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Electrochemical Behaviours of Guanine and Adenine and their Simultaneous Determination using a Three-Dimensional Porous Poly(dopamine)/Reduced Graphene Oxide-Modified Electrode

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In this work, a three-dimensional porous poly(dopamine)/graphene-modified electrode was prepared. The modified electrode was characterized by scanning electron microscopy and electrochemical methods. The electrochemical behaviours of guanine and adenine with the modified electrode were investigated by cyclic voltammetry. The experimental results showed that the modified electrode had good electrocatalytic ability for the oxidation of guanine and adenine. Differential pulse voltammetry was used to simultaneously detect guanine and adenine in 0.1 mol/L PBS (pH 7.0). The oxidation peak currents of guanine and adenine were linear with their concentrations in a range of 1.0-58 μmol/L. The linear regression equation for guanine was i_p = -1.9501- 0.0991*c* (R^2 = -0.9973) with a detection limit of 0.34 μmoL (*S/N = 3*), and the linear equation for adenine was i_p = -1.2005- 0.1049*c* (R^2 = -0.9943) with a detection limit of 0.31 μmoL (S/N = 3). In addition, this modified electrode exhibited favourable stability, reproducibility and good anti-interference ability.

Keywords: Graphene, Guanine, Adenine, Poly(dopamine), Modified electrode

1. INTRODUCTION

Guanine and adenine are important components of DNA [1] and play a fundamental role in the process of life. In addition, the expansion and modification of bases can also cause a series of lesions and cancerous changes. Therefore, it is of great significance to detect guanine and adenine in DNA or RNA [2]. At present, the most commonly used methods of base analysis are fluorescence spectrophotometry [3], high-performance liquid chromatography [4], electrophoretic separation [5], and electrochemical methods [6]. Among these methods, electrochemical methods are widely used because

of their simple operation, high sensitivity, fast detection, low cost, and easy implementation of automation [7-10].

Graphene is a new type of two-dimensional nanomaterial that has a single-layer sheet structure composed of carbon atoms and a thickness of only 0.35 nm [11,12]. This special structure demonstrates many unique properties. These properties include excellent electrical and mechanical properties and a large specific surface area. [13]. Because graphene has unique structural characteristics that influence its electrical and physicochemical properties, it has unique advantages in electrochemical analysis and can be used to prepare high-performance biosensors [11-18]. Conductive polymers are polymer films with good stability, uniformity and electrocatalytic performance, which can significantly improve the performance of modified electrodes [19].

In this paper, a poly(dopamine)/reduced graphene oxide-modified electrode was prepared. The modified electrode demonstrated strong electrocatalytic oxidation of guanine and adenine and showed good electrochemical performance when detecting guanine and adenine. Based on this, a new method for detecting guanine and adenine has been developed, which has good prospects in guanine and adenine detection applications.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

Guanine, adenine, reduced graphene oxide (RGO), and other reagents were of analytical grade and were not further purified before use. A mixture of NaH_2PO_4 and Na_2HPO_4 was used to prepare a phosphate-buffered saline (PBS) solution with a concentration of 0.1 mol/L. The pH was adjusted with H_3PO_4 and NaOH, N_2 was used to remove oxygen, and distilled water was used in all experiments.

A CHI660A electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd.) was used to conduct electrochemical measurements with a three-electrode system; a bare glass carbon electrode or a modified electrode was the working electrode, a saturated calomel electrode (SCE) was the reference electrode, and a platinum wire electrode was the auxiliary electrode.

2.2 Preparation of the poly(dopamine)/RGO-modified electrode

Before modification, the bare GCE was polished in a suspension of Al_2O_3 powder, ultrasonically cleaned in anhydrous ethanol and distilled water for 1 min each, and air-dried at room temperature. RGO (0.05 g) was added to 10 mL of water and ultrasonically dispersed for 30 min. A 10 μL dispersion was added to the surface of the treated electrode and dried at room temperature. The RGO-modified electrode was immersed in 0.1 mol/L PBS (pH 7.0) containing 0.1 mmol/L dopamine (DA) and scanned 50 times at a scan rate of 0.05 V/s in a range of -0.2- +0.6 V by cyclic voltammetry (CV). Afterward, the electrode was washed three times with water, and the obtained electrode was PDA/RGO/GCE.

3. RESULT AND DISCUSSION

3.1 Characterization of PDA/RGO/GCE

Fig. 1 shows the CVs of bare GCE (a), RGO/GCE (b), and PDA/RGO/GCE (c) in 0.1 mol/L PBS (pH 7.0). It could be seen from the figure that when PDA was modified on the electrode surface, a pair of obvious DA redox peaks appeared. The results indicated that DA had been successfully polymerized onto the electrode surface.

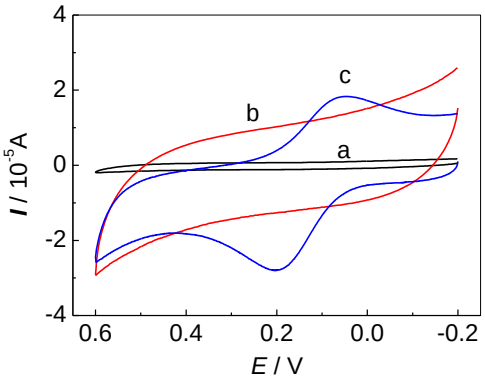

Figure 1. CV curves of the different electrodes in 0.1 M PBS (pH 7.0) with a scan rate of 50 mV/s: a- bare GCE; b- RGO/GCE; and c- PDA/RGO/GCE.

Fig. 2 shows the SEM images of the electrodes modified with different materials: RGO/GCE (a) and PDA/RGO/GCE (b). It can be seen from the figure that after DA was polymerized on the surface of RGO, a three-dimensional porous electrode interface was formed. The surface area of the electrode could be effectively increased, and the performance of the electrode might be improved.

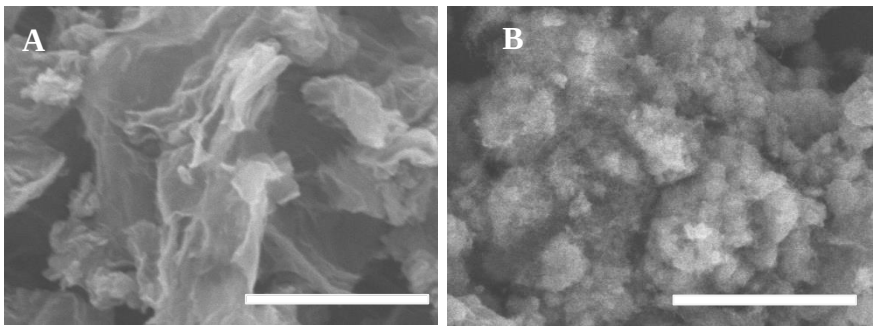

Figure 2. SEM images of the different electrodes: A- RGO/GCE and B- PDA/RGO/GCE. Scale bar: 20 μm

3.2 CV behaviours of guanine and adenine with different electrodes

Fig. 3 shows the CV curves of guanine and adenine on a bare electrode (a), RGO/GCE (b), and

PDA/RGO/GCE (c) in 0.1 mol/L PBS (pH 7.0). Two oxidation peaks appeared in CV curve which were attributed to the oxidation of guanine and adenine at the electrode surface [20], and the oxidation peaks of guanine and adenine on the bare GCE and RGO/GCE were weak. When DA was electropolymerized onto the electrode surface, the oxidation peak currents of guanine and adenine significantly increased, and the peak potentials shifted negatively. This indicated that the modified electrode had an obvious electrocatalytic effect on the two substances.

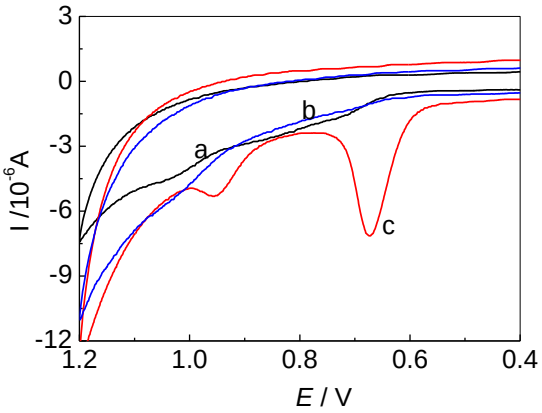

Figure 3. CV curves of guanine and adenine with the different electrodes in 0.1 M PBS (pH 7.0) and a scan rate of 100 mV/s: a- bare GCE; b- RGO/GCE; and c- PDA/RGO/GCE.

3.3 Effect of pH and scan rate

The effect of pH on the oxidation peak current of the PDA/RGO/GCE was studied, and the results are shown in Fig. 4. In the pH range of 4.0-7.0, the peak current gradually increased with increasing pH; when the pH was greater than 7.0, the peak current decreased. The modified electrode had good electrical activity when the pH was 7.0.

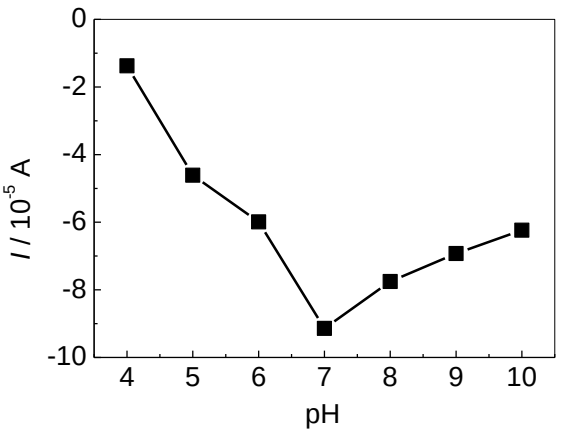

Figure 4. Relationship of the oxidation peak current of PDA/RGO/GCE at various pH values.

The effect of pH on the peak potentials of guanine and adenine was studied. The results are

shown in Fig. 5A and B. When the pH was 7.0, the peak current of guanine was at its maximum. When the pH was 6.0, the peak current of adenine was at its maximum. In addition, in a range of 5.0-9.0, as the pH increased, the peak potential shifted negatively. The peak potentials had a good linear relationship with pH (Fig. 5C). The linear equations were E = 1.4139 - 0.0663pH (R^2=-0.9974) and E = 1.0937 - 0.061pH (R^2=-0.9961). The above results showed that guanine and adenine had protons to participate in the reaction on the electrode surface, which was consistent with previous reports [21,22]. Because the pH of the human body is close to 7.0, PBS with a pH of 7.0 was selected for the solution in the next tests.

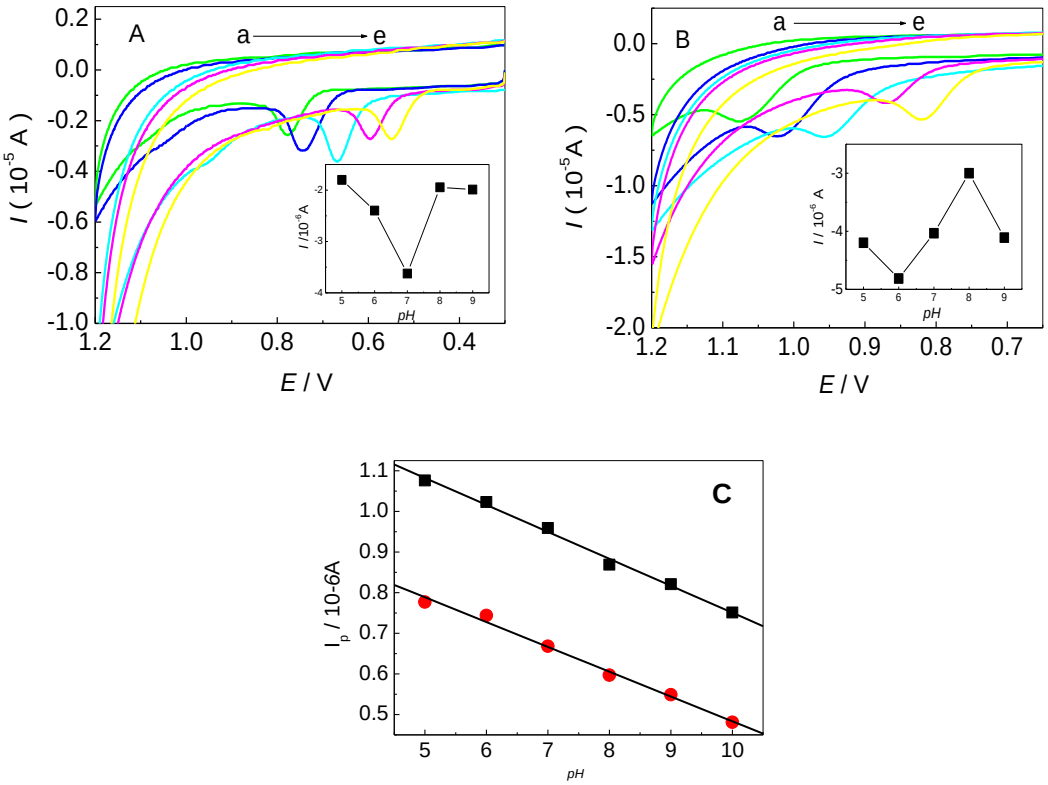

Figure 5. CV curves of adenine (A) and guanine (B) with PDA/RGO/GCE in 0.1 M PBS with a scan rate of 100 mV/s at different pH values: a-5.0, b-6.0, c-7.0, d-8.0, and e-9.0. The inset shows the oxidation peak current of adenine and guanine vs. pH. The oxidation peak current of adenine and guanine vs. pH (C).

Fig. 6 shows the effect of scan rate on the oxidation peak currents of guanine and adenine. It can be seen from the figure that as the scan rate increased, the oxidation peak currents of guanine and adenine gradually increased. Furthermore, the oxidation peak currents of guanine and adenine became linear with the square root of the scan rate. The results showed that the reaction process of the two substances on the electrode surface was controlled by adsorption.

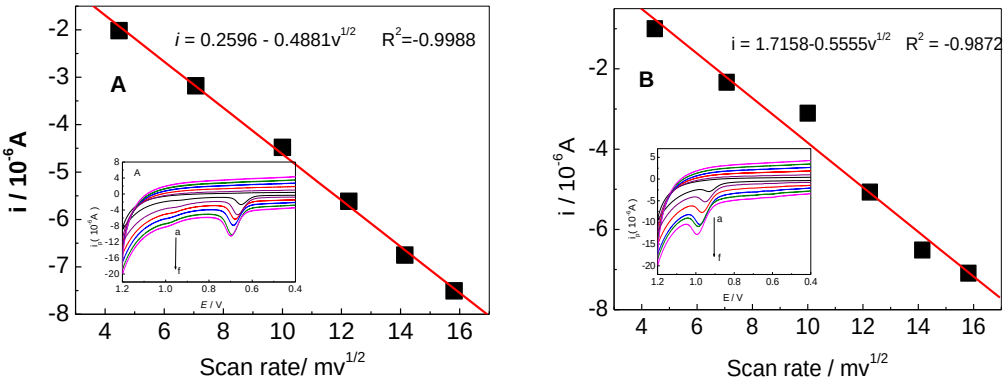

Figure 6. Effect of the scan rate on the oxidation peak current of adenine and guanine.

3.4 Separate determination of guanine and adenine

Fig. 7 shows the DPV curves of guanine (A) and adenine (B) with the modified electrodes at different concentrations. The inset shows the relationship between the oxidation peak currents of guanine and adenine and their concentrations. It can be seen from the figure that as the concentrations of the two substances increased, their oxidation peak currents gradually increased. In a range of 1 - 100 mol/L, the oxidation peak currents and their concentrations had a good linear relationship. The linear equations were i = -1.4106-0.0844c (R^2 = -0.9946) and i = -1.4192-0.09c (R^2 = -0.994) for guanine and adenine, respectively.

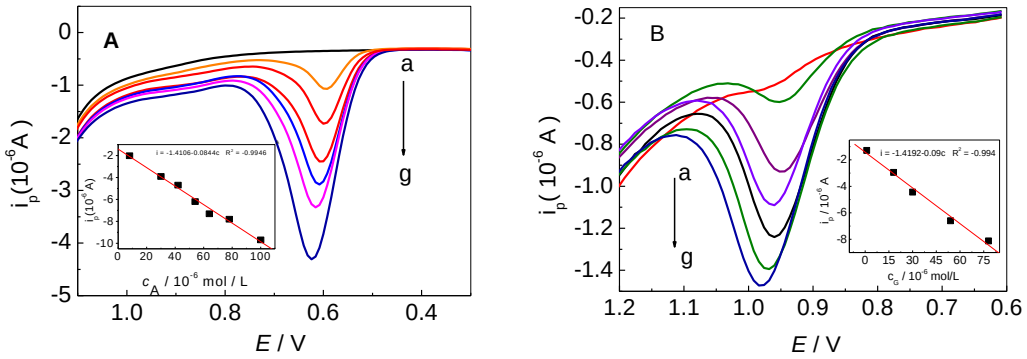

Figure 7. DPV curves of the different concentrations of guanine (A) and adenine (B) with PDA/RGO/GCE in 0.1 mol/L PBS (pH 7.0). The inset shows the oxidation peak current of guanine and adenine vs. concentration.

3.5 Simultaneous determination of guanine and adenine

DPV was used to detect a mixture of guanine and adenine. Under optimal conditions, the oxidation peak currents of guanine and adenine were measured simultaneously. The oxidation peak

currents of guanine and adenine increased with increasing concentrations. In a concentration range of 1.0-58 μmoL/L, guanine and adenine had a linear relationship with their oxidation peak currents. The linear equation for guanine was i_p = -1.9501- 0.0991*c* (R^2 = -0.9973) with a detection limit of 0.34 μmoL (*S/N = 3*), and the linear equation for adenine was i_p = -1.2005- 0.1049*c* (R^2 = -0.9943) with a detection limit of 0.31 μmoL (S/N = 3) (Fig. 8). The results showed that the electrode could detect guanine and adenine separately and simultaneously. The linear range and detection limit in this work were compared with reported electrochemical sensor [23-25], indicating the proposed method has good performances, and potential in assay guanine and adenine in biological and pharmaceutical samples.

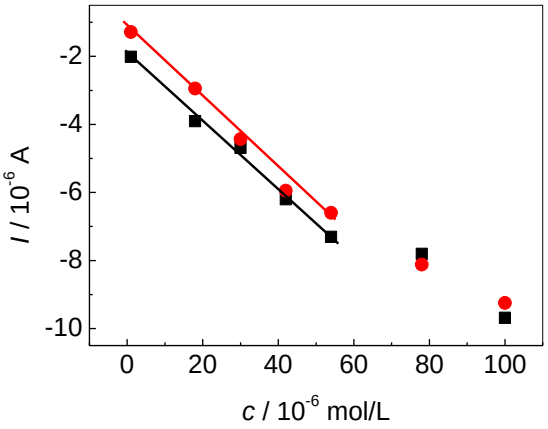

Figure 8. Oxidation peak currents of guanine and adenine vs. concentrations.

3.6 Stability and reproducibility

The modified electrode was continuously scanned 40 times in 0.1 mol/L PBS (pH 7.0) and then placed in 0.1 mol/L pH 7.0 PBS at room temperature for 3 d. It was used for the detection of guanine at the same concentration, and no obvious change in its oxidation peak current was observed. The above result indicated that the modified electrode had good stability. The same modified electrode was used for the determination of guanine at the same concentration. The determination was performed 6 times in parallel, and the relative standard deviation (R.S.D.) was 4.2%. Three modified electrodes were prepared under the same conditions for the determination of guanine at the same concentration. The relative standard deviation (R.S.D.) was 4.5%, indicating that the final modified electrode had good reproducibility.

3.7 Interference experiment and sample analysis

The effects of metal ions, thymine, and uracil on guanine and adenine detection signals were investigated. K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Al^{3+} (100 times the concentration of guanine and adenine) and concentrations of thymine and uracil (20 times the concentration of guanine and adenine) had no significant effect on the peak currents of guanine and adenine, indicating that these substances did not interfere with the detection of guanine and adenine. A calf thymus DNA sample was analysed and treated

with hydrochloric acid according to [7]. A standard addition method was used for sample determination. The measurement results showed that (G + C)/(A + T) = 0.73 (G, C, A, and T refer to guanine, cytosine, adenine, and thymine, respectively) with an R.S.D. = 4.1% (n = 3); the above value was close to the value of 0.77 from previous reports [7, 26]. This result showed that this method could be used for the analysis of actual samples. And it maybe have a potential application in studying DNA-binding anticancer agents and DNA-binding agents to regulate DNA structure and function[27].

4. CONCLUSION

Herein, a PDA/RGO-modified electrode was prepared. The properties of the modified electrode were studied. It has obvious electrocatalytic effects on guanine and adenine, and thus, can be used for the detection of guanine and adenine. In addition, the proposed electrode has good stability, reproducibility, and anti-interference ability, which supports its potential for use in real sample analyses.

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