Voltammetric behaviour of brodimoprim at mercury and its determination in biological fluids using differential-pulse polarography

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A differential-pulse polarographic (DPP) method was developed for the determination of brodimoprim (BDP) in Michaelis (MCL) buffer. The peak potential ($E_p$) occurs at $-1.33$ V (versus SCE) when the pH of the system is 4.0. The voltammetric behaviour in MCL buffer and the mechanisms of the electrode reaction were studied by, e.g., linear sweep voltammetry, cyclic voltammetry and normal-pulse polarography. The wave was confirmed to be an irreversible adsorptive wave. The relationship between peak height and concentration of BDP was linear in the range $5.0 \times 10^{-8} - 1.0 \times 10^{-7}$ mol l$^{-1}$ with a detection limit of $1.0 \times 10^{-9}$ mol l$^{-1}$, when the adsorptive accumulation time was 5 min.

**Keywords:** Brodimoprim; voltammetric behaviour; differential-pulse polarography

Brodimoprim (BDP) (Scheme 1) is an aminopyrimidine used widely as a bactericide and as a synergist of sulfanilamide. Owing to its great pharmacological activity, a highly sensitive analytical method is essential for the evaluation and administration of this drug. In 1992, Gaspari et al.\(^1\) reported the determination of BDP in human plasma, blood and urine by HPLC with a detection limit of 5 ng ml$^{-1}$. The BDP molecule has electroactive groups, but nothing appears to have been published concerning its electrochemical behaviour or its voltammetric measurement in particular. In this paper, we report on studies of the voltammetric behaviour of BDP and its determination at low concentrations in biological fluids by means of differential-pulse polarography (DPP).

**Experimental**

**Apparatus and chemicals**

An MF-1A voltammetric analyzer (Jiangsu Electroanalytical Instrument Factory, Jiangsu, China), coupled with an LZ3-304 X-Y recorder (Shanghai Dahua Instrument Factory, Shanghai, China). The three-electrode system consisted of an SH-84 hanging mercury drop electrode as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. An EG&G Princeton Applied Research (PAR) (Princeton, NJ, USA) Model 384B polarographic analyzer with an EG&G PAR Model 303A static mercury drop electrode was used. A model pH-3C pH meter was obtained from Shanghai Leici Instrument Factory (Shanghai, China). A stock standard solution of BDP ($2.0 \times 10^{-3}$ mol l$^{-1}$) in ethanol was prepared and preserved in a refrigerator. Michaelis (MCL) buffer consisted of 0.08 mol l$^{-1}$ HOAc–0.02 mol l$^{-1}$ NaOAc. All other reagents were of analytical-reagent grade. Distilled, de-ionized water was used throughout.

**Polarographic procedure**

The best voltammetric response of BDP was obtained in MCL buffer of pH 4.0. Oxygen-free nitrogen was bubbled through the solution for 10 min to deareate it while the solution was stirred. Following the deareation period, the stirring was stopped, and after 15 s a cathodic potential sweep was carried out from $-1.00$ to $-1.40$ V using DPP and normal-pulse polarography (NPP). For linear sweep voltammetric (LSV) and cyclic voltammetric (CV) methods a preconcentration period at $-1.00$ V was needed. All measurements were made at room temperature.

**Results and discussion**

Fig. 1 shows the polarograms obtained by sampled dc polarography (DCP), NPP and DPP of $2.0 \times 10^{-5}$ mol l$^{-1}$ BDP.
solutions in MCL buffer at pH 4.0. A large and defined cathodic wave around $-1.33$ V is observed, due to the reduction of the adsorbed drug. The dc wave could have been utilized for analytical purposes but the DPP wave was selected because of its increased sensitivity. The NP polarogram (versus Ag/AgCl) has a sharp cathodic peak, indicating obvious adsorptivity of the electroactive species.\(^2\)

Fig. 2 shows the cyclic voltammogram of BDP. A large cathodic peak is present in the first scan following a period of stirring at $-1.00$ V. Upon repetitive scans the cathodic peak decreases to a stable value representing the response of the blank (not shown). No peaks are observed in the anodic branch, showing that the wave is irreversible.

The non-ionic surfactant poly(vinyl alcohol) and gelatin increase the peak height considerably, the cationic surfactant ammoniumhexadecyltrimethyl bromide decreases the peak height, but when the anionic surfactant sodium dodecyl sulfonate is added to the solution the peak disappears, showing that the reaction species is adsorptive and negatively charged.

The effect of pH on the peak potential ($E_p$) and peak current ($i_p$) was studied using $1.0 \times 10^{-6}$ mol l$^{-1}$ BDP in MCL buffer at intervals of 0.3 pH unit over the pH range 3.19–6.22, the corresponding peak potentials being $-1.252$ and $-1.517$ V (Fig. 3). The peak potential shifts to more negative values according to the equation $E_p = -0.9718 - 0.0887 \times $ pH with a correlation coefficient of 0.9983. The highest peak current and least interference of the hydrogen wave were obtained at pH 4.0, which was selected for further experiments.

The effect of the potential scan rate ($v$) on the peak current and peak potential was studied. On plotting $E_p$ against $v$ there is a shift of $E_p$ to more negative values with increasing $v$ (not shown). Fig. 4 shows the relationship between $i_p$ and $v$. When the accumulation time $t_a$ is 5 s, the $i_p$ versus $v^{1/2}$ graph is linear.

**Fig. 2** Cyclic voltammogram for $1.0 \times 10^{-6}$ mol l$^{-1}$ BDP solution using a scan rate of 100 mV s$^{-1}$, with 30 s accumulation time. Other conditions as in Fig. 1.

**Fig. 3** Dependence of (A) $E_p$ and (B) $i_p$ on pH. Other conditions as in Fig. 2.

**Fig. 4** Effect of potential scan rate ($v$) on $i_p$. Accumulation time: (A) 5 and (B) 30 s. Other conditions as in Fig. 2.

**Fig. 5** Polarograms for the determination of BDP in human serum: a, blank; b, serum spiked with BDP at $2.0 \times 10^{-8}$ mol l$^{-1}$; c, d and e, standard additions of $5.0 \times 10^{-8}$, $7.0 \times 10^{-8}$ and $9.0 \times 10^{-8}$ mol l$^{-1}$ of BDP, respectively. Accumulation time, 60 s; other conditions as in Fig. 1.
indicating that the current is mainly diffusion controlled (A). However, when \( t_a \) is longer than 30 s, the \( i_p \) versus \( v \) graph is linear, indicating that the current is adsorption controlled (B).

An accumulation potential of \(-1.00 \text{ V}\), is preferred, as a defined cathodic wave and the highest peak current were obtained at this potential.

It is observed experimentally that in BDP solution the electrocapillary curve clearly falls in the negative charge region (not shown), further indicating the adsorptivity of the system.

The electrochemical behaviour of a \( 1.0 \times 10^{-6} \text{ mol l}^{-1} \) BDP solution was studied by means of LSV in MCL buffer. This allows us to calculate the electron transfer number \( n \) and the transfer coefficient \( \alpha \) according to the equation \( \frac{W^{1/2}}{2.44RT/\alpha nF} = \frac{62.5}{\alpha n} \) (25 °C), and the resulting mean value of \( \alpha n \) was 1.06, hence \( n = 2 \).

It was possible to calculate the number of protons using the slopes 0.059\( mV/\alpha n \) of the \( E_p – pH \) plot, knowing the \( \alpha n \) value. The number of protons found was 2.

The relationship between peak height and the concentration of BDP was linear in the range \( 5.0 \times 10^{-8} – 1.0 \times 10^{-7} \text{ mol l}^{-1} \) with a detection limit of \( 1.0 \times 10^{-9} \text{ mol l}^{-1} \), when the adsorptive accumulation time was 5 min at \(-1.00 \text{ V}\).

The proposed method was applied to determination of BDP in human serum and urine samples. The samples were treated as described elsewhere. An aliquot of 2.5 ml of human serum (or urine) was placed in a 10 ml centrifugal test-tube, then 0.25 \( \mu \text{g} \) of BDP and 2.5 ml of 10% trichloroacetic acid were added successively and diluted to volume. Following centrifugation for 10 min at 3000 rpm, the solution was extracted with diethyl ether (3 \( \times \) 10 ml). The aqueous phase was transferred into a 25 ml calibrated flask and diluted to volume with water. An aliquot of this solution was added to an aliquot of MCL buffer into the cell and the polarogram was recorded following the DPP procedure and using the standard additions method to minimize any matrix effect. Fig. 5 shows the polarograms for the determination of BDP in these samples. Typical results obtained by successive standard additions are summarized in Table 1.

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### Table 1 Analytical results for BDP

<table>
<thead>
<tr>
<th>Sample</th>
<th>BDP present/µg l(^{-1})</th>
<th>BDP found/µg l(^{-1})</th>
<th>Mean/µg l(^{-1})</th>
<th>RSD/%(%)</th>
<th>Recovery/%(%)</th>
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<td>Serum</td>
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<td>96</td>
<td>2.4</td>
<td>96</td>
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<tr>
<td>Urine</td>
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<td>92, 94, 96, 89, 95, 97</td>
<td>94</td>
<td>3.1</td>
<td>94</td>
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</table>

References