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Sensitive electrochemical detection of dopamine with a DNA/graphene bi-layer modified carbon ionic liquid electrode

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ABSTRACT

A DNA and graphene (GR) bi-layer modified carbon ionic liquid electrode (CILE) was fabricated by an electrodeposition method. GR nanosheets were electrodeposited on the surface of CILE at the potential of −1.3 V and then DNA was further deposited at the potential of +0.5 V on GR modified CILE. Electrochemical performances of the fabricated DNA/GR/CILE were carefully investigated. Then electrochemical behaviors of dopamine (DA) on the modified electrode were studied with the calculated electrochemical parameters. Under the optimized conditions, a linear relationship between the oxidation peak current and the concentration of DA was obtained in the range from 0.1 μmol/L to 1.0 mmol/L with a detection limit of 0.027 μmol/L (3σ). The modified electrode exhibited excellent reproducibility, repeatability, stability, validation and robustness for the electrochemical detection of DA. The proposed method was further applied to the DA injection solution and human urine samples determination with satisfactory results.

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1. Introduction

As one of the most significant catecholamines that belong to the family of excitatory chemical neurotransmitters [1], dopamine (DA) plays important roles in the central nervous, cardiovascular, renal and hormonal systems, as well as in drug addiction and Parkinson's disease [2]. So the sensitive detection of DA concentration is important for not only clinical diagnostics but also for pathological research. Because DA is an electroactive compound that can be easily oxidized on the electrode, electroanalysis of DA based on its electro-oxidation had been widely reported, which exhibits the advantages such as simplicity, high speed and sensitivity with low cost equipments. However, electrochemical determination of DA is usually affected by the oxidation of ascorbic acid (AA), which is often coexisted with DA in the biological samples and exhibits the similar oxidation potential. So the separation of electrochemical responses of DA and AA has been widely investigated by different kinds of chemically modified electrodes. Various modifiers have been used for the electrode modification and DA detection with increased sensitivity. For example, Jiang et al. applied DNA modified carbon fiber microelectrodes for DA detection with excellent anti-interference characteristics [3]. Kim et al. used a spontaneous formation of electrocatalytic poly(dopamine)-films on bare indium-tin oxide (ITO) electrode for the detection of DA [4]. Sabino et al. applied a laponite/tyrosinase modified glassy carbon electrode to determine DA [5]. Bui et al. used poly(glutamic acid) patterned single-walled carbon nanotube electrodes for the selective detection of DA [6]. Zhu et al. used a Ni/Al layered double hydroxide modified electrode for the determination of DA [7].

DNA is one of the most important biomacromolecules that is widely investigated in the field of electrochemistry and electrochemical sensors, which can not only be used as recognition element in electrochemical DNA sensor, but also exhibit efficient electroconductivity along the long chain [8]. Due to the rich p-electron of base-stacking along its double helical backbone, DNA can also be used for the sensing of bioactive species [9]. The interaction of catecholamines with DNA had also been observed, which could provide a novel way for the fabrication of neurotransmitter biosensors. Electrochemical sensors based on DNA recognition for DA, norepinephrine, uric acid in the presence of AA had been reported [10,11]. The presence of DNA film on the electrode can result in a conductive thin film with nano-structures and enhance the surface area of electrode, which can be used for the further construction of efficient biosensors [12,13].

Recently, graphene (GR) has attracted intensive research interests because of its extremely high thermal conductivity, good mechanical strength, high mobility of charge carriers, big specific surface area and upstanding electrical properties [14–16]. Because of its nanosheet structure, molecular interaction and thus electron transport through GR can be highly sensitive to the adsorbed

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molecules. Electrochemical applications of GR and its related composite have been reviewed in recent years [17,18]. Different modification procedures have been proposed for the fabrication of GR modified electrodes, which exhibit great potential for distinguishing various electroactive substances. Recently, electrochemical reduction of graphene oxide (GO) to GR has drawn considerable attention due to its fast speed and green nature [19,20]. Gao et al. directly deposited GO on the electrodes surface by electrochemical reduction and further applied the modified electrode to the rutin detection [21].

In this paper a carbon ionic liquid electrode (CILE) was fabricated by using N-hexylpyridinium hexafluorophosphate ($HPPF_6$) as the modifier, which had been reported as a working electrode with better performance [22]. CILE is prepared by incorporating ionic liquid (IL) as the binder and the modifier in the traditional carbon paste electrode (CPE), which has the advantages such as easy preparation, good reversibility, high sensitivity and the ability to lower the overpotential of electroactive compounds [23]. CILE can also be further decorated to get the modified electrode. Different kinds of the modified CILE had been prepared and used for electrochemical sensing with excellent performance [24–26]. By using CILE as the substrate electrode, GR was electrodeposited on its surface by direct electroreduction from GO solution, then a controllable thin DNA film was further deposited on GR surface to get a DNA and GR bilayer modified electrode (DNA/GR/CILE). The presence of GR on the electrode can increase the surface area and the interfacial conductivity. With the further modification of DNA on the electrode, the interaction between electroactive DA and DNA could take place on the electrode surface, which could be further used for the DA recognition and detection. Electrochemical behaviors of DA on DNA/GR/CILE were carefully investigated with the electrochemical process discussed and electrochemical parameters calculated. Compared with other reported results for electrochemistry of DA, the presence of DNA and GR on the CILE surface can largely enhance the electron transfer rate with accumulation effect. Under the optimal conditions a new electroanalytical method for the detection of DA was furthur established and applied to the DA injection samples detection.

2. Experimental

2.1. Apparatus and chemicals

Electrochemical experiments including potentiostatic reduction, cyclic voltammetry and electrochemical impedance spectroscopy (EIS) were executed on a CHI 750B electrochemical workstation (Shanghai CH Instrument, Shanghai, China). A conventional three-electrode system was used with a DNA/GR/CILE as a working electrode, a platinum wire as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. All the electrochemical experiments were conducted at the room temperature ($20\pm2\,^{\circ}C$). Scanning electron microscopy (SEM) was obtained by a JSM-7500F scanning electron microscope (Japan Electron Company, Tokyo, Japan).

N-hexylpyridinium hexafluorophosphate ($HPPF_6$, >99%, Lanzhou Greenchem. ILS. LICP. CAS., Lanzhou, China), dopamine (DA, Aladdin Reagent Co. Ltd., Shanghai, China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Co. Ltd., Shanghai, China) and double stranded DNA (dsDNA, Sigma-Aldrich Co., Shanghai, China) were used as received. Graphene oxide (GO) was synthesized by the modified Hummer's method [27]. 0.1 mol/L phosphate buffer solution (PBS) with various pH values was used as the supporting electrolyte. All the other chemicals used were of analytical reagent grade and doubly distilled water was used in the experiments.

2.2. Preparation of the modified electrode

CILE was fabricated based on the reported procedure [22]. Prior to use, a mirror-like surface can be obtained by polishing the electrode on a weighing paper. GR modified CILE was prepared by the electrochemical reduction with a potentiostatic method [21]. In brief, a freshly prepared CILE was placed in a 1.0 mg/mL GO dispersion solution with magnetic stirring and N_2 bubbling. By applying at the potential of -1.3 V for 300 s, a stable electrochemical reduced GR film could be formed on the CILE surface. The resulted electrode was denoted as GR/CILE, which was rinsed with doubly distilled water and dried in nitrogen atmosphere for the further modification. Then DNA modified electrode was prepared by immersing GR/CILE into a 0.1 mg/L dsDNA solution (in 0.05 M pH 7.0 PBS) and electrodeposition was carried out at the potential of $+0.5$ V for 1200 s. The resulting electrode was denoted as DNA/GR/CILE and other modified electrodes such as DNA/CILE, GR/CILE etc. were prepared by similar procedure for comparison.

2.3. Electrochemical procedure

All the cyclic voltammetric experiments were carried out in PBS containing certain concentration of DA at the scan rate of 100 mV/s. Differential pulse voltammograms (DPV) were recorded in the potential range from 0.6 to -0.2 V with instrumental parameters set as: pulse amplitude 0.005 V, pulse width 0.05 s, pulse period 0.2 s and quiet time 2 s.

3. Results and discussion

3.1. Characteristics of the modified electrodes

SEM was used to characterize the top views of different modified electrodes and the results are shown in Fig. 1. On the surface of CILE the layer of irregularly micrometer-sized graphite appeared (Fig. 1A), which was connected by IL with high viscosity. Fig. 1B shows the SEM image of GR nanosheets modified CILE. The typical lamellar structure of GR could be observed, which was highly beneficial in maintaining a large electrode surface area. Electrochemical reduction of GO is a convenient method for the preparation of GR with high efficient, simple, fast and green nature [19]. The large surface area of GR is helpful in increasing the sites

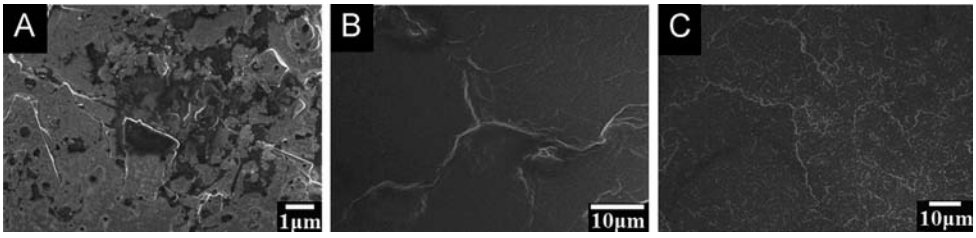

Fig. 1. SEM images of (A) CILE, (B) GR/CILE and (C) DNA/GR/CILE.

for the electrodeposition of the second DNA layer. The SEM image of DNA/GR/CILE was further recorded and shown in Fig. 1C, which exhibited many well-separated nanosized particles. Due to the presence of GR on the electrode, more DNA could be deposited on the surface of GR/CILE.

Cyclic voltammograms of different modified electrodes in a 1.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution were further recorded at the scan rate of 100 mV/s with the results shown in Fig. 2A. A pair of well-defined redox peaks could be observed on CILE with the peak-to-peak separation (ΔE_p) as 108 mV (curve b), indicating the high conductivity of CILE. While the redox peak currents of DNA/CILE decreased with the ΔE_p value increased to 187 mV (curve a), demonstrating that DNA had been immobilized on the surface of CILE. The negative charged phosphate skeletons of DNA on CILE surface exhibited a repulse force to the negatively charged $[Fe(CN)_6]^{3-/4-}$, which resulted in the decrease of the redox peak currents and the increase of ΔE_p value. On GR/CILE both redox peak currents increased obviously with ΔE_p value as 91 mV (curve d), which was due to the presence of electroreduced GR film on the electrode surface. GR has been elucidated with extremely high mobility of charge carriers, big specific surface area and excellent conductivity, which can greatly improve the electron transfer kinetics [28]. The redox peak currents on DNA/GR/CILE (curve c) were decreased again, which indicated the successful immobilization of DNA on the surface of GR/CILE. The negatively charged phosphate skeletons of DNA immobilized on the electrode surface had a repulsive force to $[Fe(CN)_6]^{3-/4-}$ anion, and therefore the current response of $[Fe(CN)_6]^{3-/4-}$ at DNA/GR/CILE was smaller than that of GR/CILE. All these results indicated the successful immobilization of DNA on the modified electrode.

Electrochemical impedance spectrum (EIS) can provide information on the electrode surface during the modification process, which is further used to probe the interface of the modified electrode. By using 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution as the electrochemical probe, the Nyquist plots of different modified electrodes were recorded with the results shown in Fig. 2B. In EIS the semicircular part at higher frequencies corresponds to the electron transfer limited process and the linear part at lower frequencies corresponds to the diffusion process. The value of electron transfer resistance (Ret) is estimated according to the diameter of the semicircle of the Nyquist plots at the high frequency region, which controls the electron transfer kinetics of redox probe at the electrode surface and reflects the interfacial electron transfer ability. Here Z′ and Z″ are the real variable and the negative value of the imaginary variable of impedance. The Ret value of bare CILE was 106.4 Ω (curve b). While on DNA/CILE the Ret value was increased to 140.1 Ω (curve a), indicating that the presence of DNA molecules with negatively charged phosphate skeletons on the surface of CILE increased the interfacial resistance. On GR/CILE the Ret value was decreased to 50.7 Ω (curve d), which was due to the presence of good conductive GR film on CILE. While on DNA/GR/CILE the Ret value was increased to 76.4 Ω (curve c), which was bigger than that of GR/CILE, indicating the successful immobilization of DNA on the GR surface.

3.2. Electrochemical behaviors of DA

Cyclic voltammograms of 1.0×10^{-4} mol/L DA on different modified electrodes were recorded with the results shown in Fig. 3. It can be seen that on all the modified electrodes a pair of well-defined redox peaks appeared, which was the typical electrochemical response of DA. Electrochemical data of DA on different modified electrodes were summarized and listed in Table 1. It can be seen that DA exhibited a draw out cyclic voltammetric curves with smallest redox peak currents and biggest peak-to-peak separation (ΔE_p) on CILE (curve a), indicating a quasi-reversible electrode process. While the redox peak currents increased with the decrease of ΔE_p value to 235 mV on GR/CILE, which could be attributed to the presence of high

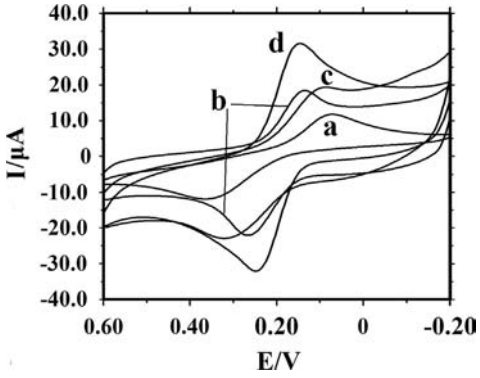

Fig. 3. Cyclic voltammograms of (a) CILE, (b) DNA/CILE, (c) GR/CILE and (d) DNA/GR/CILE in pH 6.5 PBS containing 1.0×10^{-4} mol/L DA with the scan rate as 100 mV/s.

Table 1
Electrochemical data of 1.0×10^{-4} mol/L DA on different modified electrodes.

Electrode	I_{pc} (μA)	I_{pa} (μA)	E_{pc} (V)	E_{pa} (V)	ΔE_p (V)	I_{pa}/I_{pc}
CILE	7.30	10.37	0.072	0.366	0.294	1.56
GR/CILE	13.33	15.84	0.087	0.322	0.235	1.18
DNA/CILE	12.72	14.51	0.139	0.270	0.131	1.14
DNA/GR/CILE	24.13	26.14	0.146	0.248	0.102	1.08

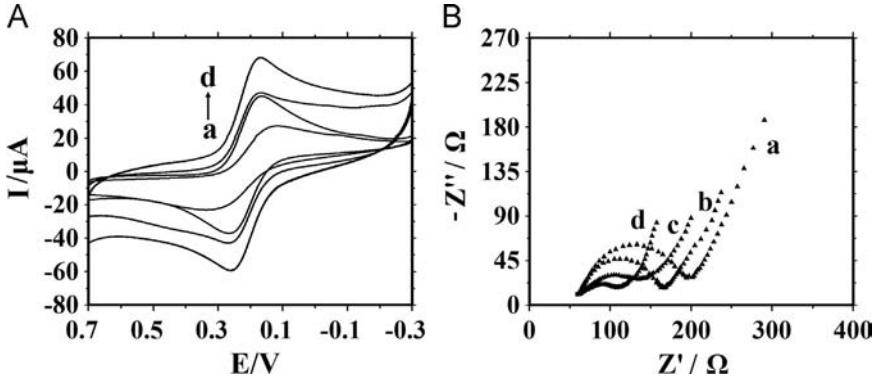

Fig. 2. (A) Cyclic voltammograms of different modified electrodes in 1.0 mmol/L $K_3[Fe(CN)_6]$ and 0.5 mol/L KCl with scan rate as 100 mV/s. (B) Electrochemical impedance spectra of different modified electrodes in 10.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol/L KCl with the frequently from 10^5 to 0.1 Hz. The electrodes from (a) to (d) were DNA/CILE, CILE, DNA/GR/CILE and GR/CILE, respectively.

conductive GR on CILE surface. Due to the specific electrochemical properties of GR with high surface area and good conductivity, electrochemical responses of DA were enhanced on the GR modified electrode. On DNA/CILE the redox peak currents also increased and ΔE_p value decreased to 131 mV (curve c), which could be ascribed to the accumulation effect of DA in the DNA structure. DA is in positive charged at pH 6.5 PBS due to its isoelectronic point (*pI*) as 8.9, which can interact with the negatively charged DNA on the electrode surface. So the presence of DNA on CILE exhibited the adsorption ability with the DA concentration increased on the electrode surface. On DNA/GR/CILE the biggest redox peak currents appeared with the smallest ΔE_p value as 102 mV (curve d). The values of redox peak currents were about 2 times larger than that of DNA/CILE and GR/CILE, which indicated that DNA/GR/CILE exhibited the best electrochemical performances for the DA detection. The results could be ascribed to the synergistic effects of GR and DNA on the modified electrode, including the high conductivity of GR and the accumulation effect of DNA to the DA molecules. The high surface area of GR on the electrode can deposit more DNA molecules on its surface, then more DA molecules could be accumulated on DNA layer with a more conductive GR surface facilitating the electron transfer. So DNA and GR bilayer modified electrode is a suitable platform for the electrochemical detection of DA with enhanced sensitivity.

3.3. Effect of buffer pH

The pH effects on the electrochemical responses of DA at DNA/GR/CILE were examined in the pH range from 5.0 to 8.0 with the results shown in Fig. S1A. It can be seen that a pair of well-defined redox peak of DA appeared on cyclic voltammograms in the selected pH range. The redox peak potentials shifted to the negative direction with the increase of buffer pH, indicating that protons involved in the electrochemical reaction. The linear relationship between the formal peak potential ($E^{0'}$) and pH was established with the equation as $E^{0'}$ (V) $=0.0511$ pH -0.512 ($\gamma=0.998$) (as shown in Fig. S1B). The slope value of 51.1 mV/pH was close to the theoretical value of 59 mV/pH, indicated that the same amounts of electrons and protons took part in the electrode reaction. The relationship of the oxidation peak current versus buffer pH was also plotted with the results shown in Fig. S1C. The maximum value appeared at pH 6.5, which was selected as optimal pH for the electrochemical detection in the following experiments, and this value was also proper to mimic the physiological environment.

3.4. Influence of scan rate

The effect of scan rate on the redox peak currents of DA at DNA/GR/CILE was also investigated with the typical cyclic voltammograms shown in Fig. S2. It can be seen that the redox peak currents increased gradually with the increase of scan rate in the range from 10.0 to 500.0 mV/s along with the changes of the redox peak potentials (Fig. S2A), which was the typical characteristics of a quasi-reversible electrochemical process. As shown in Fig. S2B, the redox peak current (I_p) exhibited a good linear relationship with the square root of scan rate ($\upsilon^{1/2}$). Two linear regression equations were got as I_{pc} (μA)$=$ 73.98 $\upsilon^{1/2}$ (V/s)-5.57 ($\gamma=0.997$) and I_{pa} (μA)$=$ -54.78 $\upsilon^{1/2}$ (V/s)-0.194 ($\gamma=0.999$), indicating a diffusional controlled electrochemical process. The redox peak potentials and $\ln \upsilon$ also exhibited good linear relationship with the regression equations calculated as E_{pa}(V)$=0.0271 \ln \upsilon+0.340$($\gamma=0.998$) and E_{pc} (V)$=$ $-0.0192 \ln \upsilon+0.101$($\gamma=0.997$) (shown in Fig. S2C). According to the following equations:

$$E_{pa} = E^{0'} + m\,[0.78 + \ln(D^{1/2}k_s{}^{-1}) - 0.5 \ln m] + \frac{m}{2} \ln \upsilon,\ m = \frac{RT}{(1-\alpha)nF} \quad (1)$$

$$E_{pc} = E^{0'} - m'[0.78 + \ln(D^{1/2}k_s{}^{-1}) - 0.5 \ln m'] - \frac{m'}{2} \ln \upsilon,\ m' = \frac{RT}{\alpha nF} \quad (2)$$

$$\log\ k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\ \alpha - \log \frac{RT}{nF\upsilon} - \frac{(1-\alpha)\alpha F \Delta E_p}{2.3RT} \quad (3)$$

The values of charge transfer coefficient (α), electron transfer number (n) and electrode reaction standard rate constant (k_s) were calculated as 0.58, 2.26 and 1.97 s^{-1}, respectively.

3.5. Analytical performance

In order to achieve the sensitive quantitative determination of low concentration DA, differential pulse voltammetry (DPV) was adopted with the typical voltammograms shown in Fig. 4A. Under the optimum experimental conditions, the oxidation peak currents (I_{pa}) had a good linear relationship with DA concentration in the range of 0.1 –1000.0 μmol/L with two sections appeared. As shown in Fig. 4B, the linear regression equations were obtained as I_{pa} (μA)$=1.667+0.156c$ (μmol/L) ($\gamma=0.996$) in the range from 0.1 to 200.0 μmol/L and I_{pa} (μA)$=24.45+0.0405c$ (μmol/L) ($\gamma=0.997$) in the range from 200.0 to 1000.0 μmol/L. The detection limit was calculated as 27.0 nmol/L (3σ). The analytical parameters of this electrode with other kinds of modified electrodes for the DA detection are compared and listed in Table 2. It can be seen that this method exhibited wider linear range and lower detection limit for DA detection.

The modified electrode exhibited good repeatability and the relative standard deviation (RSD) of 11 successive detections for 1.0×10^{-4} mol/L DA was 3.2%. Five modified electrodes were fabricated independently with the same procedure, which gave a

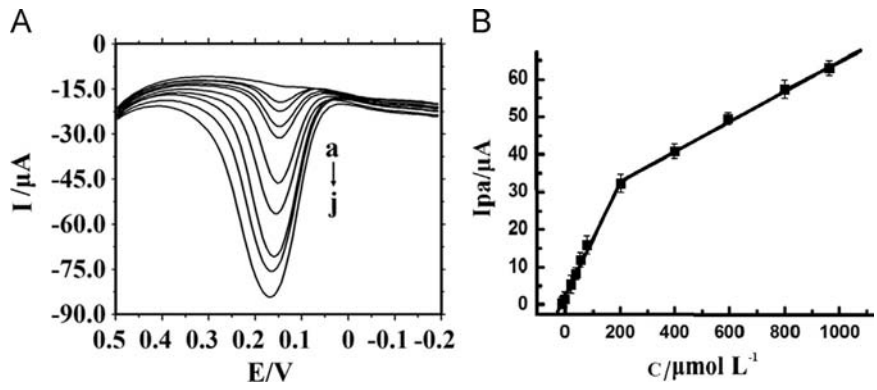

Fig. 4. (A) Differential pulse voltammograms of various concentrations DA on DNA/GR/CILE (from (a) to (j): 0.0, 20.0, 40.0, 60.0, 80.0, 200.0, 400.0, 600.0, 800.0, 1000.0 μmol/L). (B) Linear relationships of the anodic peak current with DA concentration.

Table 2
Comparison of the analytical parameters of DA on different modified electrodes.

Modified electrode	Linear range (μmol/L)	Detection limit (μmol/L)	References
DNA/PPyox/CFE[a]	0.3–10.0	0.08	[3]
ITO electrode[b]	/	0.001	[4]
GCE	4.5–400.0	2.3	[5]
LDH/CILE[c]	10–1100	5.0	[7]
DNA/Pp-ABSA/GCE[d]	0.19–13	0.088	[10]
Qu/GH/GCE[e]	0.03–400.0	0.01	[29]
RGO-Pd-NPs/GCE[f]	1–150.0	0.233	[30]
TCPP/CCG/GCE[g]	0.1–1.0	0.022	[31]
$\{AuNPs/RGO\}_{20}$/GCE[h]	1–60	0.02	[32]
DNA/GR/CILE	0.1–1000.0	0.027	This work

[a] DNA-overoxidized polypyrrole biocomposite layer modified carbon fiber electrode.
[b] Indum tin oxide electrode.
[c] Ni/Al layered double hydroxide modified carbon ionic liquid electrode.
[d] DNA/poly(p-aminobenzensulfonic acid) modified glassy carbon electrode.
[e] Quercetin-graphene composite modified glassy carbon electrode.
[f] Reduced graphene oxide and palladium nanoparticles modified glassy carbon electrode.
[g] Meso-tetra (4-carboxyphenyl) porphine/chemically reduced graphene modified glassy carbon electrode.
[h] Reduced graphene oxide/gold nanoparticles modified glassy carbon electrode.

Table 3
Determination of DA content in the injection and human urine samples ($n=6$).

Sample	Number	Specified (μmol/L)	Detected (μmol/L)	Added (μmol/L)	Total (μmol/L)	RSD (%)	Recovery (%)
Injection	1	63.28	63.12	20.0	83.01	1.82	99.4
	2	63.28	63.71	40.0	104.77	1.90	102.7
	3	63.28	63.56	60.0	122.79	2.11	98.7
Human urine	1	/	/	2.0	2.09	1.42	104.5
	2	/	/	4.0	4.14	2.15	103.5
	3	/	/	6.0	5.89	2.73	98.2

satisfactory RSD value of 2.7% for the detection of 1.0×10^{-4} mol/L DA, indicating the good reproducibility of the electrode fabrication. The stability of the modified electrode was investigated by storing in a 4 °C refrigerator for a certain time and furthur used for the detection of 1.0×10^{-4} mol/L DA. The results indicated that 96.5% of the initial response remained after 15 days storage.

The validation of electroanalysis is the process for confirming the determination procedure for a specific target that is suitable for its intended use like other analytical methods. The precision of the detection was investigated based on the intra-day and inter-day assessment with three concentrations (1ow, medium and high within the linear range) and five replicates of each concentration. The intra-day precision was based on the RSD value for the detection of 5.0, 50.0 and 500.0 μmol/L DA within the same day, which gave satisfactory results of 2.3%, 3.5% and 2.9%. Also the inter-day precision for the detection of 5.0, 50.0 and 500.0 μmol/L DA gave the RSD value of 3.7%, 4.2% and 3.1%, respectively. All results indicated that the modified electrode exhibited excellent precision for the DA detection. The accuracy of the intra-day and inter-day detection was also investigated by the standard DA solution and the recovery was in the range from 95.7% to 104.2%, indicating the high accuracy of the proposed method. The robustness of this method was evaluated by investigation on the effects of small variations of the experimental conditions such as the buffer pH, temperature and scan rate on the recovery of DA, which gave the values in the range of 96.5–98.1%. So this method had good robustness when the critical experimental conditions varied slightly.

3.6. Interference

The influences of some coexisting substances such as inorganic ions and organic compounds that commonly existed in biological samples on the determination of 1.0×10^{-4} mol/L DA were investigated with the results listed in Table S1. It can be seen that most of them did not interfere with the determination, indicating the good selectivity of the modified electrode.

Ascorbic acid (AA) is the main coexisting substance with DA in biological samples, so the electrochemical responses of DA in the presence of high concentration AA were studied with differential pulse voltammograms shown in Fig. S3. It can be seen that the oxidation peaks of AA and DA were overlapped together on CILE with a broad oxidation peak appeared (curve a), which indicated that selective determination of DA in the presence of AA was impossible on CILE. While on DNA/GR/CILE two oxidation peaks appeared at 0.195 V and 0.048 V (curve b), which corresponded to the oxidation of DA and AA respectively. The separation between these two oxidation peak potentials was calculated as 147 mV, which was large enough for the simultaneously detection of DA and AA in the mixture solution.

3.7. Samples determination

The proposed method was further applied to the determination of DA injection samples, which were purchased from Jiangsu Yabang Pharmacy Limited Company (100423) with the specified amount as 10.0 mg/mL. The samples were first diluted to 10.0 mL with water and 60.0 μL of the diluted solution was detected by the experimental procedure. The recovery of the test was analyzed by the standard addition method with the results summarized in Table 3. It can be seen that the results were satisfactory with the recovery in the range of 98.7–102.7%, indicating that the proposed electrode could be efficiently used for the determination of DA in the injection samples.

The proposed method was also used to direct analysis of DA in human urine samples, which was diluted for 300 times with pH 6.5 PBS before measurement. The results are also listed in Table 3 and the recovery was in the range of 98.2–104.5% with the RSD value less than 3%. So the proposed method can be potentially used for the real samples detection.

4. Conclusion

In the present work a DNA and GR bilayer modified CILE was fabricated by electroreduction of GO to GR and electrodeposition of DNA step-by-step on the electrode surface. The DNA/GR/CILE exhibited excellent electrochemical performances due to the synergistic effects of GR and DNA bilayer, including the accumulation and interaction of DNA with DA, the high conductive and large surface area of GR. Under the selected conditions, the anodic peak current was proportional to the DA concentration in the range from 0.1 μmol/L to 1000.0 μmol/L with the detection limit as 27.0 nmol/L (3σ). The DNA/GR/CILE showed good stability, high selectivity and excellent repeatability, which could be used for the sensitive detection of DA in the injection solution and human urine samples. Also this DNA/GR/CILE has the potential application in the electrochemical detection of other electroactive substances due to its excellent electrochemical properties.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.04.078.

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