THE DETERMINATION OF 4-SUBSTITUTED DERIVATIVES OF N-NITROSO-N-METHYL ANILINE BY FAST SCAN DIFFERENTIAL PULSE VOLTAMMETRY AT A HANGING MERCURY DROP ELECTRODE*

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Optimum conditions were found for the determination of N-nitroso-N-methyl aniline and its derivatives substituted in the 4 position by $-CH_3$, $-OCH_3$, $-Cl$, $-CN$, $-OH$ or $-NO_2$ groups by fast scan differential pulse voltammetry at a hanging mercury drop electrode in the concentration range $1 . 10^{-5}-2 . 10^{-7}$ mol l^{-1}. It was demonstrated that these techniques are useful for the analysis of a mixture of the test substances either directly or after separation by thin-layer chromatography and that false positive results can be eliminated by UV irradiation of the test sample. An attempt to further increase the sensitivity by adsorptive accumulation of the test substances on the surface of the hanging mercury drop electrode was not successful as the test N-nitroso compounds are practically not adsorbed on this electrode.

N-nitroso compounds are among the most commonly occurring chemical carcinogens and are present in trace amounts in a number of products of the chemical and even food industry, in various agricultural products and also in the living and working environment[1]. A number of analytical methods have been developed for the sensitive and selective determination of these substances even in complex matrices[2]. Polarographic and voltammetric methods[3,4] are among those fulfilling the stringent conditions on selectivity and sensitivity essential in the determination of N-nitrosamines, either in environmental analysis or in the study of their biotransformation.

The derivatives of N-nitroso-N-methyl aniline constitute an interesting group of these substances and their carcinogenic[5] and mutagenic[6] effects have recently been extensively studied; they are characterized by an unusual organ specificity leading almost exclusively to cancer of the oesophagus[7]. So far, the behaviour of these substances has been studied using DC polarography[8] and differential pulse polarography[9], employed to determine these substances in foods and other biological

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materials[10], in blood and blood serum[11] and in the urine of test animals[12]. Work was mostly carried out in mixed alcohol–water medium to ensure sufficient solubility of the test substances. Some aromatic or heterocyclic N-nitroso amines have been studied by normal pulse polarography in a mixture of water, methanol and acetonitrile[13].

This work deals with the determination of the 4-substituted derivatives of N--nitroso-N-methyl aniline (4-R–NMA, where R is H, CH_3, OCH_3, CN, Cl, NO_2 or OH) by fast scan differential pulse voltammetry (FS DPV) at a hanging mercury drop electrode (HMDE) in order to utilize this technique for analysis of a mixture of the test substances either directly or after separation by thin-layer chromatography (the combination of TLC with DC polarography was first used in ref.[14]).

EXPERIMENTAL

Reagents

The stock solutions of the test substances ($c = 1 \,.\, 10^{-3}$ mol l^{-1}) were prepared by dissolving a precisely weighed amount of the pure substance synthesized in the laboratories of the Toxicological Department of the Research Institute for Organic Synthesis in Pardubice-Rybitví[9] in freshly redistilled methanol (p.a. Lachema, Brno). Except for 4-H–NMA, which is a liquid, these substances are solid, crystalline substances that must be stored in the refrigerator because of their low stability. The content and purity of these substances was controlled by elemental analysis, melting point measurement, paper, thin-layer and high-performance liquid chromatography and by indirect coulometric titration in the dual system Ti(III)–Fe(III)[15]. Solutions with lower concentrations were prepared by precise dilution of the stock solutions with methanol. All the solutions were stored in the dark. It followed from a spectrophotometric study of the stability of these solutions that $1 \,.\, 10^{-4}$ mol l^{-1} solutions are stable for at least one month and $1 \,.\, 10^{-5}$ mol l^{-1} solutions are stable for at least one week. Solutions with lower concentrations were prepared fresh daily. The remaining chemicals were of p.a. purity (Lachema, Brno). Water was doubly distilled in a quartz apparatus.

Apparatus

Measurements were carried out on a PA3 Polarographic Analyzer with an XY-4105 recorder and SMDE 1 electrode employed as a hanging mercury drop electrode with a capillary with a diameter of 0·136 mm (all from Laboratorní přístroje, Prague). Work was carried out in a three-electrode circuit using a saturated calomel reference electrode and platinum foil auxiliary electrode. (All the potential values given are thus related to the SCE.) Where not stated otherwise, a time constant of the analog memory of 100 ms, potenital scan rate of 20 mV s^{-1}, FS DPV pulse height of −100 mV, pulse width and interval between pulses of 100 ms and maximum size of the hanging mercury drop electrode given by opening the valve for a period of 160 ms were used. Oxygen was removed from the polarographed solutions by bubbling for ten minutes with nitrogen, which was purified by passing through an alkaline solution of sodium anthraquinone-2-sulfonate and chromous ions in diluted (1 : 1) hydrochloric acid over zinc amalgam. A prebubbler containing a water–methanol (1 : 1) was placed prior to the polarographic vessel, which was made of dark glass to prevent photolysis of the N-nitrosoamines. The pH of the test solutions was measured using the PHM 64 digital pH meter (Radiometer, Copenhagen) with a combined glass-calomel electrode GK 2320c (Radiometer, Copenhagen) calibrated using acetate, borate and phosphate buffers in 50% v/v methanol[16,17]. The UV spectra were measured on a Specord UV-VIS instrument (Zeiss, Jena, F.R.G.) in quartz cuvettes with a pathlength of 0·5, 1 and 5 cm. Detection in TLC was carried out using a short-wavelength Hg discharge lampe UV 254 nm (Metra Violet, San Gabriel). Photolytic decomposition of the N-nitrosoamines was carried out using an RVL-K 250 Hg discharge lamp (Tesla, Prague).

Procedure

Calibration curves were measured in triplicate and evaluated by the least squares linear regression method. The determination limit was calculated[18] as ten times the standard deviation for determination of the analyte with a concentration of $2 . 10^{-7}$ mol l^{-1}.

The efficiency of the elution of the test substances from a Silufol UV 254 thin layer (Kavalier, Votice) was tested by applying 2—10 μl of a solution of 4-CH_3–NMA or 4-Cl–NMA in methanol ($c = 1 . 10^{-3}$ mol l^{-1}) to the start using a Hamilton microsyringe (Bonaduz); elution was immediately carried out using the descending method with methanol in an atmosphere of its saturated vapours. Elution was carried out for 20 min directly into the voltammetric vessel. After evaporation of the methanol by a stream of air at laboratory temperature, the residue was dissolved in 10·0 ml of a Britton–Robinson buffer–methanol (1 : 1) mixture at pH 3·47 and the solution obtained was studied voltammetrically in the usual manner. Volumes greater than 4 μl were applied to the thin layer in repeated small amounts, each after spontaneous vaporization of the solvent from the previous amount, at laboratory temperature. The vaporization of methanol from the thin layer or voltammetric vessel cannot be accelerated by a hot air stream as losses result from the volatility of the test substances. In a different version, the thin layer with the applied sample was developed in the ascending method with a hexane–diethyl ether–dichloromethane (10 : 6 : 0·5) mixture for a period of 45 min. Then the spot corresponding to the test substance was noted under a UV lamp, a band with dimensions of 5 × 1 cm containing this spot was cut out and the test substance was eluted in the above manner with methanol; vaporization of the methanol and transfer to the base electrolyte was followed by recording of the voltammetric curve. Irradiation during UV detection should be limited to the minimum to decrease losses through photolysis. A completely analogous procedure was employed in the separation of mixtures of 4-CH_3–NMA and 4-Cl–NMA in a molar ratio of 1 : 1.

RESULTS AND DISCUSSION

The Effect of the pH *on the Behaviour of the Test Substances in Fast Scan Differential Pulse Voltammetry at a Hanging Mercury Drop Electrode*

This effect was studied in a mixed Britton–Robinson buffer–methanol medium (1 : 1) at pH 2–13 and a depolarizer concentration of $1 . 10^{-5}$ mol l^{-1}. The results obtained are given in Table I. The pH values given in this table and in the subsequent text are always related to the pH of the resultant Britton–Robinson buffer–methanol mixture. It can be seen from Table I that the test substances in acidic medium (pH 2–6) yield a single peak that is shifted to more negative potentials with increasing pH values. In addition, 4-NO_2–NMA yields an additional peak at more positive potentials, corresponding to the reduction of the nitro-group and which is also

TABLE I

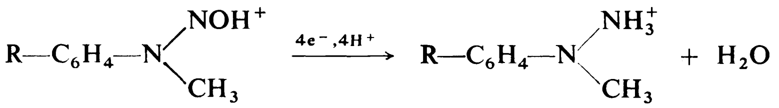
Dependence of the position (E_p) and height (I_p) of the FS DPV peak of 4-R-N-nitroso-N-methyl aniline ($c = 1 . 10^{-5}$ mol l^{-1}) on the pH in mixed Britton–Robinson buffer–methanol (1 : 1)

R	OH		CH$_3$		CN		Cl		OCH$_3$		H		NO$_2$^{a}			
pH	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA	E_p mV	I_p nA
2·47	−830	62	−775	83	−745	84	−750	83	−785	78	−800	78	−255	90	−790	82
2·77	−890	60	−810	84	−770	83	−800	83	−835	77	−845	76	−265	90	−820	80
3·47	−1 000	62	−920	81	−820	82	−870	78	−915	75	−925	78	−290	92	−870	81
4·74	−1 080	62	−1 010	80	−850	77	−970	78	−1 030	77	−1 030	80	−375	95	−1 070	84
5·95	−1 155	60	−1 085	77	−950	60	−1 060	74	−1 100	80	−1 120	65	−440	93	−1 135	88
7·35	−1 225^b	13	−1 170^b	44	−1 075	46	−1 115^b	35	−1 200	47	−1 190^b	12	−540	91	−1 225^c	21
	−1 475	10	−1 375	6			−1 395	7			−1 420	30			−1 450	12
8·15	—^{d}	—	−1 200^b	10	−1 210	62	−1 125^b	5	−1 225^b	11	−1 180^b	4	−605	85	−1 225^c	3
	−1 475	44	−1 430	36			−1 365	44	−1 430	33	−1 425	53			−1 450	62
8·76	—^{d}	—	−1 190^b	3	−1 225	72	—	—	—	—	—	—	−635	90	—	—
	−1 470	50	−1 425	56			−1 360	62	−1 440	51	−1 440	57			−1 450	83
9·20	−1 470	50	−1 425	60	−1 230	74	−1 350	63	−1 440	59	−1 405	60	−660	88	−1 440	90
10·14	−1 500	40	−1 425	56	−1 230	55	−1 360	60	−1 440	60	−1 415	56	−690	89	−1 445	85
11·11	−1 575	44	−1 425	53	−1 235^b	53	−1 370	57	−1 425	51	−1 395	50	−725	72	−1 440	84
					−1 620	6										
12·31	−1 615	30	−1 420	46	−1 230^b	57	−1 360	59	−1 425	50	−1 400	48	−785	77	−1 450	82
					−1 600	4										
12·54	−1 645	21	−1 410	48	−1 230^b	54	−1 360	59	−1 425	57	−1 400	50	−780	85	−1 415	76
					−1 600	3										
13·61	−1 640	9	−1 375	51	−1 200	41	−1 310	68	−1 435	55	−1 370	48	−750	90	−1 420	80

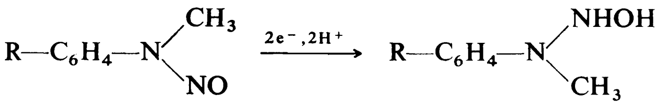
[a] First peak corresponds to reduction of the nitro group, second to reduction of the nitroso group. [b] The test substance yields two peaks at this pH. [c] The test substance 4-NO$_2$-NMA yields two peaks for the reduction of the nitroso group. [d] The pH-dependent peak disappears, leaving only the pH-independent peak.

shifted to more negative potentials with increasing pH. As the pH further increases, the height of the pH-dependent peak corresponding to the reduction of the nitroso group decreases for all the NMA derivatives and a new peak simultaneously appears at about $-1\,400$ mV. The position of this peak is practically pH-independent and its height decreases slightly with increasing pH. The height of this peak is mostly less than that of the pH-dependent peak in the acidic region. The peak for 4-OH–-NMA is an exception, where the second peak also shifts to more negative values with increasing pH, apparently connected with dissociation of the phenolic group.

The observed effect of the pH on the behaviour of the test substances in FS DPV at the HMDE is in agreement with their behaviour in DC polarography[8] and can be explained on the basis of a mechanism according to which a four-electron reduction of the protonated molecule to the corresponding hydrazine derivative occurs in acidic medium according to Eq. *A*, while a two-electron reduction to the corresponding hydroxyl amine derivative occurs in alkaline medium according to Eq. *B*:



$$R{-}C_6H_4{-}N\begin{matrix}\diagup NOH^+\\ \diagdown CH_3\end{matrix} \xrightarrow{4e^-,4H^+} R{-}C_6H_4{-}N\begin{matrix}\diagup NH_3^+\\ \diagdown CH_3\end{matrix} + H_2O \qquad (A)$$



$$R{-}C_6H_4{-}N\begin{matrix}\diagup CH_3\\ \diagdown NO\end{matrix} \xrightarrow{2e^-,2H^+} R{-}C_6H_4{-}N\begin{matrix}\diagup NHOH\\ \diagdown CH_3\end{matrix} \qquad (B)$$

The peak position for the individual substances is basically determined by the electron donor properties of the substituent in the 4-position. Figure 1 reveals acceptable correlation between the experimentally determined peak potential values and the Hammet constants σ_p of the corresponding substituents, confirming a single mechanism for the reduction of the test series of substances. The 4-nitro derivative is once again an exception; the reduction of the nitroso group is apparently complicated by the reduction of the nitro group.

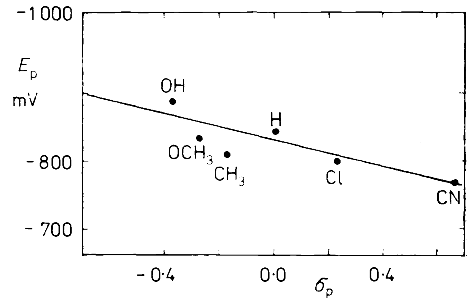


FIG. 1

Dependence of the peak potential (E_p) of 4-R-N-nitroso-N-methyl aniline on the Hammett σ_p constant of substituent R in Britton–-Robinson buffer-methanol medium (1 : 1) at pH 2·77

Analytical Utilization of Fast Scan Differential Pulse Voltammetry of the Test Substances

Determination of the individual substances. A medium with pH 3·47 was selected as optimum for the determination of the test substances; the peak height has not yet decreased with increasing pH and the danger of protolysis is minimized. At this pH value, the dependence of the peak height on the concentration is linear for all the test substances in the range $1 . 10^{-5} - 2 . 10^{-7}$ mol l^{-1}. The parameters of this dependence and the calculated determination limits are given in Table II.

An attempt to utilize adsorptive accumulation of the test substances on the surface of the hanging mercury drop to improve the sensitivity of the determination was not successful. At all the pH values (in the tested range 2 – 14), methanol concentrations (varied in the range 50 – 0% v/v) and depolarizer concentrations (varied in the range $1 . 10^{-5} - 1 . 10^{-7}$ mol l^{-1}) no dependence was observed between the

TABLE II

Parameters of the calibration curves and the determination limits for the FS DPV determination of 4-R-N-nitroso-N-methyl aniline in Britton–Robinson buffer–methanol medium (1 : 1) at pH 3·47

R	Concentration µmol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correl. coeff.	L_Q[a] mol l^{-1}
OH	2 – 10	6·1	1·3	0·9997	—
	0·2 – 1·0	6·0	−0·3	0·9991	$1{\cdot}4 . 10^{-7}$
CH_3	2 – 10	8·2	−1·7	0·9999	—
	0·2 – 1·0	8·5	−0·3	0·9984	$1{\cdot}8 . 10^{-7}$
CN	2 – 10	8·5	−5·0	0·9980	—
	0·2 – 1·0	8·3	−1·0	0·9987	$1{\cdot}8 . 10^{-7}$
Cl	2 – 10	8·0	−6·5	0·9994	—
	0·2 – 1·0	8·4	−0·1	0·9969	$2{\cdot}1 . 10^{-7}$
OCH_3	2 – 10	8·2	−0·2	0·9999	—
	0·2 – 1·0	7·9	−0·9	0·9992	$1{\cdot}4 . 10^{-7}$
H	2 – 10	7·8	−5·2	0·9995	—
	0·2 – 1·0	7·6	0·1	0·9955	$2{\cdot}2 . 10^{-7}$
NO_2	2 – 10	9·6[b]	−6·0	0·9991	—
		8·2[c]	0·8	0·9999	—
	0·2 – 1·0	9·3[b]	−0·9	0·9977	$1{\cdot}4 . 10^{-7}$
		8·8[c]	0·2	0·9964	$1{\cdot}9 . 10^{-7}$

[a] Determination limit; [b] reduction of the nitro group; [c] reduction of the nitroso group.

peak height and the time elapsed between drop formation and recording of the voltammetric curve. It can thus be concluded that the test substances are not adsorbed on the surface of the hanging mercury drop and that this technique can thus not be utilized to improve the sensitivity.

Elimination of false positive results. In the FS DPV determination of N-nitroso compounds, false positive results can be incurred through the presence of substances yielding a voltammetric peak at the same potential as the test substance. For a number of N-nitrosamines, this danger can be avoided by irradiating the sample with UV radiation[3], leading to its denitrosation through photolytic decomposition yielding polarographically inactive products. It was found by the procedure given in the experimental part that this technique can also be used for the derivatives of N--nitroso-N-methyl aniline studied here. For illustration, Fig. 2 depicts the voltammograms of a solution of 4-CH_3-NMA prior to and after irradiation of the sample, confirming the capability of eliminating false positive results in this manner.

Direct analysis of mixtures of the test substances. It followed from preliminary experiments that direct analysis of a mixture of the test substances by FS DPV is optimal in Britton–Robinson buffer at pH 3·49 containing 1% v/v or less methanol. A decrease in the methanol content from 50% v/v to 0·5% v/v leads to a shift in the peak potential to more positive values (see Table III) with an increase in the reversibility of the reduction, resulting in narrower and higher peaks. The peak height and position are practically constant in the range 0·01 – 1% methanol v/v. It follows from Table IV that, under the given conditions, the difference between the peak potentials is sufficient for direct analysis of mixtures of 4-CN–NMA or 4-NO_2–-NMA with 4-H–NMA or 4-OH–NMA. As 4-NO_2–NMA can be readily determined in the presence of the other members of the group on the basis of the reduction of the nitro group, the possibility of directly analyzing a mixture of these substances by FS DPV was verified using a model mixture of 4-CN–NMA with 4-OH–NMA and of 4-CN–NMA with 4-H–NMA. The analysis was carried out by measuring the voltammograms of solutions containing a constant concentration of one component and a varying concentration of the other component.

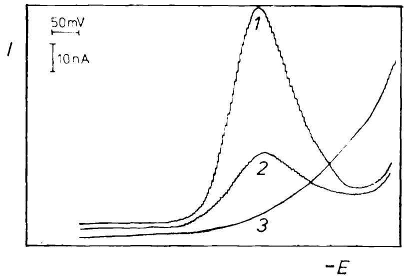


FIG. 2

FS DP voltammogram of 4-CH_3-NMA ($c = 1 \cdot 10^{-5}$ mol l^{-1}) in Britton–Robinson buffer–methanol (1 : 1) medium at pH 3·47 prior to irradiation (*1*), after 30 s (*2*) and after 60 s (*3*) irradiation with a mercury discharge lamp. Initial potential – 550 mV

The parameters of these calibration curves are given in Table V, where it can be seen that the peak height for the test substance is always proportional to the concentration of this substance, where the proportionality constant depends on the concentration of the second component of the mixture, connected with interaction between the peak heights for the two components. Mixtures of 4-OH–NMA and 4-CN–NMA can be analyzed directly only when they do not contain too great an

TABLE III

The effect of the methanol content on the position (E_p) and height (I_p) of the FS DPV peak of 4-CN–NMA and 4-OH–NMA ($c = 1 . 10^{-5}$ mol l^{-1}) at constant pH of the polarographed solution of 3·86

	R			
	4-CN–NMA		4-OH–NMA	
MeOH Content %	E_p mV	I_p nA	E_p mV	I_p nA
50	−825	86	−1 005	59
20	−745	142	−890	62
5	−680	200	−830	70
0·5	−660	250	−815	83

TABLE IV

The peak potential (E_p) and peak current (I_p) values for 4-R-N-nitroso-N-methyl aniline ($c = 1 . 10^{-6}$ mol l^{-1}) measured by FS DPV in Britton–Robinson buffer–methanol medium (99·5 : 0·5) at pH 3·43

R	E_p mV	I_p nA
OH	−815	8·3
CH_3	−780	18·0
CN	−660	25·0
Cl	−745	24·0
OCH_3	−775	24·3
H	−800	16·3
NO_2	−740	23·2
	−190[a]	20·0[a]

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[a] Reduction of the nitro group.

excess of the latter. Otherwise, a poorly discernable small peak of 4-OH–NMA is obtained as a shoulder on the 4-CN–NMA peak. Consequently, for example, 4-OH–NMA can be determined in the range (2 – 10) . 10^{-7} mol l^{-1} in the presence of 1 . 10^{-7} mol l^{-1} 4-CN–NMA; however, at a concentration of 4-CN–NMA of 5 . 10^{-7} or 1 . 10^{-6} mol l^{-1}, 4-OH–NMA can be determined only in the concentration range (2 – 20) . 10^{-6} mol l^{-1}, as at concentrations of (2 – 10) . 10^{-7} mol l^{-1} it is practically impossible to evaluate its peak on the shoulder of the 4-CN–NMA peak.

Because of the interaction between the peak heights of the two components of the mixture, the following procedure must be employed in the analysis: First the voltammogram of the test solution is measured and the height of peak of the first component (I_p^1) is measured, for which it holds that $I_p^1 = kc_1$. Then the peak height is measured

TABLE V

Parameters of the calibration curves for direct analysis of a mixture of 4-H–NMA (I), 4-OH–NMA (II) and 4-CN–NMA (III) in Britton–Robinson buffer medium at pH 3·47 by FS DPV at the HMDE

$c(A)$ mol l^{-1}	$c(B)$ mol l^{-1}	Slope mA . . mol^{-1} l	Intercept nA	Correl. coeff.	L_Q[a] mol l^{-1}
		A = I, B = III			
1 . 10^{-6}	(2 – 10) . 10^{-7}	19·2	– 1·3	0·9976	2·2 . 10^{-7}
5 . 10^{-7}	(2 – 10) . 10^{-7}	21.1	– 1·3	0·9978	2·0 . 10^{-7}
1 . 10^{-7}	(2 – 10) . 10^{-7}	22·4	– 0·8	0·9980	1·8 . 10^{-7}
		A = III, B = I			
1 . 10^{-6}	(2 – 10) . 10^{-7}	12·4	– 0·7	0·9963	2·4 . 10^{-7}
5 . 10^{-7}	(2 – 10) . 10^{-7}	14·5	– 0·7	0·9984	2·1 . 10^{-7}
1 . 10^{-7}	(2 – 10) . 10^{-7}	17·8	– 0·6	0·9997	2·0 . 10^{-7}
		A = II, B = III			
1 . 10^{-6}	(2 – 10) . 10^{-7}	17·9	– 1·0	0·9997	2·3 . 10^{-7}
5 . 10^{-7}	(2 – 10) . 10^{-7}	18·6	– 0·6	0·9963	2·0 . 10^{-7}
1 . 10^{-7}	(2 – 10) . 10^{-7}	21·0	– 0·7	0·9969	1·7 . 10^{-7}
		A = III, B = II			
1 . 10^{-6}	(2 – 10) . 10^{-6}	6·0	– 1·3	0·9939	1·8 . 10^{-6}
5 . 10^{-7}	(2 – 10) . 10^{-6}	6·8	– 0·9	0·9986	0·9 . 10^{-6}
1 . 10^{-7}	(2 – 10) . 10^{-7}	6·9	0·1	0·9996	1·6 . 10^{-7}

[a] Determination limit.

for the same component after a known standard addition, so that it holds that $I_p^{1+s} = k(c_1 + c_s)$, where c_s is the concentration corresponding to the standard addition. The proportionality constant remains a constant in these two equations as the concentration of the second component in the mixture does not change (the dilution resulting from the standard addition can be neglected). Solution of these

TABLE VI

Parameters of the calibration straight lines for the determination of a mixture of 4-CH_3-NMA (IV) and 4-Cl-NMA (V) by FS DPV in mixed Britton-Robinson buffer-methanol (1 : 1) at pH 3·47 after previous separation by TLC

Substance	Method[a]	Slope		Intercept nA	Correl. coeff.
		mA mol^{-1} l[b]	nA μg^{-1}[c]		
IV	*A*	8·4	5·6	−0·17	0·9984
	B	7·2	4·8	−0·14	0·9983
	C	6·7	4·4	−0·02	0·9966
	D	6·6	3·8	0·09	0·9950
V	*A*	8·3	4·9	−0·14	0·9969
	B	7·0	4·1	−0·12	0·9962
	C	6·0	3·5	−0·01	0·9890
	D	5·9	3·4	0·10	0·9984

[a] *A* substance added directly to the base electrolyte; *B* substance rinsed from the thin layer immediately after application; *C* after development of the chromatogram of the substance alone; *D* after development of a chromatogram of a mixture of the substances; [b] calculated on the basis of the concentration of the test substance in the analyzed solution in mol l^{-1}; [c] calculated from the absolute amount of substance applied to the thin layer in μg. The concentration of the test substance in the analyzed solution was varied in the range (2—10) . 10^{-7} mol l^{-1}.

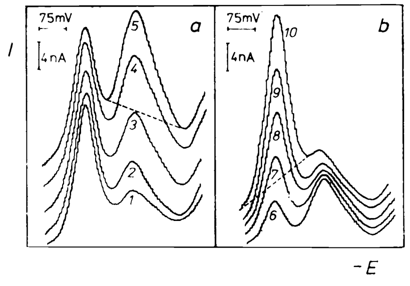

FIG. 3

FS DP voltammograms of a mixture of 4-H-NMA (I) and 4-CN-NMA (III) in Britton-Robinson buffer medium at pH 3·49. *a*: c(III) = 5 . 10^{-7} mol l^{-1}; c(I) (mol l^{-1}): *1* 2 . 10^{-7}, *2* 4 . 10^{-7}, *3* 6 . 10^{-7}, *4* 8 . 10^{-7}, *5* 10 . 10^{-7}; *b*: c(I) = 5 . 10^{-7} mol l^{-1}; c(III) (mol l^{-1}): *6* 2 . 10^{-7}, *7* 4 . 10^{-7}, *8* 6 . 10^{-7}, *9* 8 . 10^{-7}, *10* 10 . 10^{-7}. The dashed straight line corresponds to the baseline, from which the peak heights were measured. Initial potential −550 mV two equations in two unknowns (k and c_1) permits calculation of the concentration of the 1st component in the mixture. A completely analogous procedure is employed in determining the concentration of the 2nd component, i.e. its concentration is again determined through a standard addition, this time of the 2nd component to the original solution, where the concentration of the first component remains constant. For illustration, Fig. 3 depicts voltammograms of mixtures of 4-H–NMA and 4-CN–NMA at a constant concentration of one component and varying concentration of the second component.

Direct analysis of a mixture of the test substances after separation by thin-layer chromatography. The usefulness of the combination of TLC with FS DPV for analysis of a mixture of the test substances was verified on a model mixture of 4-CH_3–NMA and 4-Cl–NMA, where their potential difference in FS DPV is about 30 mV, so that direct voltammetric analysis of their mixtures is not possible.

First, the procedure described in the experimental part was employed to determine the efficiency of elution of the test substances, where the voltammograms of the test substances after elution from the thin layer were compared with voltammograms of the solutions prepared from the same amounts of the standard solutions added directly to the base electrolyte. It can be seen from Table VI that the yield of the elution is about 85%, so that a calibration curve cannot be constructed on the basis of direct addition of the stock solution of the test substance to the base electrolyte. Elution with a mixture of hexane–diethyl ether–dichloromethane (10 : 6 : 0·5) yielded an R_F value for 4-CH_3–NMA of 0·63, with a value of 0·53 for 4-Cl–NMA and it was found that development of the chromatogram further decreases the yield of these substances during elution from the thin layer, connected with spot spreading during separation and with losses connected with the rather high volatility of these substances. Accurate results can be obtained only by employing a calibration curve constructed in the same manner as used for sample treatment (i.e. application of the standard solution of the test substance, separation on the thin layer, elution, vaporization of the eluate and dissolving of the residue in the base electrolyte solution). It can be seen from Table VI that calibration curves constructed in this manner are linear in the range $(2-10) \cdot 10^{-7}$ mol l^{-1} (concentration of the test substance in the analyzed solution) or 0·3 – 1·5 μg (absolute amount of substance applied to the thin layer).

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