Adsorptive Stripping Voltammetry of Bleomycin

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In 0.05 M H_2SO_4 solution, two reductive peaks, P_1 and P_2, of bleomycin were obtained. The peak potentials E_{P_1} and E_{P_2} were −0.83 and −1.09 V (*versus* Ag/AgCl), respectively. The sensitivity of P_2 was much higher than that of P_1. The peak current of P_2 was proportional to the concentration of bleomycin over the range 1.0×10^{-9}–1.0×10^{-7} M with a detection limit of 5.0×10^{-10} M using adsorptive voltammetry at an accumulation time of 120 s (E_i = −0.80 V). The behaviour of the reduction wave was studied and applied to the determination of bleomycin in mouse serum. The reduction process of P_1 was irreversible with adsorptive characteristics and the adsorption behaviour obeyed the Frumkin adsorptive isotherm.The adsorptive coefficient β was 8.9×10^5 , the interaction factor α was 0.94 and the Gibbs energy of adsorption ΔG° was −33.93 kJ mol^{-1}. P_2 was an irreversible adsorption peak with catalytic hydrogen properties.

Keywords: *Adsorptive stripping voltammetry; bleomycin; irreversible wave*

Bleomycin (BLM) is a member of a family of structurally similar glycopeptide antibiotics recognized as antitumour drugs used in the treatment of Hodgkin's lymphoma and carcinomas of the testis, head, skin and neck. Its structure is shown in Fig. 1. The determination of BLM by gas chromatography and high-performance liquid chromatography has been reported.[1–3] However, the voltammetric behaviour of BLM and its determination have not been investigated so far. In this work, the voltammetric behaviour of BLM was studied and a method for the determination of trace amounts of BLM was developed. In 0.05 M H_2SO_4, two reductive peaks, P_1 and P_2, of BLM were obtained.The peak potentials E_{P_1} and E_{P_2} were −0.83 and −1.09 V (*versus* Ag/AgCl), respectively. The sensitivity of P_2 was much higher than that of P_1. A linear relationship held between the peak current of P_2 and the concentration of BLM in the concentration range 1.0×10^{-7}–1.0×10^{-9} M with good precision and accuracy. After concentration for 120 s, the detection limit was 5.0×10^{-10} M.The method was applied to samples of mouse serum and satisfactory results were obtained. The electrode reaction mechanism is discussed.

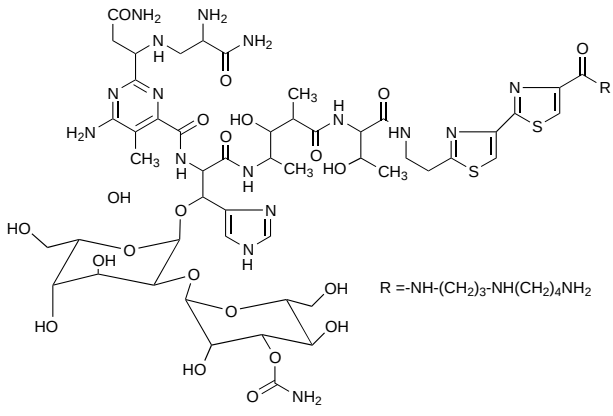

Fig. 1 Structure of BLM.

Experimental

Apparatus

A Model 370 Electrochemistry System (EG&G Princeton Applied Research, Princeton, NJ, USA) was used for linear sweep and cyclic voltammetry, with a three-electrode system consisting of a hanging mercury drop electrode (HMDE) as the working electrode, an Ag/AgCl (saturated KCl) reference electrode and a platinum counter-electrode. The electrolytic cell was a 10 ml beaker. All experiments were performed at room temperature and dissolved oxygen was removed by passing pure nitrogen through the solutions.

Reagents

BLM was obtained from the Institute of Materia Medica, Chinese Academy of Medical Science (Beijing, China), with a purity of 98%. A 2.7×10^{-4} M stock standard solution of BLM was prepared by dissolving 10.2 mg in a small volume of triply distilled water and diluting to 25.0 ml; it was stored in the dark. The supporting electrolyte was 0.5 M H_2SO_4. All chemicals were of analytical-reagent grade. Triply distilled water was used throughout.

Procedure

A 10 ml volume of 0.05 M H_2SO_4 containing a specific amount of sample solution was added to the cell and purged with purified nitrogen for 4 min to remove oxygen. The pre-concentration potential (−0.80 V) was applied to a new mercury drop for 120 s. The voltamperogram was recorded by using a linear sweep scan. The scan was terminated at −1.15 V.

Results and Discussion

In 0.05 M H_2SO_4, two reductive waves, P_1 and P_2, of BLM were obtained by dc polarography. The wave of P_2 was a sharp peak and its height was much higher than that of P_1 (see Fig. 2).

Adsorptive Properties

Repetitive cyclic voltamperograms

Fig. 3 shows repetitive cyclic voltamperograms for 1.0×10^{-6} M BLM, recorded after preconcentration at −0.60 V for 30 s. Two cathodic peaks, P_1 and P_2, are observed in the first scan (curve a) at −0.83 and −1.09 V. The peak current of P_2 was much higher than that of P_1. Subsequent scans (curves band c) exhibited a substantial decrease in the peak to a stable value, showing that BLM has adsorptive characteristics at the mercury electrode.

Effect of accumulation time

Fig. 4 shows plots of the cathodic peak currents (i_{pc}) of P_1 and P_2 in linear sweep voltammetry *versus* accumulation time (t) for different concentrations of BLM. At first, i_{pc_1} increased linearly with t, indicating that before adsorptive equilibrium is reached, the longer the accumulation time, the more BLM was adsorbed and the larger was the peak current. However, after a specific period of accumulation time, the peak current tended to level off, illustrating that adsorptive equilibrium of BLM on the mercury electrode surface was achieved.

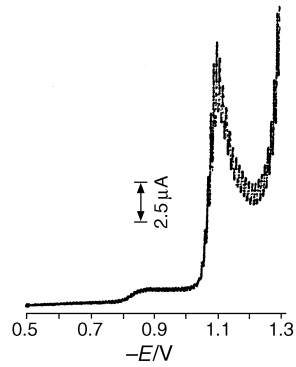

Fig. 2 Dc polarogram of BLM. Conditions: 0.05 M H_2SO_4, 6.5×10^{-5} M BLM, $V = 20$ mVs^{-1}, $t = 0.5$ s.

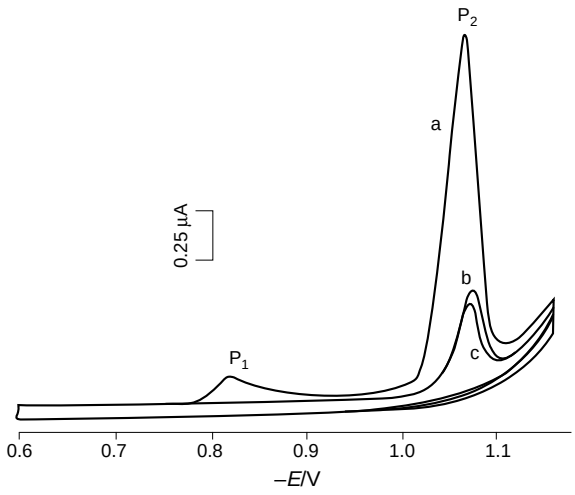

Fig. 3 Repetitive cyclic voltamperograms. Conditions: 0.05 M H_2SO_4, 1.0×10^{-6} M BLM, $V = 100$ mV s^{-1}, $t_{acc} = 30$ s.

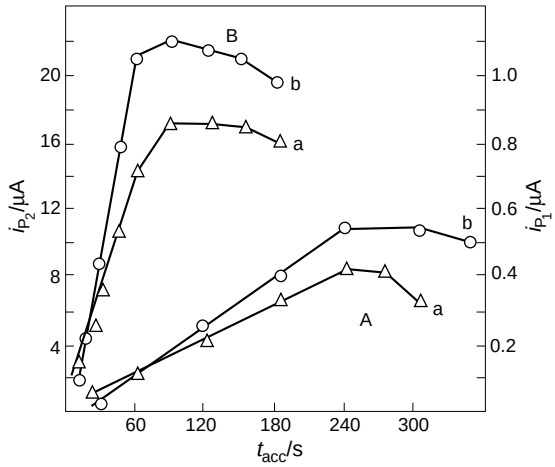

Fig. 4 Effect of accumulation time. A, P_1; B, P_2. BLM concentration: a, 1.0×10^{-7}; b, 5.0×10^{-7} M. Other conditions as in Fig. 2.

Effect of scan rate

Fig. 5 shows the effect of scan rate on the peak current. The peak currents i_{pc_1} and i_{pc_2} increased with increasing scan rate. The experiments indicate further that when $t_{acc} = 0$ s, i_{pc_1} and i_{pc_2} show a linear relationship with $v^{1/2}$, illustrating that the reduction of BLM is diffusion controlled. When $t_{acc} = 60$ s, the i_{pc_1}–v and i_{pc_2}–v curves became a straight line, suggesting that the electrode process was adsorption-controlled.[4]

Measurement of amount of BLM adsorbed

After peak P_1 in the linear sweep voltammogram, the current decreased to the background level and the area under the peak was determined and the quantity of charge (Q) transferred by reduction was calculated. The amount of BLM adsorbed per unit area (Γ) was then obtained from $\Gamma = Q/nFA$, where A is the electrode area. A plot of $nF\Gamma$ as a function of BLM concentration (c_{BLM}) is shown in Fig. 6. A linear relationship between $\ln[c_{BLM}(1 - \theta)/\theta]$ *versus* θ was found, where $\theta = \Gamma/\Gamma_{max}$ and Γ_{max} is the maximum surface coverage, showing that BLM adsorption satisfies the Frumkin isotherm $\beta c_{BLM} = \theta\exp[\alpha\theta/(1 - \theta)]$ with adsorption coefficient $\beta = 8.9 \times 10^5$ l mol^{-1} and the interaction factor $\alpha = 0.94$.[5] The Gibbs energy of adsorption $\Delta G^0 = -33.93$ kJ mol^{-1}, indicating that the

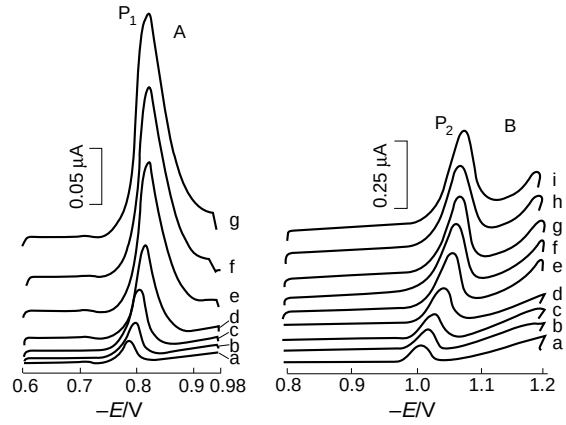

Fig. 5 Effect of scan rate (0.05 M H_2SO_4). A, P_1. Conditions: 2.0×10^{-6} M BLM, $t_{acc} = 60$ s, scan rate = a, 5; b, 10; c, 20; d, 50; e, 100; f, 150; and g, 200 mV s^{-1}. B, P_2. Conditions: 5.0×10^{-7} M BLM, $t_{acc} = 10$ s, scan rate = a, 5; b, 10; c, 20; d, 50; e, 100; f, 150; g, 200; h, 250; and i, 300 mV s^{-1}.

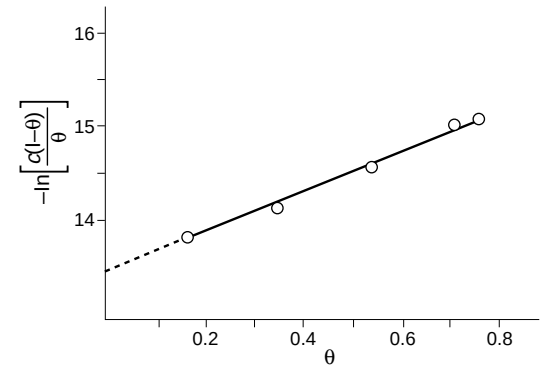

Fig. 6 Plot of $\ln[c_{BLM}(1 - \theta)/\theta]$ *versus* θ. Conditions: 0.05 M H_2SO_4, $t_{acc} = 30$ s, $v = 100$ mVs^{-1}.

reaction species has a stronger adsorption. The value of α depends primarily on the structure of the adsorbed particles and may also be a function of potential. The value of α is positive, indicating that the interactions between the adsorbed species on the electrode surface are attractive.

As mentioned above, BLM has adsorption characteristics, which can be used in an effective preconcentration step before the voltammetric measurement, hence a highly sensitive adsorptive stripping voltammetry of the drug can be obtained. Because the sensitivity of P_2 was much higher than that of P_1, peak P_2 was used in the determination of BLM.

Reversibility

In Fig. 3, it can be seen that no peaks are observed on the anodic branch of P_1 and P_2, indicating that the reduction of BLM at the Hg electrode is irreversible.

Hydrogen bubbles occur near the electrode when electrolysis is performed at the peak potential of P_2. The peak current of P_2 increased with increasing Britton–Robinson buffer capacity at the same pH value (4.35). These experiments indicated that P_2 showed catalytic hydrogen properties.

Measurement of the number of electrons transferred[6,7]

The number of electrons was determined by constant potential coulometry with a large area of the mercury cathode. Three portions of 0.05 M H_2SO_4 and concentrations of 7.7×10^{-5}, 6.5×10^{-5} and 8.5×10^{-5} M BLM were electrolysed at -0.85 V (*versus* SCE) for P_1, with consumption of 0.62, 0.52 and 0.73 C, respectively. The values of n, the number of electrons transferred, thus obtained were 1.9, 1.8 and 2.0, respectively, hence $n = 2$. For P_2,the electrolysis experiment was performed at -1.09 V after P_1 was completely electrolysed. A large current was still found after electrolysis for 200 min and indicated that the number of electrons was not determined because P_2 showed the properties of catalytic hydrogen.

Determination of α n and α

According to the Laviron equation[8] for the irreversible reduction wave:

$$W_{1/2} = 2.44RT/(\alpha nF) = 62.5/(\alpha n)\ (25\ ^\circ\mathrm{C})$$

where $W_{1/2}$ is the half-width of the peak. αn can be calculated to be 1.39 for P_1 when the scan rate $v = 100$ mV s^{-1}. P_1 of BLM undergoes a two-electron reduction, *i.e.*, $n = 2$, hence $\alpha = 0.69$.

In summary, in 0.05 M H_2SO_4, two reduction peaks of BLM, P_1 and P_2, were obtained. P_1 was an irreversible adsorption peak with a two-electron reduction process. P_2 was an irreversible adsorption peak with catalytical hydrogen properties.

Measurement by adsorptive stripping voltammetry

Selection of experimental conditions

In order to choose the optimum experimental conditions for the determination of BLM by adsorptive stripping voltammetry, a series of experiments were carried out. Various supporting electrolytes, such as H_2SO_4, HCl, NaOH, KCl, NH_3–NH_4Cl and HOAc–NaOAc buffer solutions were tested. H_2SO_4 was found to be the best because of the fairly well defined voltamperogram and reasonably high sensitivity. The effect of supporting electrolyte concentration on i_{pc_2} was examined. The results showed that i_{pc_2} increased with increasing H_2SO_4 concentration, and between 0.01 and 0.10M, i_{pc_2} remained almost constant at a high level, so 0.05 M H_2SO_4 was chosen for subsequent experiments. The effect of the preconcentration potential was also studied. i_{pc_2} increased with increasing preconcentration potential in the negative direction, then reached a constant value. A potential of -0.80 V was chosen as the optimum preconcentration potential.The stability of the system was fairly good.

Table 1 Analytical results for BLM in mouse serum

Sample No.	Found/μg ml^{-1}	Mean/μg ml^{-1}	RSD (%)
1	59.8		
	62.2	60.2	3.5
	57.4		
	61.2		
2	64.5		
	62.3	64.3	2.3
	65.8		
	64.6		
3	64.5		
	63.7	63.5	1.2
	62.7		
	63.2		

Calibration graph and detection limit

Under the optimized conditions of 0.05 M H_2SO_4, a preconcentration potential of -0.80 V and a scan rate of 100 mV s^{-1}, the peak current of BLM was found to be proportional to its concentration over the range 1.0×10^{-9}–1.0×10^{7} M when $t_{acc} = 120$ s.

According to IUPAC, the detection limit DL $= 3s/k$, where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte; k is the sensitivity, namely the slope of the calibration graph. Now, $n = 10$, $\bar{x} = 1.25 \times 10^{-2}$ μA, $s = 2.2 \times 10^{-4}$ μA, $k = 0.031 \times 10^{8}$ μA mol^{-1} l^{-1}, hence DL $= 3s/k = 3 \times 2.2 \times 10^{-4}/(0.031 \times 10^{8}) = 5.03 \times 10^{-10} \approx 5 \times 10^{-10}$ mol l^{-1}.

Analysis of Samples

Measurement of BLM in mouse serum was performed by adsorptive stripping voltammetry. The mouse serum was provided by the Biological Department in this university, which is engaged in studies of the action mechanism for anticancer drugs. BLM was administered to mice and the serum was analysed for BLM. No sample preparation was used other than dilution with the supporting electrolyte. The determination was accomplished by the standard additions method and the results of a few analyses are given in Table1. The relative standard deviation was about 1.2–3.5%. In addition, some recovery experiments were carried out and the recovery was 93.0–101.2%. The results confirm the usefulness of the proposed method for the determination of BLM.

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