

ANALYSIS OF MIXTURES OF 4-NITROTOLUENE-2-SULPHONIC ACID AND 4,4'-DINITROSTILBENE-2,2'-DISULPHONIC ACID BY TAST POLAROGRAPHY, DIFFERENTIAL PULSE POLAROGRAPHY AND THIN LAYER CHROMATOGRAPHY*

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Received June 6th, 1984

Conditions are sought for the determination of 4-nitrotoluene-2-sulphonic acid (*I*) and 4,4'-dinitrostilbene-2,2'-disulphonic acid (*II*) by TAST polarography within the concentration range of 10^{-2} – 10^{-6} mol l⁻¹ and by differential pulse polarography (DPP) within the concentration range of 10^{-3} – 10^{-8} mol l⁻¹. DPP is applied to the determination of these substances after the thin layer chromatographic separation of their mixtures. Methods are also worked out for their analysis without prior separation, based either on the combined DPP determination of *I* and the TAST polarographic determination of the sum of *I* and *II* in sulphuric acid solutions, or on the DPP determination of the C=C bond in *II* and the TAST polarographic determination of the sum of the two substances in tetramethylammonium bromide solutions.

4,4'-Nitrotoluene-2-sulphonic acid (*I*) and 4,4'-dinitrostilbene-2,2'-disulphonic acid (*II*) are fundamental intermediates in the production of stilbene-based optical brightening agents. We have worked out methods for their titrimetric¹⁻⁴, coulometric^{4,5}, polarographic^{1,5}, and spectrophotometric⁶ determination and have paid attention to the analysis of their mixtures by UV spectrophotometry and DC polarography after their separation by paper chromatography⁶. With a view to making use of its higher sensitivity and selectivity as compared with the classical DC polarography, differential pulse polarography (DPP) is employed in this work for the determination of low quantities of the two substances and it is applied to the analysis of their mixtures.

EXPERIMENTAL

Apparatus and Chemicals

The polarographic records were obtained on a PA 2 instrument interfaced to an XY 4103 recorder (Laboratorní přístroje, Prague) using the three-electrode connexion at 20°C; potential

* Part V in the series Physico-Chemical Methods in the Analysis of Dyes and Dye Intermediates; Part IV: This Journal 49, 2751 (1984).

sweep rate 2 mV s^{-1} , controlled drop time 1 s and mercury reservoir height 36 cm unless stated otherwise. The capillary parameters were $m = 2.86 \text{ mg s}^{-1}$ and $t = 2.84 \text{ s}$ at $h = 36 \text{ cm}$ in 0.1M-KCl at the applied potential of 0 V vs S.C.E. A saturated calomel reference electrode and a platinum wire auxiliary electrode were used. The $i-t$ curves were recorded on a BAK coordinate plotter (ZPA Čakovice) with a TB 1 time base. The pH was measured with a PHM 62 digital pH-meter (Radiometer, Copenhagen) equipped with a combined glass and saturated calomel electrode. Small volumes of solutions were proportioned with a $25 \mu\text{l}$ Hamilton micro-syringe or a $500 \mu\text{l}$ Agla microburette (Burrough Wellcome, London). A UV lamp (Ultraviolet, San Gabriel, USA) was used for the detection at 254 nm .

The linear dependences were processed by linear regression analysis. The detection limit was calculated based on the standard deviation of the observed values from the calculated calibration curve by the method of Skogerboe and Grant⁸ at the 99% confidence level.

Stock solutions of *I* and *II* (0.01 mol l^{-1}) in redistilled water were prepared and standardized as described previously^{1,4}. Solutions of lower concentrations were prepared by accurate dilution of the stock solutions with redistilled water. Britton–Robinson buffers were made up⁷ from chemicals of reagent grade purity and doubly redistilled water from a quartz apparatus. Oxygen was removed from the polarographed solutions by 5 min nitrogen purging; nitrogen for this purpose was purified in a train consisting of an alkaline solution of sodium anthraquinone-2-sulphonate and chromium(II) ion solutions in dilute hydrochloric acid in contact with a zinc amalgam. The thin layer separations were performed on commercial Lucefol^R Quick cellulose thin layers (Kavalier, Sázava) using an n-butanol–acetic acid–water (4 : 1 : 5) mixture as the eluting system.

RESULTS AND DISCUSSION

TAST Polarography of *I* and *II*

The dependences of the wave height on the depolarizer concentration were measured in Britton–Robinson buffers of pH 4.5 for *I* and pH 12.5 for *II*, which have been found suitable for the polarographic determination of these substances^{1,4}. The values obtained are given in Table I. The detection limit calculated according to Skogerboe and Grant⁸ is $6 \cdot 10^{-7} \text{ mol l}^{-1}$ for *I* and $3 \cdot 10^{-7} \text{ mol l}^{-1}$ for *II*. At concentrations of *I* or *II* above $2 \cdot 10^{-4} \text{ mol l}^{-1}$, a sharp maximum, which can be suppressed with gelatine, appears at the beginning of the polarographic wave. The limiting current thus has to be read at -800 mV for *I* and at -1100 mV for *II*. For *II* at concentrations lower than $10^{-5} \text{ mol l}^{-1}$ the initially single wave splits into two waves; the height of the first wave is directly proportional to the concentration whereas that of the second wave is nearly concentration-independent. The values for the first wave are given in Table I for concentrations of $(1-10) \cdot 10^{-6} \text{ mol l}^{-1}$.

Differential Pulse Polarography of *I* and *II*

Effect of pH. The dependences of the peak current (I_p) and the peak potential (E_p) on pH for the two substances are shown in Fig. 1. For substance *I*, E_p shifts to more negative values as the pH is increased over the region of pH 3–9.5; the slope

of this dependence, $\Delta E_p/\Delta \text{pH}$, is 70 mV. At higher pH, E_p remains constant. The observed decrease in I_p with increasing pH can be explained in terms of a lowered reversibility. Solutions with $\text{pH} < 6$ thus suit better for the determination of I .

For substance *II*, E_p shifts to more negative values with increasing pH over the entire pH region (Fig. 1) with a slope of $\Delta E_p/\Delta \text{pH} = 45$ mV. At $\text{pH} > 7$ the I_p

TABLE I

Parameters of the calibration straight lines for the determination of *I* and *II* by the TAST and DPP methods

Concentration range mol l^{-1}	a/s_a^a $\mu\text{A mol}^{-1}$	b/s_b^b μA	r^c	$s_{y,x}^d$ μA
Substance <i>I</i> , TAST technique				
$(1-10) \cdot 10^{-3}$	$9.4 \cdot 10^3/2.3 \cdot 10^2$	$4.8/1.5$	0.9991	1.5
$(1-10) \cdot 10^{-4}$	$1.36 \cdot 10^4/1.5 \cdot 10^2$	0/0.1	0.9998	0.1
$(1-10) \cdot 10^{-5}$	$1.36 \cdot 10^4/7.6 \cdot 10^1$	0/0.005	0.9999	0.005
$(1-10) \cdot 10^{-6}$	$1.70 \cdot 10^4/2.9 \cdot 10^2$	0/0.002	0.9996	0.002
Substance <i>II</i> , TAST technique				
$(1-6) \cdot 10^{-3e}$	$1.63 \cdot 10^4/1.4 \cdot 10^2$	$1.3/0.6$	0.9999	0.4
$(1-10) \cdot 10^{-4}$	$1.42 \cdot 10^4/9.6 \cdot 10^1$	$-0.1/0.06$	0.9999	0.06
$(1-10) \cdot 10^{-5}$	$1.55 \cdot 10^4/3.4 \cdot 10^2$	0/0.02	0.9993	0.02
$(1-10) \cdot 10^{-6}$	$1.77 \cdot 10^4/1.15 \cdot 10^2$	0/0.001	0.9999	0.001
Substance <i>I</i> , DPP technique				
$(1-10) \cdot 10^{-5}$	$2.08 \cdot 10^4/2.2 \cdot 10^2$	0/0.02	0.9998	0.014
$(1-10) \cdot 10^{-6}$	$2.65 \cdot 10^4/4.3 \cdot 10^2$	0/0.01	0.9975	0.007
$(1-10) \cdot 10^{-7}$	$1.70 \cdot 10^4/5.6 \cdot 10^2$	0/0.004	0.9984	0.001
$(1-10) \cdot 10^{-8}$	$8.55 \cdot 10^4/9.5 \cdot 10^2$	0/0.001	0.9998	0.001
Substance <i>II</i> , DPP technique				
$(1-6) \cdot 10^{-5e}$	$2.50 \cdot 10^4/2.9 \cdot 10^2$	$-0.05/0.012$	0.9999	0.008
$(1-10) \cdot 10^{-6}$	$2.12 \cdot 10^4/3.7 \cdot 10^2$	0/0.003	0.9995	0.003
$(1-10) \cdot 10^{-7}$	$2.09 \cdot 10^4/1.3 \cdot 10^2$	0/0.001	0.9942	0.001
$(1-10) \cdot 10^{-8}$	$5.40 \cdot 10^4/1.6 \cdot 10^3$	0/0.002	0.9933	0.001

^a Slope and its standard deviation; ^b intercept and its standard deviation; ^c correlation coefficient; ^d standard deviation for the scatter about the regression straight line; ^e the plot is no more linear at higher concentrations.

value drops appreciably, which is probably again associated with a lowered reversibility, and so acid solutions are again recommended for the determination.

The concentration dependences were measured over the region of $1 \cdot 10^{-4}$ to $1 \cdot 10^{-8}$ mol l⁻¹ at pH 4.5 for *I* and at pH 12.5 for *II* (although not optimum from the peak height point of view, this pH is optimum from the point of view of the extraction of *II* from the thin layer — see later). The pulse height used was 100 mV, drop time 1 s except for concentrations of $(1-10) \cdot 10^{-8}$ mol l⁻¹, where the drop time was extended to 2 s for *I* and 4 s for *II* for increasing the sensitivity of determination. The detection limit calculated according to Skogerboe and Grant was $6 \cdot 10^{-9}$ mol l⁻¹ for *I* and $1 \cdot 10^{-8}$ mol l⁻¹ for *II*.

An interesting approach to increasing the sensitivity of determination of *I* by DPP emerged from the study of the effect of 4,4'-diaminodibenzyl-2,2'-disulphonic acid (*III*), a substance structurally related with the reduction product of *II*, on the polarographic behaviour of *I*. This effect was examined in connection with the anomalous polarographic behaviour of mixtures of *I* with *II* (ref.⁹). In the presence of a fivefold molar excess of *III* with respect to *I*, the TAST wave is considerably steeper and the DP peak is about doubly as high (Fig. 2). The possibility of making use of this effect for rendering the determination of *I* (and possibly also other nitro compounds) more sensitive will be examined in detail in a further study.

DPP Determination of *I* and *II* After the Thin Layer Chromatographic Separation of Their Mixtures

The following procedure is recommended based on preliminary experiments⁹ 10 µl of solution containing 5–50 µg of either of the two acids is applied to a Luce

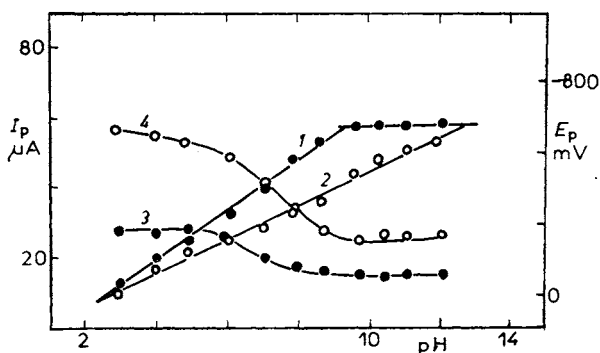


FIG. 1

Dependences of E_p 1, 2 and I_p 3, 4 on pH for substances *I* 1, 3 and *II* 2, 4. Depolarizer concentration $1 \cdot 10^{-4}$ mol l⁻¹, pulse height 100 mV

fol^R Quick thin layer by means of a Hamilton microsyringe. The chromatogram is developed for 2 h in the ascending mode using an n-butanol-acetic acid-water mixture (4 : 1 : 5) as the eluting agent in the atmosphere of its saturated vapours. Under these conditions, R_f is 0.64 for *I* and 0.46 for *II*. The spots, marked with a pencil under a UV lamp (254 nm), are cut out, washed off the metal foil together with the cellulose support using a Britton-Robinson buffer pH 4.5 for *I* or pH 12.5 for *II*, and extracted with 4×10 ml of the buffer. The cellulose is filtered off using an S2 sintered glass filter, the filtrate is diluted to 50 ml with the buffer, and the polarogram is run. The solutions for the calibration plots are obtained by adding 2, 4, 6, 8, and 10 μ l volumes of the stock solutions of *I* or *II* (0.01 mol l^{-1}) to 50 ml of buffer solution of the appropriate pH. The results obtained are given in Table II;

TABLE II
Analysis of mixtures of *I* and *II* by DPP with TLC separation

Added, μg		Found, μg		% theory	
<i>I</i>	<i>II</i>	<i>I</i>	<i>II</i>	<i>I</i>	<i>II</i>
5.06	47.43	4.85	47.24	95.8	99.6
10.12	37.94	10.12	37.94	100.0	100.0
15.19	28.45	14.75	27.66	97.1	97.2
20.25	18.97	19.60	19.54	96.8	103.0
25.32	9.48	24.56	9.64	97.0	101.7

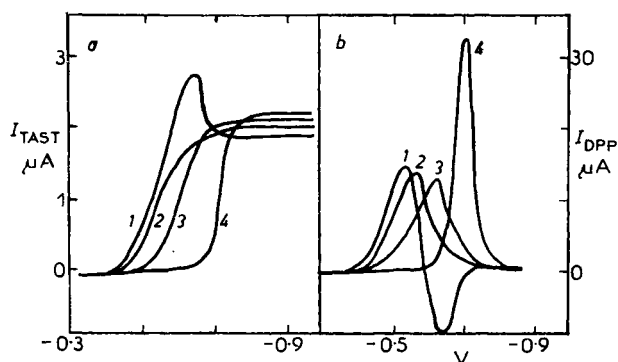


FIG. 2

TAST (a) and DP (b) polarograms of *I* ($c_I = 1 \cdot 10^{-4} \text{ mol l}^{-1}$) in the presence of *III* at pH 7.1; pulse height 50 mV. c_{III} (mol l^{-1}): 1 0 , 2 $5 \cdot 10^{-5}$, 3 $1 \cdot 10^{-4}$, 4 $5 \cdot 10^{-4}$

the relative error of determination does not exceed 5% for *I* and 3% for *II*, which is satisfactory in view of the amounts determined and the separation technique used. With regard to its simplicity and accuracy, the method can be also recommended for analyses of more complex mixtures.

*Direct Polarographic Analysis of Mixtures of I and II
in Sulphuric Acid Solutions*

The DPP behaviour of the two substances was studied with a view to eliminating the need for their prior TLC separation. The DP polarogram is not a mere sum of the polarograms of the two components. This is shown in Fig. 3 for 0.01M-H₂SO₄ solutions for which the best developed and resolved peaks were obtained. The height of the first peak, which is due to *II*, is not linearly dependent on the concentration, so that this peak is of no analytical use. The height of the other peak, which is due to *I*, on the other hand is a linear function of the concentration of *I* at a constant concentration of *II* (Fig. 4). The slope depends on the concentration of *II*, hence, the peak height for compound *I*, I_1^s , obeys the relation

$$I_1^s = kc_1^s, \quad (1)$$

where c_1^s is the concentration of *I* in the sample and k is a constant dependent on the concentration of *II*. Thus the standard additions method has to be resorted to for the

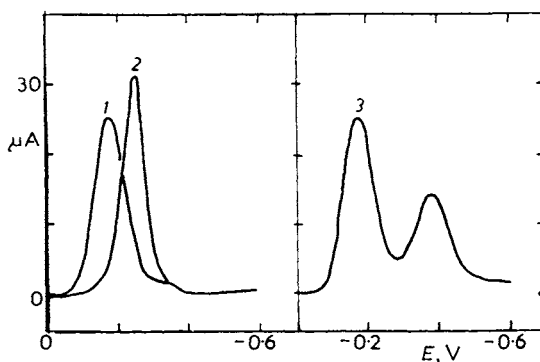


FIG. 3

DP polarograms of *II* ($c_{II} = 1 \cdot 10^{-4} \text{ mol l}^{-1}$) 1, *I* ($c_I = 2 \cdot 10^{-4} \text{ mol l}^{-1}$) 2, and a mixture of *II* and *I* ($c_{II} = 1 \cdot 10^{-4} \text{ mol l}^{-1}$, $c_I = 2 \cdot 10^{-4} \text{ mol l}^{-1}$) 3 in 0.01M-H₂SO₄ solutions. Pulse height 50 mV; 0.2 ml of 0.5% gelatine was added to 10 ml of solution

determination. After a known amount of I is added, the peak height becomes

$$I_1^{s+a} = k(c_1^s + c_1^a), \quad (2)$$

where c_1^a is the concentration of the addition of I in the final sample; the value of k remains unchanged. The c_1^s value then is solved from the system of Eqs (1) and (2).

This approach is applicable to concentrations of I of $(1-10) \cdot 10^{-4} \text{ mol l}^{-1}$ in the presence of II in concentrations of $(5-10) \cdot 10^{-5} \text{ mol l}^{-1}$. If the concentration of II is as low as $(1-5) \cdot 10^{-5} \text{ mol l}^{-1}$, the relation of I_1^s vs c_1^s is linear only over

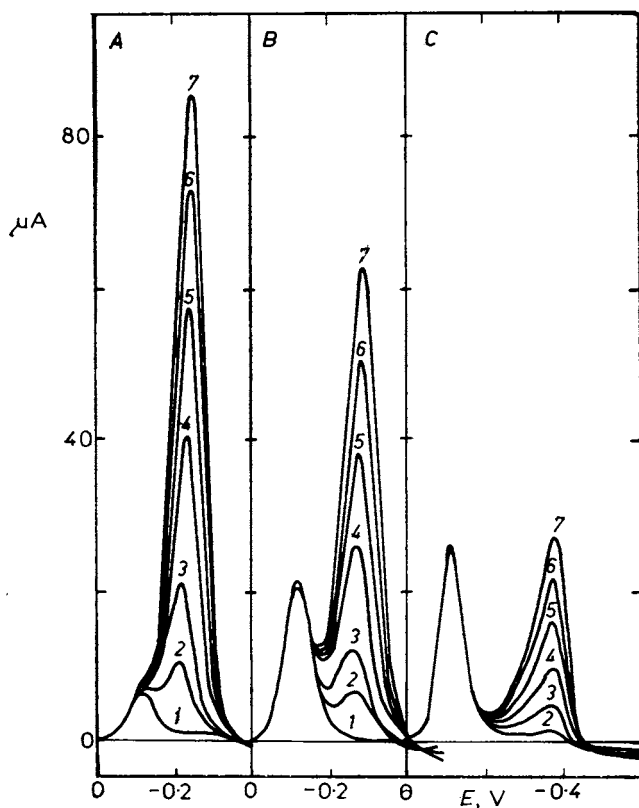


FIG. 4

DP polarograms of II at $c_{II} = 1 \cdot 10^{-5}$ (A), $5 \cdot 10^{-5}$ (B), and $1 \cdot 10^{-4} \text{ mol l}^{-1}$ (C) in the presence of I in $0.01M-H_2SO_4$ solutions; 0.2 ml of 0.5% gelatine was added to 10 ml of solution. Pulse height 50 mV , drop time 2 s . c_I (mol l^{-1}): 1.0 , $2.1 \cdot 10^{-4}$, $3.2 \cdot 10^{-4}$, $4.4 \cdot 10^{-4}$, $5.6 \cdot 10^{-4}$, $6.8 \cdot 10^{-4}$, $7.1 \cdot 10^{-3}$

the region of $(1-6) \cdot 10^{-4} \text{ mol l}^{-1}$. This implies that *I* can be determined in the presence of *II* if their molar ratio is 1 : 1 to 50 : 1, hence, if *I* is present in excess; traces of *I* in the presence of *II* cannot be determined in this manner because the former only gives rise to a minor peak on the descending part of the major peak of *II*.

Also for TAST polarography, polarograms of mixtures of *I* and *II* are other than mere sums of the polarograms of the components. While $E_{1/2}$ of *II* in the mixture coincides with that of pure *II*, the wave of *I* shifts to more negative potentials. Also, the wave height for *II* decreases and that for *I* increases with extending drop time. This indicates that *II* or its reduction products adsorb on the mercury drop electrode

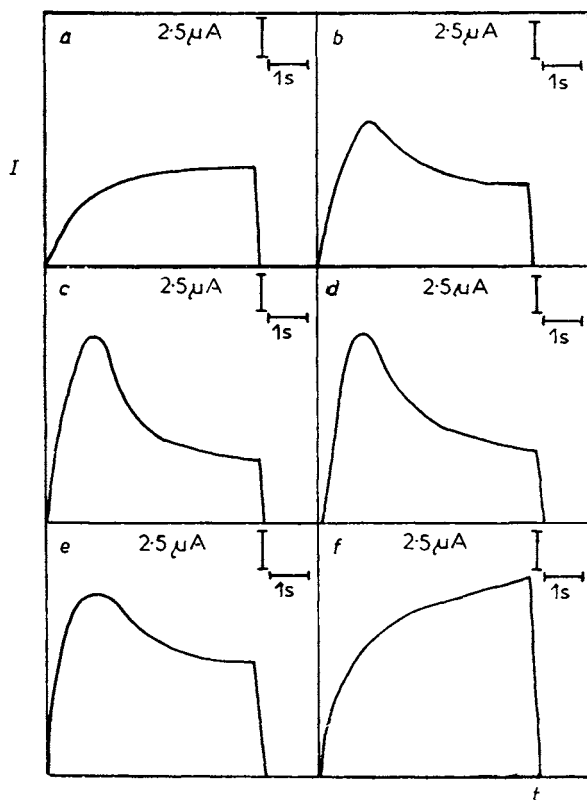


FIG. 5

I-*t* curves for the reduction of *II* on a mercury drop electrode at -250 (a), -300 (b), -350 (c), -400 (d), -450 (e), and -500 mV (f). Depolarizer concentration $1 \cdot 10^{-4} \text{ mol l}^{-1}$, pH 3, reservoir height 25 cm; 0.2 ml of 0.5% gelatine was added to 10 ml of solution

thus rendering the polarographic reduction of *I* more difficult. The *i-t* curves for *II* at various potentials (Fig. 5) show that over the region of -300 to -500 mV adsorption phenomena do occur, bringing about time decrease in the faradaic current.

TAST polarograms for mixtures of *I* and *II* in various proportions are shown in Fig. 6. The two wave heights are mutually affected and neither of them remains in a uniquely defined relation to the concentrations of the components. Only the

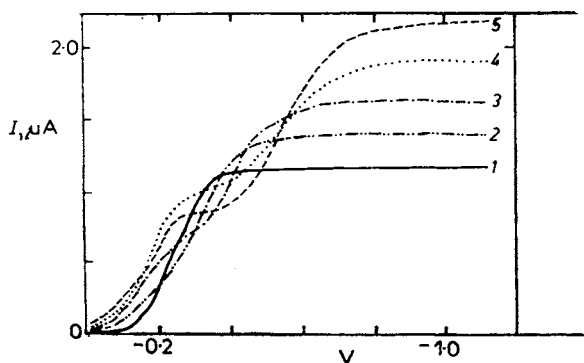


FIG. 6

TAST polarograms for *I* ($c_I = 1 \cdot 10^{-4} \text{ mol l}^{-1}$) in the presence of *II* at pH 3. Reservoir height 25 cm, drop time 4 s; 0.2 ml of 0.5% gelatine was added to 10 ml of solution. c_{II} (mol l^{-1}): 1.0, $2.5 \cdot 10^{-5}$, $3.1 \cdot 10^{-4}$, $4.15 \cdot 10^{-4}$, $5.2 \cdot 10^{-4}$

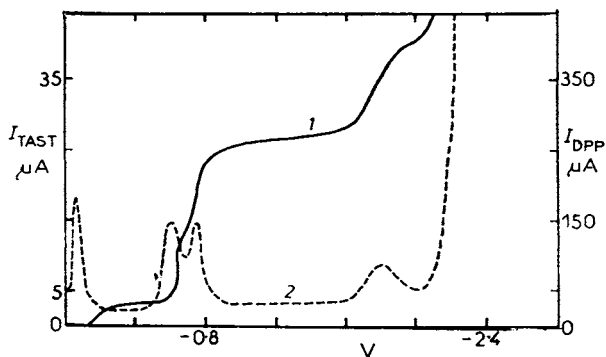


FIG. 7

TAST 1 and DPP 2 polarograms of *II* ($c_{II} = 1 \cdot 10^{-3} \text{ mol l}^{-1}$) in 0.1M tetramethylammonium bromide; pulse height 50 mV

overall limiting current over the region of -800 to $-1\ 300$ mV is a linear function of the total concentration of the two components, *i.e.*,

$$I_{lim} = k_I c_I + k_{II} c_{II}, \quad (3)$$

where the *k*'s are identical with the slopes of the calibration straight lines for solutions containing *I* or *II* solely.

Having determined the concentration of *I* by the DPP method as described above, we have c_{II} in Eq. (3) as the sole unknown which can be readily calculated. In practice,

TABLE III

Parameters of the calibration straight lines for the determination of *II* by DPP over the concentration region of $(1-5) \cdot 10^{-3}$ mol l⁻¹ in the presence of *I*. Supporting electrolyte 0.1M tetramethylammonium bromide, pulse height 100 mV

c_I mmol l ⁻¹	a/s_a^a $\mu\text{A mol}^{-1}$ l	b/s_b^b μA	r^c	$s_{y,x}^d$ μA
0	$1.75 \cdot 10^4 / 2.7 \cdot 10^2$	$-34/9$	0.9996	8.7
1	$1.75 \cdot 10^4 / 2.7 \cdot 10^2$	$-34/9$	0.9996	8.7
2	$1.74 \cdot 10^4 / 5 \cdot 10^1$	$-29/2$	0.9999	1.6
3	$1.74 \cdot 10^4 / 3 \cdot 10^2$	$-28/10$	0.9995	10.2
4 ^e	$1.68 \cdot 10^4 / 4 \cdot 10^2$	$+ 2/12$	0.9992	11.6

^{a-d} See Table I; ^e the relative error of determination of *II* exceeds 5%.

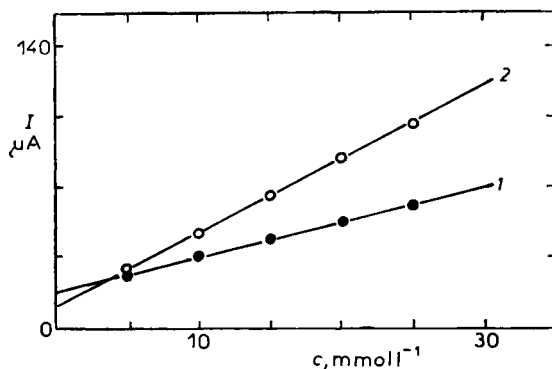


FIG. 8

Dependences of the limiting current of TAST polarography at $-1\ 300$ mV on the concentration of *I* 1 at $c_{II} = 1 \cdot 10^{-3}$ mol l⁻¹, and on the concentration of *II* 2 at $c_I = 1 \cdot 10^{-3}$ mol l⁻¹

this approach is only applicable to mixtures with *I*-to-*II* ratios of 1 : 1 to 10 : 1. With higher excess of *I* the contribution of *II* to the overall limiting current is so low that the relative error of determination of *II* is higher than 5% and it increases rapidly with decreasing mole fraction of *II*.

*Direct Polarographic Analysis of Mixtures of I and II
in Tetramethylammonium Bromide Solutions*

In tetramethylammonium bromide, *II* gives rise to two waves (Fig. 7), one corresponding to the reduction of the NO₂ groups and the other corresponding to the reduction of the C=C bond¹. The latter is the only polarographically reducible functional group by which the two compounds differ. Compound *I* gives a single wave with $E_{1/2}$ about -400 mV. Thus the wave of *II* at approximately -1 800 mV can be utilized for the determination of this substance in the presence of *I*. Actually, DPP was used for the determination with regard to the better readability of the peak height by this method (Fig. 7).

The effect of *I* on the peak height of *II* at -1 800 mV was examined, and this effect was found negligible up to a concentration of *I* of $4 \cdot 10^{-3} \text{ mol l}^{-1}$ (Table III); thus *II* can be determined in the presence of a triple molar excess of *I* over *II*.

The limiting current in the TAST polarography at -1 300 mV is a linear function of the concentration of *II* at a constant concentration of *I*, and similarly, this current is a linear function of the concentration of *I* at a constant concentration of *II*. The slopes are here again identical with those measured in the absence of the other component (Fig. 8). Hence, the TAST current obeys the relation

$$I'_{\text{lim}} = k'_I c_I + k'_{II} c_{II}, \quad (4)$$

where the constants can be obtained by measurements on pure solutions of *I* and *II*, respectively. Having determined c_{II} by DPP at -1 800 mV, we can calculate c_I from this relation. The method is applicable to mixtures of *I* and *II* in molar ratios of 1 : 10 to 3 : 1. At higher excess of *I*, *II* cannot be determined by DPP, and at an excess of *II* higher than 10 fold the contribution of *I* to the total limiting TAST current decreases so much that the relative error of determination of *I* exceeds 5% and it grows rapidly with decreasing mole fraction of *I*.

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Translated by P. Adámek.