DETERMINATION OF ACRIDINE, BENZ[*c*]ACRIDINE AND DIBENZ[*a*,*h*]ACRIDINE BY FAST SCAN DIFFERENTIAL PULSE VOLTAMMETRY AND ADSORPTIVE STRIPPING VOLTAMMETRY*

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The behaviour of the title compounds in fast scan differential pulse voltammetry and adsorptive stripping voltammetry was studied and the optimum conditions were found for their determination by FS DPV over the concentration region of $(2 - 100) \cdot 10^{-7}$ mol l^{-1} and by AdSV over the concentration regions of $(2 - 10) \cdot 10^{-8}$ mol l^{-1} for acridine and dibenz[*a*,*h*]acridine and $(2 - 100) \cdot 10^{-9}$ mol l^{-1} for benz[*c*]acridine. Practical applicability of the methods to the monitoring of the chemical efficiency of destruction of the carcinogens with potassium permanganate was tested.

Acridine benzo derivatives are among suspect chemical carcinogens[1 – 3], and International Agency for Research on Cancer (IARC) has included them among substances which are probably carcinogenic to man[4]. Substances of that kind are present in crude oil and in oil products[5,6], in various coal tar fractions[7,8] and in chemotherapeutics derived from tar[9]. They are also encountered in coal liquefaction products[10] and in asphalts[11].

The presence of such substances in air is predominantly due to diverse combustion processes[12 – 14], and they are also present in tobacco smoke[15] and in thermally treated foods with high protein contents[16,17]. In the surroundings of industrial centres, the substances have been found in sea water[18] and in sediments from the bottoms of natural reservoirs[19]. Acridine benzo derivatives are usually quantitated by means of chromatographic techniques which are powerful in the necessary separation of complex mixtures. Described have been analyses by gas chromatography[7,9,12 – 14, 17 – 19,20,21], liquid chromatography[16,22] as well as thin layer chromatography[11,23]. The use of ion exchange chromatography[24] and gel permeation chromatography[8] is rarer. In addition to flame ionization detection[12,13,17,20,21], gas chromatography also employs mass spectrometric

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detection[14,18,19], whereas fluorimetric detection is popular in HPLC for this purpose[16,22]. From among the spectrometric methods, fluorimetry has been applied to a direct determination of dibenzacridines[25].

Heteroaromatic compounds with a π-electron deficit, which include acridine and its derivatives, are characterized by low energies of the lowest occupied molecular orbital, owing to which the compounds are easy to reduce polarographically. Moreover, the polarographic reduction can be further facilitated by protonation of the nitrogen atom, which brings about additional decrease in the electron density at sites of potential electron transfer[26].

Acridine and its amino, hydroxy, chloro and sulfo derivatives are reduced in two one-electron irreversible waves whose heights are linearly concentration-dependent over the region of $10^{-3} - 10^{-5}$ mol l^{-1}. For acridine itself and for its 4-amino derivative, a third wave, presumably of adsorptive nature, has been observed[27] at concentrations in excess of 10^{-4} mol l^{-1}. The mechanism of the polarographic reduction of acridine has been studied in detail by Kay and Stonehill[28]. In purely aqueous solutions, complex anomalous polarograms were obtained due to adsorption of the semiquinoid intermediate on the mercury drop electrode. The anomalies are absent from a medium of 50% ethanol, where there remain two one-electron irreversible waves whose heights are proportional to concentration over the region of $10^{-3} - 10^{-4}$ mol l^{-1}. Polarography has also been applied to the quantitation of a number of drugs derived from acridine, in particular Mepacrin and its homologues[29 – 31], Acriflavin[32], Proflavin, Trypoflavin, Ryvanol and Atebrin[33].

Recently, the polarographic reduction of acridine and its dimethylamino derivatives has been investigated by Chodowski and coworkers[34 – 36]. A comparison of the polarographic behaviour and physiological activity of a series of acridines was the subject of paper[37]. References[38,39] include 29 aminoacridine derivatives; polarograms of virtually all of them are reproduced, the probable reduction mechanism is discussed, and a correlation of the polarographic behaviour with the antibacterial and antivirus effect of the substances is attempted.

The polarographic behaviour of acridine and its derivatives with fused benzene rings in solutions in dimethyl sulfoxide[40] and dimethylformamide[41,42] has been examined in relation to their genotoxic effects. Reference[41] suggests that the more negative the lowest occupied molecular orbital energy, the more positive the half-wave potential of the nitrogen heterocyclic compound in question. Quantum chemical calculations indicate that addition of a next aromatic ring to acridine results in an energy increase for the lowest occupied molecular orbital, viz. from –2.80 eV for acridine to –2.72 eV for benz[*c*]acridine to –2.64 eV for dibenz[*a*,*h*]acridine. This is naturally mirrored by shifts of the half-wave potentials of the polarographic waves to more negative values, viz. from –1.57 V to –1.67 V to –1.73 V vs SCE, respectively, in dimethylformamide. This shift will also be related to the higher steric hindrance in the benzo derivatives and to

the associated higher complexity of phenomena occurring during the electrode reaction[43].

So far, however, no attention has been paid to the application of the more recent electrochemical methods, viz. fast scan differential pulse voltammetry (FS DPV) and adsorptive stripping voltammetry (AdSV), to the quantitation of submicromolar concentrations of acridine and its benzo and dibenzo derivatives. This topic is therefore studied in the present work. The model substances include acridine, benz[*c*]acridine and dibenz[*a*,*h*]acridine (CAS Registry Numbers 260-94-6, 225-51-4 and 226-36-8, respectively).

In view of the ever-increasing number of laboratories working with substances of that type, IARC initiated a project concerning laboratory methods for the destruction of such genotoxic substances and decontamination of laboratories handling them[4]. Oxidation by permanganate in basic or acid medium is an efficient method of decomposition of a number of genotoxic substances. The final part of the present work pays therefore attention to the applicability of FS DPV to the monitoring of the efficiency of this destruction process.

EXPERIMENTAL

Reagents

Stock solutions of the analytes in methanol, $c = 1 \cdot 10^{-3}$ mol l^{-1} for acridine and benz[*c*]acridine and $1 \cdot 10^{-4}$ mol l^{-1} for dibenz[*a*,*h*]acridine, were prepared by dissolving the precisely weighed-in solids (Koch–Light, U.K.) in freshly distilled methanol using an ultrasonic bath. Spectroscopic monitoring gave evidence that stored in dark at 4 °C, the stock solutions keep their concentration constant within experimental error for 3 months. More dilute solutions were prepared from the stock solutions weekly (down to a concentration of $1 \cdot 10^{-5}$ mol l^{-1}) or daily ($1 \cdot 10^{-6}$ mol l^{-1} and lower). The other chemicals were of reagent grade purity (Lachema, Brno, The Czech Republic). Britton–Robinson buffers of a constant ionic strength were prepared conventionally[44]. Water was redistilled twice from a quartz still (Heraeus, Germany).

Apparatus

Voltammetric measurements were performed on a PA 4 polarographic analyzer fitted with an SMDE-1 electrode connected as a hanging mercury drop electrode with a capillary 0.136 mm in diameter. The instrument was interfaced to an XY 4106 recorder (all Laboratorni pristroje, Prague, The Czech Republic). The three-electrode connection was applied using a platinum sheet auxiliary electrode and a saturated calomel reference electrode (all electrode potentials are given relative to SCE in this paper). The analog memory time constant was 100 ms, potential sweep rate 20 mV s^{-1}, pulse height −100 mV, pulse width and interval between pulses 100 ms, the maximum size of the HMDE was determined by the valve opening period of 160 ms (all unless stated otherwise). Oxygen was removed from the solutions by 10 min nitrogen purging. Nitrogen for this was purified by passing through a solution of chromium(II) ions in dilute (1 : 1) hydrochloric acid over a zinc amalgam. Before entering the polarographic vessel, the gas was passed through a bubbler containing a water–

methanol mixture in proportions equal to the buffer–methanol proportions in the supporting electrolyte solution.

The pH of the aqueous and aqueous-methanolic solutions was measured with a PHM 62 instrument (Radiometer, Copenhagen, Denmark) equipped with a glass indicator electrode and a saturated calomel reference electrode. The calibration solutions included aqueous buffers (for work in solutions containing no more than 40 vol.% methanol), acetate, borate and phosphate buffers in 50 vol.% methanol[45] (for work in such solvent) and solutions of oxalic acid and sodium oxalate (0.01 mol l^{-1} each) in 90 vol.% methanol[46] for work in methanol of that concentration.

Procedures

Voltammetric measurements. Working solutions were prepared by pipetting the stock solution in methanol, adding the calculated volume of methanol and diluting to 10 ml with the Britton–Robinson buffer. For acridine over the concentration region of $(2 - 10) . 10^{-7}$ mol l^{-1}, tenfold dilute buffer was used to reduce the effect of impurities present in the supporting electrolyte. The calibration curves were measured in triplicate and evaluated by linear regression using the least squares method. The limit of determination was represented by the tenfold standard deviation of determination of analyte at the concentration corresponding to the lowest point of the corresponding concentration dependence[47].

Destruction of analytes with potassium permanganate in acid medium. The following procedure was used based on preliminary experiments[48]. Two ml of acridine or dibenz[*a*,*h*]acridine solution (c = 2.5 and 0.5 mg ml^{-1}, respectively) in acetonitrile or acetone were placed in a 100 ml beaker, 10 ml of a fresh solution of potassium permanganate (c = 0.3 ml l^{-1}) in sulfuric acid (c = 3 mol l^{-1}) were added, and the whole was stirred on a magnetic stirrer at 100 r.p.m. for an hour. The unreacted permanganate was eliminated by adding 8 ml of aqueous solution of oxalic acid (c = 1 mol l^{-1}) and stirring for 5 min; the solution became colourless. A 5 ml aliquot was then transferred to the polarographic vessel, 5.00 ml of methanol were added, the whole was purged for 10 min and its FS DPV curve was recorded. Successively, five 25 µl portions of acridine solution in acetone or acetonitrile (c = 0.5 mg ml^{-1}) or 50 µl portions of dibenz[*a*,*h*]acridine solution in acetone or acetonitrile (c = 0.5 and 0.02 mg ml^{-1}, respectively) were added. (The additions were such that they corresponded to 1% of the amount initially present before the destruction.) After each addition, the solution was nitrogen purged and its FS DPV curve was recorded.

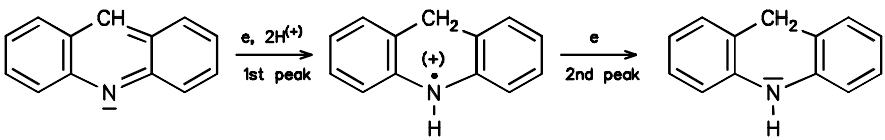
Destruction of analytes with potassium permanganate in alkaline medium. To 2 ml of acridine or dibenz[*a*,*h*]acridine solution in acetonitrile (c = 2.5 and 0.2 mg ml^{-1}, respectively) were added 10 ml of a fresh solution of potassium permanganate (c = 0.3 ml l^{-1}) in sodium hydroxide (c = 1 ml l^{-1}), and the whole was stirred on a magnetic stirrer at 100 r.p.m. for 3 h. The unreacted permanganate was eliminated with solid sodium disulfite, which was added portionwise until the violet colour vanished completely. A 5.00 ml aliquot was taken, 5.00 ml of water were added to adjust consistency, and the whole was extracted with 3 × 10 ml of cyclohexane. The combined extracts were evaporated to dryness in a vacuum rotary evaporator at a reduced pressure, and the residue was dissolved in 5.00 ml (acridine) or 9.00 ml (dibenz[*a*,*h*]acridine) of methanol with the assistance of ultrasound, and 5.00 ml of Britton–Robinson buffer at pH 5.0 (for acridine) or 1.00 of Britton–Robinson buffer at pH 2.0 (for dibenz[*a*,*h*]acridine) were added. The solution was nitrogen purged and its FS DPV curve was run. Five portions of 41.6 µl of solution of acridine (c = 0.5 mg ml^{-1}) in acetonitrile or of 16.7 µl of solution of dibenz[*a*,*h*]acridine (c = 0.1 mg ml^{-1}) in the same solvent were successively added and the FS DPV traces recorded following 1 min nitrogen purging. Again, the additions corresponded to 1% of the initially present substance in the solution prior to the destruction.

RESULTS AND DISCUSSION

Fast Scan Differential Pulse Voltammetry

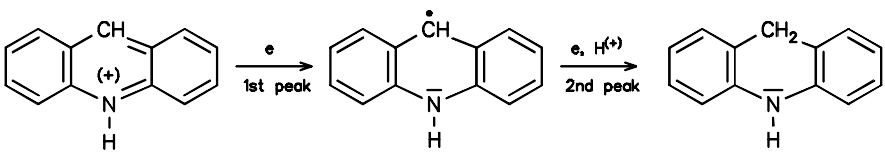
Due to the partial solubility of the analytes in water, aqueous-methanolic solutions had to be used. The methanol-to-buffer ratio was 1 : 1 for acridine and benz[*c*]acridine and 9 : 1 for dibenz[*a*,*h*]acridine. When the methanol content was lower, the analyte separated from the solution and the voltammetric curves were distorted, presumably due to adsorption of the semiquinoid intermediate product of the electrode reaction on the HMDE[28].

The effect of pH on the FS DPV traces is apparent from Tables I – III, voltammograms of the substances at selected pH values are shown in Figs 1 – 3. In dependence on pH, the substances give rise to one or two rather well-developed peaks. Additional peaks, considerably lower, appear at some pH. We suggest that the additional peaks are associated with the adsorption of the inital substance or of intermediate products of the electrode reaction on the working electrode surface. The E_p vs pH dependence for the 1st peak is linear across the region of pH 5 – 12, and the following parameters were obtained for it by linear regression (E_p in mV): $E_p = -44 - 78.8$ pH for acridine (correlation coefficient $r = 0.9978$), $E_p = -100 - 76.1$ pH for benz[*c*]acridine ($r = 0.9988$), and $E_p = -276 - 68.1$ pH for dibenz[*a*,*h*]acridine ($r = 0.9983$). The slopes indicate that an irreversible phenomenon is involved, accompanied by the exchange of different numbers of electrons and protons. We suggest that the analytes are reduced according to scheme (*A*) (additional benzene rings are fused to the basic skeleton of acridine for benz[*c*]acridine and dibenz[*a*,*h*]acridine).



(*A*)

The potential of the first peak ceases to be pH dependent at pH < 4, which indicates that protons are no more involved in the potential-determining step. We suggest that the protonated form of the analytes reduces according to scheme (*B*) (shown for acridine).



(*B*)

TABLE I
Effect of pH on FSDP voltammograms of acridine (c = 1 . 10^{-5} mol l^{-1}) in Britton–Robinson buffer–methanol 1 : 1 mixture

pH[a]	E_p, V		I_p, nA	
	1st peak	2nd peak	1st peak	2nd peak
2.8	–0.325	–[b]	29	–[b]
4.0	–0.330	–[b]	39	–[b]
4.9	–0.365	–1.130	55	–[b]
5.9	–0.420	–1.115[c]	57	29
7.1	–0.520	–1.115	50	42
8.1	–0.590	–1.115	42	42
8.8	–0.630	–1.110	41	34
9.4	–0.680	–1.115	39	46
10.3	–0.765	–1.115	33	31
11.4	–0.855	–1.115	35	33
12.2	–0.925	–1.085	35	30

[a] Of the mixed solvent; [b] overlapped by supporting electrolyte decomposition current; [c] a third peak appears at about –1.32 V, its height and position are difficult to read due to its superposition with the supporting electrolyte current.

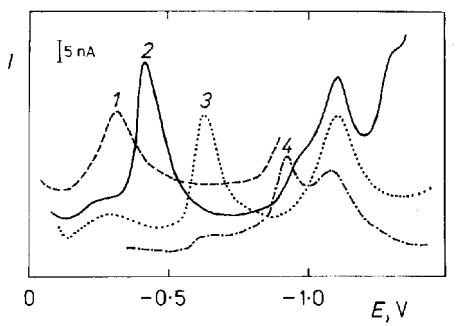


FIG. 1
FSDP voltammograms of acridine (c = 1 . 10^{-5} mol l^{-1}) in Britton–Robinson buffer–methanol mixtures 1 : 1; pH: *1* 2.8, *2* 5.9, *3* 8.8, *4* 12.2

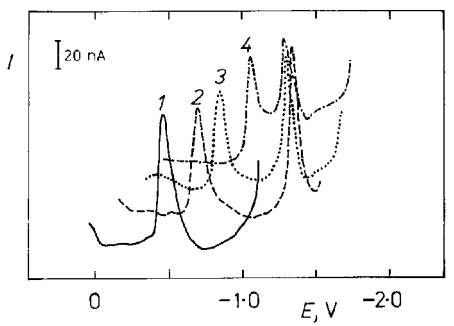


FIG. 2
FSDP voltammograms of benz[*c*]acridine (c = 1 . 10^{-5} mol l^{-1}) in Britton–Robinson buffer–methanol mixtures 1 : 1; pH: *1* 4.1, *2* 7.1, *3* 9.4, *4* 12.1

TABLE II
Effect of pH on FSDP voltammograms of benz[*c*]acridine (c = 1 . 10^{-5} mol l^{-1}) in Britton–Robinson buffer–methanol 1 : 1 mixture

pH[a]	E_p, V		I_p, nA	
	1st peak	2nd peak	1st peak	2nd peak
1.3[b]	–0.370	–[c]	76	–[c]
2.8	–0370	–[c]	96	–[c]
4.1	–0.425	–[c]	95	–[c]
5.0	–0.490	–[c]	88	–[c]
5.9	–0.550	–1.275	85	290
7.1	–0.635	–1.275	77	120
8.1	–0.710	–1.270	72	97
8.8	–0.765	–1.265	68	106
9.4	–0.810	–1.266	67	95
10.3	–0.900	–1.270	68	100
11.3	–0.950	–1.250	65	68
12.1	–1.030	–1.265	60	60

[a] Of the mixed solvent; [b] mixture of 0.1 M H_2SO_4 and methanol (1 : 1); [c] overlapped by supporting electrolyte decomposition current.

The peak potential shift towards more negative values in the acridine – benz[*c*]-acridine – dibenz[*a*,*h*]acridine series is consistent with the quantum-chemical calculations[41] and with other facts as outlined above.

From the analytical point of view it is convenient to employ for the determination the more positive of the two peaks, which is better reproducible and whose evaluation is

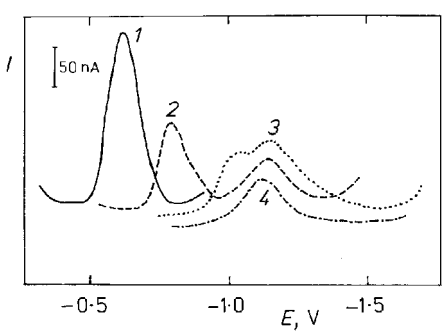


FIG. 3
FSDP voltammograms of dibenz[*a*,*h*]acridine (c = 1 . 10^{-5} mol l^{-1}) in Britton–Robinson buffer–methanol mixtures 1 : 9; pH: *1* 4.8, *2* 7.8, *3* 11.1, *4* 12.2

not complicated by superposition with the current due to the decomposition of the supporting electrolyte. The optimum pH chosen was pH 5.9 for acridine, 4.1 for benz[*c*]acridine and 4.8 for dibenz[*a*,*h*]acridine, and was adjusted with Britton–Robinson buffers of pH 5.0, 3.0 and 2.0, respectively, using the appropriate additions of methanol.

The dependence of the height of the FS DPV peak on the concentration of acridine is linear over the concentration regions of $(2 - 10) \cdot 10^{-7}$ and $(2 - 10) \cdot 10^{-6}$ mol l^{-1}, whereas it is bent within the region of $(2 - 10) \cdot 10^{-5}$ mol l^{-1}. For benz[*c*]acridine and dibenz[*a*,*h*]acridine the dependences are bent within the region of concentrations as low as $(2 - 10) \cdot 10^{-6}$ mol l^{-1} and is only linear across the region of $(2 - 10) \cdot 10^{-7}$ mol l^{-1}. The parameters of the linear concentration dependences along with the calculated limits of determination are given in Table IV.

In order to assess whether the bending of the concentration dependences is due to a separation of the analyte from solution or to passivation of the electrode caused by the adsorption of the analyte or reduction products on its surface, the stability of the solutions measured was examined. At concentrations of $1 \cdot 10^{-4}$, $1 \cdot 10^{-5}$ and $1 \cdot 10^{-6}$ mol l^{-1}, the peak height of acridine and benz[*c*]acridine is constant (within the limits of

TABLE III
Effect of pH on FSDP voltammograms of dibenz[*a*,*h*]acridine ($c = 1 \cdot 10^{-5}$ mol l^{-1}) in Britton–Robinson buffer–methanol 1 : 9 mixture

pH[a]	E_p, V		I_p, nA	
	1st peak	2nd peak	1st peak	2nd peak
1.2[b]	–0.580	–[c]	185	–[c]
4.8	–0.620	–[c]	303	–[c]
6.1	–0.690	–1.150	230	63
6.8	–0.735	–1.150	200	68
7.8	–0.795	–1.140	150	68
9.0	–0.875	–1.150	113	68
9.9	–0.945	–1.150	113	80
10.2	–0.975	–1.110	98	75
10.5	–0.990	–1.125	100	80
11.0	–1.025	–1.150	113	138
11.5	–1.060	–	88	–
12.2	–1.120	–	80	–

[a] Of the mixed solvent; [b] mixture 3 M H_2SO_4 and methanol (1 : 9); [c] overlapped by supporting electrolyte decomposition current.

experimental error) for 120 min from the solution preparation. Concentrated solutions of dibenz[*a*,*h*]acridine are somewhat less stable, which is apparently related to the low solubility of this solute and the associated slow separation from the solution. The decrease, however, is not very marked and cannot be responsible for the concentration dependence nonlinearity: at a concentration of $1 \cdot 10^{-5}$ mol l^{-1} the peak decreases 7%, 10% and 13% in 30, 60 and 120 min, respectively. The alternative explanation in terms of passivation of the electrode surface, on the other hand, is supported by the fact that the concentration dependences measured by differential pulse polarography at a static mercury drop electrode whose surface is renewed during the measurement are linear also across those concentration regions for which FS DPV at a HMDE whose surface is not renewed gives bent dependences.

Adsorptive Stripping Voltammetry

For the determination of acridine derivatives in low concentrations, the feasibility of their preliminary accumulation by adsorption on the hanging mercury drop was investigated. All of the substances examined were found to accumulate on the HMDE, although to different extents.

TABLE IV
Calibration straight line parameters and limits of determination of acridine (*I*), benz[*c*]acridine (*II*) and dibenz[*a*,*h*]acridine (*III*) in Britton–Robinson buffer–methanol mixtures 1 : 1 by FS DPV and AdSV

Substance	Technique	pH	*c*, mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	r^a	$L_Q{}^b$ mol l^{-1}
I	FS DPV	5.9	$(1 - 10) \cdot 10^{-6}$	5.05	4.30	0.9956	–
		6.0[c]	$(2 - 10) \cdot 10^{-7}$	4.45	–0.31	0.9993	$1.4 \cdot 10^{-7}$
	AdSV[d]	–[e]	$(2 - 10) \cdot 10^{-8}$	88.0	0.28	0.9975	$2.4 \cdot 10^{-8}$
		–[f]	$(2 - 10) \cdot 10^{-8}$	175.0	–0.37	0.9986	$1.8 \cdot 10^{-8}$
II	FS DPV	4.1	$(2 - 10) \cdot 10^{-7}$	9.1	–0.58	0.9957	$2.0 \cdot 10^{-7}$
	AdSV[d]	–[e]	$(2 - 10) \cdot 10^{-8}$	77.5	–0.03	0.9975	–
		–[e]	$(2 - 10) \cdot 10^{-9}$	117.8	–0.03	0.9984	$3.4 \cdot 10^{-9}$
		–[f]	$(2 - 10) \cdot 10^{-9}$	305.1	–0.01	0.9911	$4.4 \cdot 10^{-9}$
III	FS DPV	4.8[g]	$(2 - 10) \cdot 10^{-7}$	58.8	–1.55	0.9992	$1.4 \cdot 10^{-7}$
	AdSV[d]	–[e]	$(2 - 10) \cdot 10^{-8}$	345.3	–0.71	0.9980	$2.3 \cdot 10^{-8}$
		–[f]	$(2 - 10) \cdot 10^{-8}$	594.2	–0.29	0.9982	$2.0 \cdot 10^{-8}$

[a] Correlation coefficient; [b] limit of determination; [c] tenfold diluted Britton–Robinson buffer–methanol mixture 1 : 1; [d] conditions of accumulation given in Table V; [e] unstirred solution; [f] stirred solution; [g] Britton–Robinson buffer–methanol mixture 1 : 9.

As expected, dibenz[*a*,*h*]acridine accumulates well owing to its low solubility. In a medium identical with that where the FS DPV was performed without accumulation (a 1 : 9 mixture of Britton–Robinson buffer at pH 2.0 with methanol, resulting pH 4.8), the substance gave a single, well-developed peak. Benz[*c*]acridine can also be accumulated in the system in which the FS DPV determination without accumulation was accomplished, i.e. in a 1 : 1 mixture of Britton–Robinson buffer at pH 3.0 with methanol, but a system containing the Britton–Robinson buffer at pH 7.0 and methanol in the 1 : 1 ratio (resulting pH 8.1) is preferable because the supporting electrolyte baseline is more favourable, owing to which the limit of determination is lower. Acridine sorbs to the least extent due to its higher polarity, and thus a higher solubility, in the mixed aqueous-methanolic solvent. In solution containing 50 vol.% methanol, acridine sorbs to a considerably lower extent than the impurities present. This did not improve by tenfold dilution of the buffer with redistilled water. Therefore, the methanol content was reduced to 10 vol.%, and based on the shapes of the base electrolyte line, the Britton–Robinson buffer with pH 9.0 was chosen.

The time for which the substance was to be accumulated was determined by evaluating the FSDP voltammograms of the depolarizer solution at a concentration of $1 \cdot 10^{-7}$ mol l^{-1}, with or without stirring, in various time intervals from the formation of the drop (Fig. 4). For the stirred solution, the trace was only recorded

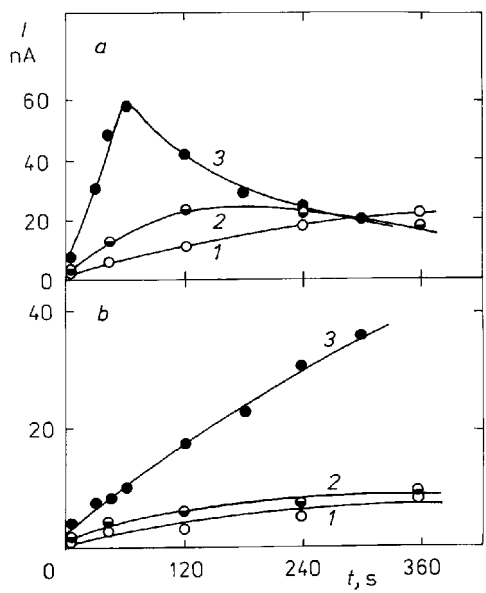


FIG. 4
Dependences of FS DPV peaks of acridine (*1*), benz[*c*]acridine (*2*) and dibenz[*a*,*h*]acridine (*3*) ($c = 1 \cdot 10^{-7}$ mol l^{-1}) on time of accumulation in stirred (*a*) and unstirred (*b*) solutions; for conditions of accumulation see Table V

15 s after discontinuing the stirring. The dependences of the peak height on the time of accumulation for the three substances are first linear but they bend at higher times of accumulation; the bending is particularly marked for the stirred solutions, which is presumably related to the maximum possible covering of the electrode surface by the adsorbing substance. The observed decrease in the peak height of benz[*c*]acridine after long times of accumulation in stirred solution is probably due to the electrode surface passivation by the electrode reaction products. The times of accumulation applied in the subsequent measurements correspond to the time interval for which the maximum current signal was attained. The accumulation parameters for the acridine derivatives are given in Table V.

Under such conditions, the concentration dependences of the substances studied were measured in stirred and unstirred solutions. Over the concentration region of $(2 - 10) \cdot 10^{-8}$ mol l^{-1}, the dependences were linear for all the three substances in unstirred solution, whereas if the solution was stirred, only acridine and dibenz[*a,h*]acridine gave linear dependences, whereas the dependence for benz[*c*]acridine was bent. The bending, which for the remaining two substances appeared at concentrations of $(2 - 10) \cdot 10^{-7}$ mol l^{-1}, is apparently related to the highest possible coating of the electrode by the adsorbed substance. Towards lower concentrations, the concentration dependence for benz[*c*]acridine was also linear within the $(2 - 10) \cdot 10^{-9}$ mol l^{-1} region.

The parameters of the linear concentration dependences, along with the calculated limits of determination, are given in Table IV. FSDP voltammograms of benz[*c*]acridine and dibenz[*a,h*]acridine after adsorptive accumulation are shown in Fig. 5.

TABLE V
Conditions chosen for the accumulation of acridine (*I*), benz[*c*]acridine (*II*) and dibenz[*a,h*]acridine (*III*)

Substance	MeOH–buffer	Buffer, pH	Mixture[a], pH	t_{acc}, s	E_{acc}, V
I	1 : 9	9.0[b]	9.0	360[c], 120[d]	–0.5
II	1 : 1	7.0	8.1	360[c], 360[d]	–0.6
III	9 : 1	2.0	4.1	300[c], 60[d]	–0.3

[a] pH value of resulting aqueous-methanolic medium; [b] in this case, buffer was diluted tenfold with redistilled water; [c] unstirred solution; [d] stirred solution.

Application of FS DPV to the Monitoring of the Efficiency of Chemical Destruction of Acridine and Dibenz[a,h]acridine with Potassium Permanganate

Acid Medium

Figure 6 demonstrates that oxidation of acridine or dibenz[*a*,*h*]acridine solution in acetone by the action of potassium permanganate in acid solution, as described in the Experimental, gives rise to a small quantity of polarographically active products. Their peaks, however, are sufficiently well separated from those of the initial substances. Moreover, comparison of the FS DPV curves after standard additions corresponding to 1 – 5% of the initially present amount of analyte before the destruction gives evidence that the analyte concentration after destruction is lower than as corresponds to the first addition (1%), hence, the efficiency of the destruction is higher than 99% (methanol was added to the solution measured because in aqueous solutions the FSDP voltammograms of acridine and dibenz[*a*,*h*]acridine are ill-developed and ill-reproducible).

The results using acetonitrile in place of acetone were similar. The heights of the peak in the range about –0.5 V, recorded immediately after degradation and after standard additions, indicate that no less than 99% acridine was oxidized. The situation is somewhat more complicated with dibenz[*a*,*h*]acridine in acetonitrile, for which the peak height increase in the –0.7 V range in less pronounced due to the considerably

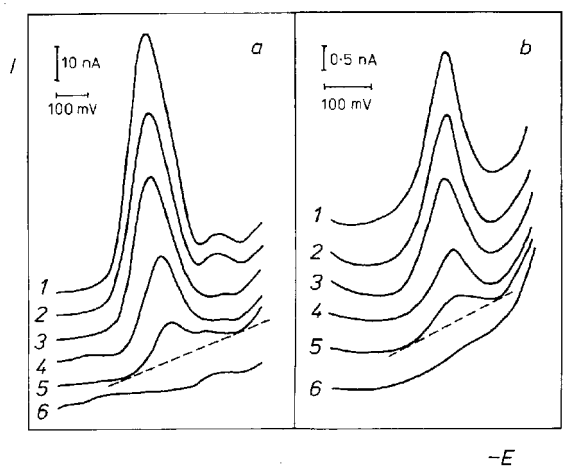


FIG. 5

FSDP voltammograms of dibenz[*a*,*h*]acridine (*a*) and benz[*c*]acridine (*b*) recorded following adsorptive accumulation; *a* accumulated for 300 s in a stirred solution of Britton–Robinson buffer–methanol mixture 1 : 9, pH 4.1, starting potential –0.3 V, $c \cdot 10^8$ (mol l^{-1}): *1* 10, *2* 8, *3* 6, *4* 4, *5* 2, *6* supporting electrolyte; *b* accumulated for 360 s in stirred solution of Britton–Robinson buffer–methanol mixture 1 : 1, pH 8.1, starting potential –0.6 V, $c \cdot 10^9$ (mol l^{-1}): *1* 10, *2* 8, *3* 6, *4* 4, *5* 2, *6* supporting electrolyte. Broken line is the baseline from which the peak height was read

lower initial analyte concentration as a consequence of its low solubility. The peak height had to be read from a line parallel to the axis of potentials at the minimum before the peak. In this manner it was found that a minimum of 96% of the substances was removed by the procedure.

Alkaline Medium

The destruction of the substances with potassium permanganate in alkaline solutions, as described in the Experimental, does not give clear solutions because insoluble hydrate manganese(IV) oxide emerges. The analysis of the sample after destruction must be therefore preceded by extraction. Cyclohexane was chosen as the extractant based on preliminary results. The destruction of acridine by the method described in the Experimental was efficient to a minimum of 99.4%. For dibenz[*a*,*h*]acridine, however, about 10% of the initially present substance remained in the solution after 24 h of action of excess permanganate, which indicates that the method is not very well suited to the destruction of this substance.

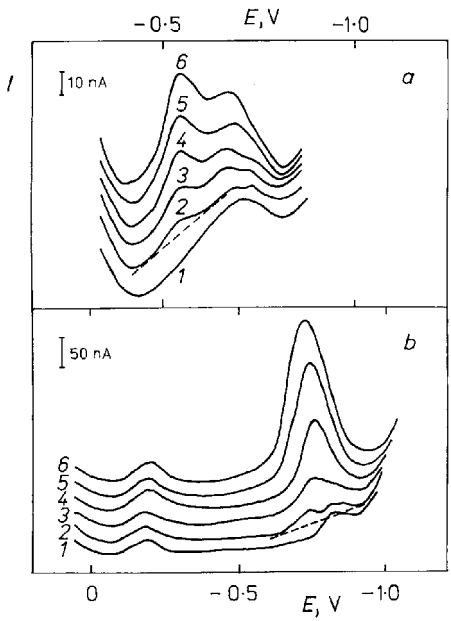


FIG. 6
FS DPV monitoring of efficiency of destruction of acridine (*a*) and dibenz[*a*,*h*]acridine (*b*) in acetone. Standard addition (in %) corresponds to fraction of the initially present substance before destruction of: *1* 0, *2* 1, *3* 2, *4* 3, *5* 4, *6* 5. Broken line is the baseline from which the peak height was read

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