THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 5-(3-PHENOXYSULPHENYLAZO)-6-HYDROXY-3-CYANO-1,4-DIMETHYL-2-PYRIDONE*

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The polarographic reduction of the title azodye has been studied, a mechanism was proposed and optimal conditions were found for the determination of this substance by TAST polarography in the range $5 \cdot 10^{-5} - 2 \cdot 10^{-6}$ mol l^{-1} and differential pulse polarography at a dropping mercury electrode in the range $5 \cdot 10^{-5} - 2 \cdot 10^{-8}$ mol l^{-1}. The sensitivity was further increased by adsorptive accumulation of the determined substance on the surface of a hanging mercury drop electrode with linear scan voltammetry (determination limit $5 \cdot 10^{-10}$ mol l^{-1}). The selectivity was increased by prior separation of the determined azodye using thin layer chromatography and by transferring the substance adsorbed on the surface of the hanging mercury drop to a new base electrolyte solution.

So far, primarily spectrophotometric methods have been used for the analysis of dyes and dye intermediates and for their determination in waste waters and in the working or natural environment, while polarographic and voltammetric methods have been rather neglected. Nonetheless, their sensitivity, applicability to an unusually wide concentration range and also their low cost and availability are highly satisfactory for requirements in toxicology, ecotoxicology and environmental regulation enforcement. Modern polarographic and voltammetric methods can even be used to control the purity of special dyes, which is essential for their use in modern applications, such as electronics, liquid crystals, lasers, solar cells etc. The broad usefulness of polarography in the analysis of dyes and dye intermediates follows from the fact that all organic dyes can be reduced polarographically and most dye intermediates can be reduced or oxidized electrochemically under suitable conditions[1].

This work is devoted to the polarographic and voltammetric determination of 5-(3-phenoxysulphenylazo)-6-hydroxy-3-cyano-1,4-dimethyl-2-pyridone (formula *I* in Eq. (*A*)), which is an industrially manufactured azodye. Because of the limited solubility of this substance in water, its titanometric[2], spectrophotometric[3] and

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polarographic[4] determinations have been studied in acetonitrile medium. The polarographic behaviour of variously substituted azopyridines has been studied in water[5], acetonitrile[6] and other aprotic solvents[7], and the effect of substituents and the solvents on the polarographic behaviour of variously substituted azocompounds with a pyridine ring has been studied[8,9]. The polarographic behaviour of various types of azocompounds is surveyed in monographs[10-13]. However, no reference has been found to the polarographic behaviour of the title compound. Thus, this work describes a detailed study of the polarographic behaviour of this compound in mixed water-methanol medium, ensuring sufficient solubility, that is easier to use than anhydrous acetonitrile medium. Polarographic techniques that have been found to be useful for the analysis of azodyes were employed[14,15], namely TAST polarography, differential pulse polarography (DPP), fast scan differential pulse voltammetry (FS DPV) and linear scan voltammetry (LSV).

EXPERIMENTAL

Reagents

The stock solution of the studied azodye ($c = 1 . 10^{-4}$ mol l^{-1}) was prepared by dissolving the pure substance (Research Institute of Organic Synthesis, Pardubice-Rybitví) in p.a. methanol. Solutions with lower concentrations were prepared by precise dilution of the stock solution with methanol. All the solutions were stored in the dark. The content and purity of the substance were controlled titanometrically[2], by thin-layer chromatography[3] and elemental analysis. Britton–Robinson buffer solutions were prepared in the usual manner[16]. The actual pH value of the methanol–buffer mixture (1 : 1) was determined using a combined glass-calomel electrode, GK 2320c (Radiometer, Copenhagen) calibrated using acetate, borate and phosphate buffers in 50% (v/v) methanol[17,18]. The remaining chemicals and solvents were of p.a. purity (Lachema, Brno). The water used was doubly distilled in a quartz apparatus.

Apparatus

Polarographic and voltammetric measurements were carried out using a PA 3 polarographic analyzer with an XY-4105 recorder (Laboratorní přístroje, Prague) in a three-electrode arrangement with a saturated calomel reference electrode (SCE) and platinum wire auxiliary electrode. All the potential values are related to the SCE. Where not stated otherwise, measurements at the classical dropping mercury electrode (i.e. DC, TAST, DP and AC techniques) were carried out at a polarization rate of 5 mV s^{-1}, electronically controlled drop time of 1 s, mercury reservoir height of 36 cm and amplitude modulation in DPP of −100 mV. The dropping mercury electrode had the following parameters: at a mercury reservoir height of $h = 36$ cm, the drop time $\tau = 3.91$ s and the mercury flow rate $m = 1.53$ mg s^{-1}. (The drop time was measured in 0·1 mol l^{-1} KCl at a potential of 0 V). The working electrode in LSV, CVA and FS DPV was a static mercury drop electrode SMDE 1 (Laboratorní přístroje, Prague) with a capillary diameter of 0·136 mm connected as a hanging mercury drop electrode (HMDE). Where not stated otherwise, a polarization rate of 20 mV s^{-1} and maximal drop size determined by opening the valve for 160 ms were used.

Oxygen was removed from the analyzed solutions by bubbling for ten minutes with nitrogen that was purified by passing through an alkaline solution of sodium anthraquinone-2-sulphonate and an acid solution of chromium(II) ions over zinc amalgam. A prebubbler with a methanol–water mixture (1 : 1) was placed prior to the polarographic vessel.

Coulometric measurements were carried out using an OH 404 coulometric analyzer (Radelkis, Budapest) permitting automatic compensation of the residual current and digital integration of the current passed. An all-glass vessel with a volume of 200 ml was employed, with cathode and anode spaces separated by a frit. The mercury pool was used as the cathode, with a saturated calomel reference electrode and platinum foil auxiliary electrode. During the determination, the solution was stirred with a magnetic stirrer and an inert atmosphere was ensured by passing nitrogen over the surface of the solution.

Spectrophotometric measurements were carried out on a Pye Unicam PU 8800 instrument (Philips, Cambridge) in 0·5 and 1 cm quartz cuvettes.

All the measurements were carried out at laboratory temperature.

Procedure

The calibration curves were measured in triplicate and evaluated by the method of linear regressions. The detection limit was found by the method of Skogerboe and Grant[19] as the value ts/a, where s is the standard deviation of the experimental points from the calculated calibration straight line, a is the slope of this line and t is the Student coefficient at the 99% confidence level, which depends on the number of points used to construct the calibration curve.

Coulometry at constant potential was carried out by measuring 50 ml of the given buffer into the coulometric vessel with 40 ml of methanol and bubbling with nitrogen. Pre-electrolysis was commenced simultaneously at a preset potential. After about 20 min, the residual current value decreased to below 0·2 mA and no longer changed. Then the appropriate parameters of the circuit were set for automatic residual current compensation and 10·0 ml of a $1 \,.\, 10^{-4}$ mol l^{-1} solution of the studied substance in methanol, which had also been prebubbled with nitrogen, was added with constant stirring and bubbling with nitrogen. The electrolysis was terminated when the current decreased to the residual value (c. 50 min) and the charge passed was determined by digital integration of the current. The reaction was followed spectrophotometrically and polarographically by removing 10 ml of the solution from the coulometric vessel at a given time and measuring its TAST polarographic curve and UV-VIS spectrum. Sampling was carried out before commencing the coulometric reduction and after reduction of 25, 50, 75 and 100% of the studied substance (calculated assuming exchange of 4 electrons).

In measuring the electrocapillary curves the drop time was measured using an electronic stopwatch as the average time for 10 drops. The surface tension was calculated from the measured drop time τ using the relationship $\Delta\sigma/\sigma = 0{\cdot}973\, \Delta\tau/\tau$, where σ and τ are the values for the corresponding aqueous solution of 0·1 mol l^{-1} $NaNO_3$ and $\Delta\sigma$ and $\Delta\tau$ are the changes in these quantities on transfer to the studied solution[20].

Thin-layer chromatography was carried out on commercial Silufol UV 366 thin layers (Kavalier, Votice) by the ascending technique in an atmosphere saturated with the vapours of the chloroform methanol (3 : 2) elution mixture. An amount of 5 to 125 µl of a solution of the azodye in methanol ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) was applied to the thin layer using a Hamilton microsyringe. (Volumes of 50 µl and more were applied repeatedly after evaporation of the previous amount using hot air). After development and drying of the chromatogram, the band of the determined substance was cut out and eluted by the descending method using methanol in an atmosphere saturated with methanol vapours directly into the polarographic vessel. (Detection was not necessary because of the intense yellow colour of the determined substance.) After vaporization of the solvent with hot air, 10·00 ml of the base electrolyte were added (Britton–Robinson buffer –

– methanol (1 : 1), pH 7·09) and the solution was polarographed in the usual manner using the FS DPV technique at an HMDE. For the applied amounts of azodye, the concentration in the polarographed solution varied from $5 \cdot 10^{-8}$ to $1{\cdot}25 \cdot 10^{-6}$ mol l^{-1}.

RESULTS AND DISCUSSION

The Polarographic Behaviour of the Studied Azodye

The effect of the pH on the TAST and DP polarograms of the studied substance is given in Table I. At all the pH values, the studied substance exhibited a single wave or peak, apparently corresponding to the reduction of the azogroup. The value of the slope of the dependence of the half-wave potential on the pH calculated by linear regression for the TAST polarographic results has a value of $dE_{1/2}/dpH = -66{\cdot}8$ mV for the pH range 2–8 and $dE_{1/2}/dpH = -17{\cdot}5$ mV in the range pH 9–13. A dependence of the peak potential on the pH of $dE_p/dpH = -64{\cdot}6$ mV for pH 2–8 and $dE_p/dpH = -12{\cdot}3$ mV for pH 9–13 was found for DP polarography. This shift of the $E_{1/2}$ or E_p value to more negative values with increasing pH has already been explained[15]. The dependence of the wave height on the pH

TABLE I

The effect of the pH on the TAST and DP polarograms of the test azodye ($c = 5 \cdot 10^{-5}$ mol l^{-1}) in Britton–Robinson buffer – methanol medium (1 : 1)

pH	$E_{1/2}$ mV	I_{lim} μA	E_p mV	I_p nA	χ^a mV
2·33	−375	124	−350	352	73·3
2·55	−385	126	−370	397	83·4
3·83	−465	128	−450	362	81·3
4·84	−540	133	−520	383	62·9
5·76	−600	133	−580	383	100·2
6·98	−685	142	−660	353	106·4
8·03	−750	159	−715	495	76·6
8·56	−775	195	−730	513	74·5
9·18	−785	186	−750	418	44·1
9·83	−800	183	−755	320	38·9
10·65	−820	180	−760	335	64·3
11·42	−835	180	−775	320	61·9
11·61	−845	163	−780	313	50·7
12·69	−835	128	−780	240	49·5

[a] Slope of the dependence of E on $\log (I_{lim} - I)/I$.

in TAST polarography is not very marked. It cannot be attributed to a change in the number of exchanged electrons, but rather to different diffusion coefficient values. The effect of aggregation of the azodye molecules, which could occur at these concentrations, cannot be excluded. The dependence of the peak height on the pH in DPP reflects both a change in the wave height in TAST polarography and a change in its reversibility.

For analytical applications, the highest and best developed wave or peak is obtained at pH = 8·56, and all subsequent dependences were measured at this value. In this medium, the wave height is diffusion-controlled, as was confirmed by measuring the dependence of the DC wave height on the square root of the mercury reservoir height (a straight line passing through the origin was obtained) and by verifying the constancy of the expression $k = I_{\mathrm{lim}}/m^{2/3}\tau^{1/6}$ in the case of TAST polarography (in the range $m = 1-4\ \mathrm{mg\ s^{-1}}$ and $\tau = 1-4\ \mathrm{s}$, it holds that $k = (0{\cdot}29 \pm 0{\cdot}01)\ \mathrm{mA\ g^{-2/3}\ s^{1/2}}$). The diffusion character of the measured currents is also confirmed by the linearity of the concentration dependences.

It followed from logarithmic analysis of the TAST polarographic curves and from the observed dependence of $E_{1/2}$ on the concentration of the test substance that the process is not reversible. This fact was also confirmed by DPP with positive and negative pulse polarity[21] and by cyclic voltammetry at a hanging mercury drop[22].

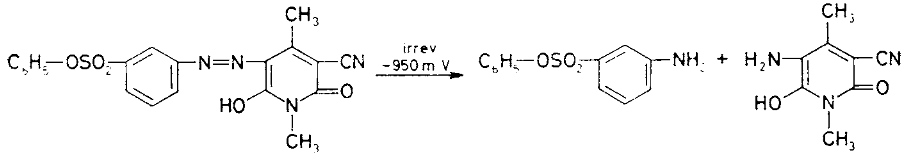
In the former case, the dependence of the height and position of the DP peak was measured for variation of the height and polarity of the modulation pulses at pH 4·84, 8·56 and 10·65. It was found that the ratio of the peak height for anodic and cathodic polarity of the modulation pulses is less than 1, whereas the difference in the peak potential for cathodic and anodic polarity of the modulation pulses corresponds to the modulation amplitude. According to the criteria derived by Birke[21] for simple charge transfer, this behaviour corresponds to an irreversible system.

In study of the reversibility of the system using cyclic voltammetry, no anodic peak was observed in the pH range 2 to 13 at polarization rates of $1-500\ \mathrm{mV\ s^{-1}}$. It follows from the measured effect of the polarization rate on the height and position of the cathodic peak at pH 8·61 that the peak height increases with the square root of the polarization rate. However, this dependence is not linear, as required by the equations[22] for a simple, totally irreversible process. It could be assumed that the studied substance is adsorbed on the surface of the working electrode.

Adsorption of the studied azodye on the mercury electrode was also confirmed by AC polarography[23] and by measuring the electrocapillary curves[24]. A decrease on the AC polarographic current in the presence of the studied substance in the region from 0 to −0·3 V below the value for the base electrolyte can be seen in Fig. 1, indicating[23] that the initial substance is adsorbed on the surface of the droping mercury electrode. Similarly, the decrease in the surface tension in the presence of the studied substance (Fig. 2) indicates that it is adsorbed on the working electrode. This fact is also confirmed by the dependence of the peak height in FS DPV or LSV at the HMDE on the time elapsed between formation of the drop and recording of the voltammogram.

It was found by coulometry at constant potential of −950 mV, corresponding to the limiting current in the Britton–Robinson buffer – methanol (1 : 1) medium, pH 8·56, for the studied substance, that a total of 4 electrons are exchanged under these conditions (see Fig. 3). It can be seen from the TAST polarographic study of the coulometric reduction (Fig. 4) that the polarographically active group disappears and the product formed is not polarographically active in the studied potential range.. It follows from the spectrophotometric study of the coulometric reduction (Fig. 5) that it involves reduction of the chromophore, i.e. the azo group. Apparently, the double bond between the oxygen and carbon in the dihydropyridine ring is not reduced.

The above observations indicate that the studied azodye undergoes irreversible diffusion-controlled four-electron reduction under the above conditions, according to Eq. (*A*).



(*A*)

I

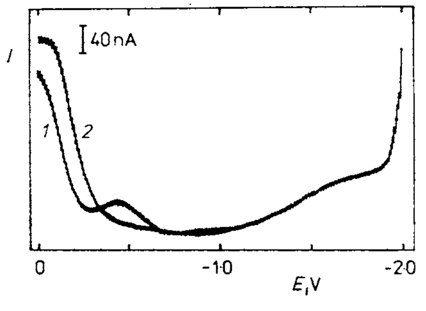


FIG. 1

The AC polarogram of a 5 . 10^{-4} mol l^{-1} solution of the studied substance (*1*) in Britton–Robinson buffer – methanol medium (1 : 1), pH 8·56 and the curve for the base electrolyte (2)

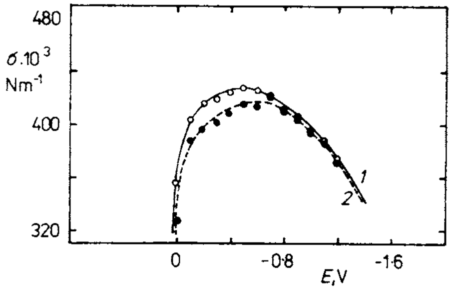


FIG. 2

The dependence of the surface tension on the potential for the base electrolyte (*1*) and for a solution of the studied substance ($c =$ $= 5 . 10^{-5}$ mol l^{-1}) in a base electrolyte of Britton–Robinson buffer – methanol (1 : 1), pH 8·56 (2)

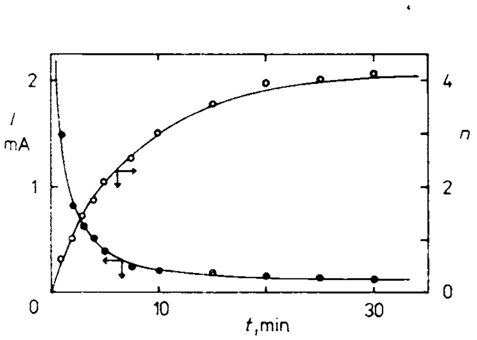


FIG. 3

The dependence of the electrolytic current I and number of exchanged electrons n on time during the reduction of the studied substance by constant potential coulometry at -950 mV in Britton–Robinson buffer – methanol medium (1 : 1), pH 8·56

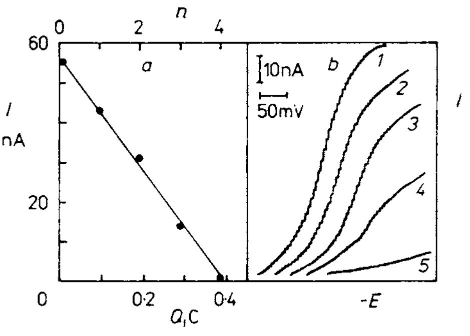


FIG. 4

TAST polarographic study of the reduction of the test substance by constant potential coulometry at -950 mV in Britton–Robinson buffer – methanol medium (1 : 1), pH 8·56. *a* Dependence of the limiting current on the charge passed, Q, recalculated to the number of electrons, n, per molecule of reduced substance; *b* TAST polarograms of the solutions after passage of charge corresponding to $n = 0$ (*1*), 1 (*2*), 2 (*3*), 3 (*4*) and 4 (*5*). Initial potential -650 mV

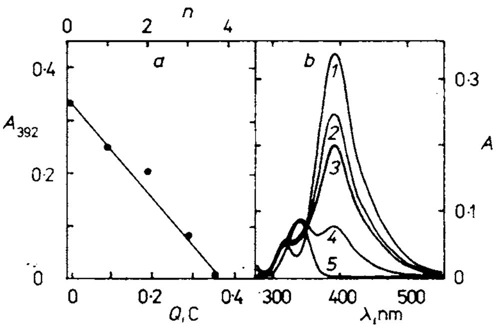


FIG. 5

Spectrophotometric study of the reduction of the test substance by constant potential coulometry at -950 mV in Britton–Robinson buffer – methanol medium (1 : 1), pH 8·56. *a* Dependence of the absorbance at 392 nm on the charge passed, Q, recalculated to the number of electrons, n, per molecule of substance reduced; *b* spectra of the solution after passage of charge corresponding to $n = 0$(*1*), 1(*2*), 2(*3*), 3(*4*), and 4(*5*)

Polarographic and Voltammetric Determination of the Studied Azodye

It followed from spectrophotometric study of the stability of the stock solution of the studied substance in methanol ($c = 10^{-4}$ mol l^{-1}) that its absorbance did not change by more than ±0·2% in the region of the maximum at 429 nm over 30 days and did not decrease by more than 1·2% over 60 days. The molar absorptivity was found to equal 40 500 mol^{-1} l cm^{-1}. The absorbance of a solution of the studied azodye with $c = 10^{-5}$ mol l^{-1} in methanol did not decrease by more than 0·2% over 30 days and 1% over 60 days. More dilute solutions were prepared fresh daily. All the solutions were stored in the dark.

The stability of 10^{-5} to 10^{-7}M solutions of the azodye in the polarographic medium (i.e. Britton–Robinson buffer – methanol (1 : 1), pH 8·56) was studied using DPP at the DME. It was found that the peak height did not change over 30 minutes after preparation of a 10^{-5} to 10^{-6}M solution and, after 60 and 90 minutes, respectively, decreased by 0·6 and 1·2% for a 10^{-5}M solution and 2 and 3% for a 10^{-6}M solution. The decrease for a 10^{-7}M solution was 0, 3, 6 and 8% after 15, 30, 60 and 90 minutes, respectively. Thus, it is necessary to polarograph more dilute solutions at the shortest possible, constant time after preparation.

During TAST polarography in Britton–Robinson buffer – methanol solution (1 : 1), pH 8·56, the dependence of the wave height on the concentration is linear in the range 5 . 10^{-5} to 2·10^{-6} mol l^{-1}. The parameters of the calibration straight line calculated by the least squares method together with the calculated determination limit are given in Table II. The half-wave potential of the studied azodye shifts with decreasing concentration to more positive values (roughly by 10 mV per concentration order), which is apparently connected with the irreversible character of the reaction.

The calibration curves for DPP at the DME in this medium are linear in the concentration range 1 . 10^{-5} to 2 . 10^{-8} mol l^{-1} and their parameters and the determination limits are given in Table II. The peak height was evaluated relative to the line connecting the minima on both sides.

In FS DPV at the HMDE, the effect of the pH was verified at a depolarizer concentration of 1 . 10^{-6} mol l^{-1}. The recording was carried out 5 s after drop formation. It was found (see Table III) that the dependence of the peak height and position on the pH is roughly the same as for DPP at the DME. The slope of the dependence of the peak potential on the pH has the value $dE_p/dpH = -65·3$ mV in the range pH 2–8 and $dE_p/dpH = -32·1$ mV for pH 9–13. The peak height is optimal in medium with pH 7·09 and all the subsequent dependences were measured at this value. Under these conditions, the calibration straight lines are linear in the concentration range 10^{-5} to 10^{-7} mol l^{-1} (see Table II).

It was verified that the peak height recorded using the FS DPV technique at the HMDE increases with time elapsed between drop formation and recording of the

TABLE II

Parameters of the calibration curves and detection limits for various methods of determining the test azodye

Method	c µmol l^{-1}	a/s_a [a] nA mol l^{-1}	b/s_b [b] nA	$s_{1.C}$ [c] nA	r[d]	DL[e] mol l^{-1}
TAST at DME	10—50	$37 . 10^5/58 . 10^3$	−1/1·9	1·8	0·9996	—
TAST at DME	2—10	$44 . 10^5/192 . 10^3$	−1·2/1·3	1·2	0·9971	$1·3 . 10^{-6}$
DPP at DME	10—50	$102 . 10^5/75 . 10^3$	−1·6/2·9	2·4	0·9999	—
DPP at DME	2—10	$105 . 10^5/287 . 10^3$	4·3/1·9	1·8	0·9988	—
DPP at DME	0·2—1	$119 . 10^5/200 \cdot 10^3$	0·8/0·3	0·2	0·9996	—
DPP at DME	0·02—0·1	$167 . 10^5/957 . 10^3$	−0·1/0·1	0·1	0·9951	$1·8 . 10^{-8}$
FS DPV at HMDE	2—10	$2·7 . 10^7/9·3 . 10^5$	−3·3/6·2	5·9	0·9983	—
FS DPV at HMDE	0·2—1	$2·7 . 10^7/5·8 . 10^5$	1·8/0·4	0·4	0·9973	$6 . 10^{-8}$
FS DPV at HMDE[f]	0·02 —0·1	$5·9 . 10^8/2·8 . 10^7$	−3·4/1·9	1·8	0·9966	$1·4 . 10^{-8}$
LSV at HMDE	2—10	$20 . 10^6/7·5 . 10^5$	14/4·9	4·8	0·9978	$1·1 . 10^{-6}$
LSV at HMDE[g]	0·2—1	$51 . 10^6/7·6 . 10^5$	0·1/0·5	0·5	0·9996	$5 . 10^{-8}$
LSV at HMDE[h]	0·02—0·1	$6·8 . 10^8/4·0 . 10^7$	3·8/2·7	2·6	0·9947	$1·8 . 10^{-8}$
LSV at HMDE[h]	0·002—0·01	$5·6 . 10^8/3·8 . 10^7$	0·6/0·2	0·6	0·9765	$3·1 . 10^{-9}$
LSV at HMDE[i]	0·0002—0·001	$2·1 . 10^9/3·3 . 10^8$	0·2/0·2	0·2	0·9644	$4·6 . 10^{-10}$

[a] Slope/its standard deviation; [b] intercept/its standard deviation; [c] standard deviation of the measured points from the calculated straight line; [d] correlation coefficient; [e] detection limit; [f] adsorptive accumulation 300 s in stirred solution; [g] 60 s without stirring; [h] 120 s in stirred solution; [i] 10 min in stirred solution.

curve and can be increased by stirring (see Table IV). This phenomenon can be explained by adsorptive accumulation of the determined substance on the surface of the hanging mercury drop electrode. It is known[25] that adsorptive accumulation can depend on the composition of the solution and potential of the working electrode. Thus the effect of the methanol concentration on the increase in the peak height was measured at an azodye concentration of $2 \,.\, 10^{-8}$ mol l^{-1} and 300 second accumulation with stirring. The following values were found:

Methanol content, % (v/v)	0·2	10	20	30	40	50
I_p, nA	7·5	15	9	12	8	4·5

The effect of the methanol content on the shape and height of the peak under these conditions is illustrated in Fig. 6, indicating that a methanol content of 30% (v/v) is optimal, as the peaks obtained are symmetrical and can be most readily evaluated. On the other hand, it was found that the accumulation potential does not affect the peak height. The calibration straight line for 300 s adsorptive accumulation was

TABLE III

The dependence of the position (E_p) and height (I_p) of the peak of the studied substance ($c = 1 \,.\, 10^{-6}$ mol l^{-1}) on the pH in FS DPV and LSV at the HMDE in Britton–Robinson buffer – methanol medium (1 : 1)

pH	$E_p^{\text{FS DPV}}$ mV	$I_p^{\text{FS DPV}}$ nA	E_p^{LSV} mV	I_p^{LSV} nA
2·45	−235	10	−300	10
2·76	−265	18	−325	11
4·58	−380	26	−445	15
6·05	−475	21	−540	16
7·09	−550	34	−610	18
8·04	−600	30	−660	21
8·89	−625	28	−685	20
9·04	−640	29	−710	24
9·67	−665	25	−730	26
10·21	−685	26	−760	30
11·17	−715	30	−790	27
11·86	−725	27	−805	20
12·88	−760	16	−835	12

measured in Britton–Robinson buffer – methanol medium (7 : 3), pH 7·09. Its parameters and the calculated determination limit are given in Table II.

For LSV at the HMDE, the effect of the pH on the position and height of the peak were first studied in a Britton–Robinson buffer – methanol medium (1 : 1) at an azodye concentration of $1 \cdot 10^{-6}$ mol l^{-1}. It can be seen from the data in Table III that the dependence of the peak potential E_p on the pH has the same character as for DPP at the DME and FS DPV at the HMDE. The slope of the dependence of E_p

TABLE IV

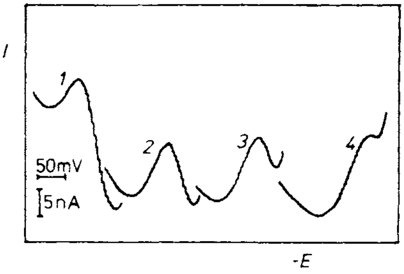
The dependence of the peak height (I_p) on the time of accumulation of the substance (t_a) on the surface of the HMDE in Britton–Robinson buffer – methanol medium (1 : 1), pH 7·09 (FS DPV) or pH 10·21 (LSV). Accumulation potential 0 V; azodye concentration $2 \cdot 10^{-7}$ mol l^{-1}

t_a s	I_p, nA			
	FS DPV[a]	FS DPV[b]	LSV[a]	LSV[b]
5	7·0	10	6·0	12·5
10	8·3	14	6·5	13·5
20	8·7	19	7·5	17·5
30	9·2	26	8·0	21·0
40	10·3	32	8·3	29·5
50	11·0	37	9·7	37·5
60	12·3	43	10·0	45·0
90	15·0	60	12·0	70·0
120	18·5	77	13·5	80·0
180	24·2	110	17·5	61·5
300	34·2	154	25·0	46·0

[a] Accumulation in unstirred solution; [b] accumulation in stirred solution; recorded 10 s after termination of stirring.

FIG. 6

The effect of the methanol content on the shape and height of the peaks of a $2 \cdot 10^{-8}$ mol l^{-1} solution of the studied substance measured by the FS DPV method at the HMDE. Methanol content, %(v/v): *1* 10, *2* 30, *3* 40, *4* 50; pH 7·09; adsorptive accumulation at 0 V with stirring for 300 s; initial potential −350 mV



on the pH has the value $dE_p/dpH = -64.9$ mV in the range pH 2–8 and $dE_p/pH = -36.6$ mV for pH 9–13. The highest and best developed peak was obtained in a medium with pH 10·21, and all subsequent dependences were measured at this value. In LSV an increase in the peak height as a result of adsorptive accumulation was also found (see Table IV), but this dependence was not monotonous. The observed decrease in the peak height for accumulation with stirring for periods of greater than 120 s is apparently connected with the maximal possible coverage of the electrode surface by the adsorbed substance and possible blockage of its surface by the products of the electrochemical reduction process. In contrast, the following dependence of the LSV peak height on the accumulation time was found at an azodye concentration of $2 \cdot 10^{-9}$ mol l^{-1}:

t_a, min:	2	5	10
I_p, nA:	0·9	2·7	6·2

Thus, longer accumulation times can be employed at lower concentrations of the studied substance without danger of blockage of the electrode surface. Study of the effect of the potential of the working electrode for 120 s accumulation with stirring and azodye concentration of $2 \cdot 10^{-7}$ mol l^{-1} yielded the following values:

E_p, mV:	0	−100	−200	−300	−400	−500
I_p, nA:	81	74	64	60	48	38

Thus, accumulation was carried out at a potential of 0 V in all the subsequent measurements. The following values were obtained in a study of the effect of the methanol concentration (φ) on the height of the LSV peak for adsorptive accumulation for 120 s in a stirred solution at an azodye concentration of $2 \cdot 10^{-7}$ mol l^{-1}:

φ, % (v/v):	2	5	10	20	30	40	60	80	90
I_p, nA:	100	102	98	95	92	83	72	61	42

The observed decrease in the peak height with increasing methanol content can be explained both by simultaneous adsorption of methanol on the surface of the working electrode and also because it increases the solubility of the studied azodye in the polarographed solution. For both these reasons, a decrease in the methanol concentration increases the adsorption of the azodye on the working electrode.

The concentration dependences for the LSV technique at the HMDE were measured in the range $1 \cdot 10^{-5}$ to $2 \cdot 10^{-10}$ mol l^{-1}. The calibration curve was measured without accumulation in the concentration range $(2-10) \cdot 10^{-6}$ mol l^{-1} in Britton–Robinson buffer – methanol medium (1 : 1), pH 10·21. In the concentration range $(2-10) \cdot 10^{-7}$ mol l^{-1}, the same medium was employed with 60 s adsorptive accumulation in unstirred solution. In the range $1 . 10^{-7}$ to $2 . 10^{-9}$ mol l^{-1}, 120 s adsorptive accumulation was employed in stirred solution containing 2% (v/v) methanol at pH 10·44. In the lowest concentration range $(2-10) . 10^{-10}$ mol l^{-1}, 10 minute adsorptive accumulation was employed in a stirred solution containing 2% (v/v) methanol at pH 10·21. The Britton–Robinson buffer employed was diluted 100-fold with distilled water to decrease the detrimental effect of impurities present in the buffer on the shape of the base electrolyte curve. The parameters of these calibration curves and the calculated determination limits are listed in Table II. Fig. 7 gives the voltammograms for the lowest concentration ranges. The peak height was always measured from the line connecting the minima on both sides of the peak.

Practical Applications

An attempt to increase the selectivity of the voltammetric determination of the studied azodye led to a study of the possibility of preliminary separation by thin-layer chromatography. Various amounts of substance (5 – 125 µl of a 10^{-4}M solution) were applied to the thin layer and, after development of the chromatogram, were eluted into the polarographic vessel by the procedure given under Experimental. After evaporation of the methanol, the substance was dissolved in 10·00 ml of base electrolyte. An R_F value of 0·73 was found using a chloroform–methanol (3 : 2) elution system. The height of the FS DPV peak was compared with the peak height obtained by measuring solutions prepared by addition of 5 to 125 µl of 10^{-4} mol l^{-1} solution of the studied substance directly to 10·00 ml of the base electrolyte. It can be seen from Table V that very good results can be obtained, especially at lower concentrations. At higher concentrations, the yield decreases slightly, apparently as a result of imperfect elution of the substance from the thin layer.

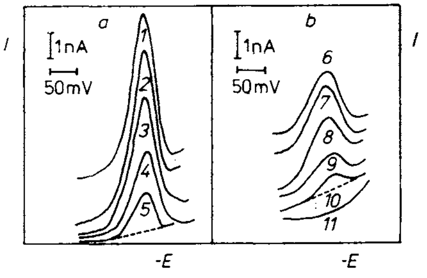


FIG. 7

LS voltammograms of the studied azodye at the HMDE. *a* Accumulation with stirring for 120 s: Britton–Robinson buffer – methanol medium (49 : 1), pH 10·44; azodye concentration (nmol l^{-1}): *1* 10, *2* 8, *3* 6, *4* 4, *5* 2; initial potential –600 mV. *b* Accumulation with stirring for 10 min; medium 100-fold diluted Britton–Robinson buffer – methanol (49 : 1), pH 10·21; azodye concentration (nmol l^{-1}): *6* 1, *7* 0·8, *8* 0·6, *9* 0·4, *10* 0·2, *11* 0; initial potential –650 mV. The dashed line is the baseline from which the peak height was evaluated

Adsorption of the studied substance on the surface of the working electrode can be employed to increase the selectivity of the determination by transferring the adsorbed substance to a pure solution not containing interferents. (This approach can, of course, be used to eliminate interference only from those substances that do not adsorb on the mercury electrode surface.) The usefulness of this technique for the studied azodye was verified as follows: The calibration straight line was measured in the concentration range $(2-10) . 10^{-7}$ mol l^{-1} in Britton–Robinson buffer – – methanol medium (9 : 1), pH 10·21, using LSV at the HMDE with 60 s adsorptive accumulation in unstirred solution. These calibration curves were then compared

TABLE V

Determination of the test azodye by FS DPV at the HMDE after prior separation by TLC

Added μg	Concentration[a] mol l^{-1}	Found % of theoretical
2·12	$5 . 10^{-8}$	101·0
4·24	$1·0 . 10^{-7}$	99·9
10·6	$2·5 . 10^{-7}$	100·2
21·2	$5·0 . 10^{-7}$	98·5
31·8	$7·5 . 10^{-7}$	99·4
42·4	$1·0 . 10^{-6}$	95·2
53·0	$1·25 . 10^{-6}$	94·9

[a] Values correspond to the concentration of the test substance in the voltammetric solution.

TABLE VI

Parameters of the calibration straight lines for the determination of the studied substance in the concentration range $(2-10) . 10^{-7}$ mol l^{-1} by LSV at the HMDE

Method[a]	a/s_a[b] nA mol^{-1} l	b/s_b nA	$s_{1,C}$ nA	r
A	$42·9·10^6/98·10^4$	1·2/0·7	0·6	0·9992
B	$39·6·10^6/60·10^4$	1·3/0·4	0·4	0·9996
C	$42·3·10^6/19·10^5$	0·1/1·3	1·2	0·9969

[a] *A* accumulation for 60 s without solution transfer, *B* accumulation for 60 s from 10 ml of solution with transfer to fresh base electrolyte, *C* the same procedure with 1 ml of solution; [b] significance of the other symbols as in Table II.

with those obtained in the same manner, but with replacement of the original solution by pure base electrolyte. The good agreement of the parameters obtained with and without solution exchange (see Table VI) confirms that it is possible to transfer the electrode with the adsorbed substance to a new base electrolyte solution. Practically the same results were obtained for accumulation of the determined azodye from solution with volumes of 10 ml or 1 ml; thus the amount of solution requited for the analysis can be decreased.

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