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**THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 1-PHENYL-5-HYDROXY-3-CARBAMOYLPYRAZZOL-4-AZO-(4'-ETHOXYCARBONYLBENZENE)\***

Jiří BAREK and Dagmar CIVIŠOVÁ

*Department of Analytical Chemistry, Charles University, 128 40 Prague 2*

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The polarographic reduction of the title azodye has been studied, a mechanism has been proposed for this process and conditions have been found for the determination of this substance by TAST polarography, with a detection limit of  $7 \cdot 10^{-7} \text{ mol l}^{-1}$ , differential pulse polarography at a dropping mercury electrode with a detection limit of  $3 \cdot 5 \cdot 10^{-7} \text{ mol l}^{-1}$ , fast scan differential pulse voltammetry at a static mercury drop electrode with a detection limit of  $6 \cdot 9 \cdot 10^{-9} \text{ mol} \cdot \text{l}^{-1}$  and voltammetry with linearly increasing voltage at a hanging mercury drop with a detection limit of  $1 \cdot 6 \cdot 10^{-9} \text{ mol l}^{-1}$ .

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Chemical dyes are some of the mostly widely manufactured products of the chemical industry and are becoming extensively scattered throughout the environment. Study of their ecotoxicological properties requires sufficiently sensitive and selective analytical methods. Consequently, this work is devoted to the study of the polarographic behaviour of 1-phenyl-5-hydroxy-3-carbamoylpyrrazol-4-azo-(4'-ethoxy-carbonylbenzene) (see formula I, Eq. (A)), which is an industrially manufactured azodye. A volumetric titrimetric method has been developed<sup>1</sup> for the determination of this substance and its spectrophotometric<sup>2</sup> and polarographic<sup>3</sup> behaviour in acetonitrile medium has been studied. Details on the polarographic behaviour of azodyes can be found in monographs<sup>4-6</sup> and ref.<sup>7</sup> describes the application of differential pulse techniques to the determination of low concentrations of azocompounds.

The polarographic behaviour of the above azodye has been studied in a mixed methanol - Britton-Robinson buffer (1:1), ensuring sufficient solubility. In addition to TAST polarography and differential pulse polarography (DPP), the reversibility was studied using cyclic voltammetry (CVA) and fast scan differential pulse voltammetry (FS DPV) at a static mercury drop electrode (SMDE)<sup>8</sup> and voltammetry with linearly increasing voltage (LSV) at a hanging mercury drop electrode (HMDE) have been used for the determination of very low concentrations of this substance. The detection limit in the latter method was further decreased using preliminary accumulation of the studied substance on the surface of the working electrode<sup>9</sup>.

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## EXPERIMENTAL

### Reagents

The stock solution of the studied azodye in methanol ( $10^{-4} \text{ mol l}^{-1}$ ) was prepared by dissolving 0.0379 g of the pure substance (Research Institute for Organic Synthesis, Pardubice-Rybitví) in *p.a.* solvent and dilution to 1 l. Solutions with lower concentrations were prepared by precise dilution of the stock solution with methanol. All the solutions were stored in the dark. Britton–Robinson buffer solutions were prepared in the usual manner<sup>10</sup>. All the chemicals and solvents were of *p.a.* purity (Lachema, Brno). Water was doubly distilled in a quartz apparatus.

### Apparatus

Polarographic curves at a dropping mercury electrode (DME) were recorded using a PA2 polarograph with an XY-4103 recorder (both from Laboratorní přístroje, Prague) in a three-electrode arrangement (DME, SCE, platinum auxiliary electrode) with electronically controlled drop time. (DME parameters: flow rate  $1.16 \text{ mg s}^{-1}$  at a reservoir height of 25 cm, drop time 8.4 s in Britton–Robinson buffer – methanol (1 : 1) medium with pH 5.0 at an applied potential of 0 V and mercury reservoir height of 25 cm). Temperature measurements were carried out in an all-glass tempered vessel using a U3 thermostat (Mechanik Prüfgeräte, Medingen, G.D.R.) for temperature maintenance.

DPP and FS DPV recordings at the SMDE, LSV, and CVA measurements at the HMDE were recorded using a PA3 instrument combined with an XY-4105 recorder (both from Laboratorní přístroje, Prague) in a three-electrode arrangement (SMDE/HMDE, SCE, platinum auxiliary electrode). The static or hanging mercury drop electrode was the SMDE/HMDE electrode (Laboratorní přístroje, Prague) using a capillary with an internal diameter of 0.136 mm. All experiments employed a maximum drop size produced by opening the valve for 160 ms.

Coulometry at constant potential was carried out using an OH 404 coulometric analyzer (Radelkis, Budapest) with digital integration of the charge passed and automatic compensation of the residual current. A mercury pool was used as the cathode, with an OH 935 platinum electrode with an area of  $5 \text{ cm}^2$  as an auxiliary electrode separated by a glass frit and a saturated calomel reference electrode. The solution was mixed using an electromagnetic stirrer. Oxygen was removed from the polarographic solution by bubbling for ten minutes with nitrogen. The nitrogen purification line contained an alkaline solution of sodium anthraquinone-2-sulphonane and a solution of chromium(II) ions in dilute hydrochloric acid over zinc amalgam. Prior to entering the polarographic vessel, the nitrogen was passed through methanol. The pH was measured using a pHM 62 instrument (Radiometer, Copenhagen) with a combined glass and saturated calomel electrode. Spectrophotometric measurements were carried out using a Specord UV VIS instrument (Zeiss, Jena) in glass cuvettes with a thickness of 0.5 cm.

### Procedure

In polarographic measurements, the calibration curve was measured in triplicate and statistically evaluated using linear regression. The detection limit was found by the method of Skogerboe and Grant<sup>11</sup> as described in ref.<sup>12</sup>. In agreement with ref.<sup>13</sup>, the determination limit was considered to be three times this value.

Coulometry at constant potential was carried out by measuring 40 ml methanol and 50 ml Britton–Robinson buffer, pH 4, into the coulometric vessel (the final methanol solution had a pH of 5); the solution was then bubbled with nitrogen. 15 minute pre-electrolysis at constant potential of  $-550 \text{ mV}$  corresponding to the limiting current was followed by addition of 10.00 ml

of a  $10^{-4} \text{ mol l}^{-1}$  solution of the studied substance in methanol that had also been bubbled with nitrogen. Electrolysis was terminated after about 40 minutes when the electric current decreased to the residual current recorded prior to addition of the depolarizer.

## RESULTS AND DISCUSSION

The stability of a  $10^{-4} \text{ mol l}^{-1}$  solution of the studied azodye in methanol was controlled spectrophotometrically. No marked change was observed in the absorbance at a wavelength of 430 nm (Fig. 1) after sixty days ( $\epsilon = 24\,700 \text{ mol}^{-1} \text{ l cm}^{-1}$ ).

### *DC and TAST Polarography at a Dropping Mercury Electrode*

First the effect of the pH on the polarographic behaviour of the studied substance was measured. Experiments were carried out at a reservoir height of 81 cm, polarization rate of 2 mV/s and, in TAST polarography, an electronically controlled drop time of 1 s. Table I gives the determined dependences of  $E_{1/2}$ ,  $I_{\text{lim}}$  and the slope of the logarithmic analysis on the pH. The observed shift in  $E_{1/2}$  with increasing pH towards negative values can be explained by prior protonation of the azogroup, resulting in a decrease in the electron density in the region around the double bond between the nitrogen atoms, leading to facilitation of the reduction in acid medium. The practical independence of  $I_{\text{lim}}$  on the pH indicates that the studied substance exchanges the same number of electrons over the whole pH range. The observed increase in the slope of the logarithmic dependence in the pH region 8–10 apparently is connected with a decrease in the reversibility of the studied system, also reflected in a decrease in the peak current ( $I_p$ ) in DPP. The value of the slope of the logarithmic analysis and the coulometrically determined number of electrons exchanged (see below) indicate that the studied process is irreversible, as was also confirmed by cyclic voltammetry at the HMDE (Fig. 2).

A maximum appears on the polarographic wave of the studied substance especially in alkaline medium and complicates its evaluation. This maximum can be suppressed by addition of 0.4 ml of 0.5% gelatine solution to 10 ml of polarographed solution. From an analytical point of view, the best developed curve was obtained in medium of pH 5, in which all the subsequent dependences were studied.

In agreement with the Ilkovich equation, the limiting current measured in DC polarography was found to be linearly dependent on the square root of the height of the mercury reservoir. It was found in TAST polarography that the limiting current increases with increasing reservoir height in agreement with the relationship  $I_{\text{lim}} = k \cdot m^{2/3} t^{1/6}$ . At  $m$  in the range  $1\text{--}4 \text{ mg s}^{-1}$  and  $t$  in the range  $1\text{--}4 \text{ s}$ ,  $k$  remains constant ( $78 \pm 4 \mu\text{A g}^{-2/3} \text{ s}^{1/2}$ ) at pH 5. Both these facts reflect the diffusion character of the observed limiting current, as does the value of the temperature coefficient (2.3%) found in the usual manner<sup>14</sup>.

TABLE I  
The effect of the pH on the DC and DP polarograms of substance I<sup>a</sup>

pH	$E_{1/2}$ mV	$I_{lim}$ $\mu$ A	Slope $a^b$ mV	$E_p$ mV	$I_p$ $\mu$ A
4.11	-248	0.23	28	-250	0.50
5.10	-313	0.23	31	-320	0.51
6.02	-379	0.23	31	-380	0.51
7.20	-448	0.25	35	-465	0.51
8.15	-520	0.29	50	-540	0.43
8.95	-574	0.29	70	-575	0.34
9.56	-629	0.31	74	-650	0.39
10.70	-652	0.30	34	-695	0.55
11.71	-722	0.32	31	-730	0.64

<sup>a</sup>  $c_I = 5 \cdot 10^{-5} \text{ mol l}^{-1}$ , methanol-Britton-Robinson buffer (1:1) medium; <sup>b</sup> slope of the logarithmic analysis.

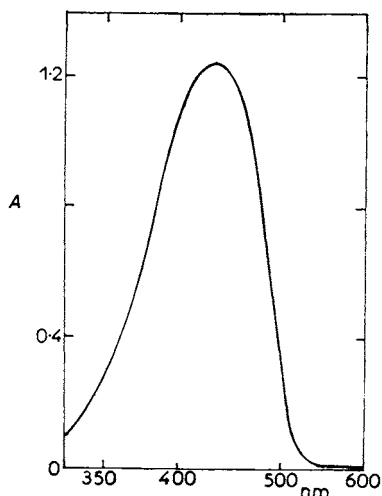


FIG. 1  
The visible spectrum of a  $10^{-4} \text{ mol l}^{-1}$  solution of the studied substance in methanol. Specific cuvette thickness, 0.5 cm

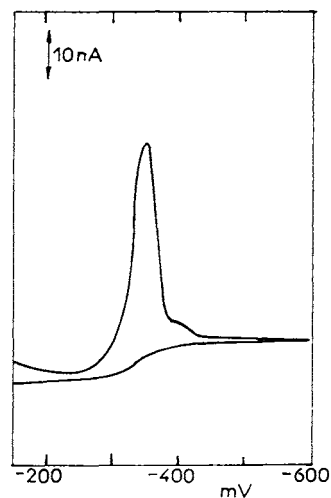


FIG. 2  
Cyclic voltammogram at the HMDE of a  $10^{-5} \text{ mol l}^{-1}$  solution of the studied substance in methanol-Britton-Robinson buffer (1:1) medium at pH 5 with a polarization rate of  $5 \text{ mV s}^{-1}$

The parameters of the concentration dependence ( $I_{lim} = a \cdot c + b$ ) are listed together with the detection limit in Table II. The linearity of this dependence confirms the diffusion character of the limiting current and permits its analytical utilization.

#### Determination of the Number of Exchanged Electrons

It has been found by coulometry at constant potential that 4 electrons ( $n = 4.2 \pm 0.15$ ) are exchanged in Britton–Robinson buffer–methanol medium (1 : 1) at pH 5 at a potential of  $-550$  mV, corresponding to the limiting current, corresponding to complete reduction of the studied azocompound to the corresponding amines according to Eq. (A):

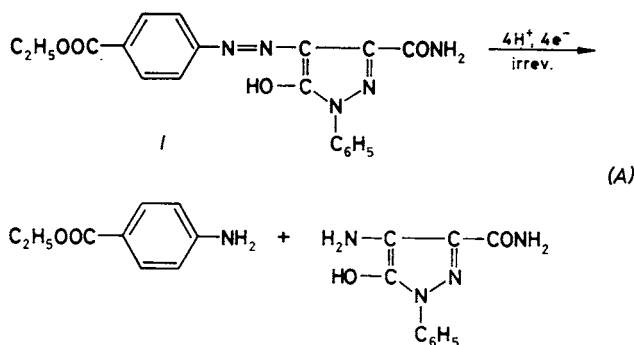


TABLE II

The parameters of the calibration straight lines and calculated detection limits for various methods of determination of the studied substance.

Method	$c$ $\text{mol l}^{-1}$	$a/s_a$ $\text{mA mol}^{-1} \text{ l}$	$b/s_b$ $\text{nA}$	$s_{T,c}$ $\text{nA}$	$r$	$dl$ $\text{mol l}^{-1}$
DC at DME	$(1-5) \cdot 10^{-5}$	4.75/0.39	-2.91/1.18	1.52	0.9998	$1.3 \cdot 10^{-6}$
TAST at DME	$(1-10) \cdot 10^{-6}$	4.99/0.23	1.21/0.79	0.76	0.9965	$7.0 \cdot 10^{-7}$
DPP at DME	$(1-10) \cdot 10^{-7}$	9.57/1.15	-1.28/0.77	0.73	0.9788	$3.5 \cdot 10^{-7}$
DPP at SMDE	$(1-10) \cdot 10^{-8}$	25.5/0.95	0.13/0.06	0.06	0.9979	$8.9 \cdot 10^{-9}$
FS DPV at SMDE	$(1-10) \cdot 10^{-8}$	18.1/1.20	0.2/0.80	0.80	0.9965	$6.9 \cdot 10^{-9}$
LSV at HMDE	$(1-10) \cdot 10^{-9}$	253.0/22.2	0.40/0.15	0.14	0.9886	$1.6 \cdot 10^{-9}$

$a/s_a$  — slope/its standard deviation;  $b/s_b$  — intercept/its standard deviation;  $S_{T,c}$  — standard deviation of the experimental points from the calculated straight line;  $r$  — correlation coefficient;  $dl$  — detection limit.

### Differential Pulse Polarography at a Dropping Mercury Electrode

The dependence of the position ( $E_p$ ) and height ( $I_p$ ) of the peak on the pH retains the same character as in DC polarography (see Table I). It was also found that, in agreement with the theory, the peak height increases with increasing modulation amplitude, drop time and height of the mercury reservoir. The dependence of the peak height on the depolarizer concentration in the range  $5 \cdot 10^{-5} - 10^{-7} \text{ mol l}^{-1}$  is linear. Table II lists the parameters of this dependence measured at a mercury reservoir height of 25 cm, drop time of 1 s, modulation amplitude of 50 mV and polarization rate of  $2 \text{ mV s}^{-1}$ , along with the calculated detection limit.

### Differential Pulse Polarography and Fast Scan Differential Pulse Voltammetry at a Static Mercury Drop Electrode

These techniques were employed to measure the concentration dependence in the range  $10^{-6} - 10^{-8} \text{ mol l}^{-1}$ . The DPP measurements were carried out at a drop time of 1 s, modulation amplitude of 50 mV and polarization rate of  $10 \text{ mV s}^{-1}$ . FS DPV was carried out using the automatic program with a modulation amplitude of 50 mV and polarization rate of  $20 \text{ mV s}^{-1}$ . This program permits maintenance of a constant "pre-sweep delay" of 4 s in all measurements. The dependence of the peak height on the depolarizer concentration was found to be linear in the studied

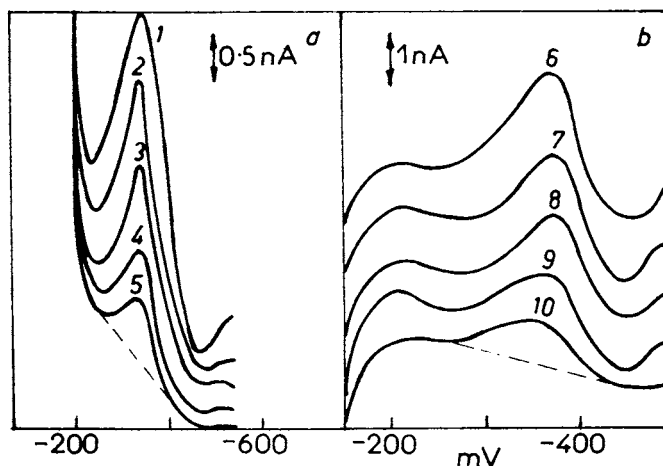


FIG. 3

FS DPV at SMDE (a) and LSV at HMDE (b) recordings. Conditions given in the text. Depolarizer concentration ( $\mu\text{mol l}^{-1}$ ): 1 0.1; 2 0.08; 3 0.06; 4 0.04; 5 0.02; 6 0.01; 7 0.008; 8 0.006; 9 0.004; 10 0.002. The dashed line represents the base line from which the peak heights were calculated

range in both methods and its parameters are given in Table II together with the detection limit. It can be seen that use of the SMDE, especially in combination with FS DPV, leads to a substantial decrease in the detection limit with simultaneous decrease in the analysis time.

### *Voltammetry with Linearly Increasing Potential at a Hanging Mercury Drop Electrode*

This method permits determination of very low concentrations of the studied substance because of the low noise level compared to pulse techniques and also because of the possibility of increasing the signal through accumulation of the depolarizer through adsorption on the surface of the working electrode. Preliminary experiments indicated that the peak height increases with increasing time from the formation of the drop up to recording of the voltammetric curve. The maximal signal value was attained after 60 second accumulation in a stirred solution and a rest period of 30 seconds in quiescent solution. The optimal polarization rate was  $20 \text{ mV s}^{-1}$ . Table II lists the parameters of this concentration dependence and the calculated detection limit.

Fig. 3 depicts for the purposes of illustration the FS DPV recordings at the SMDE and LSV at the HMDE for the lowest concentration range and the method employed in evaluation. The selectivity of these electrochemical methods can be increased by combination with thin layer chromatography<sup>2</sup>.

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