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Advanced biosensor for dopamine detection based on polyglutamic acid modified electrode

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Rapid and accurate dopamine (DA) detection is of great significance in clinical practice. In this study, polyglutamic acid modified graphite electrode (PGA/GE) was fabricated via one-step electrodeposition, and was employed to construct electrochemical sensor for DA detection. By systematic optimization, PGA/GE based sensor showed good performance with a wide linear range (0.5-120 μM) and especially a low limit of detection (0.0714 μM). Besides, PGA/GE base sensor presented many merits such as low cost, excellent reproducibility and anti-interference ability, allowing actual urine samples detection with a favorable recovery rate. So, PGA/GE based sensor has good application prospect in clinical diagnosis.

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Keywords: Polyglutamic acid, Modified electrode, Dopamine, Electrochemical detection

1. Introduction

Neurotransmitters are crucial signaling carriers that influence a lot of psychological and neurophysiological functions in living organism. Dopamine (DA), as one of important neurotransmitter in human brain [1], is mainly responsible for transmitting signals of pleasure and excitement. It is associated with human physiological functions such as maintaining the dynamic balance of nerve conduction, precisely regulating the rhythm of cardiovascular operation, and ensuring the stability of kidney metabolic function [2]. However, when DA concentration in human body is in abnormal levels, many serious diseases such as depression, schizophrenia, Parkinson's disease, Alzheimer's disease, pituitary tumors, and other neurological disorders [3-7] would occur. So, the development of a simple and accurate DA sensing technology can not only deepen the pathophysiological analysis of its neuroregulatory mechanism, but also provide a reference for the clinical diagnosis and treatment of neurodegenerative diseases, which is of great research significance for clinical practice [8].

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The traditional methods for DA detection include spectrophotometry [9], micro dialysis [10], high performance liquid chromatography [11], and fluorescence spectroscopy [12] etc. These techniques have their own disadvantageous such as complex operation and high costs, so they usually cannot be seen as appropriate methods in a real-time or on-site determination of DA. Electrochemical analysis [13-14], a highly favored technology owing to its rapid response, good sensitivity, reliability, low cost, and convenient operation, and specially the capable of being developed as portable devices for real-time monitoring, thereby widely applied in detection of bioactive molecules. Generally, an advanced electrochemical sensor highly depends on the preparation of high-performance electrode materials. The conventional commercialized electrode such as graphite electrode, glassy carbon electrode usually cannot provide a sufficiently high sensitivity in DA detection [15]. Therefore, electrode surface modification is necessary for improving the performance of the electrode. Many finely prepared materials such as carbon-based materials (carbon fibers, carbon nanotubes, graphene oxide etc.) [16,17], MOF [18], metal oxides [19], and even some precious metal materials metal nanoparticles (Au, Pd etc.) [20-21], were reported as electrode modifiers applied in DA detection, achieving positive results to some extent.

Recently, biocompatibility and fast-to-prepare polymerized amino acids had received widespread attention in advanced biosensor applications due to their unique electrochemical properties [22]. As one of the most prevalent amino acids, glutamic acid is regarded as a promising electrode modifier, which can easily form a polymer film (PGA) on the electrode surface by electrochemical polymerization. During this process, functional groups of α-amino in one monomer and β-carboxylic acid in another can form an amino bond that polymerizes the glutamic acid monomers together [23]. As a biodegradable and biocompatible polymer, PGA had been widely used is in many sectors such as food, water treatment, cosmetics, and medicine [24-26]. Furthermore, the characteristics of PGA such as abundant active sites, strong adherence to electrode, good stability and selectivity make it quite suitable in biosensor applications. Owing to its unique structure that the γ-peptide linked polymer skeleton contains a large number free carboxyl groups capable of binding various compounds [27], PGA can effectively promote electron transfer and serve as conducting bridges.

In this study, polyglutamic acid modified graphite electrodes (PGA/GEs) were successfully fabricated thought a simple on-site electrodeposition method and employed for constructing high-performance electrochemical sensor for DA detection. By systematic experimental optimization, the PGA/GE based sensor exhibited good performance toward DA detection, achieving a wide linear detection range (0.5 to 120 μM) and a low detection limit (0.0714 μM). We attributed the improvement to the analyte enrichment and the promoting effect of charge interface transfer from PGA. Besides sensitivity, the sensor exhibited outstanding stability, reproducibility and anti-interference ability, allowing actual urine samples detection with favorable recovery rates of 96.74% to 101.80%. The present work deepens the understanding of PGA in the field of electrochemical sensing, meanwhile provides a feasible strategy for DA detection, which can be seen a meaningful effort for the clinical diagnosis and public health safeguarding.

2. Experimental

2.1. Materials and apparatus

Dopamine (DA), uric acid (UA), ascorbic acid (AA), potassium ferricyanide, potassium ferrocyanide, tris (hydroxymethyl) aminomethane hydrochloride, potassium chloride and sodium chloride were bought form Shanghai Aladdin Biochemical Technology Co., LTD. Sulfuric acid and hydrochloric acid were provided by Sinopharm Group Chemical Reagent Co., LTD. Other reagents like potassium sulfate, sodium sulfate and glacial acetic acid came from Tianjin Comiao Chemical Reagent Co., LTD. All the reagents were analytical grade and used as received. The electrochemical characterizations were conducted on a CHI 660E workstation (Shanghai Chenhua Instrument Co., LTD). Graphite electrode with a diameter of 4 mm came from Shanghai Ledun Industrial Co., LTD. Platinum wire and saturated calomel electrode (SCE, 0.2412 V vs. SHE) were also provided by Shanghai Chenhua Instrument Co., LTD. A digital pH meter (PHSJ-3F, Shanghai Instrument & Electrical Scientific Instrument Co., LTD) was used for the determination of solution pH values.

2.2. Preparation of polyglutamic acid modified graphite electrode

A simple on-site electrodeposition method was used for the preparation of polyglutamic acid modified graphite electrode (PGA/GE). In brief, the graphite electrodes (GE) were successively polished in circles with sandpapers of different mesh sizes (500 mesh, 1000 mesh, 2000 mesh) until surface smooth. Then, they were rinsed with deionized water and placed in sulfuric acid solution (0.5 M) for cyclic voltammetry (CV) scanning in the range -1.0~1.0 V. After scanning for 5 circles, the electrode was taking out, washing with deionized water and drying naturally. Next, the pre-treated GE was placed in 2 mM glutamic acid solution (pH = 4.5, with 0.1 M KCl as the supporting electrolyte) and was carried out CV scanning for the formation of polyglutamic acid film on the electrode surface, thereby fabricating PGA/GE. The optimization of the preparation condition was conducted by changing the scanning voltage range and the number of cyclic scanning cycles.

2.3. Electrochemical testing

Electrochemical investigations were conducted on a common three-electrode system in which the PGA/GE or the bare GE served as the working electrode, the platinum wire acted as the counter electrode, and the SCE was used as the reference electrode for recording the potential of the working electrode. The electrochemical performances of PGA/GE were evaluated by CV scanning first in $[Fe(CN)_6]^{3-/4-}$ solution and then in different DA solutions, respectively. For the purpose of improving detection sensitivity, the differential pulse voltammetry (DPV) was adopted to optimize the detection conditions (acidity, buffer solution, supporting electrolyte) and draw the standard curves. The main parameters of DPV were set as the amplitude of 0.05 V, the potential increment of 0.004 V, the sampling interval of 0.02 s, the pulse width of 0.05 s and the pulse period of 0.5 seconds.

3. Results and discussion

3.1. Electrochemical behaviors of GE and PGA/GE in $[Fe(CN)_6]^{3-/4-}$ and DA solutions

The electrochemical behaviors of GE and PGA/GE electrodes in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution (with 0.1 M KCl as the supporting electrolyte) were evaluated by CV at a scanning rate of 100 $mV \cdot s^{-1}$ in the range -1.0~1.0 V, results shown as Fig. 1a. Despite the redox peaks of $[Fe(CN)_6]^{3-}$

$^{/4-}$ can be clearly seen on the both CV curves of GE and PGA/GE electrodes, by comparation, the one on PGA/GE was more symmetrical and was of higher peak current (approximately 1.3 times that that on the GE electrode). It indicated that the PGA/GE electrode has better interface charge transfer capability towards $[Fe(CN)_6]^{3-/4-}$ than that of GE. The electrochemical performance of GE and PGA/GE electrodes on DA detection were characterized in 100 μM DA solution (pH = 5.0 PBS, with 0.1 M KCl as the supporting electrolyte), similarly employing CV method. Fig. 1b shows the resulting CV diagrams. By comparing with the test in blank solution, we can confirm that the redox peaks located around 0.2 V shown on both curves were corresponding to the oxidation-reduction process of DA. Its oxidation mechanism was reported involving the processes of two electrons and two protons transferring in acidic environment, as illustrated in Fig. 2, where DA is first protonated forming an intermediate and then oxidized to the oxidation state of dopamine quinone (DAQ) [28]. Although both GE and PGA/GE demonstrated detection capabilities, there were obvious differences in detection sensitivity. Compared with GE, PGA/GE exhibits smaller redox peak potential difference and much higher peak current for instance the oxidation peak current on PGA/GE was around 3.2 times that that on GE, which indicated that the PGA/GE had much more sensitive response to DA under the same conditions. As we know, there are a large number free carboxyl groups on the polymer skeleton, which can work as active sites. The chemical affinity between carboxyl groups in PGA and the hydroxyl group on the benzene ring of DA not only promotes the enrichment of analytes on the surface of the electrode but also accelerates the transfer rate of the electrons crossing the interface. Considering that the oxidation peak current is significantly higher than the reduction peak current, therefore the oxidation peak was selected as the object for the subsequent DA detection studies.

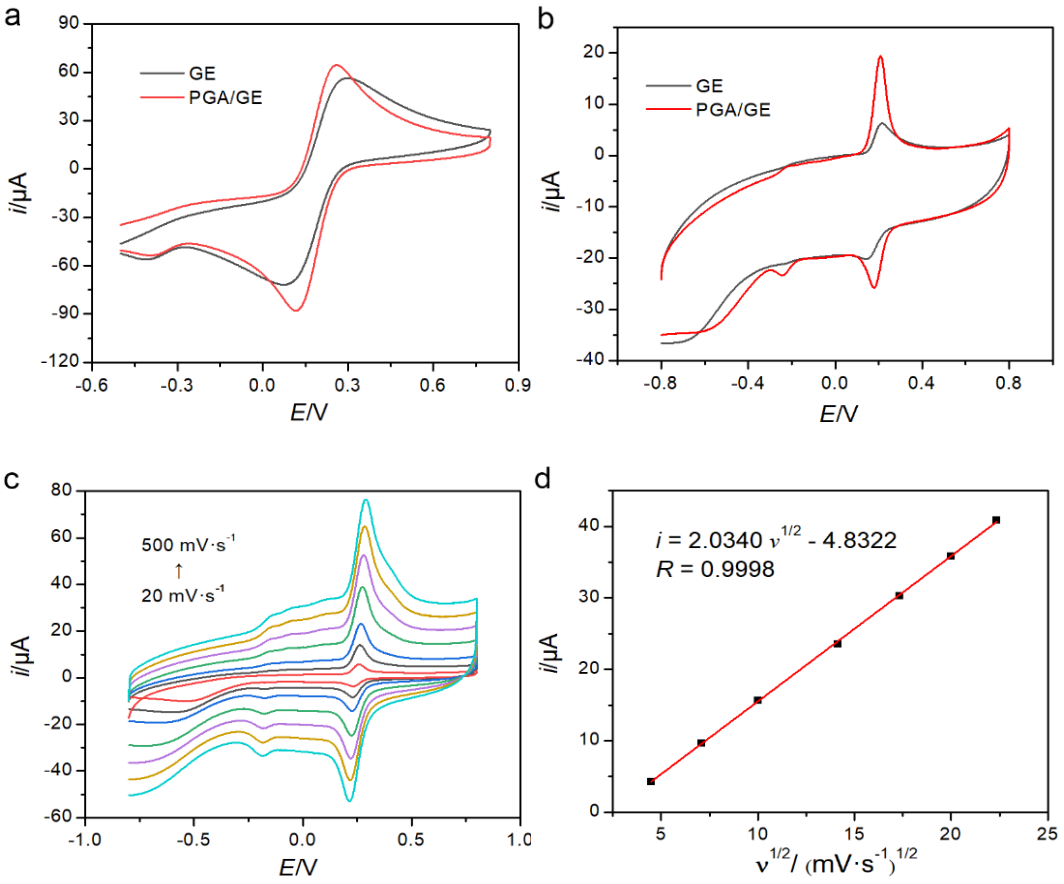

Fig. 1. CV curves of GE and PGA/GE in $[Fe(CN)_6]^{3-/4-}$ solution (5 mM, with 0.1 M KCl as the supporting electrolyte) (a) and DA solution (100 μM, PBS with pH = 5.0, with 0.1 M KCl as the supporting electrolyte) (b) at a scanning rate of 100 $mV{\cdot}s^{-1}$. CV curves of PGA/GE in above DA solution with different scanning speeds (c), and the as exhibited relationship between the oxidation peak current i and speed square root $v^{1/2}$.

(d).

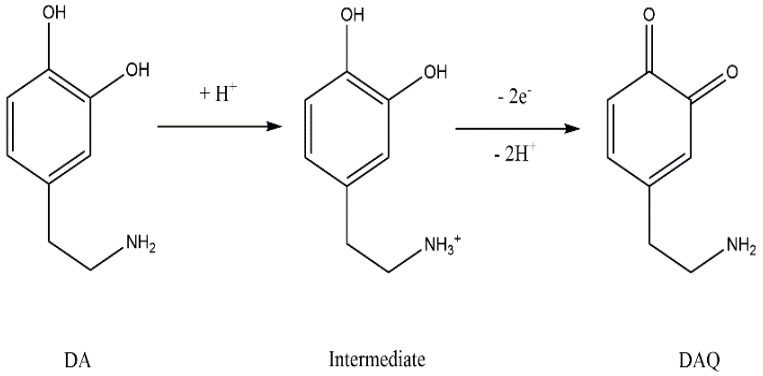

Fig. 2. The schematic diagram of electrochemical oxidation mechanism about DA.

In order to further acknowledging the reactive dynamic behaviors of PGA/GE, we explored the effect of the scanning speeds on the electrochemical behavior of DA. The tests were separately conducted at different scanning speeds (20, 50, 100, 200, 300, 400, 500 $mV \cdot s^{-1}$) in 100 μM DA solution (pH = 5.0 PBS, 0.1 M KCl), the results shown in Fig. 1c. It suggests that with increasing the scanning rates, the current of oxidation peak enhanced synchronously. As displayed in Fig. 3d, a good linear relationship was found between the peak current *i* and speed square root $v^{1/2}$. The linear equation was fitted as i (μA) = 2.0340 $v^{1/2}$ - 4.8322, with a linear correlation coefficient 0.9998. Such a dynamic behavior indicated the anodic oxidation process of DA on PGA/GE surface controlled by mass diffusion.

3.2. Optimization of detection conditions

Modification potential range is one of the key factors affecting the film-forming effect of glutamic acid polymerization. Fig. 3a presents the DPV curves tested in 100 μM DA (pH = 5.0 PBS, 0.1 M KCl) at different modification potential ranges. When fixing the positive potential at 1.5 V and changing negative potential from -1.0 to -1.2 V, the oxidation peak current value undergone first increase and then decrease, with a maximum value at -1.1 V. While fixing the negative potential at -1.1 V and changing positive potential from 1.4 to 1.6 V, the oxidation peak current value went through the similar process of first increasing and then decreasing, and appeared a maximum value at 1.5 V (47.21 μA). In all, -1.1 to 1.5 V is the preferred modification potential range.

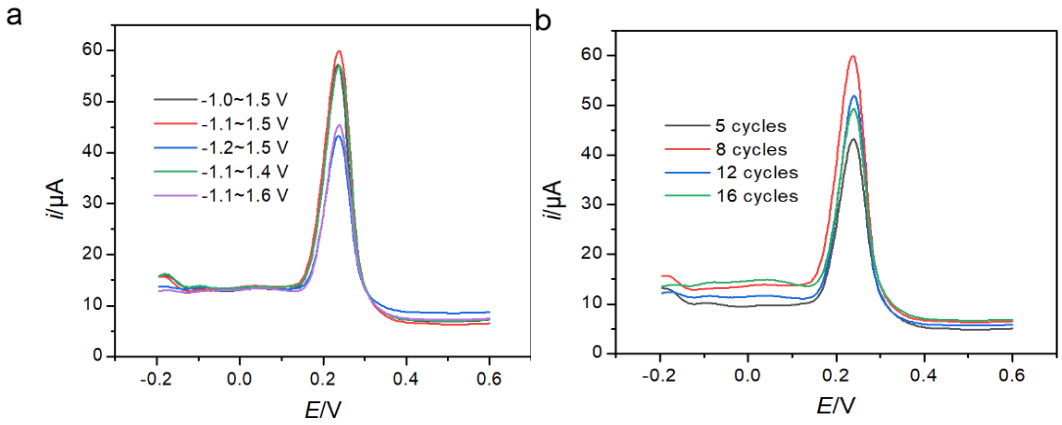

Fig. 3. (a)The DPV curves of DA on PGA/GE under different potential ranges at a fixed modification cycles 8. (b) The DPV curves of DA on PGA/GE under different modification cycles in the range -1.1 to 1.5 V. Experiment conditions: 100 μM DA, pH = 5.0 PBS, 0.1 M KCl.

The number of modification scan cycles determines the thickness of the polymerized film layer, which in turn affects its electrochemical response to DA. Fig. 3b gives DPV curves generated by PGA/GE electrodes separately prepared by different modification scan cycles in 100 μM DA (pH = 5.0 PBS, 0.1 M KCl). It suggests that when increasing the number of modification cycles, the peak current showed a trend of increasing first and then decreasing, and give a maximum value (47.32 μA) at 8 cycles. The results indicate that when the number of modification cycles reached 8, the electrode surface were almost completely covered by polyglutamic acid film. At this point, if the modification amount is further increased, it will not only fail to further increase the number of active sites, but also the overly thick modified film layer reaction would increase the ohmic impedance of electron transfer. Consequently, 8 times is a relatively appropriate number of modification circles.

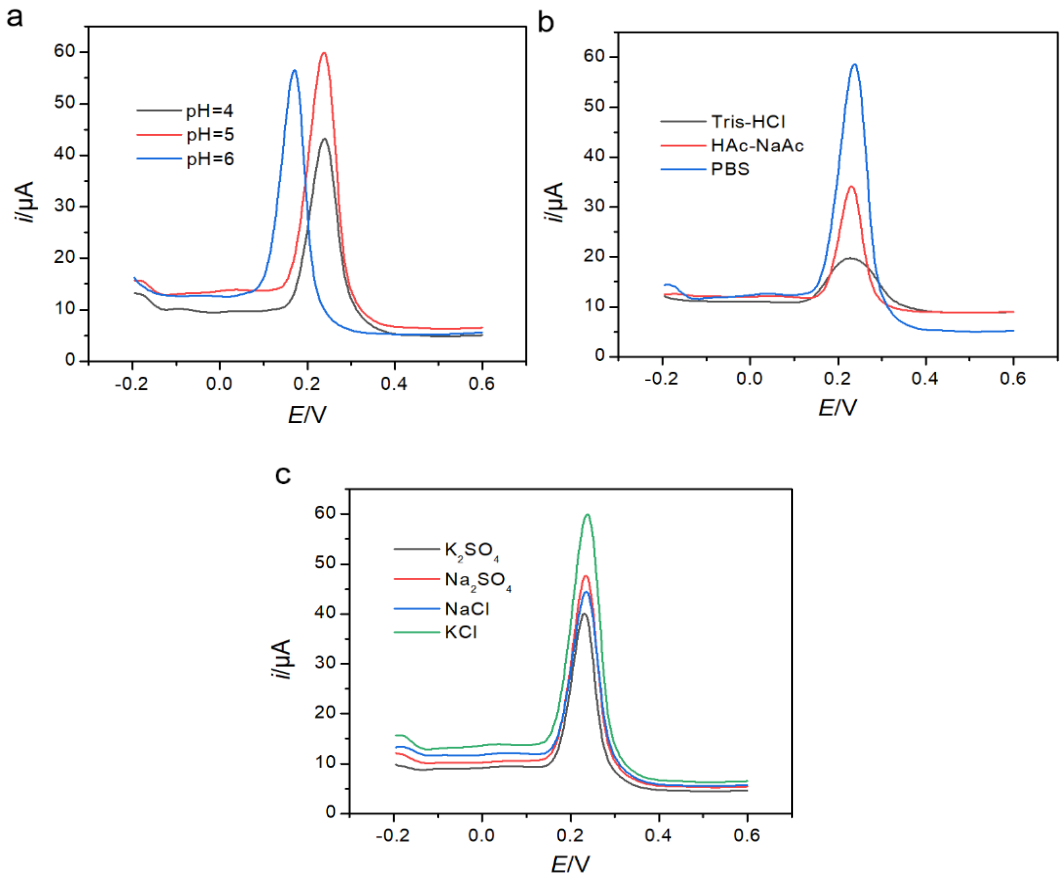

Fig. 4. The DPV curves of DA on PGA/GE at different pH (fixed 100 μM DA, PBS, 0.1 M KCl) (a), in different buffer solutions (fixed 100 μM DA, 0.1 M KCl, pH = 5.0) (b), and in different electrolyte (fixed 100 μM DA, pH = 5.0 PBS) (c), respectively.

The acidity of the test environment is one of crucial factors determining the electrocatalytic performance of the electrode. Fig. 4a shows the DPV curves obtained in 100 μM DA solutions (PBS, 0.1 M KCl) at different pH (4.0, 5.0, 6.0). We found that both the current and potential of the oxidation peak showed some differences at different pH values. The peak potentials were 0.240 V 0.235 V, and 0.170 V, meanwhile the peak currents were 39.48 μA, 47.35 μA, and 46.91 μA for pH = 4.0, pH = 5.0, and pH = 6.0, respectively. Since we pay more attention to the magnitude of the

current, pH = 5.0 was confirmed as the suitable acidity condition. Besides acidity, we also observed that the buffer solution types exerted big influence on DA detection. Three types of buffer solution systems as PBS, Tris-HCl, and HAc-NaAc were investigated, respectively, at the same acidity condition of pH = 5.0. Fig. 4b draws the resulting DPV curves. Although there was no obvious difference in peak potential, the peak current values vary greatly (47.28 μA, 9.61 μA, and 23.27 μA for PBS, Tris-HCl, and HAc-NaAc, respectively). In other words, the peak current values of DA in PBS buffer solution were approximately 5 times and 2 times than that of Tris-HCl and HAc-NaAc buffer solutions, respectively. The reason is probably that the mass diffusion ability of DA to the electrode surface varies in different buffer solution systems. Obviously, PBS buffer solution was more suitable for DA detection. The type of electrolyte can also exert a significant influence on the electrochemical performance of PGA/GE electrode in DA detection. Fig. 4c presents the DPV curves separately tested in 100 μM DA (pH = 5.0 PBS) solution with different supporting electrolytes (K_2SO_4, Na_2SO_4, NaCl, and KCl). The results suggest that the oxidation peak current in the KCl supporting electrolyte is the largest (47.25 μA) among them, which was approximately 1.5 times, 1.2 times, and 1.4 times than that in K_2SO_4, Na_2SO_4, and NaCl, respectively. Consequently, KCl was selected as the preferred supporting electrolyte for the subsequent DA determination.

3.3. Standard curve

Under the optimal experimental conditions (pH = 5.0 PBS, 0.1 mol·L^{-1} KCl), PGA/GE electrodes were employed for DA determination. Fig. 5a exhibits the DPV curves investigated in different DA concentrations (0.5, 1, 2, 5, 10, 20, 40, 60, 80, 100, 120, 200, 300, 400 and 500 μM). We found the oxidation peak current value gradually enhanced along with the increasement of DA concentration. Within the range of 0.5-120 μM, the DA concentration showed a good linear relationship with the peak current (Fig. 5b). The linear equation can be given as i (μA) = 0.4734 c (μM) + 1.4052, with a correlation coefficient R = 0.9986. The limit of detection (LOD) was estimated as 0.0714 μM (S/N=3). Compared with the previous research works on DA detection (Table 2), PGA/GE presents competitive detection performance of a relatively wide linear range and especially a low LOD.

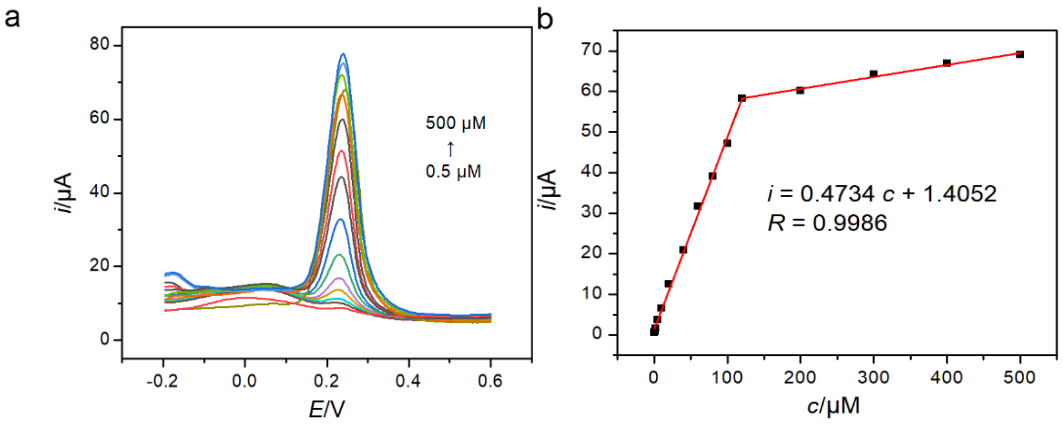

Fig. 5. Dose response curves (a) in DA solutions (pH = 5.0 PBS, 0.1 M KCl) and the linear relationship graph (b).

Table 1. Performance comparison of modified electrodes used in DA electrochemical detection.

Electrodes	Liner Range (μM)	LOD (μM)	Reference
AuNP/GR/OPPy-MIP/GCE	0.5-8	0.01	[29]
GSCR-MIPs	10-830	10	[30]
GR-MIP/Au	0.1-10	0.033	[31]
Apt-CFE	2-10	0.60	[32]
CNT/ CFE	5-120.6	10.78	[33]
AuNPs@NBSAC	1-50	20.53	[34]
Apt-Au-N-RGOF	1-100	0.50	[35]
PGA/GE	0.5-120	0.071	This work

3.4. Anti-interference and reproducibility

To verify the anti-interference capability of PGA/GE in DA determination, the electrochemical behaviors when analogues such as uric acid (UA) and ascorbic acid (AA) in presence were investigated, the results shown as Fig. 6a.

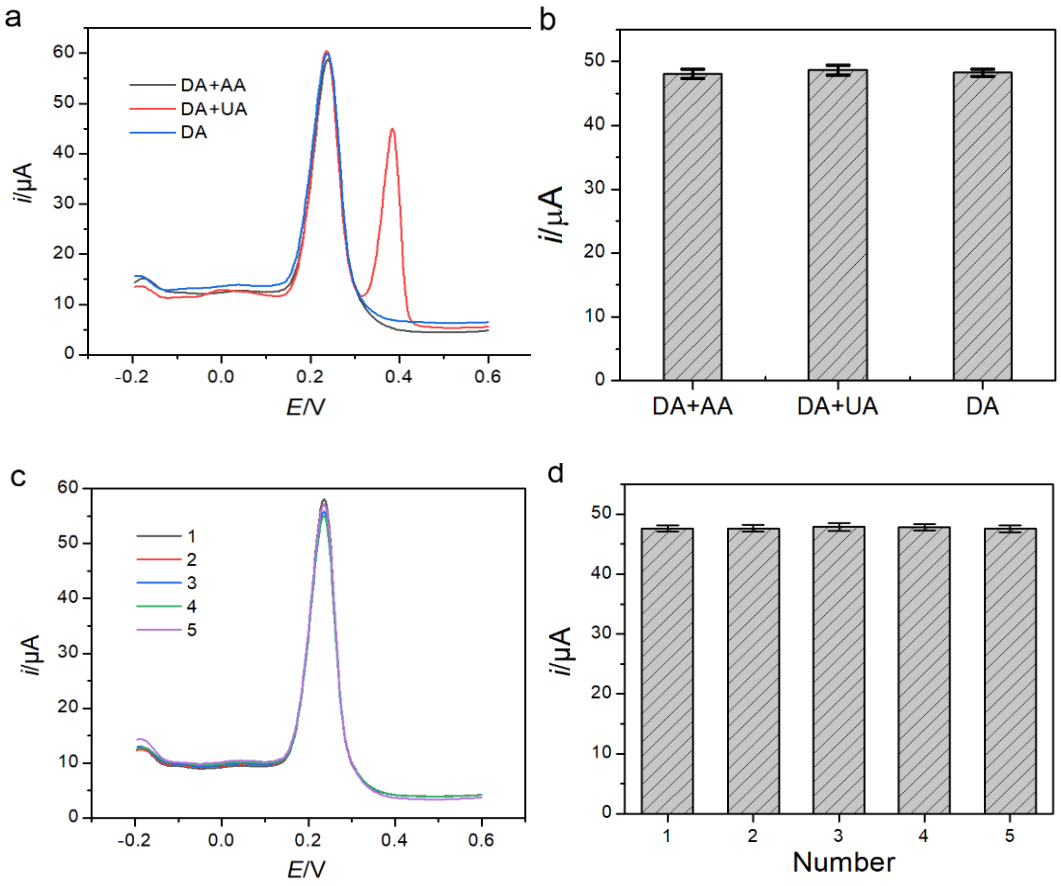

Fig. 6. The DPV curves (a) and the corresponding oxidation peak currents histogram (b) in anti-interference experiments, in which the added AA and UA were both 100 μM. The DPV curves (c) and the corresponding oxidation peak currents histogram (d) of 5 PGA/GEs parallel measurement in DA determination. Experiment conditions: 100 μM DA, pH=5 PBS, 0.1 M KCl.

In the experimental, the interferences uric acid and ascorbic acid both kept the same concentration as DA (100 μM). The results demonstrated that the oxidation peaks were almost coincident whether there was distraction or not. The oxidation peak current value varied within ± 5%, indicating that PGA/GE has a certain anti-interference ability for DA detection. Parallel experiments were carried out using five repeatedly prepared electrodes to verify the reproducibility of PGA/GE in DA determination. Each electrode was measured in parallel in the 100 μM DA solution (pH = 5.0 PBS, 0.1 M KCl) for six times. It (Fig. 6b) showed that the five generated oxidation peaks almost overlapped with each other, and the relative standard deviation (RSD) of average current value was 2.38% (Fig. 6b). These results confirmed the good reproducibility and stability of PGA/GE in DA sensing.

3.5. Determination of actual samples

Urine samples taken from healthy individual was preprocessed by filtering, and adjusting the pH to 9 (by Na_2CO_3). Then the clarification liquid was adjusted with hydrochloric acid to pH 3-4, and was subsequently diluted to twice with PBS. Next, the content of DA in the pre-treated sample was quantitatively determined by PGA/GE, and the result showed the value as 0.60 μM (corresponding the content in original urine sample 1.20 μM). For ensuring the reliability of the above analysis result, spiked recovery experiments were carried out and the data were detailed in Table 2. It showed favorable recovery rates of spiked samples within the range of 96.74% to 101.80%. These results confirmed that PGA/GE could be effectively used for DA detection in actual urine samples.

Table 2. Spiked Recovery experiments of actual samples.

No.	Spiked (μM)	Total (μM)	Recovery (%)	RSD (%, n=3)
1	0	0.60	/	/
2	10	10.78	101.80	1.14
3	20	20.53	99.65	1.07
4	50	48.97	96.74	0.59
5	80	80.45	99.81	2.63

Note: Spiked recovery rate = (Actual detected concentration/Added concentration) × 100%
Actual detected concentration = Detected concentration - 0.60

4. Conclusion

In this study, PGA/GE was prepared by on-site electrodeposition method using glutamic acid as the polymerization monomer, and was employed for the DA detection. Through the optimization experiments, we ensured the optimal modification condition was cyclic scanning 8 times in the range -1.1 to 1.5 V, and the optimal test conditions was in pH = 5.0 PBS with 0.1 M KCl as the supporting electrolyte. PGA/GE based sensor showed a wide linear response to DA within the range of 0.5-120 μM, with a LOD of 0.0714 μM. The linear equation can be fitted as i (μA) = 0.4734 c (μM) + 1.4052 (R = 0.9986). Compared with other electrochemical technologies, the detection performance of PGA/GE based sensor is considerable and progressive. We attributed the improvement to the suitable chemical affinity between carboxyl groups in PGA and the hydroxyl group on the benzene ring of DA, which effectively promoted the enrichment of analytes and

accelerated the electron transfer. Besides sensitivity, PGA/GE exhibited a certain anti-interference ability and good reproducibility, which all throw lights on the reliability of PGA/GE in DA determination. Actual urine samples detection suggested the recovery rate of spiked samples within the range of 96.74% to 101.80%, proving that PGA/GE has great ability in actual samples detection and is expected to be widely applied in clinical practice.

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