Polarographic Study of 3-(4,5-Dimethyl-2-thiazolylazo)-2,6-diaminopyridine in Aqueous Methanol Medium

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The polarographic behaviour of the azo dye 3-(4,5-dimethyl-2-thiazolylazo)-2,6-diaminopyridine (DMTADAP) in methanol - water (65 + 35) at 20 °C was studied using various electrochemical techniques. In acidic media the reduction process involved two electrons, was diffusion controlled and showed a certain level of reversibility, whereas in neutral and alkaline media four electrons were consumed. In the latter instance a strong adsorption effect was apparent and the irreversibility of the process increased. The linear relationship between limiting diffusion current and DMTADAP concentration allowed the polarographic determination of DMTADAP over a wide concentration range, from 8.0×10^{-7} to 6.8×10^{-5} M at pH 6.3, with a relative standard deviation of 1.2% (ten determinations at the 5.0×10^{-6} M level).

Keywords: *3-(4,5-Dimethyl-2-thiazolylazo)-2,6-diaminopyridine determination; differential-pulse polarography; cyclic voltammetry*

Azo dye compounds are widely used as additives in many areas, such as foods, drugs and cosmetics.[1] Their potential carcinogenicity[2,3] and the possibility of their inhibiting the growth of animal tumours[4] has led to studies of the mechanism of their polarographic reduction, which parallels the metabolism of these compounds *in vivo*.[5]

Aromatic azo dyes have been the subject of many polarographic investigations, which have shown that even the simplest of these compounds has a complicated polarographic behaviour[6,7] and that the introduction of substituent groups into the aromatic rings can completely change the nature of the electrode reaction.[8,9]

Polarographic studies of heterocyclic azo compounds, especially those containing the thiazolyl group, are scarce.[10-12] The aim of this work was to study the polarographic behaviour of the azo dye 3-(4,5-dimethyl-2-thiazolylazo)-2,6-diaminopyridine (DMTADAP) and to develop a polarographic method for its determination. DMTADAP, synthesized in our laboratories,[13] has known chromogenic[14] and possible anti-cancer[15] properties, which have increased the importance of its determination.

Experimental

Apparatus

Experiments were carried out using a PAR Model 174 polarographic analyser equipped with a PAR Model 303 SMDE cell and a Houston Omnigraphic *X* - *Y* recorder. A three-electrode semi-micro cell with a working volume of 10.0 ml was employed, containing a dropping mercury electrode (capillary tube of i.d. 0.015 mm) as the indicator electrode, saturated Ag - AgCl - KCl as the reference electrode and a platinum wire as the auxiliary electrode.

Polarography was performed in the sampled d.c., normal and differential-pulse polarographic (DPP) modes. A pulse of 25 mV was applied using a drop time of 0.5 s; the electrode area was 0.96 mm² (average value standardised by EG & G Princeton Applied Research for the GO 17K capillary used). Scans were recorded from -0.10 to -1.0 V (*vs.* Ag - AgCl) at a scan rate of 5 mV s^{-1} with a full range of 1.5 V.

Cyclic voltammetric measurements were made using a PAR Model 174A polarograph coupled with a PAR 175 unit and used together with a PAR SMDE 303 working electrode and an Ag - AgCl reference electrode.

Coulometric measurements at a controlled potential were made with a PAR 179 digital coulometer connected to a PAR 173 potentiostat. A platinum cylinder (diameter 3.6 cm, height 2 cm) was used as the working electrode.

All the experiments were performed at 20 ± 1 °C. Dissolved air was removed from the solutions by bubbling oxygen-free nitrogen through the cell for 8 min.

The pH of the solutions was monitored with a Radiometer pHM64 digital pH - voltmeter using a combined glass - calomel electrode.

Reagents

Analytical-reagent grade chemicals and solvents were used throughout without further purification.

DMTADAP was synthesized by a modification of the Jansen technique,[16] as described by Gallo *et al.*[13] The purity was $99.08 \pm 0.01\%$.[13] A stock solution of DMTADAP (10^{-3} M) was prepared in methanol. A Britton - Robinson buffer consisting of 0.04 M boric acid, 0.04 M phosphoric acid and 0.04 M acetic acid was used as the supporting electrolyte, the pH being adjusted by addition of 1 M potassium hydroxide solution and 1 M hydrochloric acid. The ionic strength was adjusted to 0.25 M with potassium chloride.

Results and Discussion

The effect of pH on the current - voltage peaks in DPP was investigated by recording polarograms of aqueous methanolic solutions of DMTADAP at a concentration of 4×10^{-5} M in Britton - Robinson buffers. Differential pulse polarograms obtained over the pH range 2.7–11.9 are shown in Fig. 1.

DMTADAP exhibits a single polarographic peak over the whole pH range studied. The peak potential, E_p, is shifted linearly to more negative potentials when the pH is increased, indicating that hydrogen ions are consumed in the reduction
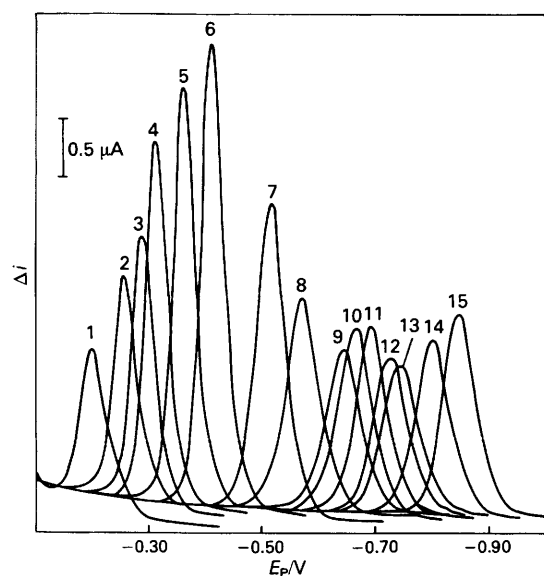
Fig. 1. Differential-pulse polarograms of DMTADAP (4.0×10^{-5} M) in 0.25 M potassium chloride solution and methanol - water ($65 + 35$) at different pH values. pH: 1, 2.68; 2, 3.29; 3, 3.66; 4, 3.96; 5, 4.50; 6, 5.10; 7, 6.21; 8, 7.23; 9, 7.63; 10, 8.13; 11, 8.80; 12, 9.44; 13, 9.74; 14, 10.68; and 15, 11.88
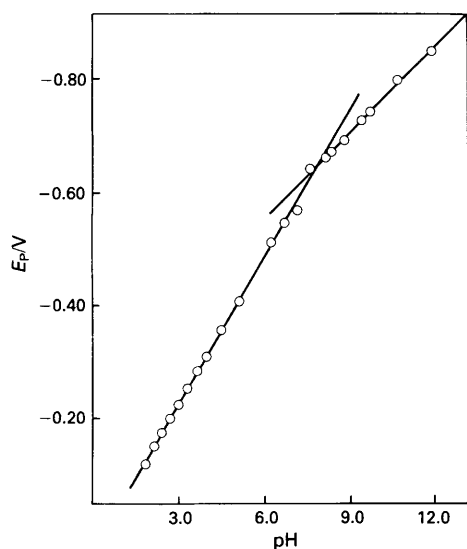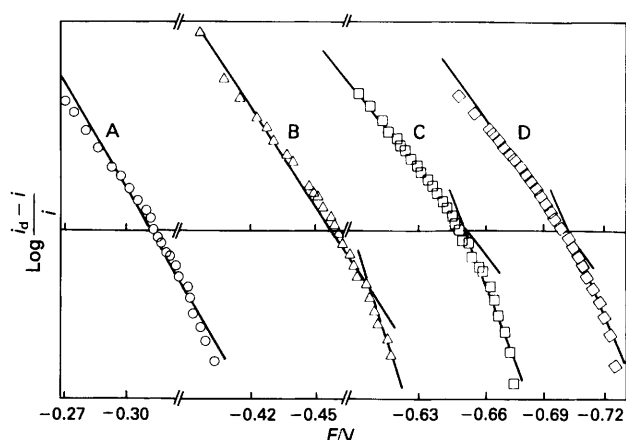

Fig. 3. Logarithmic analysis of DMTADAP (4.84×10^{-5} M) in 0.25 M potassium chloride solution and methanol - water ($65 + 35$) at different pH values. pH: A, 3.23; B, 4.98; C, 7.72; and D, 8.69

Fig. 2. Influence of pH on DMTADAP peak potentials. Conditions as in Fig. 1

Table 1. α_n values obtained from logarithmic treatment in sampled d.c. polarography for different pH values

pH	α_n
3.33	1.89
4.98*	1.89, 3.78
7.72*	1.42, 4.01
8.69*	1.53, 2.83

* At these pH values there are two α_n values, as there are two linear portions in the plot of $\log[(i_d - i)/i]$ versus E.

process. The plot of E_p vs. pH (Fig. 2) shows two linear portions with a break at pH 7.9, corresponding to the dissociation constant of the azo dye reduction product.[17] In the first linear portion (pH range 2.0–7.9), the peak potential may be expressed by the equation

$$E_p = -0.03 - 0.086\text{pH} \qquad .. \quad .. \quad (1)$$

and in the second portion (pH range 7.9–12.0) by

$$E_p = -0.26 - 0.050\text{pH} \qquad .. \quad .. \quad (2)$$

The peak current is also pH dependent, the highest current being obtained at pH $\approx$ 5.

The reversibility of the electrode process was studied using sampled d.c., normal pulse and differential pulse polarography and cyclic voltammetry, applying different criteria depending on the technique chosen.

From logarithmic analyses $\{\log[(i_d - i)/i]$ vs. $E\}$[18] of the sampled d.c. polarograms, α_n values were obtained. As can be seen in Fig. 3, the α_n values are pH dependent. At pH < 4 the

plot shows a single straight line, whereas at pH > 4 two linear portions appeared. The α_n values summarised in Table 1 indicate the tendency of the system to be more reversible at pH < 4, whereas at higher pH values the process becomes more irreversible.

Normal pulse polarographic criteria[19] showed the greater reversibility of the reduction process in an acidic medium. According to the criteria of Birke et al.[20] for DPP, a certain level of reversibility close to quasi-reversibility is observed over the whole pH range studied, although for pH > 4 the irreversibility increased.

The existence in cyclic voltammetry[21] of both anodic and cathodic waves (Fig. 4) at pH < 4 indicates the reversibility of the process. For higher pH values the anodic wave disappears, which can be explained by the irreversibility of the system or an adsorption effect of the compound on the electrode surface. This effect can be demonstrated by the asymmetric shape of the wave and the tensammetric cathodic peak existing in the cyclic voltammograms (Fig. 5).

In conclusion, all the criteria applied indicated that the reduction showed a tendency towards reversibility in acidic media, but the reversibility decreased as the pH increased.

The reduction process is mainly diffusion controlled, as deduced from the linear relationship between peak current and concentration of DMTADAP. For neutral and alkaline media a strong adsorption component is added to the diffusion control.

The number of electrons involved in the reduction process, determined by controlled-potential coulometry, was pH dependent, in agreement with Florence,[8,9] being equal to 2 at pH 3.0 and 4 between pH 7 and 9.

Mechanism of the Reduction Process

At pH < 4, the process involves two protons and two electrons, the reduction product being the corresponding hydrazo compound. When the pH is increased, the hydrazo
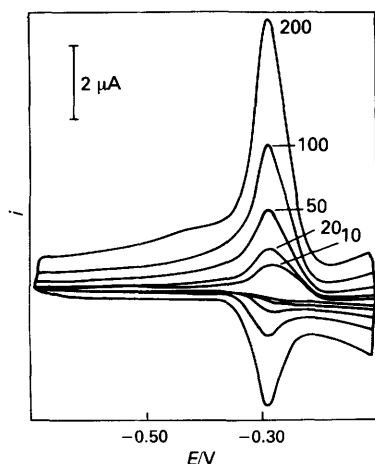
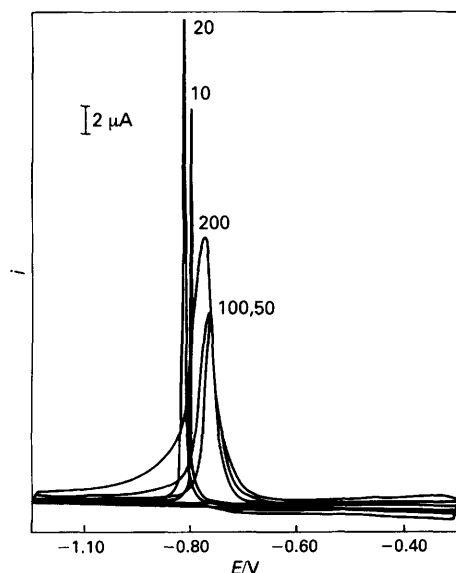**Fig. 4.** Cyclic voltammograms of DMTADAP at pH 3.0 for different scan rates


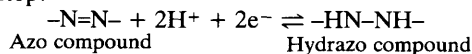**Fig. 5.** Cyclic voltammograms of DMTADAP at pH 9.0 for different scan rates

compound formed in the first step of the reduction is capable of being reduced further to the corresponding amine, involving four electrons, as was calculated by coulometry and logarithmic analysis $\{\log[(i_d - i)/i]$ vs. $E\}$. This treatment showed two linear portions, which indicated the two steps of the reduction.

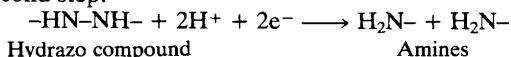The reduction process at pH 3.0–4 can be expressed as

$$-N=N- + 2H^+ + 2\ e^- \rightleftharpoons -HN-NH-$$

Azo compound   Hydrazo compound
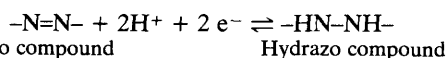
In neutral and alkaline media, the process can be represented as follows:

First step:

$$-N=N- + 2H^+ + 2e^- \rightleftharpoons -HN-NH-$$

Azo compound   Hydrazo compound

Second step:

$$-HN-NH- + 2H^+ + 2e^- \longrightarrow H_2N- + H_2N-$$

Hydrazo compound   Amines

This reduction mechanism is in agreement with Florence et al.[10] and Arias et al.[11] for the reduction of pyridyl and thiazolylazo compounds that have electron-releasing substituents. Florence[9] reported the influence of the pH on the stability of the hydrazo compound, being higher in acidic media.

Polarographic Determination of DMTADAP

From the relationship between peak current and concentration in DPP, a polarographic method for the determination of DMTADAP was developed.

Calibration graphs were constructed at two pH values, 4.2 and 6.3, and were linear over the concentration ranges 1.5×10^{-6}–3.6×10^{-5} M at pH 4.2 and 8.0×10^{-7}–6.8×10^{-5} M at pH 6.3 with relative standard deviations of 1.0% and 1.2%, respectively (ten determinations at the 5.0×10^{-6} M level). The slopes of the calibration graphs at pH 4.2 and 6.3 were 5.72×10^4 and 4.83×10^4 μA l mol^{-1}, respectively, with correlation coefficients of 0.9994 and 0.9994, respectively.

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