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Electrochemical biosensor for simultaneous determination of guanine and adenine based on dopamine-melanin colloidal nanospheres–graphene composites

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Abstract Dopamine-melanin colloidal nanospheres (Dpa-melanin CNDs)–graphene composites-modified glassy carbon electrode (GCE) was prepared by a simple procedure and then successfully used to simultaneously determine guanine and adenine. Scanning electron microscopy (SEM) images and transmission electron microscopy (TEM) were used to characterize the morphology of the Dpa-melanin CNSs–graphene composite. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to characterize the electrode modifying process. Differential pulse voltammetry (DPV) was used to study the electrocatalytic activity toward the electrochemical oxidation of guanine and adenine. The modified electrode exhibited enhanced electrocatalytic behavior and good stability for the simultaneous determination of guanine and adenine compared with bare GCE. The electrochemical biosensor exhibited wide linear range of 0.5 to 150 μM with detection limit of 0.05 and 0.03 μM for guanine and adenine detection ($S/N=3$), respectively. Furthermore, the biosensor showed high sensitivity, good selectivity, good reproducibility, and long-term stability to guanine and adenine detection. At the same time, the fabricated electrode was successfully applied for the determination of guanine and adenine in denatured DNA samples with satisfying results. These results demonstrated that Dpa-melanin CNSs–graphene composite was a promising substrate for the development of high-performance electrochemical biosensor.

Introduction

Deoxyribonucleic acid (DNA) is an important biological macromolecule which plays a crucial role in the storage of genetic information and protein biosynthesis [1]. Guanine (G) and adenine (A) are two important purine bases existed in DNA, which play fundamental roles in life process. They have participated in processes as distinct as energy transduction, metabolic cofactors, and cell signaling [2]. The abnormal changes of the bases in organism suggest the deficiency and mutation of the immunity system and may indicate the presence of various diseases including cancer, epilepsy, and HIV infection. Moreover, their concentration levels are considered as important parameter for diagnosis of cancers, AIDS, myocardial cellular energy status, disease progress, and therapy responses [3]. Therefore, the determination of guanine and adenine is of great significance for clinical diagnosis as well as insight into fundamental mechanisms of genetic information [4].

In recent years, a large number of methods have been developed for the detection and quantification of guanine and adenine in nucleic acids, such as mass spectrometry [5], spectroscopic methods [6, 7], capillary electrophoresis [8], high-performance liquid chromatography [9], ion-pairing liquid chromatography [10], and flow injection chemiluminescence [11]. However, some of these mentioned methods exhibit the disadvantages of time-consuming, expensive instruments, and complicated pretreatment. Compared to these methods, the electrochemical technique has attracted a great deal of interest owing to its high sensitivity, simple operation, fast response speed, low cost, and real-time detection in situ condition. And some electrochemical methods have been developed for the determination of the

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guanine and adenine [12–17]. Huang et al. reported a graphene-COOH-modified electrode for the simultaneous detection of adenine and guanine sensor [18]. Wang et al. fabricated a β-cyclodextrin/nanotube (MWNT)-modified electrode for simultaneous determination of guanine and adenine multi-walled with detection limits of 6.6×10^{-7} and 1.5×10^{-6} M, respectively [19]. Sun et al. constructed a carbon ionic liquid (N-butylpyridinium hexafluorophosphate) electrode for the simultaneous detection of adenine and guanine [20]. Despite such progress, to develop simple, novel, reliable and efficient methods for sensitive analysis of guanine and adenine is still great challenge.

Graphene, a two-dimensional carbon nanomaterial, has attracted considerable attention in nanoelectronics, nanocomposites, chemical sensors, and biosensors due to its high thermal conductivity, good mechanical strength, high mobility of charge carriers, high specific surface area, and excellent electric conductivity [21–25]. Furthermore, graphene can greatly promote the electrochemical reactivity of biomolecules and improve the detection sensitivity on the modified electrode surface. These make it extremely attractive as a support material for designing nanoelectronic applications [22, 26]. The graphene-based hybrid materials have shown greater versatility as enhanced electrode materials for electrochemical sensors and biosensors applications [27–29].

Dopamine-melanin colloidal nanospheres (Dpa-melanin CNSs) have stimulated intense research interest in the past few years owing to its cost-effectivity, low toxicity, well-dispersion, good stability, and biodegradability [30–32]. It was reported that Dpa-melanin CNSs have been used as an efficient near-infrared photothermal therapeutic agent for in vivo cancer therapy [32]. However, there have been very few reports about Dpa-melanin CNSs as modified electrode materials. In this work, we have constructed a simple, convenient, and sensitive method for simultaneous determination of guanine and adenine based on Dpa-melanin CNSs–graphene composites. The fabricated modified electrode exhibited remarkable electrocatalytic activity towards adenine and guanine oxidation and showed good sensitivity, stability, and a low detection limit. Moreover, the proposed method was applied to the detection of the content of guanine and adenine in DNA sample with excellent analytical performance.

Experimental

Chemicals and materials

Graphite powder (99.95 %, 325 mesh) was purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Guanine and adenine were purchased from Sigma-Aldrich Chemical Company (St. Louis, Mo, USA). Dopamine hydrochloride was obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). All other chemicals of analytical grade

were supplied by China National Medicines Co. Ltd. (Beijing, China) and without further purification. All the water used in this work was obtained from a Millipore Milli-Q water purification system (Billerica, MA, USA) with electrical resistance >18.2 MΩ.

Apparatus

All electrochemical measurements were performed with a CHI 660D electrochemical analyzer (CHI Inc. Shanghai, China) at room temperature by using a three-electrode system consisting of a KCl-saturated calomel reference electrode (SCE), a platinum counter electrode, and a modified glass carbon electrode (GCE, 3 mm diameter) as the working electrode. Scanning electron microscopy (SEM) images were recorded on a Hitachi S-4800 scanning electron microscope. Transmission electron microscopy (TEM) was performed using a JEM-2010 transmission electron microscopy.

Synthesis of Dpa-melanin CNSs

Dpa-melanin CNSs were synthesized by the oxidation and self-polymerization of dopamine in a mixture containing water, ethanol, and ammonia at room temperature [31]. In a typical synthesis of Dpa-melanin CNSs, 2 mL ammonia aqueous solution was firstly mixed with 40 mL ethanol and 90 mL ultrapure water under mild stirring at 30 °C for 30 min. Then 0.5 g dopamine hydrochloride was dissolved in 10 mL ultrapure water and injected into the above mixture solution rapidly. The color of the solution immediately turned to chalky yellow and gradually changed to dark brown. And the solution was incubated at 30 °C for 24 h. The reaction mixture was purified by repeated centrifugation at 10, 000 rpm for 10 min and rinsing with ultrapure water. Finally, black powder of Dpa-melanin CNSs was obtained by dried in vacuum.

Preparation of graphene

Graphene oxide was prepared by a modified Hummers method using graphite powder as starting material [33]. Graphene was prepared from reduction of exfoliated graphite oxide. In a typical preparation of graphene, 100 mL graphene oxide dispersion was mixed with 70 μL hydrazine solution (50 wt% in water) and 0.7 mL ammonia solution (28 wt% in water). And the mixture was stirred at 95 °C for 1 h. Then, the final product was isolated by filtration, rinsed thoroughly with ultrapure water and ethanol, and dried in vacuum. Finally, the graphene was obtained in the form of black powder.

Preparation of modified electrode

Before the modification, GCE (3 mm in diameter) were polished to a mirror-like with 0.3 and 0.05 μm of alumina

slurry in sequence on a polishing cloth, and then washed with ultrapure water, anhydrous ethanol, and ultrapure water in an ultrasonic bath, and the GCE was dried under the stream of high-purity nitrogen for further use. For electrode preparation, 1 mg Dpa-melanin CNSs and 1 mg graphene were dispersed in 2 ml DMF with ultrasonic treatment for 1 h to get a black homogenous suspension. Then, 5 μL of the mixture was dropped on the pretreated GCE, and then dried for 24 h in air before use. The obtained electrodes were noted as Dpa-melanin CNSs–graphene/GCE.

Preparation of DNA sample

Thermally denatured ds-DNA was prepared according to the previous reported literature method [20]. Briefly, the native herring sperm ds-DNA solution was heated in a boiling water bath at 100 °C for 10 min, and followed by rapid cooling in an ice bath. Generally, thermal denaturation involved the rupture of hydrogen bonds, the disturbance of stacking interaction but not any breakage of covalent bond. So, this kind of thermally denatured ds-DNA can be used as single-stranded DNA (ss-DNA).

Experimental procedure

The electrochemical measurements were performed in 0.1 M HAc-NaAc buffer solutions (ABS, pH 4.5) with different concentrations of guanine or adenine or their mixtures. The accumulation procedures were carried out on open circuit for 100 s. After 2 s of quiet period, differential pulse voltammograms were recorded at the scan rate of 100 mV s^{-1}.

Results and discussion

Characterization

Dpa-melanin CNSs were synthesized by the oxidation and self-polymerization of dopamine in a mixture solution containing water, ethanol, and ammonia at room temperature. The morphology of the Dpa-melanin CNSs and Dpa-melanin CNSs–graphene composite were studied by the means of SEM and TEM. Figure 1a, b showed the SEM and TEM image of Dpa-melanin CNSs, illustrating a 3D sphere-like architecture. The average particle size of Dpa-melanin CNSs was about 200 nm. The surface morphology of Dpa-melanin CNSs–graphene composites had also been investigated. Figure 1c clearly illustrated sphere Dpa-melanin CNSs were compactly embedded on the graphene substrate. Figure 1d showed the typical TEM of graphene nanosheet and spherical Dpa-melanin CNSs. Furthermore, it was observed that Dpa-melanin CNSs were supported on the surface of graphene nanosheets.

### Electrochemical properties of Dpa-melanin CNSs–graphene composites

Figure 2a showed the behavior of cyclic voltammograms (CV) response of [Fe(CN)$_6$]$^{3-/4-}$ on the different modified electrodes surface. As shown in Fig. 2a curve a, it could been seen that the bare GCE exhibited a couple of quasi-reversible, well-defined redox peaks of [Fe(CN)$_6$]$^{3-/4-}$. Compared with bare GCE, the peak currents at graphene/GCE (curve b), Dpa-melanin CNSs/GCE (curve c), and Dpa-melanin CNSs–graphene/GCE (curve d) all increased. And the peak current of Dpa-melanin CNSs–graphene/GCE (curve d) exhibited the biggest, which indicates that Dpa-melanin CNSs–graphene composites possessed the requisite surface structure and electronic properties to support rapid electron transfer for this particular mechanistically complicated redox system.

Electrochemical impedance spectroscopy (EIS) is an effective technique for studying the change of interface properties of different modified electrode. The EIS included a semicircle portion and a linear portion. The semicircle diameter at higher frequencies corresponds to the electron transfer resistance (R_{et}), and the linear part at lower frequencies corresponds to the diffusion process. From Fig. 2b, it was observed that the EIS of graphene/GCE (curve b), Dpa-melanin CNSs/GCE (curve c), and Dpa-melanin CNSs–graphene/GCE (curve d) all displayed decreased semicircle at higher frequencies compared with bare GCE (curve a). However, the EIS of Dpa-melanin CNSs–graphene/GCE displayed almost straight lines in the Nyquist plot of impedance spectroscopy, demonstrating its excellent electron conductivity.

Electrochemical behavior of adenine and guanine

The electrochemical behavior of guanine and adenine on different modified electrodes was investigated in 0.1 M ABS buffer solution (pH 4.5) by differential pulse voltammograms (DPV). As shown in Fig. 3, just two small peaks on the bare GCE could be observed (curve a). Compared to the DPV response of the bare GCE, the oxidation peak currents of guanine and adenine on the graphene/GCE obviously increased (curve b in Fig. 3). And the oxidation peak potential of guanine and adenine negatively shifted to 0.83 and 1.13 V, respectively. It indicated that graphene could facilitate the electron transfer of guanine and adenine. However, curve c showed that the oxide peaks of guanine and adenine at Dpa-melanin CNSs/GCE were at approximately 0.85 and 1.17 V, respectively. A positive shift of oxidation potential and increase peak current to guanine and adenine were clearly seen. The changes revealed that Dpa-melanin CNSs-modified GCE exhibited a good catalytic activity toward guanine and adenine, and CNSs were dominating participator in the electrocatalytic reaction. However, the oxidation peak currents on Dpa-melanin CNSs–graphene/GCE enhanced significantly

Fig. 1 a SEM images of Dpa-melanin CNSs; **b** TEM images of Dpa-melanin CNSs; **c** SEM images of Dpa-melanin CNSs–graphene composites; **d** TEM images of Dpa-melanin CNSs–graphene composites
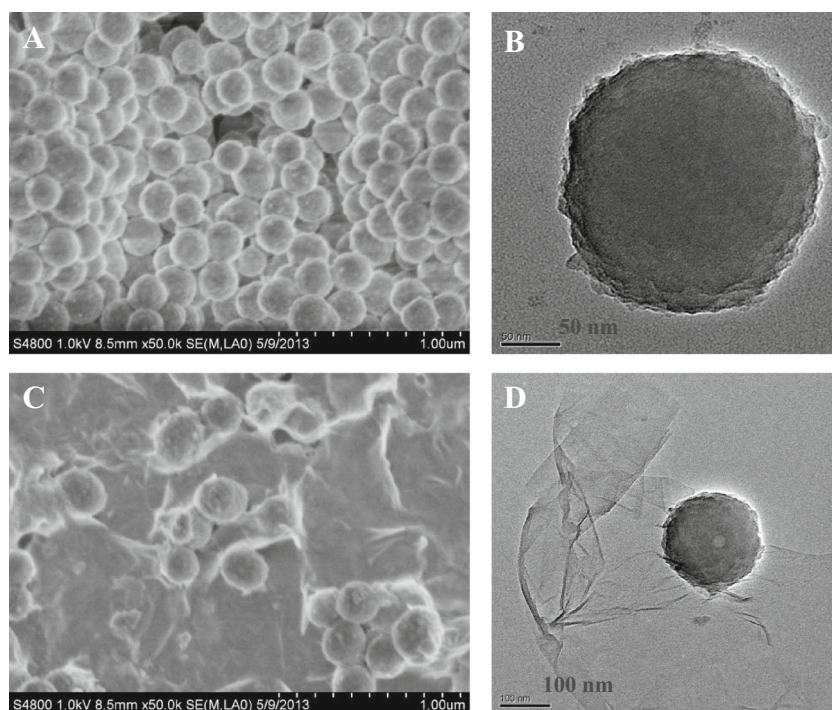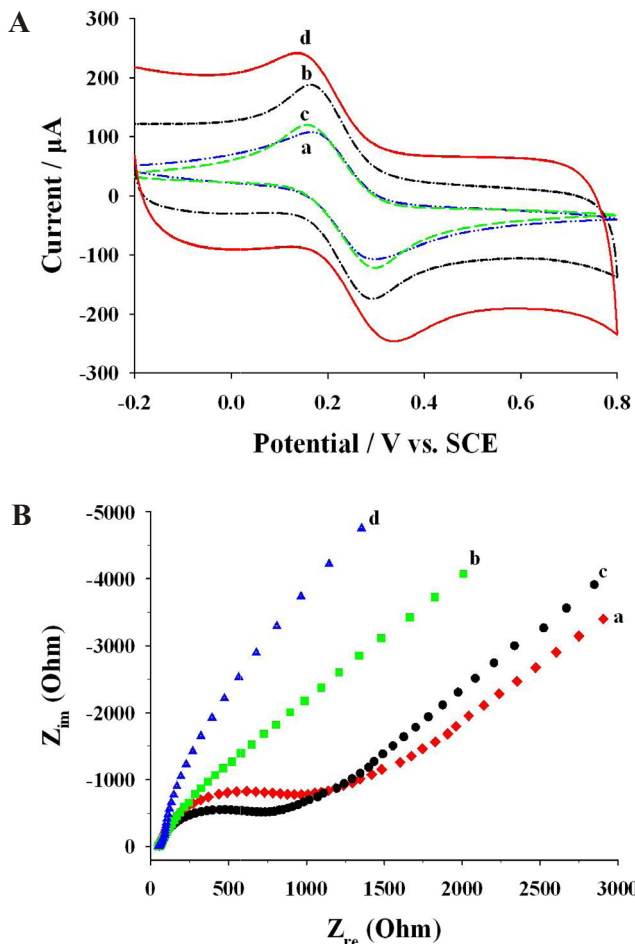

compared to all the above electrodes. It was ascribed to that Dpa-melanin CNSs–graphene significantly increased the effective surface area and the electrocatalytic activity towards the oxidation of guanine and adenine. Furthermore, the peak-to-peak separation of guanine and adenine was 0.32 V. Such a large peak separation could effectively decrease the mutual interfering interactions between guanine and adenine in simultaneous measurement of the two compounds and increase the determination selectivity.

To obtain the kinetic parameters of guanine and adenine redox at Dpa-melanin CNSs–graphene/GCE, the effect of scan rates on the peak currents was investigated by using cyclic voltammetry. As shown in Fig. 4, only oxidation peaks were observed, which indicated that the electrooxidation of guanine and adenine was a totally irreversible electrode process. It was found that the oxidation peak currents of guanine and adenine increased in a linear fashion as the scan rate increased from 50 to 600 mV s^{-1}. The relationship between the oxidation peak current and scan rate was established with linear regression equations as I_{pa} (μA)$=1.6748+0.0161$ v (mV s^{-1}) ($R=0.9951$) and I_{pa} (μA)$=4.0118+0.0668$ v (mV s^{-1}) ($R=0.9995$) for guanine and adenine, respectively. It suggested that the electrooxidation process of guanine and adenine on the Dpa-melanin CNSs–graphene/GCE was an adsorption-controlled process.

The adsorbed amount of guanine and adenine on the surface of Dpa-melanin CNSs–graphene/GCE could be further calculated by the following equation [34]: $I_p=nFQv/4RT=n^2F^2Av\Gamma_c/4RT$, where n is the number of electron transferred, F (C mol^{-1}) is the Faraday's constant, A (cm^2) is the area of the electrode, Γ_c (mol cm^{-2}) is the surface concentration of the guanine and adenine, Q (C) is the quantity of charge consumed during the electrooxidation reaction, and v (V s^{-1}) is the scan rate. Based on the relationship of I_p with v, the values of the electron transfer number (n) of the guanine and adenine were calculated as 2.14 and 2.28, respectively. The surface concentration of the guanine and adenine (Γ_c) were obtained with the results as 2.83×10^{-10} and 2.79×10^{-10} mol cm^{-2}, respectively. Furthermore, the charge transfer coefficient was calculated to be 0.51 for guanine and 0.54 for adenine, respectively. The results revealed that the electrochemical oxidation of guanine and adenine on the Dpa-melanin CNSs–graphene/GCE was a two-electron and two-proton process.

The effect of buffer pH on the electrooxidation of guanine and adenine was also investigated in the range of pH 3.0–6.0, as shown in Fig. 5. It was observed that peak current of guanine and adenine increased with increasing pH value until it reached 4.5, and then it decreased when the pH increased further. Both the maximum current responses of guanine and adenine were obtained at pH 4.5. Therefore, a pH value of 4.5 was selected for the subsequent analytical experiments. In addition, the oxidation peak potential of guanine and adenine shifted negatively with the increment of the solution pH. There was a good linear relationship between the peak potential and the solution pH with the linear regression equation as E_{pa} (V)$=1.194-0.055$ pH ($R=0.9961$) and E_{pa} (V)$=1.498-0.060$ pH ($R=0.9985$) for guanine and adenine, respectively. The slope value of 55 mV pH^{-1} and 60 mV pH^{-1} implied that the electron transfer was accompanied by an equal number of protons in the electrode reaction process [35].
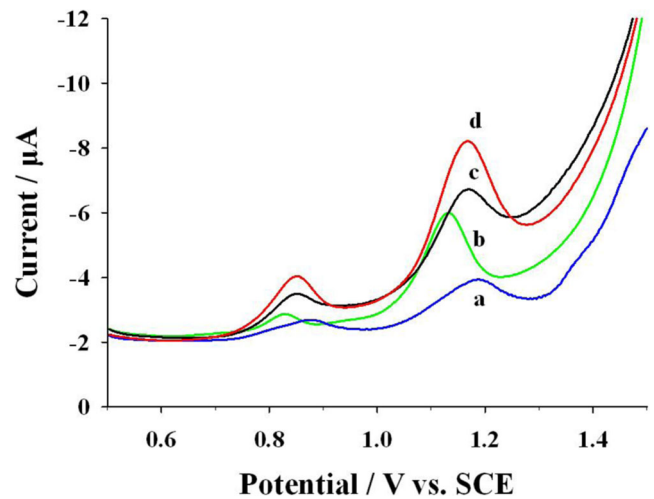
A

Fig. 3 Differential pulse voltammograms of 20 μM guanine and 20 μM adenine on bare GCE (*a*), graphene/GCE (*b*), Dpa-melanin CNSs/GCE (*c*), and Dpa-melanin CNSs–graphene/GCE (*d*) in 0.1 M HAc-NaAc buffer solution (pH 4.5)

linear segment increased from 1 to 20 μM with the regression equation of I_{pa} (μA)$=-0.9094-0.0536\ C$ (μM) ($R=0.9933$)

B

Fig. 2 **a** Cyclic voltammograms of bare GCE (*a*), graphene/GCE (*b*), Dpa-melanin CNSs/GCE (*c*), and Dpa-melanin CNSs–graphene/GCE (*d*) in a PBS (pH 7.0) solution containing 5 mM Fe(CN)$_6^{3-/4-}$ and 0.1 M KCl. **b** Nyquist plots of bare GCE (*a*), graphene/GCE (*b*), Dpa-melanin CNSs/GCE (*c*), and Dpa-melanin CNSs–graphene/GCE (*d*) in a PBS (pH 7.0) solution containing 5 mM Fe(CN)$_6^{3-/4-}$ and 0.1 M KCl. The frequency range was form 0.1 to 10^5 Hz with perturbation amplitude of 5 mV

The effect of accumulation time on the oxidation peak current of guanine and adenine was investigated in 0.1 M HAc-NaAc buffer solution (pH 4.5) under stirring. The peak current of guanine and adenine increased with accumulation time growing form 0 to 100 s, but after 100 s they kept almost unchanged, indicating the saturated adsorption of analytes. Thus, the optimal accumulation time of 100 s was employed in the further experiments.
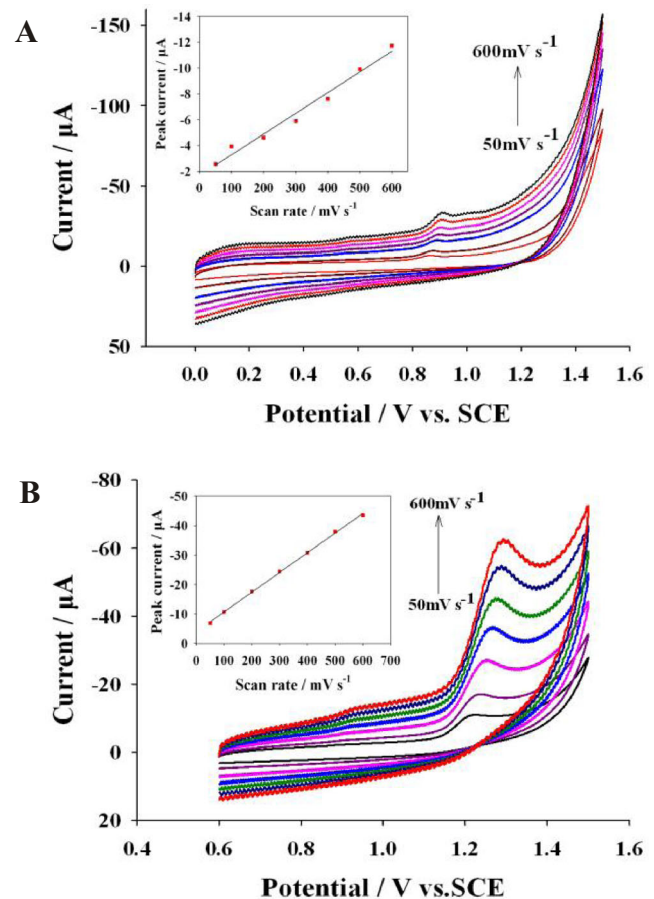
Individual determination of adenine and guanine

Under the optimal conditions, the electrochemical sensing performance of the Dpa-melanin CNSs–graphene/GCE towards guanine and adenine detection was investigated by DPV in 0.1 M HAc-NaAc buffer solution. As shown in Fig. 6, it could be seen that the peak current of guanine increased with the increasing concentration. The calibration curve for guanine exhibited two linear segments: the first
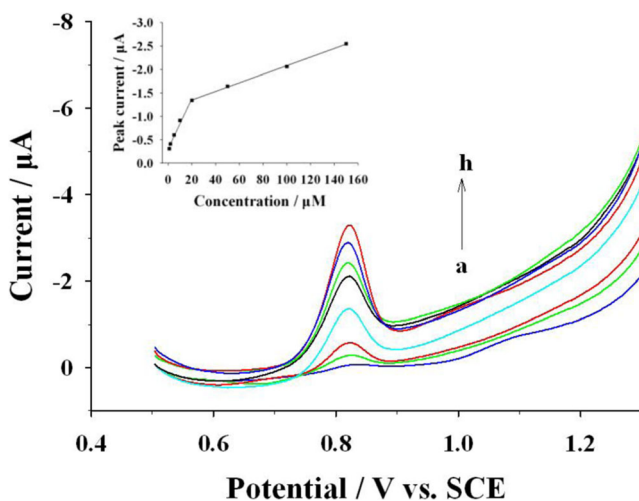

Fig. 4 Cyclic voltammograms of 20 μM guanine (**a**) and 20 μM adenine (**b**) on Dpa-melanin CNSs–graphene/GCE in 0.1 M HAc-NaAc buffer solution (pH 4.5) at different scan rates from 50 to 600 mV s^{-1}. The *inset* shows the plot of the oxidation peak current vs. scan rates
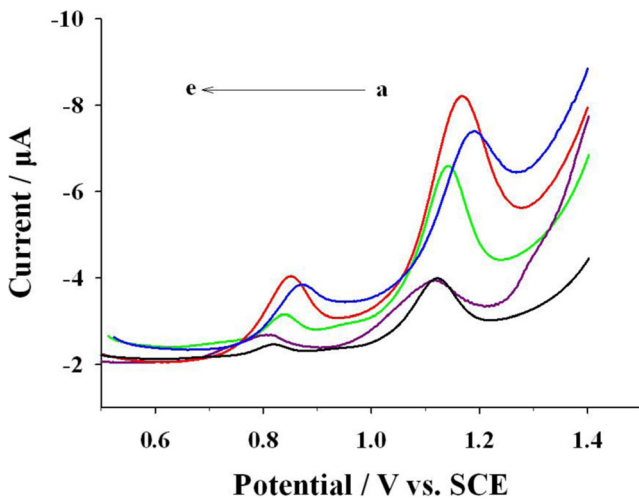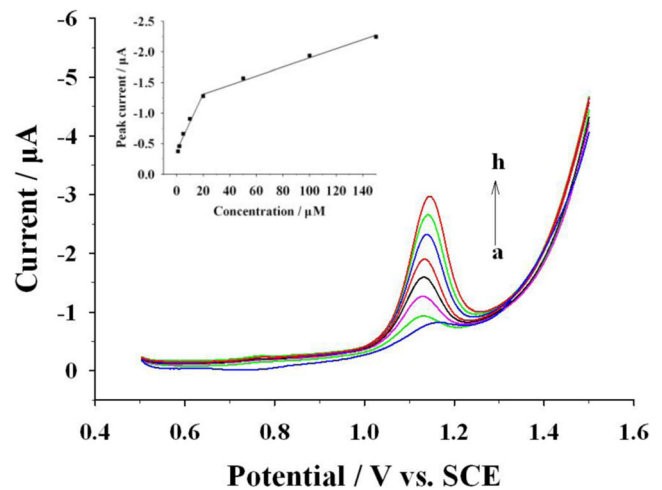
Fig. 5 Differential pulse voltammograms of 20 μM guanine and 20 μM adenine at Dpa-melanin CNSs–graphene/GCE in 0.1 M HAc-NaAc buffer solution (pH 4.5) with different values of pH (from *a* to *e*: 3.0, 4.5, 5.0, 5.5, 6.0)

Fig. 7 Differential pulse voltammograms of adenine (from *a* to *h*: 1, 2, 5, 10, 20, 50, 100 and 150 μM) at Dpa-melanin CNSs–graphene/GCE in 0.1 M HAc-NaAc buffer solution (pH 4.5). *Inset*: calibration plots of the oxidation peak current versus different concentration of adenine

and the second linear segment increased up to 150 μM with the regression equation of I_{pa} (μA)$=-1.1664-0.0091\,C$ (μM) ($R=0.9995$). As to adenine, the same appeared to be the case with the concentration of adenine in the range of 1 to 150 μM (shown in Fig. 7). The linear regression equations were I_{pa} (μA)$=-0.3817-0.0468\,C$ (μM) ($R=0.9927$) and I_{pa} (μA)$=-1.1608-0.0074\,C$ (μM) ($R=0.9972$) at the turn of 20 μM. It has also been observed in earlier reports that the calibration curve of guanine and adenine detection usually divided into two linear regions [4, 36]. The first linear region could be attributed an absorption process of guanine or adenine on the modified electrode surface, and the second linear region might be ascribed to a diffusion process on the monolayer-covered surface [36].
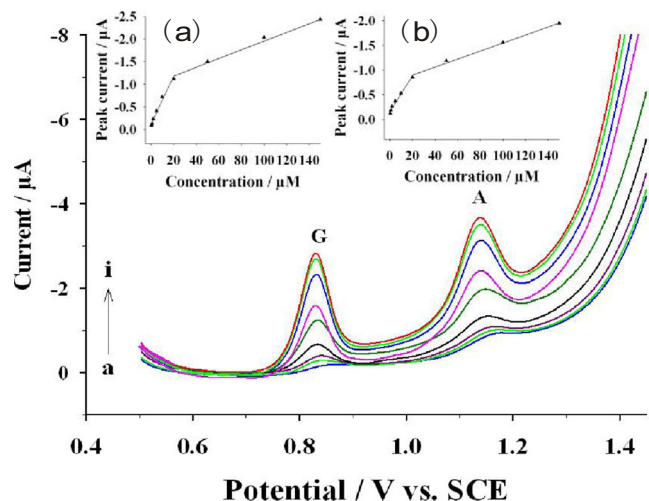
Simultaneous determination of adenine and guanine

In order to evaluate the feasibility of the Dpa-melanin CNSs–graphene/GCE for guanine and adenine determination, the fabricated electrode was applied to detect guanine and adenine by simultaneously changing their concentrations. Figure 8 showed the DPV for simultaneous determination of guanine and adenine. It could be seen that two well-defined oxidation peaks appeared at about +0.848 and +1.168 V, corresponding to the oxidation of guanine and adenine, respectively. The peak-to-peak separation was 320 mV, which suggested that the individual or simultaneous determination of these analytes

Fig. 6 Differential pulse voltammograms of guanine (from *a* to *h*: 1, 2, 5, 10, 20, 50, 100, and 150 μM) at Dpa-melanin CNSs–graphene/GCE in 0.1 M HAc-NaAc buffer solution (pH 4.5). *Inset*: calibration plots of the oxidation peak current versus different concentration of guanine

Fig. 8 Differential pulse voltammograms for the simultaneous determination of guanine and adenine in 0.1 M HAc-NaAc buffer solution at Dpa-melanin CNSs–graphene/GCE with guanine and adenine concentration ranging from 0.5 to 150 μM (from *a* to *i*: 0.5, 1, 2, 5, 10, 20, 50, 100 and 150 μM). *Inset*: (*a*) calibration plots of the peak current versus different concentration of guanine, and (*b*) calibration plots of the peak current versus different concentration of adenine

was possible without any interference and suitable for detecting these species in less or equal concentrations, respectively. The peak currents also exhibited two linear segments with the guanine and adenine concentration in the range of 0.5 to 150 μM. The regression equations for guanine were I_{pa} (μA)=−0.1147−0.0531 C (μM) (R=0.9932) and I_{pa} (μA)=−0.9917−0.0096 C (μM) (R=0.9952) at the turn of 20 μM. The regression equations for adenine were I_{pa} (μA)=−0.1612−0.0355 C (μM) (R=0.9928) and I_{pa} (μA)=−0.7341−0.0081 C (μM) (R=0.9965) at the turn of 20 μM. The linear detection range for both guanine and adenine was 0.5 to 150 μM. The detection limit for guanine and adenine was calculated to be 0.05 and 0.03 μM (S/N=3), which was significantly lower and comparable with many previously reported electrochemical sensors (as shown in Table 1). Thus, this proposed method for simultaneously sensitive determination of guanine and adenine was realized by using Dpa-melanin CNSs–graphene/GCE.

Reproducibility, stability, and interference

The reproducibility of the modified electrode was estimated by determining 20 μM guanine and 20 μM adenine with five modified electrodes which were made at the same electrode. The relative standard deviation (RSD) of 3.6 % indicated good reproducibility of the fabrication protocol.

The stability of the proposed sensor was evaluated by ten successive scans in the mixed solution containing 20 μM guanine and 20 μM adenine. The RSD values were found to be 2.86 and 2.92 % for guanine and adenine, respectively, indicating excellent stability of the modified electrode. The long-term stability of the electrochemical sensor was investigated by examining its current response during storage in a refrigerator at 4 °C for 2 weeks. The results showed that the analytical performance had no obvious decline (RSD≤ 5 %), demonstrating that the modified electrode had good stability.

Possible interferences for the detection of DNA bases were investigated by carrying out the determination of 20 μM guanine and 20 μM adenine in 0.1 M HAc-NaAc buffer solution (pH 4.5) in the presence of different concentrations of the interferences. The results indicated that Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Al^{3+}, Cl^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, glucose, cysteine, ascorbic acid, uric acid, and dopamine had no significant interference (signal change<5 %). Therefore, the sensor could be used for the assessment of guanine and adenine contents in real-life samples.

Real-life sample analysis

In order to evaluate the validity of the proposed method, the fabricated electrode was applied to detect the guanine and adenine concentration of thermally denatured DNA. The determination of guanine and adenine concentrations was performed by standard addition methods according to previous reports [20]. From the differences between the peak currents of guanine and adenine, the concentration of guanine and adenine in DNA could be obtained by the calibration curves. The contents of adenine and guanine in thermally denatured DNA were calculated as 22.1 and 27.5 % (in the molar ratio, mol%), respectively. The value (G + C) / (A + T) was calculated as 0.80 for thermally denatured DNA sample, which was close to the standard value of 0.77 [40].

Table 1 Comparison of different modified electrodes for the detection of guanine and adenine

Modified electrodes	Analytes	Dynamic range (μM)	LOD (μM)	Ref.
Multi-walled carbon nanotubes/ poly(new fuchsin)/GCE	G	100–8,500	97.92	[13]
	A	100–8,500	95.76	
Multi-walled carbon nanotubes/Au/ hydroxypropyl-β-cyclodextrin /GCE	G	1,170–2,940	90	[14]
	A	70–570	70	
TiO$_2$-graphene nanocomposite/GCE	G	0.5–200	0.15	[15]
	A	0.5–200	0,10	
Graphene-COOH/GCE	G	0.5–200	0.05	[18]
	A	0.5–200	0.025	
CeO$_2$ nanoparticles/multi-walled carbon nanotubes/GCE	G	5–50	0.01	[37]
	A	5–35	0.02	
Graphene/ionic liquid/chitosan/GCE	G	2.5–150	0,75	[38]
	A	1.5–350	0,45	
Graphitized mesoporous carbon/GCE	G	25–200	0.76	[39]
	A	25–150	0.63	
Dpa-melanin CNSs–graphene/GCE	G	0.5–150	0.05	This work
	A	0.5–150	0.03	

Conclusions

In conclusion, we have demonstrated a novel electrochemical sensor for the sensitive determination of guanine and adenine based on Dpa-melanin CNSs–graphene-modified GCE. The as-prepared Dpa-melanin CNSs–graphene-modified glassy carbon electrode exhibited remarkable electrocatalytic activity towards adenine and guanine oxidation. The results were attributed to the good adsorptivity and the good conductivity of Dpa-melanin CNSs–graphene composites. The fabricated sensor exhibited acceptable reproducibility, high sensitivity, long-term stability, and low detection limit. In addition, the proposed method was further used for the detection of guanine and adenine in thermally denatured DNA with satisfactory results. Therefore, the Dpa-melanin CNSs–graphene composites could provide a promising platform for the development of biosensor and electrochemical sensor.

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