Simultaneous Determination of Dopamine, Uric Acid and Guanine at Polyadenine Film Modified Electrode

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A polyadenine film (PAE) modified glassy carbon electrode (GCE) was employed for the simultaneous determination of dopamine (DA), uric acid (UA) and guanine (GA). Experimental results showed that this modified electrode had good electrocatalytic properties for the oxidation of DA, UA and GA. Under optimal conditions, DA, UA and GA reflected their electrochemical response into three separated and well-defined oxidation peaks, whose currents increased 47-, 12-, and 7-fold, respectively, compared with those at bare electrode. Moreover, their oxidation peak currents were linear proportional to their concentrations within the ranges of 2.5 – 75, 10 – 750, and 7.5 – 75 μmol L⁻¹, respectively, and the limits of detection (LOD) (S/N = 3) were 0.075, 0.35, and 0.025 μmol L⁻¹, respectively. Compared with a variety of modified electrodes, this designed sensor had a wider linear range and lower LOD. Furthermore, the sensor exhibited good stability and reproducibility.

Keywords Adenine, determination, dopamine, guanine, uric acid

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voltammetric peaks corresponding to their oxidation reactions, and the oxidation peak currents increased 47-, 12-, and 7-fold compared to those at the bare electrode, respectively. Compared to reported analytical methods, this paper provided a more simple method for detecting these three substances, and achieved the purpose for their simultaneous determination. Furthermore, adenine as the modifier material, was advantageous to research the interaction between DNA and DA, UA and GA.

Experimental

Reagents and chemicals

DA, UA, GA, AE were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Glucose, sucrose, epinephrine and ascorbic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade. Triple distilled water was used in all experiments. We also used 0.1 mol L⁻¹ phosphate buffer solution (PBS) with different pH adjusted with 0.1 mol L⁻¹ Na₂HPO₄, 0.1 mol L⁻¹ NaH₂PO₄ and 0.1 mol L⁻¹ H₃PO₄.

Apparatus

Electrochemical experiments were performed on a CHI 660D electrochemical workstation (Shanghai Chenhua CHI Co., Ltd., China), which had a three electrode system consisting of a PAE/GCE working electrode, a platinum counter electrode and a saturated calomel reference electrode (SCE). Scanning electron micrograph (SEM) measurements were carried out on a scanning electron microscope (JSM-6700F, 15.0 kV, Japan). The square wave voltammetry (SWV) measurements and linear sweep voltammetry (LSV) measurements were conducted in 0.1 mol L⁻¹ PBS (pH 5.5). Instrumental parameters of SWV were: step potential of 4 mV, modulation amplitude of 25 mV and frequency of 15 Hz. Instrumental parameters of LSV were sample interval of 1 mV and quiet time of 2 s.

Fabrication of the PAE/GCE

Before modification, GCE was polished in 0.05 μm alumina slurry on chamois leather, ultrasonically washed for 30 min in anhydrous ethanol and triple distilled water successively in order to remove impurity particles on the electrode surface, then rinsed several times with anhydrous ethanol and triple distilled water, dried in the air and the clean GCE was obtained. Finally, cyclic voltammetry (CV) was conducted in a 10-mL mixture containing 5.0 mmol L⁻¹ AE and 0.1 mol L⁻¹ KNO₃ by using clean GCE as the working electrode with potential range of 0 - 1.2 V for 20 cycles at scan rate of 100 mV s⁻¹; after that, the PAE/GCE was prepared.

Results and Discussion

The surface morphology of bare GCE and PAE/GCE

Scanning electron microscope was employed to characterize the surface morphology of the bare GCE and PAE/GCE. As can be seen from Fig. 1 inset, no particles were observed on the bare GCE, indicating that the electrode was clean before modification. After the clean electrode was immersed in polymerization solution containing 5.0 mmol L⁻¹ AE and 0.1 mol L⁻¹ KNO₃, two small hump peaks of UA (0.40 V) and GA (0.79 V) were obtained at the bare GCE (curve a). Furthermore, when using PAE/GCE as a working electrode, three remarkable oxidation peaks of DA (0.26 V), UA (0.40 V) and GA (0.79 V) appeared (curve b), and the oxidation peak currents of DA, UA, and GA were enhanced.
47-, 12-, and 7-fold, respectively, compared with those at the bare electrode, which was attributed to the enlarged active surface by PAE film. A larger surface area could adsorb more analyte molecules, and more active sites were advantageous to the electronic transmission, so PAE could enhance the current response of the three analytes. Perhaps PAE cannot change the kinetics of oxidation reactions, so a noticeable shift of peak potential was not observed.

**Effect of scan rate**

LSV was employed to investigate the effect of scan rate on electrochemical oxidation of DA, UA and GA. These experiments were conducted at PAE/GCE in 0.1 mol L$^{-1}$ PBS of pH 5.5 containing 0.1 mmol L$^{-1}$ DA, 0.1 mmol L$^{-1}$ UA or 0.1 mmol L$^{-1}$ GA, as shown in Figs. 3(A), 3(B), and 3(C), respectively. The oxidation peak currents of DA, UA and GA($I_{pa}$) linearly increased with the scan rates ($v$) in the range of 40 – 220, 20 – 300, and 40 – 320 mV s$^{-1}$, respectively, and the relationships between them can be expressed as three equations: $I_{pa} = 0.0158v + 2.348$ ($r = 0.9937$) for DA, $I_{pa} = 0.0115v + 1.684$ ($r = 0.9936$) for UA and $I_{pa} = 0.0095v + 3.415$ ($r = 0.9961$) for GA, which indicated that the oxidation reactions of DA, UA and GA at the PAE/GCE were adsorption controlled processes.

**Effect of pH**

PBS (0.1 mol L$^{-1}$) with different pH (3.0 – 6.0) were used to investigate the effect of pH on the electrochemical oxidation of 10 μmol L$^{-1}$ DA, 100 μmol L$^{-1}$ UA and 10 μmol L$^{-1}$ GA at PAE/GCE. As can be seen from Fig. 4, the oxidation peak currents of DA and UA increased gradually with pH increasing from 3.0 to 5.5, and reached a maximum value at pH 5.5, then fell with the pH further increasing. However, as for GA, the current response always increased with pH changing from 3.0 to 6.0. Considering that the current responses of DA and UA were both weaker than GA, 5.5 was chosen as the optimum pH for their simultaneous determination.

Furthermore, the oxidation peak potentials of DA, UA and GA shifted negatively with an increase in pH, which indicated that protons took part in their electrode reaction processes. The relationships between oxidation potentials ($E_{pa}$) and pHs (Figs. s1(A), (B), and (C), Supporting Information) correspond with the following equations: $E_{pa} = -0.0503pH + 0.5353$ ($r = 0.9991$) for DA, $E_{pa} = -0.0620pH + 0.7370$ ($r = 0.9972$) for GA, and $E_{pa} = -0.0503pH + 0.5353$ ($r = 0.9991$) for UA.
These results demonstrated that proton transfer number was equal to electron transfer number in three electrode reactions. These conclusions were consistent with the electrochemical reaction mechanisms of DA, UA and GA as shown in Scheme s1 (Supporting Information).27–29

Calibration curve of DA, UA and GA

The electrochemical sensing performance of PAE/GCE for simultaneous determination of DA, UA and GA was researched by SWV in 0.1 mol L⁻¹ PBS (pH 5.5) (Fig. 5). When we fixed UA at 250 μmol L⁻¹ and changed DA from 2.5 μmol L⁻¹ to 75 μmol L⁻¹, the oxidation peak currents of DA were linearly proportional to its concentrations (shown in Fig. 5(A)), and the calibration curve equation was as follows: \( I_{pa} (\mu A) = 0.1282C_{DA} (\mu mol L^{-1}) + 1.2784 (r = 0.9916) \), with LOD of 0.075 μmol L⁻¹ (S/N = 3); the LOD was calculated by using three times the signal (S)-to-noise (N) ratio.

Similarly, when we fixed DA at 25 μmol L⁻¹ and changed UA from 10 μmol L⁻¹ to 750 μmol L⁻¹, the oxidation peak currents of UA and its concentrations accorded with the following calibration curve equation (shown in Fig. 5(B)): \( I_{pa} (\mu A) = 0.0423C_{UA} (\mu mol L^{-1}) + 5.5276 (r = 0.9905) \), and the LOD was 0.35 μmol L⁻¹ (S/N = 3).

As for GA, we individually changed its concentration ranging from 7.5 to 75 μmol L⁻¹, and found that the relationship between its oxidation peak currents and its concentrations could be expressed as a linear regression equation (shown in Fig. 5(C)): \( I_{pa} (\mu A) = 0.1260C_{GA} (\mu mol L^{-1}) + 4.2407 (r = 0.9945) \), with LOD of 0.025 μmol L⁻¹ (S/N = 3).

Figure 6 exhibited the electrochemical behavior of DA, UA and GA with simultaneously changing concentration at PAE/GCE under the optimal conditions. As can be seen, DA, UA and GA had detached oxidation peak potentials, and the oxidation peak currents increased with their concentrations. Therefore, PAE/GCE can be used to simultaneously detect DA, UA and GA in a mixture, and do not interfere with each other.

The remarkable electrochemical sensing performance of PAE/GCE can be attributed to a large active surface and good adsorption of the PAE film, which provided a favorable microenvironment for oxidation reactions of DA, UA and GA.30

Determinations of DA, UA and GA at different modified electrodes were compared in Table 1.23–25,31–41 As can be seen from the comparison results that PAE/GCE had relative wider linear range and lower LOD, which further proved that
Interference of coexistences

The selectivity of PAE/GCE was analyzed by the determination of 50 μmol L⁻¹ DA, 100 μmol L⁻¹ UA and 50 μmol L⁻¹ GA in 0.1 mol L⁻¹ PBS (pH 5.5) simultaneously containing important biological substances with different concentrations. An approximately ±5% relative error was defined as the tolerance limit of the foreign substances. As can be seen from Table 2, K⁺, Na⁺, Mg²⁺, Ca²⁺, glucose and sucrose had no significant interference. However, Cu²⁺ and Fe²⁺ had a small effect for the determination of DA, UA and GA. Moreover, ascorbic acid and epinephrine had a large impa

Stability and reproducibility of PAE/GCE

The stability of PAE/GCE was evaluated by examining the current responses of DA, UA and GA. After storage in a refrigerator at 4°C for two weeks, the oxidation peak currents of DA, UA and GA decreased to 92.7, 90.5, and 92.9% of their original values, respectively, which indicated that this sensor had good stability.

Successful determinations of DA, UA and GA at the same modified electrode were carried out to characterize its reproducibility. After 10 repetitive measurements, the relative standard deviations (RSDs) (n = 7) were calculated as 3.5, 1.8, and 2.0% for DA, UA and GA, respectively. Moreover, three parallel modified electrodes were employed to detect the same solution, and the RSDs (n = 7) were 3.6, 4.2, and 2.8% for DA, UA and GA, respectively. These results suggested good reproducibility of this electrochemical sensor for the response of three compounds.

Recovery test

In order to investigate the practical application of PAE/GCE, we carried out the determination of DA, UA and GA in human serum samples. The human blood samples were collected from a hospital laboratory, then, centrifuged for 30 min to get the upper serum. The obtained serum samples were diluted 10 times with PBS (0.1 mol L⁻¹, pH 5.5), then spiked with standard concentrations of DA (5, 10, and 15 μmol L⁻¹), UA (10, 15, and 20 μmol L⁻¹) and GA (10, 15, and 20 μmol L⁻¹) for confirmation, respectively. The standard addition method can be used to test the accuracy of determination and the results are shown in Table 3. The recovery was ranging from 99.20 – 104.20, 99.85 – 103.10% for DA, UA and GA, respectively, which showed this modified electrode could be used for determining DA, UA and GA in human serum samples.
successfully used for the determination of DA, UA and GA in human serum, and further proved this modified electrode had good practical application.

Conclusions

A novel biosensor for simultaneous determination of DA, UA and GA was easily prepared by electro-polymerizing AE on GCE with CV. Under optimal conditions, this sensor exhibited a wider linear range and lower detection limit compared with reported modified electrodes. An interference test was carried out, and the results demonstrated that the modified electrode had good anti fouling ability for most important biological substances. Moreover, this sensor exhibited excellent stability and reproducibility. This example of applying life gene substance (adenine) as an electrode modifier, which maybe play an important role in future studies.

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Supporting Information

This section including two parts: Fig.s1 and Scheme s1. Figure s1 shows the linear relationship between oxidation peak potential ($E_p$) and pHs. Scheme s1 shows the electrochemical oxidation mechanisms of DA, UA and GA. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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