POLAROGRAPHIC DETERMINATION OF 4′-SUBSTITUTED DERIVATIVES OF 3-CARBOXY-4-HYDROXY-6-ACETYLAMINOAZOBENZENE

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Received December 21, 1993
Accepted August 25, 1994

Tast polarography, differential pulse polarography and cyclic voltammetry were applied to the examination of 7 derivatives of 3-carboxy-4-hydroxy-6-acetylaminoazobenzene with H, CH_3, OCH_3, Cl, $COCH_3$, NO_2 and $NHCOCH_3$ substituents in position 4′. A general reduction mechanism was suggested for the substances. The half-wave potentials obtained from tast polarographic measurements were correlated with the Hammett σ constants of the substituents in the *para*-position. The plot of this dependence is linear, which suggests that the reduction mechanism is identical within the entire series. The optimum conditions for quantitation of the substances were found within the concentration regions of $1 . 10^{-4}$ to $1 . 10^{-6}$ mol l^{-1} for tast polarography and $1 . 10^{-4}$ to $1 . 10^{-7}$ mol l^{-1} for differential pulse polarography.

Azo dyes constitute the most important class of dyes: their share in commercial sales of dyes is nearly one-half, despite the fact that many of them exhibit adverse genotoxic or ecotoxic effects[1]. 4′-Substituted derivatives of 3-carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-R-CHAAB, see *I* in Eq. (*A*)) form an interesting group of azo dyes. Some of them were examined previously[2] with respect to their application as dyes for polyamide fabrics; the study cited was also aimed at finding relations between the nature of substituent R and some physical and spectral properties of the substances.

The present paper is concerned with the polarographic and voltammetric behaviour of 7 substances from this group. The 4′-substituents were R = H, CH_3, OCH_3, Cl, $COCH_3$, NO_2 and $NHCOCH_3$. We aimed at identifying the optimum conditions for determination of the substances in low concentrations, with regard to the requirements of toxicological testing of new kinds of azo dyes or their metabolites in the blood or urine of test animals. Dyes are among organic substances that are being monitored for toxicity. Traces of dyes in ecosystems in the surroundings of industrial plants, in waters, etc., can be conveniently measured by the modern polarographic methods.

The polarographic behaviour of azo compounds has been studied extensively[3–5], minor attention, however, has been paid to azo pigments insoluble in water[6–10].

For finding the optimum conditions for their quantitation by the sensitive polarographic and voltammetric techniques, their behaviour in the conventional DC polarography must be known. The same results can be obtained by the tast polarographic technique[11], which was employed for a basic study of the azo dyes. Differential pulse (DP) polarography was used as well; this technique is considerably more sensitive than DC or tast polarography[12–14]. In addition to analytical applications, the present paper deals with the mechanism of polarographic reduction of the compounds and with the relation between their structure and polarographic activity. In addition to potentiostatic coulometry, cyclic voltammetry at a hanging mercury drop electrode[15] and correlation of the half-wave potentials with the Hammett substituent constants[16] were employed to investigate the polarographic reduction mechanism. The examination was performed in aqueous-methanolic systems, in which the substances are sufficiently well soluble.

EXPERIMENTAL

Reagents

The azo dyes were synthesized at the Department of Industrial Organic Chemistry, Torino University, Italy, by diazotization and coupling in alkaline solutions[2] and purified by double crystallization from ethanol. Their purity was checked by elemental analysis, melting temperature measurement, thin layer chromatography and UV-VIS spectral measurement. The following substances were studied:

3-Carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-H-CHAAB); $C_{15}H_{13}N_3O_4$; CAS Name: benzoic acid, 4-(acetylamino)-2-hydroxy-5-(phenylazo); CAS Registry Number: 88850-49-1.

4′-Methyl-3-carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-CH_3-CHAAB); $C_{16}H_{15}N_3O_4$; CAS Name: benzoic acid, 4-(acetylamino)-2-hydroxy-5-((4-methylphenyl)azo); CAS Registry Number: 88850-54-8.

4′-Methoxy-3-carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-OCH_3-CHAAB); $C_{16}H_{15}N_3O_5$; CAS Name: benzoic acid, 4-(acetylamino)-2-hydroxy-5-((4-methoxyphenyl)azo); CAS Registry Number: 88850-69-5.

4′-Chloro-3-carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-Cl-CHAAB); $C_{15}H_{12}ClN_3O_4$; CAS Name: benzoic acid, 4-(acetylamino)-5-((4-chlorophenyl)azo)-2-hydroxy; CAS Registry Number: 88850-74-2.

4′-Acetyl-3-carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-$COCH_3$-CHAAB); $C_{17}H_{15}N_3O_5$; CAS Name: benzoic acid, 4-(acetylamino)-5-((4-acetylphenyl)azo)-2-hydroxy; CAS Registry Number: 88850-59-3.

4′-Nitro-3-carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-NO_2-CHAAB); $C_{15}H_{12}N_4O_6$; CAS Name: benzoic acid, 4-(acetylamino)-2-hydroxy-5-((4-nitrophenyl)azo); CAS Registry Number: 88850-64-0.

4′-Acetylamino-3-carboxy-4-hydroxy-6-acetylaminoazobenzene (4′-$NHCOCH_3$-CHAAB); $C_{17}H_{16}N_4O_5$; CAS Name: benzoic acid, 4-(acetylamino)-5-((4-acetylaminophenyl)azo)-2-hydroxy; CAS Registry Number: 88850-84-4.

Stock solutions of the substances in methanol were prepared by dissolving weighed amounts of the substances in 100 ml volumetric flasks and diluting to the mark. The concentration of the stock solutions was 1 mmol l^{-1} for 4′-H-CHAAB, 4′-OCH_3-CHAAB and 4′-Cl-CHAAB, 0.4 mmol l^{-1} for 4′-CH_3-CHAAB, and 0.2 mmol l^{-1} for 4′-$COCH_3$-CHAAB, 4′-NO_2-CHAAB and 4′-$NHCOCH_3$-CHAAB.

The concentrations were limited by the solubilities of the substances. More dilute solutions were prepared by diluting the stock solutions with methanol. All solutions were stored in dark.

All the other chemicals used, viz. methanol, acetic acid, phosphoric acid, boric acid, sodium hydroxide and potassium chloride, were of reagent grade purity (Lachema Brno, The Czech Republic).

Britton–Robinson buffers were prepared conventionally[17].

Apparatus

A PA 3 polarographic analyzer interfaced to an XY 4101 recorder (Laboratorni pristroje Praha, The Czech Republic) was used. In DC polarography, tast polarography, and differential pulse (DP) polarography with a dropping mercury electrode (DME), the potential sweep rate was 5 mV s^{-1}, controlled drop time 1 s, mercury reservoir height 64 cm, and DPP modulation amplitude −100 mV. The drop time of the DME in 0.1 M KCl was $\tau = 3.45$ s at a mercury reservoir height of 64 cm and a potential of 0 V vs SCE; the mercury flow rate at that reservoir height was $m = 1.42$ mg s^{-1}.

The three-electrode connection involving a saturated calomel reference electrode (SCE) and an auxiliary platinum wire electrode was used. All potential data reported are versus SCE. Oxygen was removed from the solutions by 10 min nitrogen purging. Nitrogen was purified by passing it through a solution of chromium(II) ions in dilute hydrochloric acid above zinc amalgam.

Cyclic voltammetric measurements were performed by using an SMDE 1 static mercury drop electrode (Laboratorni pristroje Praha, The Czech Republic) equipped with a capillary 0.136 mm in diameter, connected as a hanging mercury drop electrode (HMDE). The maximum drop size was used, determined by the valve opening period of 160 ms. The scan rate was varied over the region of 5 to 100 mV s^{-1}.

Constant potential coulometry was performed with an OH 404 coulometric analyzer (Radelkis, Budapest, Hungary) in a 100 ml vessel. The mercury bottom served as the cathode, and the anode and cathode compartments were separated with a frit. An SCE and an auxiliary platinum electrode were used. The solution was stirred with an electromagnetic stirrer during measurement, nitrogen was fed constantly to form an inert atmosphere.

Acidity was measured with an PHM 62 pH-meter (Radiometer, Copenhagen, Denmark) fitted with a glass electrode and an SCE.

Spectrophotometric measurements were carried out on a Pye Unicam 8800 instrument (Cambridge, U.K.) using quartz cells 1 cm optical pathlength.

All measurements were accomplished at room temperature.

Procedures

Solutions in Britton–Robinson buffer–methanol mixed solvents were investigated. The methanol content was chosen so that the substances were sufficiently well soluble in the systems and the best evaluable polarographic curves were obtained. Given the analyte concentration of 0.1 mmol l^{-1}, Britton–Robinson buffer–methanol 1 : 1 mixtures (henceforth “the 1 : 1 mixed solvent”) were suitable for the compounds with the 4′-substituents R = H, OCH_3, Cl, $COCH_3$, and $NHCOCH_3$, whereas Britton-Robinson buffer–methanol 1 : 9 mixtures (henceforth “the 1 : 9 mixed solvent”) were chosen for the compounds with R = CH_3 and NO_2.

For polarographic/voltammetric measurement, the adequate volume of solution of the substance in methanol was added to a volumetric flask, the system was diluted to 5 or 9 ml with methanol, and the whole was completed to 10 ml with the buffer of the desired pH. The solution was transferred to the polarographic vessel and freed from oxygen by 10 min nitrogen purging, and the polarographic/voltammetric curve was recorded. Each measurement was performed in triplicate and statistically processed. The calibration dependences were subjected to linear regression treatment. The limit of determination L_Q was calculated as the tenfold standard deviation for 7 determinations of analyte at the concentration corresponding to the lowest point of the calibration straight line[18].

For constant potential coulometry, 40 ml of methanol and 50 ml of the Britton–Robinson buffer of the desired pH were added to the measuring vessel and nitrogen purged. Pre-electrolysis at a constant potential was commenced simultaneously. In 20 – 30 min, the current decreased to the residual value and remained constant. The circuit parameters were then adjusted for automatic residual current compensation, and 10 ml of analyte stock solution in methanol was added with constant nitrogen purging. Termination of electrolysis was indicated by current decrease to its residual value. The time of electrolysis was 60 to 90 min. The charge passed was found by digital integration of the passing current.

RESULTS AND DISCUSSION

Stability of Stock Solutions and Working Solutions of the Analytes

The stability of stock solutions of the analytes was monitored spectrophotometrically at an optical pathlength of 1 cm. The absorbances were measured at the wavelengths of the absorption maxima (λ_{max}). No appreciable absorbance decrease was observed in 3 months.

The stability of the polarographed solutions (c = 0.1 mmol l^{-1}) in Britton–Robinson buffer–methanol solutions was examined by DPP. The peak heights were constant at least for 1 h after preparation of the solutions.

Effect of pH on the Polarographic Behaviour of the Substances

The effect of pH on the substances with R = H, OCH_3, Cl, and $NHCOCH_3$ (c = 0.1 mmol l^{-1}) was examined in the 1 : 1 mixed solvents over the region of pH 2.9 – 12.6. 4′-H-CHAAB gave a single wave whose limiting current decreased with increasing pH while the half-wave potential ($E_{1/2}$) shifted to more negative values (Fig. 1*a*). At some pH values the wave was distorted by a maximum, which complicated evaluation and made the logarithmic analysis impossible. The behaviour of the substance in DPP was consistent with that in tast polarography (Fig. 1*b*). The tast polarographic and DPP data are given in Table I. The logarithmic analysis slopes indicate that the phenomenon involved is irreversible. The parameters of the pH-dependences of $E_{1/2}$ and E_p calculated by linear regression are given in Table II.

The shift of $E_{1/2}$ and E_p in the negative direction with increasing pH (Fig. 2) can be explained in terms of a preceding protonation of the azo group, which brings about decrease in the electron density at the bond between the two nitrogen atoms, facilitating thus the reduction. The sigmoid I_{lim} vs pH plot shows that the number of exchanged electrons is twice as high in the acid region than in the basic region. At high pH values the $E_{1/2}$ and E_p potentials are nearly pH-independent.

For 4′-CH_3-CHAAB, the effect of pH was examined in the 1 : 9 mixed solvent at c = 0.1 mmol l^{-1} over the region of pH 4.3 – 12.6. Several polarographic waves were

TABLE I

Effect of pH on the $E_{1/2}$ (mV), I_{lim} (μA), E_p (mV) and I_p (μA) values in tast and DP polarography of the substances at $c = 0.1$ mmol l^{-1} in Britton–Robinson buffer–methanol 1 : 1 mixed solvent

R	Wave/Peak parameters	pH											
		2.9	4.1	5.0	6.1	7.3	8.3	8.8	9.2	10.1	10.9	11.7	12.6
H	$E_{1/2}$	–185	–270	–330	–415	–480	–525	–550	–575	–615	–725	–735	–735
	I_{lim}	0.65	0.67	0.66	0.60	0.42	0.35	0.35	0.34	0.35	0.34	0.35	0.32
	E_p	–170	–245	–305	–385	–440	–475	–505	–535	–575^a –680^a	–705	–700	–695
	I_p	1.85	1.86	1.94	1.83	1.21	1.10	1.07	0.96	0.48^a 0.30^a	0.71	0.77	0.78
OCH_3	$E_{1/2}$	–240	–335	–400	–470	–550	–600	–615	–640	–685	–695^a –825^a	–815	–815
	I_{lim}	0.60	0.59	0.59	0.57	0.54	0.53	0.41	0.39	0.32	0.07^a 0.13^a	0.30	0.30
	E_p	–230	–310	–375	–440	–510	–550	–575	–600	–650^a –780^a	–670^a –795^a	–795	–780
	I_p	1.63	1.59	1.74	1.80	1.82	1.23	1.04	0.92	0.51^a 0.20^a	0.15^a 0.53^a	0.69	0.68

TABLE I
(*Continued*)

R	Wave/Peak parameters	pH											
		2.9	4.1	5.0	6.1	7.3	8.3	8.8	9.2	10.1	10.9	11.7	12.6
Cl	$E_{1/2}$	–200	–290	–355	–445	–490	–535	–565	–595	–640	–715	–740	–755
	I_{lim}	0.61	0.63	0.63	0.56	0.37	0.34	0.33	0.33	0.32	0.32	0.32	0.30
	E_p	–185	–270	–320	–385	–445	–495	–520	–565	–600[a] –690[a]	–705	–715	–705
	I_p	1.66	1.67	1.62	1.38	1.08	1.07	1.07	0.91	0.49[a] 0.36[a]	0.74	0.88	0.81
$NHCOCH_3$	$E_{1/2}$	–210	–310	–380	–490	–530	–570	–580	–600	–645	–745	–765	–765
	I_{lim}	0.53	0.51	0.50	0.46	0.39	0.28	0.26	0.24	0.24	0.23	0.24	0.23
	E_p	–195	–300	–350	–445	–485	–540	–550	–570	–615[a] –715[a]	–740	–740	–725
	I_p	1.49	1.48	1.50	1.41	1.07	0.93	0.89	0.84	0.42[a] 0.26[a]	0.71	0.75	0.77

[a] Split wave/peak.

TABLE II
Parameters of the $E_{1/2}$ or E_p vs pH plots at c = 0.1 mmol l^{-1}

R	Solvent	pH	$E_{1/2}$ vs pH			E_p vs pH		
			slope mV pH^{-1}	intercept mV	correl. coeff.	slope mV pH^{-1}	intercept mV	correl. coeff.
H	BR buffer–MeOH 1 : 1	2.9 – 10.1	–56.7	–38.1	0.9711	–57.4	–13.8	0.9724
OCH_3	BR buffer–MeOH 1 : 1	2.9 – 8.3	–66.7	–57.8	0.9979	–57.1	–79.3	0.9975
Cl	BR buffer–MeOH 1 : 1	2.9 – 8.3	–59.3	–12.6	0.9818	–56.6	–31.9	0.9982
$NHCOCH_3$	BR buffer–MeOH 1 : 1	2.9 – 7.3	–75.7	0.4	0.9897	–76.5	23.6	0.9970
CH_3	BR buffer–MeOH 1 : 9	8.5 – 12.6[a]	–4.9	–340.8	0.7657	–6.7	–317.6	0.7057
		4.3 – 9.5[b]	–60.7	–81.4	0.9987	–39.2	–214.9	0.9897
$COCH_3$	BR buffer–MeOH 1 : 1	4.3 – 12.6[a]	–69.3	168.4	0.9954	–69.7	212.7	0.9936
NO_2	BR buffer–MeOH 1 : 9	4.3 – 12.6[a]	–53.3	50.8	0.9946	–57.7	95.0	0.9991

[a] First wave or peak; [b] second wave or peak.

observed (Fig. 3). The limiting current of the highest wave dropped rapidly with increasing pH and the shape of the wave was distorted by a maximum at nearly all pH values, which made the determination of the $E_{1/2}$ and I_{lim} data difficult. The parameters of the pH-dependences of the $E_{1/2}$ and I_{lim} values (in tast polarography) and E_p and I_p values (in DPP) are given in Table III.

When using a tenfold lower concentration, 4′-CH_3-CHAAB gave only 2 polarographic waves, which decreased and shifted towards more negative potentials with increasing pH. This suggests that the higher number of waves/peaks for this substance, which contains a single polarographically active functional group, is due to adsorption phenomena rather than to faradaic phenomena.

The effect of pH on the polarographic behaviour of 4′-$COCH_3$-CHAAB (c = 0.1 mmol l^{-1}) was examined in the 1 : 1 mixed solvent over the region of pH 2.9 – 12.6. The substance gave 2 polarographic waves which were free from distortion by maxima

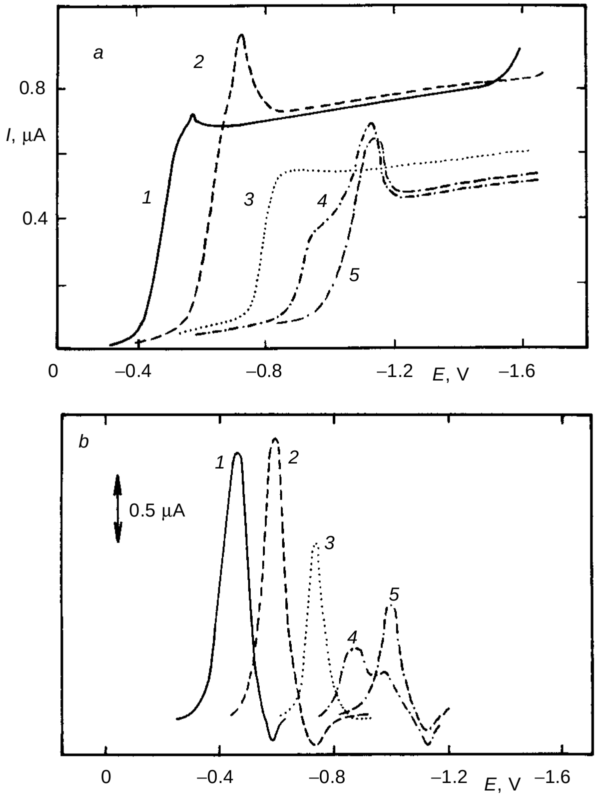


FIG. 1
Tast (*a*) and DP (*b*) polarograms of 4′-H-CHAAB, c = 0.1 mmol l^{-1}, in Britton–Robinson buffer–methanol 1 : 1 mixed solvent; pH: *1* 2.9, *2* 5.0, *3* 7.3, *4* 10.1, *5* 11.7

TABLE III
Effect of pH on the $E_{1/2}$ (mV), I_{lim} (μA), E_p (mV) and I_p (μA) values in tast and DP polarography of 4′-CH_3–CHAAB at c = 0.1 mmol l^{-1} in Britton–Robinson buffer–methanol 1 : 9 mixed solvent

Wave/Peak	Wave/Peak parameters	pH											
		4.3	5.8	6.6	7.5	8.5	9.5	9.9	10.2	10.6	11.1	11.9	12.6
1st wave	$E_{1/2}$	–	–	–	–	–380	–395	–385	–395	–385	–400	–395	–405
	I_{lim}	–	–	–	–	60	90	150	110	110	105	135	225
2nd wave	$E_{1/2}$	–340	–440	–480	–530	–605	–655	–660	–665	–670	–670	–	–
	I_{lim}	955	900	910	840	420	400	310	225	110	75	–	–
3rd wave	$E_{1/2}$	–	–	–	–	–	[a]	[a]	[a]	–980	–990	–980	–990
	I_{lim}	–	–	–	–	–	[a]	[a]	[a]	205	240	265	240
4th wave	$E_{1/2}$	–	–1 270	–1 280	–1 290	–1 240	–	–	–	–	–	–	–
	I_{lim}	–	20	65	125	260	–	–	–	–	–	–	–
1st peak	E_p	–	–	–	–	–360	–390	–385	–400	–390	–390	–390	–400
	I_p	–	–	–	–	30	50	60	80	80	80	100	110
2nd peak	E_p	–375	–440	–475	–505	–565	–605	–615	–615	–625	–625	–	–
	I_p	945	1 400	1 510	1 370	900	540	390	260	110	70	–	–
3rd peak	E_p	–	–	–	–	–1 105	–1 040	–1 010	–1 020	–980	–1 000	–930	–995
	I_p	–	–	–	–	130	240	220	250	180	260	230	260
4th peak	E_p	–	–1 270	–1 315	–1 295	–1 295	–1 300	–1 285	–1 305	–1 305	–1 290	–1 280	–1 290
	I_p	–	20	50	90	140	150	120	130	40	120	50	110

[a] Wave could not be evaluated.

(Fig. 4). The half-wave potentials of the second wave encroached on the supporting electrolyte decomposition region, due to which this wave, which is apparently associated with the reduction of the carbonyl C=O double bond in the $CH_3C=O$ group, was difficult to evaluate. The polarographic parameters are given in Table IV.

For 4′-NO_2-CHAAB, the effect of pH was examined in the 1 : 9 mixed solvent at $c = 0.1$ mmol l^{-1} over the region of pH 4.3 – 12.6. Several polarographic waves, some of which were difficult to evaluate, were obtained (Fig. 5). The half-wave potentials shifted to more negative values with increasing pH (Table V). The dependence of $E_{1/2}$ and I_{lim} on pH at an analyte concentration of 0.01 mmol l^{-1} was of a similar nature[19].

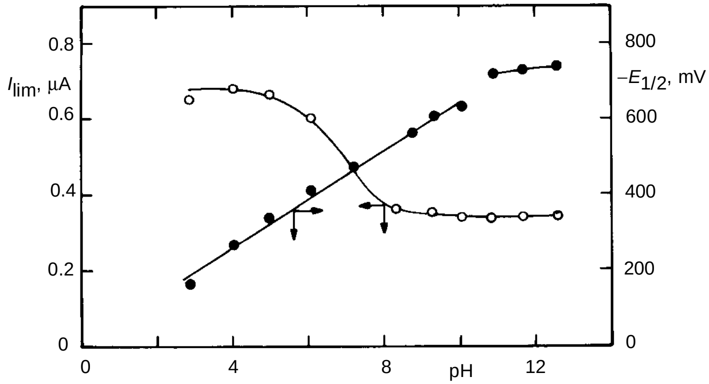


FIG. 2
Dependences of I_{lim} and $E_{1/2}$ on pH for 4′-H-CHAAB, $c = 0.1$ mmol l^{-1}, in Britton–Robinson buffer–methanol 1 : 1 mixed solvents

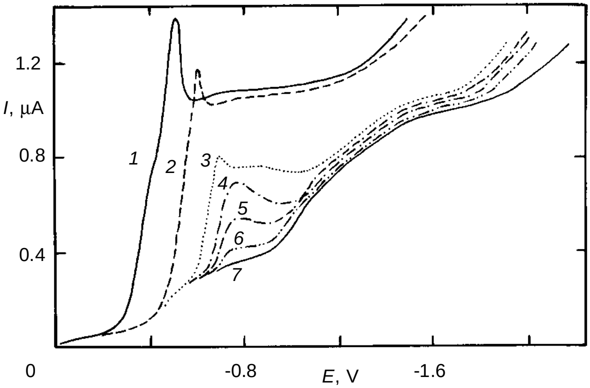


FIG. 3
Tast polarograms of 4′-CH_3-CHAAB, $c = 0.1$ mmol l^{-1}, in Britton–Robinson buffer–methanol 1 : 9 mixed solvent; pH: *1* 4.3, *2* 6.6, *3* 8.5, *4* 9.5, *5* 10.2, *6* 11.1, *7* 12.3

TABLE IV
Effect of pH on the tast and DP polarographic parameters of 4′-$COCH_3$–CHAAB at c = 0.1 mmol l^{-1} in Britton–Robinson buffer–methanol 1 : 1 mixed solvent

pH[a]	1st wave		2nd wave		1st peak		2nd peak	
	$E_{1/2}$ mV	I_{lim} nA	$E_{1/2}$ mV	I_{lim} nA	E_p mV	I_p nA	E_p mV	I_p nA
2.9[b]	–145	335	–	[c]	–100	870	–1 075	175
	–585	75	–	[c]	–570	170		
4.1	–235	365	–	[c]	–190	910	–1 250	150
5.0	–290	350	–1 325	300	–245	890	–1 320	300
6.1	–325	330	–1 430	230	–310	845	–1 460	155
7.3	–440	295	–1 550	120	–385	840	–1 525	50
8.3	–490	270	–1 785	250	–440	800	–1 775	200
8.8	–490	270	–1 775	230	–445	755	–1 765	235
9.2	–515	260	–1 780	275	–465	775	–1 760	295
10.1	–565	280	–1 790	280	–545	670	–1 775	245
10.9	–620	270	–1 805	265	–600	705	–1 790	115
11.7	–665	365	–1 800	140	–610	655	–1 780	60
12.6	–715	250	–1 745	105	–675	610	–1 725	50

[a] pH of the mixture; [b] wave is split; [c] wave is overlapped by the supporting electrolyte decomposition current.

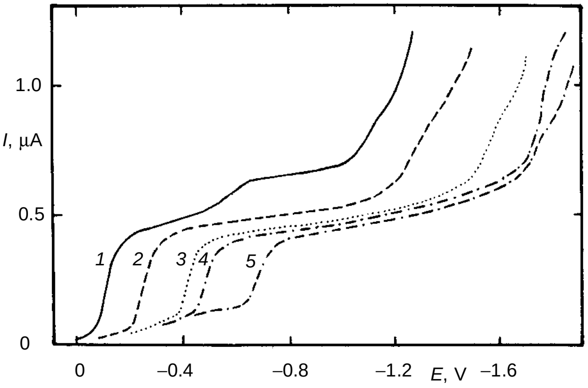


FIG. 4
Tast polarograms of 4′-$COCH_3$-CHAAB, c = 0.1 mmol l^{-1}, in Britton–Robinson buffer–methanol 1 : 1 mixed solvent; pH: *1* 2.9, *2* 5.0, *3* 7.3, *4* 9.2, *5* 12.6

TABLE V
Effect of pH on the tast and DP polarographic parameters of 4′-NO_2–CHAAB at c = 0.1 mmol l^{-1} in Britton–Robinson buffer–methanol 1 : 9 mixed solvent

pH[a]	1st wave		2nd wave		3rd wave		2nd and 3rd waves[b]	1st peak		2nd peak		3rd peak	
	$E_{1/2}$ mV	I_{lim} nA	$E_{1/2}$ mV	I_{lim} nA	$E_{1/2}$ mV	I_{lim} nA	I_{lim} nA	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA
4.3[d]	–160	340	–	[c]	–	[c]	1 380	–150	510	–680	600	–1 215	30
								–560	570				
5.8[d]	–265	460	–	[c]	–	[c]	1 280	–245	1 110	–790	230	–1 260	40
								–535	820				
6.6[d]	–305	500	–	[c]	–	[c]	1 310	–290	1 310	–870	430	–1 280	40
								–550	650				
7.5[d]	–345	510	–	[c]	–	[c]	1 230	–330	1 440	–890	1 390	–1 310	110
								–575	1 070				
8.5[d]	–410	570	–975	740	–1 335	220	–	–395	1 530	–940	750	–1 340	230
								–610	610				
9.5[d]	–475	640	–1 015	450	–1 355	460	–	–450	1 370	–970	460	–1 325	390
								–620	60				
9.9	–485	630	–1 005	360	–1 330	500	–	–480	1 290	–970	380	–1 315	460
10.2	–485	590	–1 005	310	–1 325	510	–	–485	1 260	–970	340	–1 310	480
10.6	–500	580	–1 010	250	–1 325	540	–	–495	1 290	–970	320	–1 305	520
11.1	–565	560	–1 005	210	–1 315	530	–	–505	1 330	–965	290	–1 295	520
11.9	–570	390	–1 000	190	–1 305	570	–	–525	1 150	–970	290	–1 295	540
12.6	–605	380	–1 005	160	–1 280	560	–	–545	860	–965	260	–1 265	530

[a] pH of the mixture; [b] summary wave height; [c] waves merge; [d] 1st peak is split.

Mechanism of Polarographic Reduction of the Substances

The observed linear dependence of I_{lim} on analyte concentration (see below) indicates a diffusion nature of the current. This is also borne out by the dependence of the DC polarographic limiting current on the mercury reservoir height root.

Logarithmic analysis of the tast polarographic curves gave evidence that the phenomenon involved is not reversible. This was also confirmed by cyclic voltammetry at the HMDE, where a cathodic peak only was observed. 4′-$COCH_3$-CHAAB and 4′-NO_2-CHAAB are exceptions: the former exhibited a hint of an anodic peak at higher polarization rates, the latter gave 2 cathodic peaks owing to the presence of 2 polarographically reducible groups.

The effect of the polarization rate on the cathodic peak height and position was examined for 4′-H-CHAAB, 4′-OCH_3-CHAAB, 4′-Cl-CHAAB, and 4′-$NHCOCH_3$-CHAAB in the 1 : 1 mixed solvent at pH 2.9. For 4′-$COCH_3$-CHAAB, the pH was 5.0, for 4′-CH_3-CHAAB and 4′-NO_2-CHAAB, the 1 : 9 solvent was used and the pH was 4.3 and 5.8, respectively. Such media gave the best evaluable peaks and are recommended for polarographic quantitation. For the majority of substances the peak height increased with increasing polarization rate root more slowly than as corresponds to a linear dependence, which points to some complications in the phenomenon due to analyte adsorption on the electrode surface.

4′-$COCH_3$-CHAAB and 4′-NO_2-CHAAB are exceptions in this group of substances. For the former, the cathodic peak height was a linear function of the polarization rate root, corresponding to a diffusion-controlled irreversible phenomenon. The anodic peak was also observable at higher polarization rates (50 and 100 mV s^{-1}), indicating that the system began to behave quasi-reversibly. 4′-NO_2-CHAAB gave 2 peaks. The height of the first peak was a linear function of the polarization rate root, indicating a diffusion-

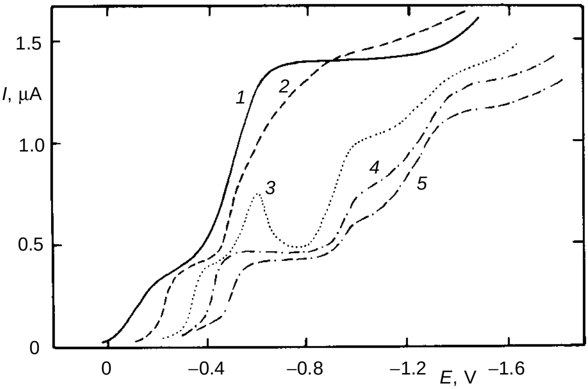


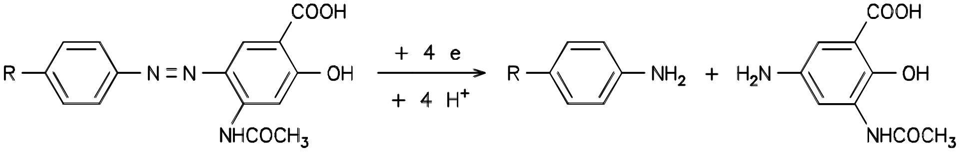
FIG. 5

Tast polarograms of 4′-NO_2-CHAAB, c = 0.1 mmol l^{-1}, in Britton–Robinson buffer–methanol 1 : 9 mixed solvent; pH: *1* 4.3, *2* 6.6, *3* 8.5, *4* 9.9, *5* 11.5

controlled irreversible phenomenon. For the second peak the $I_p/\nu^{1/2}$ ratio was not constant, which indicates that the phenomenon is rather complex, involving analyte adsorption on the electrode surface. In analogy with the nitroazo compounds studied previously[20] we suggest that the first wave/peak corresponds to the reduction of the azo group whereas the second and third waves/peaks are associated with the stepwise reduction of the nitro group.

Potentiostatic coulometry at a mercury pool electrode revealed that in the 1 : 1 mixed solvent, 4′-H-CHAAB exchanges 3.6 electrons at pH 2.9 and at a constant potential of –0.45 V, and 2.5 electrons at pH 10.9 and a constant potential of –1.05 V (Fig. 6).

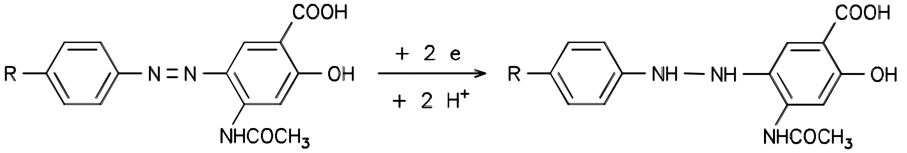
The above results suggest that the four-electron irreversible reduction according to Eq. (*A*) occurs in the acid region,



I

(*A*)

whereas only a two-electron reduction occurs in the basic regions.



(*B*)

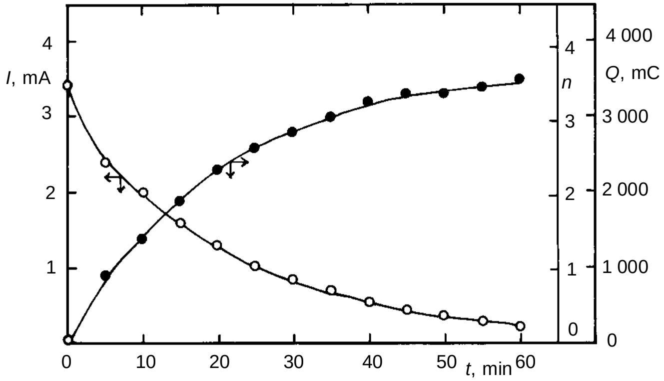
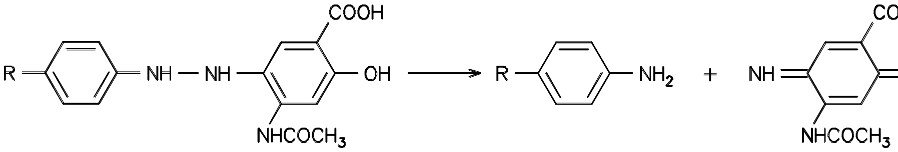


FIG. 6

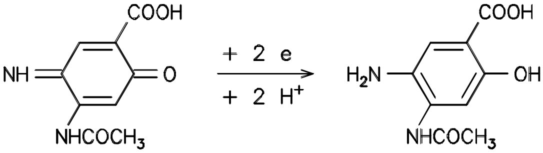
Dependences of current *I* and charge *Q* on time during the coulometric reduction of 4′-H-CHAAB in Britton–Robinson buffer–methanol 1 : 1 mixed solvent at pH 2.9; applied potential –0.45 V

The fact that the observed number of exchanged electrons, $n = 2.5$, is higher than the expected value of $n = 2$, is apparently associated with the possible disproportionation of the formed hydrazo compound by reaction (*C*) giving the quinonediimine (*II*), which is reduced instantaneously to the corresponding amine by reaction (*D*). Since *II* is reduced at more positive potentials than the starting azo pigment, no new polarographic wave appears. Presumably, the reduction mechanism in the acid system is similar, the acid-catalyzed disproportionation rate, however, is so high that the observed wave height corresponds to a four-electron reduction.



(*C*)

II



(*D*)

The effect of substituents in position 4′ on the tast polarographic behaviour of the substances was examined at a concentration of 0.01 mmol l^{-1} in the 1 : 1 mixed solvent at pH 2.9. The $E_{1/2}$ values were plotted against the Hammett substituent constants σ_p (Fig. 7). The linearity of the plot suggests that under the conditions applied, the substances in the series studied are all reduced by the same mechanism.

Analytical Application of the Polarographic Reduction

The concentration dependences were measured in conditions giving the best developed waves or peaks. The systems used and the calculated parameters of the linear segments of the calibration plots are given in Table VI.

For 4′-H-CHAAB, the dependences in tast polarography were measured over the region of $1.5 \cdot 10^{-4}$ to $1 \cdot 10^{-6}$ mol l^{-1} and in DPP, over the region of $1.5 \cdot 10^{-4}$ to $1 \cdot 10^{-7}$ mol l^{-1}; the 1 : 1 mixed solvent at pH 2.9 was used in both cases. In tast polarography as well as DPP, the plot deviates from linearity at concentrations higher than $8 \cdot 10^{-5}$ mol l^{-1} (ref.[19]). Therefore the dependence of absorbance at 342 nm on concentration in the above system was measured over the region of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ mol l^{-1}, where the plot is linear[19]. It is reasonable to assume that the bending of the

TABLE VI
Parameters of the calibration straight lines for the determination of the substances

R	Method	*c* . 10^5 mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	*r*[a]	L_Q[b] mol l^{-1}
H[c]	Tast	1 – 8	7.02	12.5	0.9992	–
		0.1 – 1	7.12	3.7	0.9995	1.7 . 10^{-6}
	DPP	1 – 8	18.54	165.2	0.9998	–
		0.1 – 1	23.22	–3.1	0.9992	–
		0.02 – 0.1	21.07	–1.7	0.9980	2.8 . 10^{-7}
CH_3[d]	Tast	1 – 10	–	–	–	[i]
		0.1 – 1	8.20	–0.3	0.9989	1.4 . 10^{-6}
	DPP	1 – 10	–	–	–	[i]
		0.1 – 1	31.20	–1.7	0.9972	–
		0.02 – 0.1	32.40	–0.6	0.9986	1.8 . 10^{-7}
OCH_3[c]	Tast	1 – 8	5.94	10.7	0.9979	–
		0.1 – 1	6.30	–4.8	0.9955	1.8 . 10^{-6}
	DPP	1 – 6	18.40	25.0	0.9992	–
		0.1 – 1	18.00	1.0	0.9995	–
		0.02 – 0.1	19.30	–0.4	0.9990	2.6 . 10^{-7}
Cl[c]	Tast	1 – 8	6.25	42.4	0.9999	–
		0.1 – 1	6.35	0	0.9990	1.9 . 10^{-6}
	DPP	1 – 8	18.88	3.6	0.9999	–
		0.1 – 1	16.12	–9.7	0.9997	–
		0.02 – 0.1	12.75	–3.1	0.9909	4.3 . 10^{-7}
$COCH_3$[e]	Tast	1 – 10	3.20	–9.2	0.9990	–
		0.1 – 1	2.80	–4.1	0.9896	2.8 . 10^{-6}
	DPP	1 – 10	8.50	–51.2	0.9982	–
		0.1 – 1	5.30	–5.4	0.9959	–
		0.02 – 0.1	3.00	–0.2	0.9980	3.2 . 10^{-7}
NO_2[f]	Tast[g]	1 – 10	3.80	20.1	0.9922	–
		0.1 – 1	5.20	–4.0	0.9941	2.6 . 10^{-6}
	Tast[h]	1 – 10	14.30	–21.4	0.9993	–
		0.1 – 1	15.10	–2.0	0.9978	1.5 . 10^{-6}
	DPP[g]	1 – 10	14.00	34.7	0.9725	–
		0.1 – 1	15.60	–19.2	0.9958	–
		0.02 – 0.1	16.00	–3.7	0.9946	2.9 . 10^{-7}
	DPP[h]	1 – 10	9.70	20.6	0.9852	–
		0.1 – 1	11.10	–17.5	0.9937	–
		0.02 0.1	12.20	–1.3	0.9961	3.1 . 10^{-7}

TABLE VI
(*Continued*)

R	Method	c . 10^5 mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	r[a]	L_Q[b] mol l^{-1}
$NHCOCH_3$[c]	Tast	1 – 8	5.80	–2.1	0.9977	–
		0.2 – 1	3.30	–1.2	0.9743	2.2 . 10^{-6}
	DPP	1 – 8	16.00	–8.0	0.9982	–
		0.1 – 1	14.10	–8.6	0.9989	–
		0.02 – 0.1	12.60	–1.4	0.9999	2.1 . 10^{-7}

[a] Correlation coefficient; [b] limit of determination; [c] Britton–Robinson buffer–methanol 1 : 1 mixed solvent at pH 2.9; [d] Britton–Robinson buffer–methanol 1 : 9 mixed solvent at pH 4.3; [e] Britton–Robinson buffer–methanol 1 : 1 mixed solvent at pH 5.0; [f] Britton–Robinson buffer–methanol 1 : 9 mixed solvent at pH 5.8; [g] 1st wave or peak; [h] 2nd wave or peak; [i] curves were not evaluated because of poor reproducibility.

concentration dependences in tast polarography and DPP within the concentration range in question is associated with the electrode process (electrode passivation or analyte adsorption) rather than separation of the substance from the aqueous-methanolic solvent due to its low solubility, or aggregation of its molecules.

The situation with 4′-CH_3-CHAAB is similar. Tast polarography and DPP gave poorly reproducible results over the concentration region of 1 . 10^{-4} to 1 . 10^{-5} mol l^{-1},

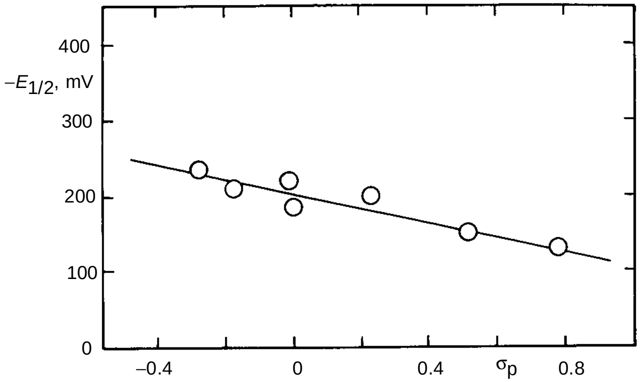


FIG. 7
Plot of $E_{1/2}$ vs Hammett σ_p substituent constants for the substances, c = 0.01 mmol l^{-1}, in Britton–Robinson buffer–methanol 1 : 1 mixed solvent at pH 2.9

although spectrophotometry at 368 nm provides linear and well-reproducible dependences. Deviations from linearity were also observed for 4′-OCH_3-CHAAB, viz. at concentrations in excess of 8 . 10^{-5} mol l^{-1} in tast polarography and 6 . 10^{-5} mol l^{-1} in DPP. For 4′-Cl-CHAAB and 4′-$NHCOCH_3$-CHAAB, the linearity limit is 8 . 10^{-5} mol l^{-1} in both polarographic techniques, for 4′-$COCH_3$-CHAAB and 4′-NO_2-CHAAB the limit is 1 . 10^{-4} mol l^{-1}.

This research was financially supported by the Grant Agency of the Czech Republic (Grant No. 203/93/0050).

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Translated by P. Adamek.