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Direct determination of guanine in Aciclovir Dispersible Tablets solution by acylpyrazolone modified glassy carbon electrode

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Abstract

In this article, the investigation of electrochemical determination of guanine(GU) by 1-phenyl-3-methyl-4-(2-furoyl)-5-pyrazolone (HPMαFP) modified glassy carbon electrode (HPMαFP/GCE) in Aciclovir Dispersible Tablets solution was reported. The direct electrochemical behavior of GU was carefully studied in pH 5.5 phosphate buffer solution. Cyclic voltammogram results indicated that a well-defined irreversible redox peak was obtained with the formal potential of 0.92 V. The electrochemical parameter such as the electron transfer number (n) was calculated as 2. The HPMαFP modified electrode showed good electrocatalytic ability to GU. The concentration linear equation is as follows: iP (μA)=4.3767C (μmol l^{-1})+9.51332 with the detection limit of 1.2×10^{-7} mol/l.

Keywords: acylpyrazolone; Aciclovir Dispersible Tablets; cyclic voltammetry; guanine; modified electrode.

Introduction

Guanine (GU) is an important component found in deoxyribonucleic acid (DNA), which is vital in various biological processes. The level of GU in plasma has been suggested to be related to carcinoma or liver diseases (Wang et al. 2002, Xu et al. 2002). The determination of GU concentrations in DNA is important for the measurement of nucleic acid concentration itself (Ferapontova 2004) and detection of these analytes is necessary in pharmacological (Humphrey et al. 1987, Tacconi et al. 2003) and biological agents (Sheng and Ni 1991, Wang et al. 1996, Chiti et al. 2001, Ibrahim 2001). Thus, it is important to establish sensitive and simple methods for GU detection. Some methods for the measurement of GU based on their oxidation at pyrolytic graphite, glassy carbon, and carbon paste electrodes have been developed (Dryhurst 1971, 1972, Yao et al. 1977, Kenley et al. 1985, Gilmartin and Hart 1992, Oliveira Brett and Matysik 1997). The previously attempted methods for determination of these analytes have problems such as irreversible adsorption of purine bases on the electrode surface and have led to surface fouling (Dryhurst 1972). Recently, the electroactive groups of DNA oxidation were adenine residues (Wang et al. 2000), which exhibited slow direct electron transfer on the bare working electrode with low sensitivity for DNA detection. The modification can improve the surface state of the electrode, which can lead to significant reduction of overpotentials and increase of the electron transfer (ET) rate constant of desired redox reactions (Zare and Golabi 1999, Zhao et al. 2005, Raoof et al. 2006, Zhou and Wang 2006) so that it obtains more attention.

A literature survey reveals that there were no attempts to use the 1-phenyl-3-methyl-4-(2-furoyl)-5-pyrazolone (HPMαFP) modified glassy carbon to examine GU. HPMαFP belongs to the acylpyrazolones, which are an interesting class of β-diketones, containing a pyrazole fused to a chelating arm and usually studied in synthetics or as ligands (Li et al.). The neutral acylpyrazolones (Figure 1) can exist with several possible tautomeric forms as reported previously (Nishihama et al. 2001, Marchetti et al. 2005). The OH enol form of the acylpyrazolones display weak acidic, as a surfactant, which is suitable for electrochemical analysis (Ghoneim et al. 2008).

In this paper, GU is firstly determined by HPMαFP modified glassy carbon electrode (GCE) and the results revealed that peak current enhanced significantly. GU electrochemistry behavior and optimization condition are also discussed. This method shows good selective, high sensitivity and low limit on determining GU.

Materials and methods

Reagents

HPMαFP was synthesized according to the method proposed by Jensen (1959) (yield 73%, m.p. 374–375 K) with further purification, and HPMαFP standard solution was prepared by dissolving it into anhydrous ethanol. Guanine was purchased from Shanghai Reagent Company (Shanghai, China) and was used without further purification. Standard guanine stock solution was prepared by dissolving it into double distilled water. Phosphate buffer solution was prepared from 0.1 mol/l KH_2PO_4 and 0.1 mol/l K_2HPO_4 and adjusted pH with 0.1 mol/l HCl or 0.1 mol/l NaOH. Other reagents were of analytical grade. All solutions were prepared with double distilled water.

Apparatus

All electrochemical measurements were measured on a CHI-650A electrochemical workstation (CH Instrument

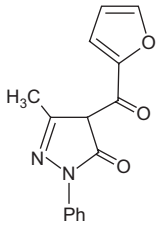

Figure 1 The structure of HPMαFP.

Inc., Shanghai, China). A conventional three-electrode system was used in the experiment. With a bare or HPMαFP film modified GCE (3 mm in diameter) was used as the working electrode. A saturated calomel electrode was used as a reference electrode and a Platinum wire as an auxiliary electrode. A 10-ml cell was used for electrochemical measurements.

Preparation of the HPMαFP/GCE modified electrode

The GCE was disposed by polishing to the mirror with wet emery papers with 0.05 μm Al_2O_3 powder. Then it was rinsed with double distilled water and anhydrous ethanol several times and dried in air. The treated GCE was modified with HPMαFP (1.0 μl, 0.01 mol/l) solution by dropping and dried in the desiccator. This pretreated electrode was on standby in the experiment.

Preparation of Aciclovir Dispersible Tablets solution

The Aciclovir Dispersible Tablets (TongHe Medicine Corporation, Chongqing, China) was a commercial medication. Two tablets was weighed and dissolved in 0.5 mol/l NaOH solution. Then the mixture solution was filtered by a funnel and clear liquid was taken. The solution was diluted with buffer solution. The treated solution was used as stock for the experiments.

Experiments

The electrochemical experiments were performed in a cell with 10 ml solutions in which oxygen was removed by purging high-purity nitrogen before voltammograms so that all measurements were carried out under nitrogen atmosphere. GU stocked solution was added according to the requirement. Voltammograms were recorded after a 6-s quiescence period. All measurements were performed at a circumambience temperature (25±2°C).

Results and discussion

Cyclic voltammograms of GU at bare and HPMαFP/GCE

The response of GU in pH 5 phosphate buffer solution on bare and HPMαFP modified GCE in the potential range of

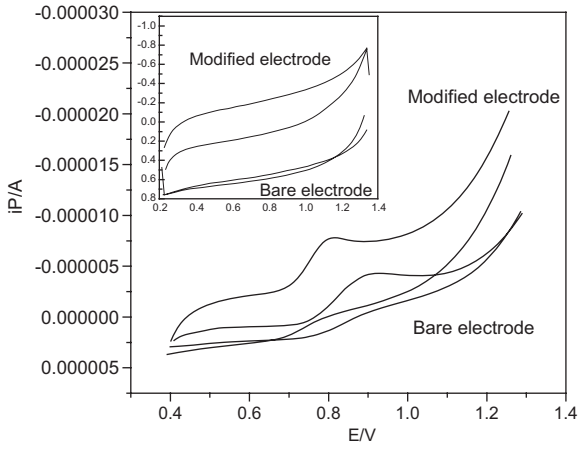

Figure 2 Cyclic voltammograms of bare electrode and modified electrode.

0.3 to 1.4 V at a scan rate of 0.1 V/s is illustrated in Figure 2. As can be seen, the oxidation peaks potential at 0.92 V on bare GCE and 0.81 V on HPMαFP/GCE. The oxidation signal of GU at bare electrode is weak yielding a small peak, whereas the modified electrode obtained a well-defined enhanced peak. The oxidation peak current value increased almost two times more than the bare electrode and the shape of the peak turn sharper. This result could be attributable to the HPMαFP that activated the GCE surface (Marchetti et al. 2005) and promoted the electron-transfer of GU on the electrode. There was no reduction peak in reverse scan in cyclic voltammograms, which provides evidence that the electrochemical reaction is an irreversible process. The same situation was found in the only buffer solution as shown in the Figure 2 insert.

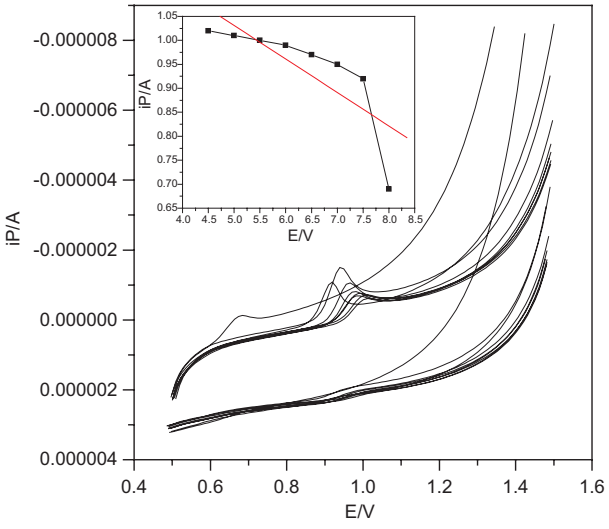

Figure 3 Influence of different pH values on the cyclic HPMαFP/GCE in pH=5.5. Phosphate buffer solution voltammograms and 0.1 mM GU (figure insert: only phosphate buffer solution on cyclic voltammograms of bare GCE and HPMαFP/GCE); scan rate: 0.1 V/s.

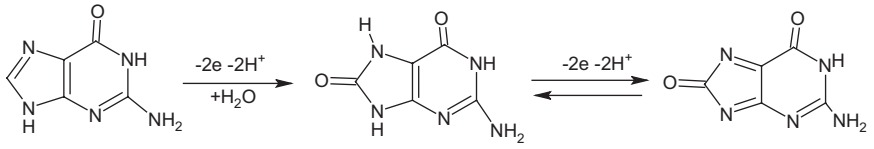

Figure 4 The electro-oxidation reaction mechanism of the GU mechanism of GU in solution.

Effect of solutions pH

The pH dependence of peak potential (Ep) obtained for 0.1 mM GU was studied in the pH range between 1 and 7 with the potential from -0.4 V to 1.6 V observed in Figure 3. The results revealed that peak potential shifted negatively vs. the increase of pH value. A linear dependence over the entire pH range studied was found. The E-pH relationship can be expressed by the equation: Ep(V)=-0.06976 pH+1.37976 with a correlation coefficient of -0.99887, which indicates that protons are involved in the electrode reaction. According to (Nicholson 1965), the equation: 59.0x/n=69.76, where x is the hydrogen ion participating in the electrode reaction and n is the electron transfer number, and thus the loss of electrons was accompanied by the loss of an equal amount of protons and x=n=2. The result revealed that two protons were involved in the electrode reaction. The mechanism (Wang et al. 2006), shown in Figure 4 (similar to Figure 3), revealed that the oxidation peak current value reached the highest as pH=5.5 and thus we chose a pH of 5.5 in our experiments.

Effect of scan rates

As can be seen in Figure 5, the oxidation peak currents increased gradually Vs. the increase of the scan rate. The relationship of oxidation peak current with scan rate was plotted with the equation: iP (μA)=1.069 V (mV/s)+0.124 (r=0.98449) in the range of 0.01 to 0.25 V/s. Linear correlations were obtained which confirmed that the electrode processes of the investigated GU were mainly controlled by adsorption (Dong 1981).

Effect of HPMαFP amount

Figure 6 revealed that the peak current increased along with the quality of HPMαFP. The peak current value increased quickly from 0.2 to 1.2 μl. This indicates that the increment of quality of HPMαFP promotes the peak current value. When it is more than 1.2 μl, the peak current was unsteady and the shape became abnormal. This could be due to the electrode surface enrichment being too thick, which hindered GU and the electrode exchange electronics.

Effect of concentration

GU with different concentrations was performed on the bare electrode and the HPMαFP/GCE. As can be seen from Figure 7, there is a linear relationship between peak current value and GU concentration. The linear regression equation at bare electrode was: iP (μA)=1.66996C (μmol/l) +1.80828 (r=0.99917). The linear regression equation at modified electrode was: iP (μA)=4.3767C (μmol/l)+9.51332

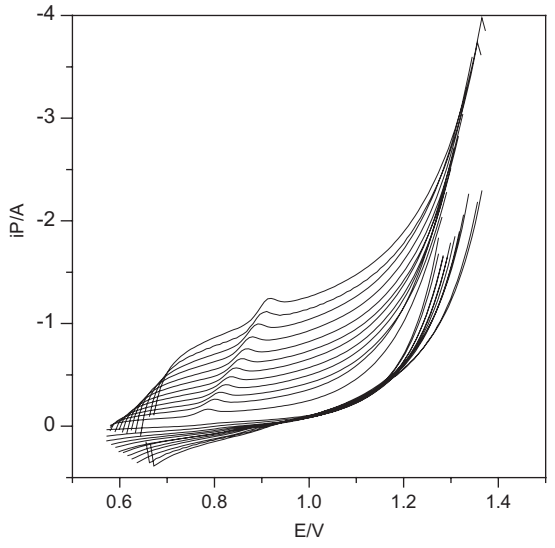

Figure 5 Cyclic voltammograms of GU on modified electrode at different scan rate.

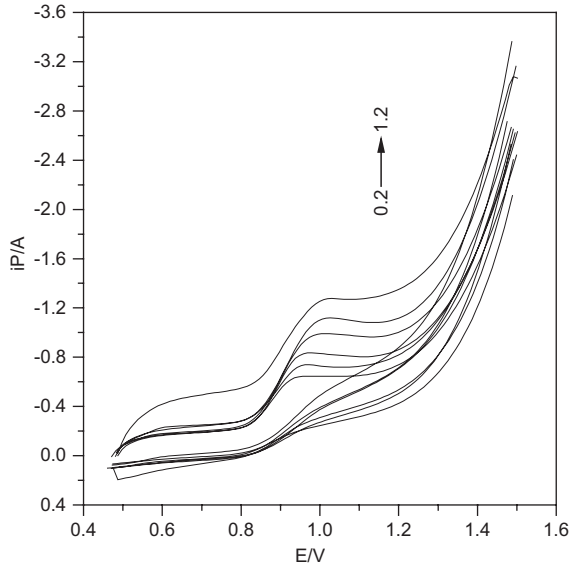

Figure 6 Influence of different HPMαFP amount on cyclic voltammograms by every 20 mV and HPMαFP/GCE at scan rates from 0.01 to 0.25 V/s.

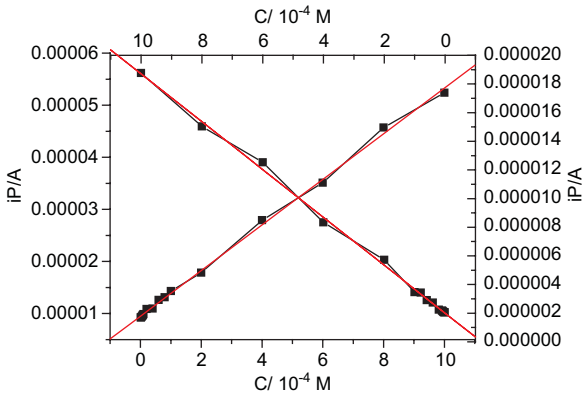

Figure 7 GU concentration vs. peak current on the bare electrode and HPMαFP/GCE.

(r=0.99919). Concentration range was measured from 10^{-6} to 10^{-4} mol/l. The detection limit is 1.2×10^{-7} mol/l.

Stability and reproducibility of the modified electrode

After the HPMαFP/GCE was stored for 1 week in the desiccator at ambient temperature, only a small decrease of peak current sensitivity with a relative standard deviation (RSD) of 2.11±0.05% for 0.1 mM GU was observed, which exhibited the excellent stability of the modified electrode. Furthermore, the reproducibility of the determination was performed with several successive scans in the solution containing 0.1 mM GU. The RSD values were found to be 3.21±0.02% in the experiment, which indicates good reproducibility of the modified electrode.

Interference

Through best experimental conditions, the effects of some small biomolecules, such as DL-serine, L-histidine, DL-phenylalanine, glycine, L-leucine, L-lysine, and tyrosine, were examined using standard addition. The results showed that 60 times content of these biomolecules have no influence on the determination of GU.

Sample determination

Taking 2.5 ml of treated solution as is done in 2.4 into 10 ml phosphate buffer solution (pH=5.5) was measured by HPMαFP/GCE using standard addition by cyclic voltammetry. The results are shown in Table 1. The recovery and RSD were acceptable proving that the proposed methods could be efficiently used for the determination of GU in the real sample.

Table 1 Results for determinations of GU in the Aciclovir Dispersible Tablets solutions.

Recovery of sample (n=3)	Added ($\times10^{-5}$ g/l)	Found/g/l ($\times10^{-3}$ g/l)				Recovery (%)	RSD (%)
		1	2	3	Average		
Injection	0	1.56	1.57	1.60	1.58	–	2.12
	1.88	3.48	3.50	3.47	3.49	101.6	1.73
	4.69	6.21	6.26	6.25	6.24	99.4	2.65

Conclusion

This paper primary discussed electrocatalytic oxidation reactions and measuring condition of GU in Aciclovir Dispersible Tablets by HPMαFP modified electrode. The experiment was determined by cyclic voltammetry. The results revealed that the HPMαFP/GCE presented high catalytic activities towards GU in Aciclovir Dispersible Tablets solution. In summary, the determination of GU reveals high sensitivity, good selectivity, reproducibility, and simplicity.

Acknowledgements

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