. Compared with other GQDs used to modify GCE [27-29], the as-prepared GQDs in this study has a simpler preparation process and a more uniform particle size, and the uniform particle size makes itself have more excellent performance.

3.2. Electrochemical responses of the electrochemical sensor (GCE modified with GQDs) to UA

The electrochemical performance of the electrochemical sensor (GCE modified with GQDs) decides its application for the detection UA. Figure 2 showed the differential pulse voltammetry (DPV) and cyclic voltammetry (CV) of working electrode (GCE modified with GQDs) in 0.1 mol/L PBS (10 mL, pH=6.5). As shown in Figure 2A, when UA was not added into PBS, there was no peak current at 0.51 V on DPV curve (a); after adding UA into PBS, the DPVcurve (b) showed an obvious peak current at 0.51 V, indicating that GCE modified with GQDs prepared in this study could show a significant electrochemical response to UA in PBS with pH=6.5. Cyclic voltammetry (CV) was further used to investigate the electrochemical response of the working electrode in PBS (pH=6.5) containing UA, as shown in Figure 2B. The anodic peak current of UA on CV curves increased with the increase of scanning rate, and there was a good linear relationship between the value of peak anodic current and the square root of scanning rate in the range of 0.01~0.7 V/s. The corresponding linear regression equation was I_{pa}=0.6117+5.244$v^{1/2}$ with R=0.9956. Considering the electrochemical response of DPV and CV to UA in PBS (pH=6.5), the corresponding response of DPV curve to UA was more suitable for the quantification of UA. In terms of electrochemical response, compared with other electrochemical sensors used for the detection of UA [30-32], the electrochemical sensor prepared in this study is more sensitive to UA.

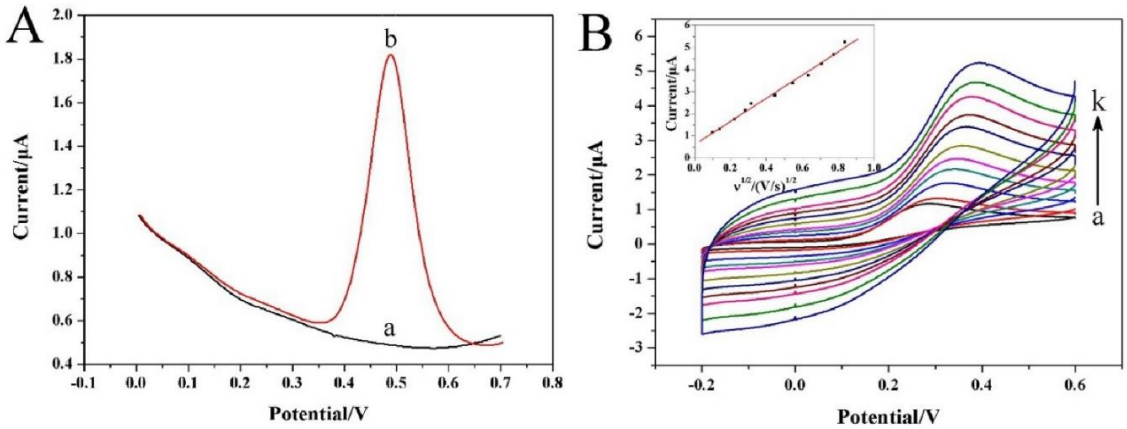


Figure 2. (A) DPV curves of GCE modified with GQDs in 0.1 mol/L PBS (pH=6.5) without UA (a) and with UA (b). (B) CV curves of GCE modified with GQDs in PBS (pH=6.5) with 200 μmol/L UA at different scanning rates (from a to k: 0.01, 0.02, 0.05, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 V/s). The inset showed the linear relation between the value of anodic peak current and the square root of scanning rates.

3.3. Identification of UA by the electrochemical sensor (GCE modified with GQDs)

The amperometric responses of the working electrode (GCE modified with GQDs) for glucose, ascorbic acid (AA), epinephrine, glutathione (GSH), Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{3+} were investigated to determine the selectivity of the electrochemical sensor, as shown in Figure 3. After UA was successively added into the testing solution (0.1 mol/L PBS with pH=6.5), the amperometric responses of the electrochemical sensor gradually increased with the increasing of UA in the PBS. When the concentration of UA reached 250 μmol/L in the PBS, some substances commonly found in the blood were successively added into the same PBS as interferents, including glucose, AA, epinephrine, GSH, Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{3+}. The concentration of all the interferents was 1 mmol/L in the PBS. The amperometric responses did not increase with the addition of the interferents in the electrochemical detection system with 250 μmol/L UA. Then, when UA was added into the electrochemical detection system again, the amperometric responses increased significantly with the increasing of UA in the PBS. The as-prepared electrochemical sensor had no response to the interferents, indicating that the electrochemical detection system had good specificity and selectivity for the detection of UA.

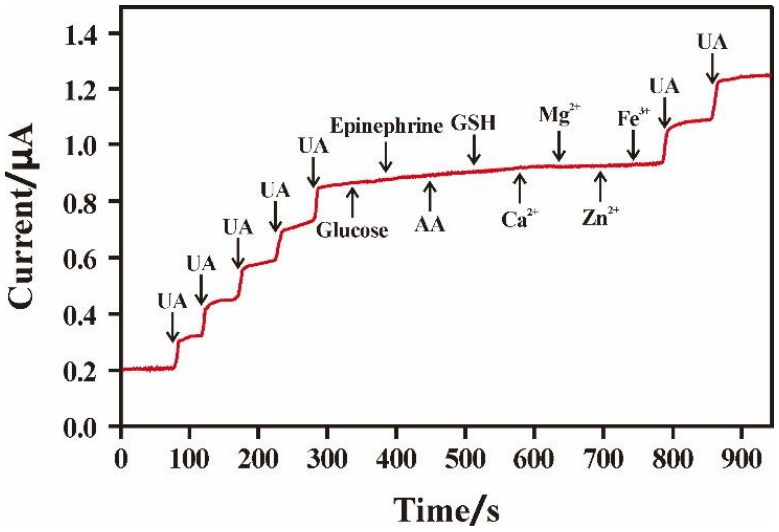


Figure 3. Amperometric responses of the as-prepared electrochemical sensor for the successive addition of UA, glucose, epinephrine, AA, GSH, Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{3+} in the electrochemial detection system (0.1 mol/L PBS with pH=6.5).

3.4. Detection of UA at different concentrations by the electrochemical sensor

The electrochemical sensor (GCE modified with GQDs) was used to detect a series of different concentration of UA in 0.1 mol/L PBS (10 mL, pH=6.5) under optimal conditions. As shown in Figure 4, a succession of DPV curves was obtained with the successive addition of UA and anodic peak current of DPV curves at 0.51 V gradually increased with the increase of UA concentration. Obviously, the peak current of DPV curves increased linearly with the increasing of UA concentration from 10 to 1000 μmol/L with a linear regression equation (I_p(μA)=1.0227+0.2343C_{UA}, R^2=0.9998). The limit of detection were 107 nmol/L for UA with a signal to noise ratio of 3 (S/N=3).

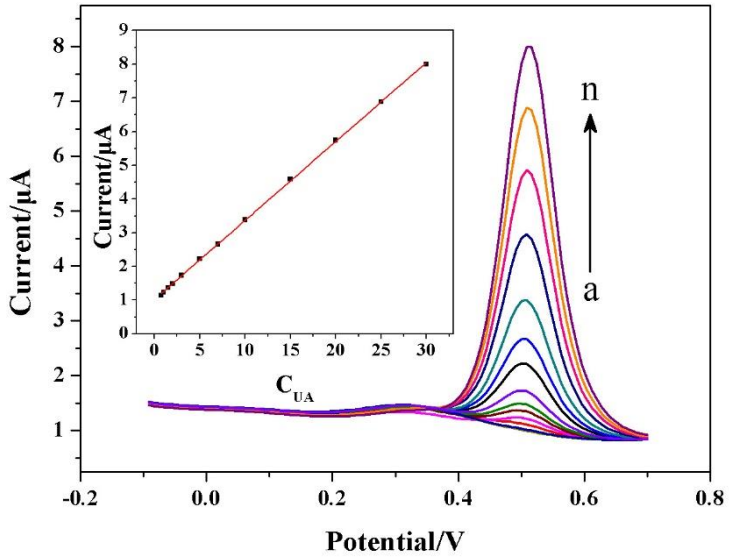


Figure 4. DPV curves of the different concentration of UA in 0.1 mol/L PBS (10 mL, pH=6.5) by the electrochemical sensor. From a to n, the concentrations were successively 10, 20, 40, 80, 100, 150, 200, 400, 500, 600, 700, 800, 900, and 1000 μmol/L. The inset showed the standard curve of UA concentrations versus peak current of DPV curves.

3.5. Detection of UA in human serum by the electrochemical sensor

Based on the simple preparation process and excellent detection performance of the developed sensor system, the electrochemical sensor was used to detect and analyze UA in human serum, with a view to becoming an alternative for detecting UA in clinical specimens. The detection limit of the developed electrochemical method was 107 nmol/L for UA, which was much lower than the conventional concentration of UA in the human serum (several hundred μmol/L), making the large-fold dilution of the sample feasible in the pretreatment. After 10-fold dilution of the serum specimens, the developed electrochemical sensor was used to analyze UA in the serum specimens. After the addition of standard UA, the concentration of UA was in the range of 302-507 μmol/L, as shown in Table 1. In the spiked recovery study, a known amount of UA was added into the serum specimens and diluted by the same multiple (10-fold) for determination. Satisfactory recoveries were obtained by the as-prepared electrochemical sensors, ranging from 95.8% to 104%, meaning that the electrochemical sensor can be used for the detection of UA in serum specimens. The reliable results obtained in the spiked recovery study were mainly attributed to the high sensitivity and selectivity of the electrochemical detection scheme for UA. These preliminary results indicated that the sensor had potential application value in the analysis and detection of UA in clinical specimens.

In order to verify the accuracy, 35 human serum specimens containing different concentration of UA were detected by the as-prepared electrochemical sensor, and the results were compared with those obtained by the clinical laboratory (Health Examination Department in Affiliated Hospital of Putian University). The concentration of UA detected by the sensor ranged 150 μmol/L from 300 μmol/L with RSD (n=3) in the range of 0.3%~8.5%, which fully demonstrated that the electrochemical sensor could detect UA content in serum specimens well.

Table 1. The accuracy of the as-prepared electrochemical sensor for the detection of UA in clinical specimens was verified by adding different amounts of UA standard solution into two human serum specimens.

Detected UA in unspiked human serum (μmol/L)	Added UA (μmol/L)	Expected total UA (μmol/L)	Detected total UA (μmol/L)	Recovery (%)
152	150	302	295	97.7
	200	352	366	104.0
	250	402	391	97.3
257	150	407	417	102.5
	200	457	438	95.8
	250	507	518	102.2

4. DISCUSSION

UA, despite being a major antioxidant in the human plasma, both correlates and predicts development of obesity, hypertension, and cardiovascular disease, conditions associated with oxidative stress [33]. Several lines of evidence suggest that increased serum uric acid may be a significant modifiable risk factor [34]. Therefore, we developed an electrochemical sensor based on graphene quantum dots for the detection of UA.

Firstly, we investigated that the as-prepared GQDs could be uniformly dispersed through TEM, which laid a foundation for the further preparation of the electrochemical sensor (GCE modified with GQDs), as shown in Figure 1. Then, the as-prepared modified electrode was used to investigate the electrochemical property of UA, and we could clearly observe that significant electrochemical signals appeared after the addition of UA, as shown in Figure 2A. The results indicated the GCE modified with GQDs could be used to detect UA. In Figure 2B, the cyclic voltammetry of UA showed that the oxidation of UA on the as-prepared modified electrode was a typical diffusion-controlled process, the relation of which accorded with Cottrell equation. The above experimental results also adequately showed the special properties of GQDs prepared by the pyrolysis method, as reported in the literature [35].

The molecular recognition of electrochemical sensor (GCE modified with GQDs) was a greatly important factor for the detection of clinical specimens. Common coexisting electrochemical active substances, such as glucose, epinephrine, AA, GSH, Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{3+}, may seriously affect the detection of UA in the electrochemical detection system. And the normal physiological level of glucose, epinephrine, AA, GSH, Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{3+} is higher than the physiological level of UA. As shown in Figure 3, these results suggested that the electrochemical sensor possessed acceptable specificity for the detection of UA. The result in Figure 4 adequately demonstrated that the GCE modified with GQDs could be used for the quantitative analysis of UA, and there was a good linear relationship between the concentrations of UA and the peak current value of DPV.

The standard addition recovery test showed that the electrochemical sensor could be used to

detect UA in human serum, and the satisfactory recoveries ranged from 95.8% to 104%. In addition, the detecting results of UA in clinical specimens by the electrochemical sensor were basically consistent with those by ELISA, indicating that the sensor could be used to accurately detect the level of UA in human serum. Although the proposed sensor had a wider linear range than spectrophotometric method, the electrochemical sensor provides an alternative choice for the detection of UA in human serum. The excellent performance of the electrochemical sensor for UA was mainly attributed to the specific catalytic action of the as-prepared GQDs on UA, which also showed that the electrochemical technology was well combined with carbon nanomaterials in this study.

Table 2. Comparison of the electrochemical sensor with other similar electrochemical sensor for the detection of UA.

Nanomaterials	Detection method	Detection platform	Linearity range (μmol/L)	LOD (μmol/L)	Clinical specimens	Ref.
Graphene	I-T	CFE	0.194~49.68	0.132	No	[36]
SWCNTs	DPV	Gold (Au) wire	0.002~0.01	0.00505	No	[37]
RGO–ZnO nanorods composite	I-T	GCE	1~800	0.312	Serum/urine	[38]
Poly(PDA)/SiO_2@Fe_3O_4	DPV	CPE	1.2~8.2	0.4	Serum/urine	[39]
Fe_3O_4@SiO_2/MWCNT nanocomposite	SWV	CPE	0.6~100	0.13	Serum/urine	[40]
Au-Ag NPs/GO/TH composites	SWV	GCE	1~100	0.3	Serum/urine	[41]
MC-GO-Fe_3O_4	DPV	GCE	0.5~140	0.17	Urine	[42]
Porous g-C_3N_4 and MWCNTs	DPV	GCE	0.2~20	0.139	Serum	[43]
CNCo	DPV	GCE	2~110	0.83	Serum	[44]
HADC-BIL	I-T	LPG	2~1050	1.27	Serum	[45]
GQDs	DPV	GCE	10~1000	0.107	Serum	This work

RGO: reduced graphene oxide; I-T: amperometric response; CFE: carbon fiber electrode; CPE: carbon paste electrode; LPG: lead pencil graphite; PDA: dipicolinic acid; SWV: square wave voltammetry; MWCNT(s): multi-walled carbon nanotubes; SWCNTs: single-walled carbon nanotubes; HADC: heteroatoms (S, N, P, and O) doped carbon; BIL: benzimidazolium-1-acetate ionic liquid; CNCo: N,Co-doped porous carbon; MC-GO-Fe_3O_4: methylcellulose/graphene oxide/iron oxide nano hydrogel; Au-Ag NPs/GO/TH: Au-Ag nanoparticle/graphene oxide/thionine composites

A variety of electrochemical sensor based on the modified electrode for the detection of UA have been reported in the literatures. Therefore, based on nanomaterials, detection methods, detection electrodes, linearity range, LOD, and clinical specimens, we performed a detailed comparison of reported techniques of detecting UA with our work, as shown in Table 2. There are various electrochemical methods that can be used to detect UA in clinical specimens, but we can clearly find

that each detection method for UA had its own characteristics and advantages. However, the best method to detect UA is to meet the detection requirements of clinical specimens. In contrast, the preparation method of GQDs in this study is simpler, highlighting the advantages in the preparation of modified nanomaterials. In addition, the selectivity, sensitivity, accuracy and linear range of GCE modified with GQDs for UA are very suitable for the detection of UA in clinical specimens. Considering the production cost, the as-prepared electrochemical sensor for detecting UA in this study is more suitable for primary medical institutions, or some medical institutions in poor areas. Therefore, the electrochemical sensing technology was applied to detect UA in serum, which opened up a new option for unconventional detection of UA, provided an experimental basis for the development of portable UA detection technology, and further expanded the application of electrochemical sensing technology in the field of laboratory medicine.

5. CONCLUSIONS

In this study, GQDs was successfully synthesized from CA by pyrolysis method and used to modify GCE to prepare an electrochemical sensor for the detection of UA. The as-prepared sensor for UA showed good selectivity, high sensitivity and acceptable precision, and was successfully applied to determinate UA in PBS (pH=6.5) without the influence of other interferents. It was further confirmed that the electrochemical sensor could be used to detect UA in human serum samples by the standard recovery test and the analysis and determination of UA in blood samples, indicating the sensor can be considered as a valuable tool for the detection of UA in human serum. Compared with reported electrochemical sensor techniques for UA detection, the electrochemical sensing platform used to detect UA in this study has lower preparation cost and is more suitable for primary medical institutions and medical institutions in poor areas.

The study provides an alternative for the detection of UA by the electrochemical technology in human serum and expends the application of GQDs in electrochemical analysis. At the same time, the study combines carbon nanomaterials well with electrochemical technology, which provides a new idea for the development of determination technology. In addition, this work also promotes the interdisciplinary development of laboratory medicine and other disciplines, especially carbon nanotechnology.

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DECLARATION OF INTEREST
All authors declared that there was no potential conflict of interest.

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