

## THE POLAROGRAPHIC DETERMINATION OF 1-[4'-(PHENYLAZO)PHENYL]-3,3-DIMETHYLTRIAZENE\*

Jiří BAREK<sup>a</sup>, Dana DŘEVÍNKOVÁ<sup>a</sup>, Jiří ZIMA<sup>a</sup> and Arnold FOGG<sup>b</sup>

<sup>a</sup> UNESCO Laboratory of the Environmental Electrochemistry, Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia

<sup>b</sup> Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire LE 11 3TU, Great Britain

Received November 27, 1991

Accepted March 8, 1992

---

A study has been made of the polarographic behaviour of genotoxic 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene in mixed water-methanol medium. A mechanism has been proposed for its polarographic reduction and optimal conditions have been found for its determination by fast polarography in the concentration range  $1 \cdot 10^{-4} - 2 \cdot 10^{-6}$  mol l<sup>-1</sup> and using differential pulse polarography in the concentration range  $1 \cdot 10^{-4} - 2 \cdot 10^{-7}$  mol l<sup>-1</sup>.

---

The derivatives of 1-phenyl-3,3-dimethyltriazene are genotoxic substances<sup>1</sup> and a number of them are simultaneously carcinostatics<sup>2,3</sup>. Thus, there is a need for sensitive analytical methods for the determination of trace amounts of these substances. Because of the ready reducibility of triazenes<sup>4</sup>, modern polarographic methods would seem suitable for this purpose; these methods have been found useful, e.g., for the determination of trace amounts of pharmaceutically important carboxyl amide derivatives of 1-phenyl-3,3-dimethyltriazene<sup>5-7</sup>. A number of other works dealing with the mechanism or analytical utilization of the polarographic reduction of various types of triazenes can be found in the monograph<sup>4</sup> or in ref.<sup>6</sup>.

This work describes the polarographic behaviour of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene (see I, Eq. (A)), which contains two polarographically active groups (azo and triazene) in the *p*-position of the central aromatic ring, permitting their electronic interaction. The mechanism of the polarographic reduction of the azo group, which is described in detail, e.g. in monographs<sup>8-10</sup>, can be strongly affected by this interaction. Consequently, a study was made of the effect of the pH on the behaviour of the test substance in fast polarography and differential pulse polarography (DPP) at a

---

\* Part XXI in the series Analysis of Chemical Carcinogens; Part XX: Collect. Czech. Chem. Commun. 57, 1230 (1992).

classical dropping mercury electrode (DME) and in cyclic voltammetry at a hanging mercury drop electrode. In addition, potentiostatic coulometry at a large-area mercury electrode was employed to determine the number of electrons exchanged. The reduction of the test substances during potentiostatic coulometry was followed using fast polarography, spectrophotometry in the UV and visible regions and high performance liquid chromatography with electrochemical detection. The information obtained was employed to determine the optimal conditions for the polarographic determination of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene. The experiments were carried out in mixed Britton–Robinson buffer–methanol medium (1 : 1), to ensure sufficient solubility of the test substance.

## EXPERIMENTAL

### Reagents

The stock solution of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene in methanol ( $c = 1 \cdot 10^{-3} \text{ mol l}^{-1}$ ) was prepared by dissolving an exactly weighed amount of the substance (Research Institute of Organic Synthesis, Pardubice–Rybitví) in p.a. solvent. The purity of the substances used was controlled by paper and thin-layer chromatography and by measuring the spectra of a methanol solution in the UV and visible regions<sup>11</sup>. More dilute solutions were prepared by exact dilution of the stock solution. All the solutions were stored in the dark. It followed from a spectrophotometric study of the stability of these solutions that the solution with a concentration of  $1 \cdot 10^{-3} \text{ mol l}^{-1}$  must be prepared fresh once a month,  $1 \cdot 10^{-4} \text{ mol l}^{-1}$  every week, and  $1 \cdot 10^{-5} \text{ mol l}^{-1}$  or  $1 \cdot 10^{-6} \text{ mol l}^{-1}$  daily. The other chemicals employed were of p.a. purity (Lachema, Brno). Water was doubly distilled in a quartz apparatus.

### Apparatus

A polarographic analyzer PA 4 was used with an XY 4105 recorder (Laboratorní přístroje, Praha). Work was carried out in a three-electrode system using a classical dropping mercury electrode, saturated calomel reference electrode and platinum wire auxiliary electrode. All the potential values are referred to the saturated calomel electrode. At a mercury reservoir height of  $h = 49 \text{ cm}$ , the flow rate was  $m = 0.967 \text{ mg s}^{-1}$  and the drop time was  $\tau = 6.5 \text{ s}$  (at an applied voltage of 0 V in 0.1M-KCl). Where not stated otherwise, work with the DME was carried out at a polarization rate of  $5 \text{ mV s}^{-1}$ , controlled drop time of 1 s, mercury reservoir height of 49 cm and modulation amplitude in differential pulse polarography of  $-100 \text{ mV}$ . Oxygen was removed from the polarographic solution by bubbling for ten minutes with nitrogen, which was purified by passing through a solution of chromium(II) ions in dilute hydrochloric acid over zinc amalgam. A prebubbler containing a water–methanol mixture in the same ratio as the polarographed solution was placed prior to the polarographic vessel.

The coulometric and spectrophotometric measurements and cyclic voltammetry were carried out using the instrument described in ref.<sup>12</sup>.

The HPLC measurements were carried using a HPP 5001 pump, ICI 30 injector with a  $3 \mu\text{l}$  loop, ADLC 2 amperometric detector combined with a voltammetric detector with a carbon fibre<sup>13</sup> and a TZ 4200 linear recorder (all from Laboratorní přístroje, Praha).

The acidity of the solution was measured using a PHM 62 instrument (Radiometer, Copenhagen) with glass and saturated calomel electrodes.

All the measurements were carried out at laboratory temperature.

### Procedures

The calibration curves were always measured in triplicate and evaluated by the least squares linear regression method. The determination limit was calculated as ten times the standard deviation for the determination of the analyte with a concentration corresponding to the lowest point on the given calibration line<sup>14</sup>.

The procedure for the determination of the number of electrons exchanged using potentiostatic coulometry at a large-area mercury electrode was analogous to that employed previously<sup>15</sup>, with the difference that 50 ml of Britton–Robinson buffer with the given pH and 40 ml of methanol were transferred to the coulometric vessel; after pre-electrolysis at a selected constant potential, 10.00 ml of solution of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-3} \text{ mol l}^{-1}$ ) in methanol were added.

The progress of the coulometric reduction was studied at set time intervals using *tast* polarography and spectrophotometry by removing 10 ml of solution from the coulometric vessel using an injection syringe through a septum; this solution was transferred to a cuvette or polarographic vessel rinsed with nitrogen and also closed by a septum and the *tast* polarogram or spectrum in the UV and visible regions was recorded. (This procedure was designed to prevent oxidation of the products of the coulometric reduction by atmospheric oxygen.)

The coulometric reduction was studied by HPLC with electrochemical detection using an ODS 18 column, 10  $\mu\text{m}$  with a length of 15 cm and internal diameter of 3.4 mm (Tessek, Praha) with a mobile phase methanol (40% (v/v))–aqueous solution of 0.1 mol l<sup>-1</sup> sodium acetate (60% (v/v)), pH 3.6 at a flow rate of 0.3 ml min<sup>-1</sup>. An amount of 3  $\mu\text{l}$  of solution was injected after the coulometric reduction and the potential of the working electrode was adjusted to +1.2 V.

### RESULTS AND DISCUSSION

#### *Study of the Mechanism of the Polarographic Reduction of 1-[4'-(Phenylazo)-phenyl]-3,3-dimethyltriazene*

*Tast polarography.* It can be seen from Table I that the test substance yields one to three waves in dependence on the pH. The first wave is analytically most useful as it is best developed and its height does not change in the pH range 4 – 13. The half-wave potential varies with the pH in the range 4 – 13 according to the relationship  $E_{1/2}^1 \text{ (mV)} = -36 - 58.4 \text{ pH}$ . The height of this wave for pH 4 – 13 is the same as that of the wave of azobenzene measured under the same conditions, in accordance with the results of the potentiostatic coulometry, indicating the exchange of two electrons. The slope of the logarithmic analysis equalled 40 – 50 mV, so that it can be assumed that the studied process is not completely reversible. The height of this wave increases in acid medium at pH < 2, achieved using a mixture of perchloric acid with methanol in a ratio of 1 : 1, indicating that a reaction involving exchange of four electrons begins to become important. At pH < 5, a further wave can be observed on the polarograms, somewhat lower than the first wave; its drawn out character indicates an irreversible process. At pH > 5, this wave coalesces with the third wave. The height of the third wave is also difficult to measure in the range pH 1 – 5 as the limiting current of the second polarographic wave is not parallel with the potential axis. The overall value of the limiting

current of the third wave measured against the curve for the base electrolyte remains approximately constant at pH 1 – 7 and corresponds to approximately four times the limiting current of the first wave in the region pH 4 – 13. In the alkaline region, the limiting current of the third wave decreases with increasing pH and the limiting current also greatly decreases at very negative potentials (about -1.6 V) (see Fig. 1). As the pH increases, the potential at which this decrease begins to appear is shifted to more positive potentials, the length of the limiting current plateau decreases and the wave gradually assumes the shape of a broad peak, whose height decreases with increasing pH. At pH > 13, the third wave has completely disappeared. The variation of the half-wave potential of the third wave with pH in the range pH 3 – 13 is given by the equation  $E_{1/2}^3$  (mV) = -814 - 45.6 pH. The slope of the logarithmic analysis is approximately 60 V for pH 8 – 10. At lower pH values, the slope of the logarithmic analysis varies between 90 and 150 mV, connected with the coalescence of the second and third waves at pH 6 – 8 and the very drawn-out shape of the third wave at pH < 6. In contrast

TABLE I

The effect of the pH on the fast polarographic behaviour of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4}$  mol l<sup>-1</sup>) in mixed Britton-Robinson buffer-methanol medium (1 : 1)

pH <sup>a</sup>	$E_{1/2}^1$ mV	$I_{lim}^1$ μA	$E_{1/2}^2$ mV	$I_{lim}^2$ μA	$E_{1/2}^3$ mV	$I_{lim}^3$ μA
0.5 <sup>b</sup>	-100 <sup>e</sup>	0.50	-460 <sup>f</sup>	0.02 <sup>f</sup>	-775 <sup>f</sup>	0.36 <sup>f</sup>
1.2 <sup>c</sup>	-100 <sup>e</sup>	0.36	-460 <sup>f</sup>	0.10 <sup>f</sup>	-845 <sup>f</sup>	0.39 <sup>f</sup>
1.7 <sup>d</sup>	-140 <sup>e</sup>	0.34	-535 <sup>f</sup>	0.10 <sup>f</sup>	-920 <sup>f</sup>	0.39 <sup>f</sup>
2.7	-155 <sup>e</sup>	0.35 <sup>e</sup>	-550 <sup>f</sup>	0.10 <sup>f</sup>	-940 <sup>f</sup>	0.40 <sup>f</sup>
4.2	-260	0.24	-645 <sup>f</sup>	0.15 <sup>f</sup>	-1 010 <sup>f</sup>	0.40 <sup>f</sup>
4.8	-315	0.24	-725 <sup>f</sup>	0.15 <sup>f</sup>	-1 050 <sup>f</sup>	0.45 <sup>f</sup>
5.7	-370	0.23	- <sup>g</sup>	- <sup>g</sup>	-1 025	0.83
6.9	-450	0.23	- <sup>g</sup>	- <sup>g</sup>	-1 125	0.80
7.9	-500	0.23	- <sup>g</sup>	- <sup>g</sup>	-1 180	0.55
8.6	-540	0.22	- <sup>g</sup>	- <sup>g</sup>	-1 215	0.49
9.7	-600	0.24	- <sup>g</sup>	- <sup>g</sup>	-1 270	0.51
10.1	-630	0.22	- <sup>g</sup>	- <sup>g</sup>	-1 285	0.46
10.7	-700	0.24	- <sup>g</sup>	- <sup>g</sup>	-1 320	0.20
11.8	-730	0.24	- <sup>g</sup>	- <sup>g</sup>	-1 325	0.06
13.4	-785	0.24	- <sup>g</sup>	- <sup>g</sup>	- <sup>h</sup>	- <sup>h</sup>

<sup>a</sup> Final pH of the mixture of the buffer or perchloric acid with methanol. <sup>b</sup> Achieved using 2M-HClO<sub>4</sub>. <sup>c</sup> Achieved using 0.2M-HClO<sub>4</sub>. <sup>d</sup> Achieved using 0.02M-HClO<sub>4</sub>. <sup>e</sup> Approximate values because of the drawn-out wave, with a shape corresponding to the coalescence of two poorly distinguishable waves. <sup>f</sup> Approximate values because of the difficult evaluation of the very drawn-out strongly irreversible wave. <sup>g</sup> The 2nd and 3rd waves coalesce. <sup>h</sup> The more negative wave completely disappears.

to the carboxyl amido derivative 1-phenyl-3,3-dimethyltriazene, studied in earlier works<sup>6,7</sup>, the height of the observed waves practically did not change over 60 min after preparation of the solutions in the studied pH range. It follows that the azo group in the *p*-position relative to the triazene group greatly increases its resistance to acid-catalyzed hydrolysis. It follows from the measured dependence of the wave height on the square root of the height of the mercury reservoir in DC polarography that the heights of all the observed waves at pH 4.0, 8.2 and 9.4 are diffusion-controlled. However, the dependence of the current values on the square root of the mercury reservoir height for the region of current decrease on the plateau of the third wave does not pass through the origin, indicating mixed diffusion-kinetic control. In the region of the minimum, the current value is practically independent of the mercury reservoir height, indicating that kinetic control of the observed current is predominant.

It can be assumed that the substance adsorbed on the electrode surface undergoes preliminary protonation and that the observed decrease in the limiting current in the region around  $-1.6$  V at pH  $> 7$  is connected with the decreased rate of surface protonation as a consequence of desorption of the test substances at potentials that are far more negative than the potential of the electrocapillary zero.

*Constant potential coulometry.* Potentiostatic coulometry at a large-area mercury electrode at pH 13.4 and a constant potential of  $-1.00$  V yielded a value of  $n = 2.0$  for the number of exchanged electrons. The spectrophotometric study of the coulometric reduction in this medium (Fig. 2a) revealed that the absorption band at about 380 nm disappeared; this band can be attributed to the azo group in conjugation with the triazene group. On the other hand, a new band was formed at about 310 nm and can be attributed to the hydrazo group. The band at about 340 nm probably corresponds to the triazene group and apparently coalesces with the band of the azo group in the spectrum of 1-[4'(phenylazo)phenyl]-3,3-dimethyltriazene as a consequence of the bathochromic shift connected with conjugation of the azo and triazene groups. If the solution is left to stand in the presence of atmospheric oxygen after completion of the coulometric reaction, the band corresponding to the azo group reappears in the spectrum. It follows

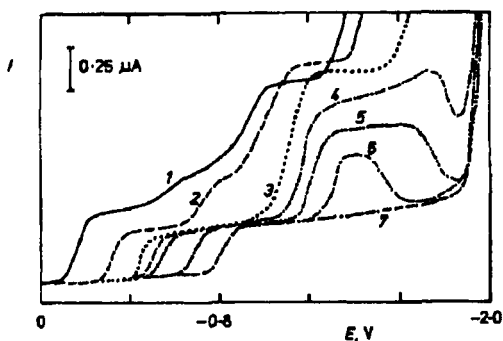
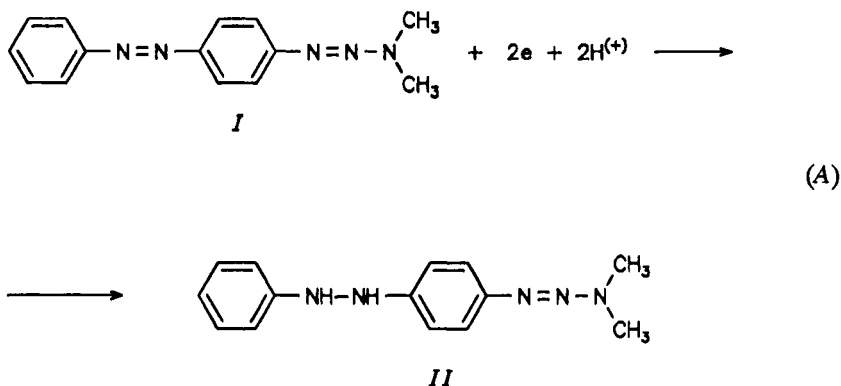


FIG. 1  
Tact polarograms of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4}$  mol  $l^{-1}$ ) in mixed Britton-Robinson buffer-methanol medium (1 : 1) at pH 2.7 (1), 4.8 (2), 6.9 (3), 7.9 (4), 8.6 (5), 10.1 (6) and 13.4 (7)

from a study of the coulometric reduction by fast polarography (Fig. 2*d*) that an anodically oxidizable substance is formed in this medium, and constitutes an almost reversible pair with the initial substance. The total height of the cathodic, cathodic-anodic or anodic wave remains constant during the coulometric experiment, indicating identity of the diffusion coefficients of the oxidized and reduced forms and the quantitiveness of the reaction. The half-wave potential of the cathodic wave is about 25 mV more negative than the half-wave potential of the anodic wave, i.e. the pair is not completely reversible. If the solution is allowed to stand in the air after completion of the coulometric reduction (curve 6 in Fig. 2*d*), then the cathodic wave reappears, with a height and position corresponding to that of the original cathodic wave of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazeno. In addition, a new cathodic wave appears at a potential of about -1.2 V. The oxidation of the product of the coulometric reduction by atmospheric oxygen does not lead only to regeneration of the original substance, but apparently also to the formation of a mixture of further polarographically active substances.

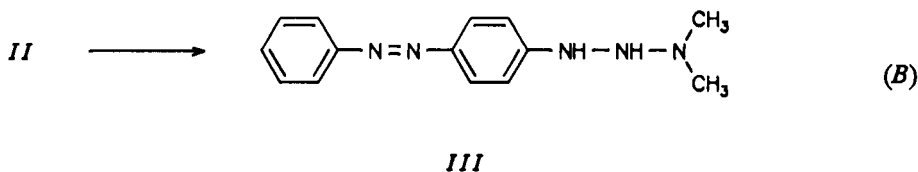
It follows from the above observations that, in alkaline medium, the test substance undergoes quasireversible reduction according to equation (A).



In medium with pH 8.0 and constant potential of -0.70 V, a value of  $n = 2.70$  was found for the number of exchanged electrons. It follows from the spectrophotometric study of the reduction (Fig. 2*b*) that the absorption band at 380 nm corresponding to the azo group disappears. Similarly, the absorption band at about 340 nm attributed to the triazene group, which appeared during the coulometric reduction, has practically disappeared by the end of the reduction. Only the absorption band at about 240 nm corresponding to the aromatic nucleus and that at 290 nm, which can be assigned to the hydrazo group in the aromatic ring, remain in the spectrum. Similarly, the fast polarographic study of the coulometric reduction (Fig. 2*e*) indicated that an anodically oxidizable product is formed (probably a hydrazo compound). Simultaneously, the

more negative wave, with a half-wave potential far more negative than that at which the coulometric reduction was carried out, disappears. It is thus apparent that the coulometric reduction involves interaction of the azo and triazene groups. It is possible that the triazene group is decomposed after the azo group is reduced to the hydrazo group, so that the stabilizing effect connected with the conjugation of the azo and triazene groups is no longer present. This assumption would also explain the observed fact that the triazene group disappears only after the wave of the azo group practically disappears. It should be realized that the time required for the coulometric reduction is of the order of tens of minutes, so that even relatively slow decomposition of the triazene group can be important. This concept is also supported by the fact that, if the coulometric reduction at  $-0.70$  V is followed by a change in the potential to  $-1.35$  V, then a total value of  $n = 4.6$  is obtained, i.e. much lower than when the coulometric reduction is carried out directly at a value of  $-1.35$  V (see below). The decomposition of the triazene group also explains the fact that the oxidation of the products of the coulometric reduction at pH 13.4 with atmospheric oxygen does not involve regeneration of only the initial substance, but also of additional polarographically active compounds. Reoxidation of the products of the coulometric reduction by atmospheric oxygen also occurs at pH 8, but is much slower.

The determined value  $n = 2.7$  at the potential corresponding to the limiting current of the two-electron reduction of the azo group to the hydrazo group can be connected with the tautomeric rearrangement of the hydrazo compound formed according to Eq. (B), as the azo compound formed (III) would apparently also be reduced at a potential of  $-0.70$  V.



The nonquantitativeness of the tautomeric rearrangement connected with the assumed decomposition of the intermediate (II) leads to the determination of an experimental value smaller than 4.

A value of  $n = 8.78$  was found at a constant potential of  $-1.35$  V in medium with pH 8. The spectrophotometric study of the coulometric reduction (Fig. 2c) revealed that the band corresponding to the azo and triazene groups disappeared. The absorption bands at 240 and 285 nm, visible even after completion of the reduction, can be assigned to the aromatic amine or hydrazine. It follows from the fast polarographic study of the reaction (Fig. 2f) that the azo and triazene groups are simultaneously electrochemically reduced with formation of an anodically oxidizable product (probably a hydrazo compound or *p*-phenylene diamine). However, if the potential is changed to  $-1.35$  V after completion of the electrolysis at  $-0.70$  V, a value of  $n = 4.6$  is found, confirming

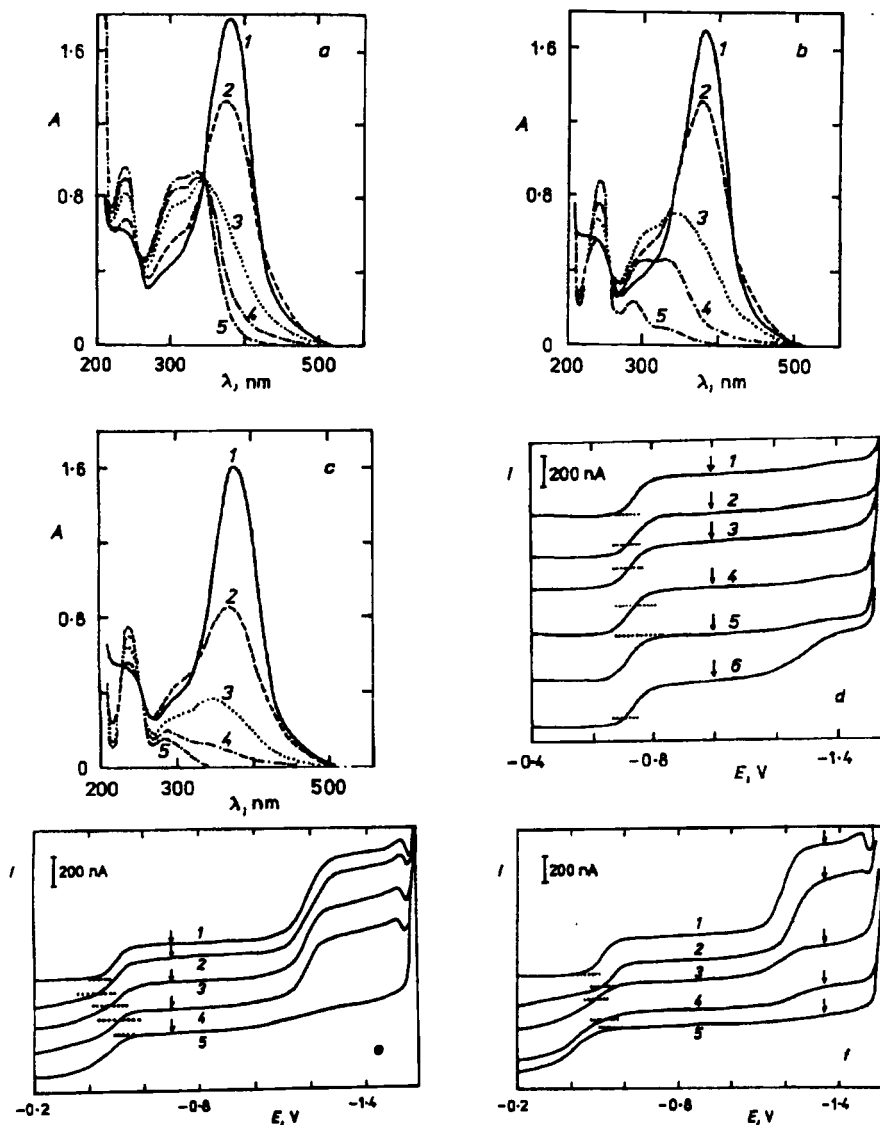


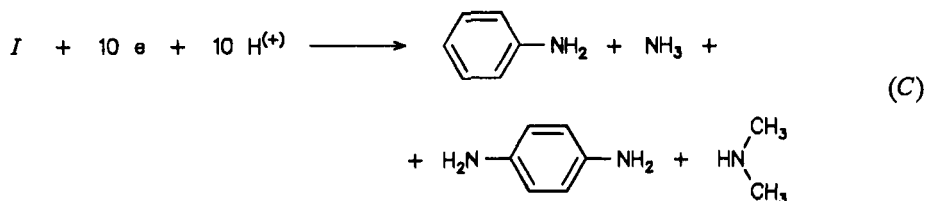
FIG. 2

Spectrophotometric (a - c) and fast polarographic (d - f) study of the coulometric reduction of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ) at a constant potential of  $-1.00 \text{ V}$  (a, d),  $-0.70 \text{ V}$  (b, e) and  $-1.35 \text{ V}$  (c, f) vs SCE in mixed Britton-Robinson buffer-methanol medium (1 : 1) at pH 13.4 (a, d) and 8.0 (b, c, e, f). Charge required for the reduction (calculated relative to the determined  $n$  value) in %: (1) 0, (2) 25, (3) 50, (4) 75, (5) 100. Curve 6 corresponds to the solution after completion of the coulometric reduction, left to stand for 1 h in the presence of the air. The arrow denotes the potential at which the coulometric reduction was carried out; the dotted line corresponds to the zero current position for the given curve; cuvette thickness 0.5 cm

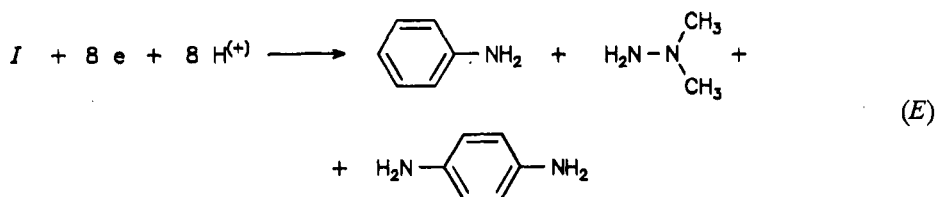
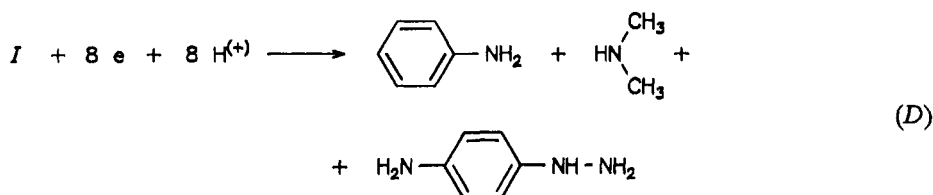


that subsequent reactions occur in potentiostatic coulometry, leading to an increase in the number of exchanged electrons determined coulometrically.

The exchange of more than 8 electrons per molecule of test substance could be explained only in terms of simultaneous four-electron reduction of the azo group and six-electron reduction of the triazene group according to Eq. (C).



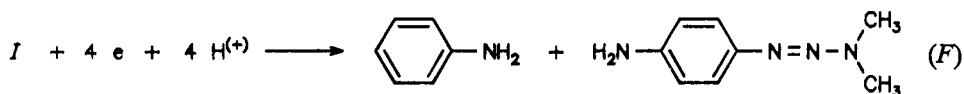
However, because the presence of ammonia could not be demonstrated in the reaction mixture using the Nessler reagent, it must be assumed that a maximum of an eight-electron reduction occurs according to Eq. (D) or (E).



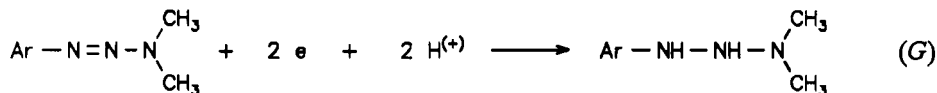
The determined higher  $n$  value is apparently connected with the relatively high residual current in the given medium at a potential of  $-1.35$  V, which cannot be completely compensated by reduction of the background electrolyte alone prior to the addition of the test substance, as it can be affected by the products of the coulometric reduction (e.g. catalytic evolution of hydrogen in the presence of these substances). This concept is also supported by the fact that azobenzene demonstrably exchanges 2 electrons and yields a value of  $n = 2.43$  under the same conditions at a constant potential of  $-1.35$  V.

A value of  $n = 3.04$  was found in medium with pH 2.7 at a constant potential of  $-0.30$  V, where the spectrum of the solution after reduction is practically identical with

that obtained after coulometric reduction at  $-0.7$  V in medium with pH 8.0. (The observed hypsochromic shift in the maximum of the remaining peaks by about 5 nm can be explained on the basis of protonation of nitrogen atoms.) It follows from the fast polarographic study of the coulometric reduction (Fig. 3, curves 1 and 2) that the heights of the 2nd and 3rd waves attributed to step-wise reduction of the triazene group decrease simultaneously with the reduction of the azo group. This can be explained in terms of acid-catalyzed decomposition of the triazene group in the absence of the stabilizing effect of the azo group. The fact that the determined  $n$  value is greater than would correspond to the reduction of the azo group is apparently connected with partial reduction of the hydrazo group to two aromatic amines according to Eq. (F). This concept is supported by the greater height of the first wave at pH 2.7 compared with that at pH 4 – 13, and smaller height of the anodic wave after the reduction compared with the first cathodic wave prior to the reduction.



A value of  $n = 3.87$  was found at a constant potential of  $-0.70$  V and at pH 2.7. The spectrum of the solution after this reduction is analogous to the spectrum of the solution after reduction at  $-0.30$  V and the fast polarogram of the solution after the reduction (curve 3, Fig. 3) also indicates disappearance of the triazene group. The anodic wave is then higher than for reduction at  $-0.30$  V, possibly connected with partial reduction of the triazene group according to Eq. (G), where Ar is  $\text{C}_6\text{H}_5\text{-NH-NH-C}_6\text{H}_4\text{-}$  for two-electron reduction of the azo group or  $\text{H}_2\text{N-C}_6\text{H}_4\text{-}$  for four-electron reduction of this group.



At a constant potential of  $-1.10$  V in medium with pH 2.7, a value of  $n = 10.19$  was found and the UV spectra and fast polarograms are similar to those obtained for reduction at more positive potentials. If exhaustive electrolysis is carried out at  $-0.70$  V and the potential is then changed to  $-1.10$  V, then the value found is only  $n = 6.42$ , once again indicating the existence of subsequent reactions. As ammonia was not found in the solution even under these conditions and azobenzene yielded a value of  $n = 3$

under the same conditions, it can once again be assumed that the high value of  $n$  found is connected with poor compensation of the residual current. The eight-electron reduction according to Eqs (D) or (E) once again seems more probable.

In order to decide between these two possibilities, the solution obtained from the coulometric reduction at pH 2.7 at a constant potential of  $-1.10$  V was analyzed using HPLC with electrochemical detection, permitting especially sensitive determination of aromatic amines and diamines<sup>16</sup>. Aniline was demonstrated to be present by the procedure described in the experimental section; the retention time of this substance under the given conditions is equal to 390 s. *p*-Phenylene diamine, with a retention time of 234 s, is also present. Thus, it follows that the test substance is reduced according to Eq. (E) under the given conditions.

*Cyclic voltammetry at a hanging mercury drop electrode.* At pH 2.5, the test substance yields 2 peaks, the first of which ( $E_p = -195$  mV) corresponds to the reduction of the azo group and the second of which ( $E_p = -900$  mV) corresponds to the reduction of the triazene group (Fig. 4a). The small peak ( $E_p = -100$  mV) prior to the first peak is apparently caused by adsorption of the test substance on the surface of the working electrode as its height is directly proportional to the polarization rate. The shoulder on the descending part of the second peak is probably connected with the stepwise mechanism of the reduction of the triazene group, which is demonstrably irreversible. The anodic peak ( $E_p = -140$  mV) apparently corresponds to oxidation of the hydrazo group formed. If the polarization direction is reversed at  $-1.30$  V, then this peak is lower than the cathodic peak at  $-295$  mV ( $I_p^a/I_p^c = 0.38$ ) and would appear to correspond to a stepwise process, involving simultaneous oxidation of the substance formed by reduction of the triazene group. In contrast, when the polarization direction is reversed at  $-0.35$  V, the ratio of the heights of the anodic and cathodic peaks is equal to  $I_p^a/I_p^c = 0.87$  and the difference in their potentials is  $E_p^a - E_p^c = 90$  mV (see Fig. 4b), so that the process is somewhat more, but not completely, reversible. When the cycle is repeated, the small peak prior to the main peak disappears and simultaneously the

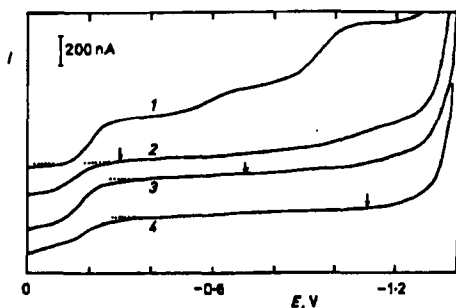


FIG. 3

Tact polarographic curves of 1-[4'-(phenylazo)-phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4}$  mol  $l^{-1}$ ) in mixed Britton-Robinson buffer-methanol medium (1 : 1) at pH 2.7 prior to the reduction (1) and after complete reduction at constant potential of  $-0.30$  V (2),  $-0.70$  V (3) and  $-1.10$  V (4) vs SCE. The arrow denotes the potential at which the coulometric reduction was carried out; the dotted line corresponds to the zero current position for the given curve

maximum of this peak is shifted to more negative potential values, accompanied by a decrease in the  $I_p^a/I_p^c$  ratio (Fig. 4c), possibly connected with passivation of the electrode by the products of the electrode reaction. This passivation is especially marked at low polarization rates, where it appears as a change in the shape of the curve in the first cycle (Fig. 4d). The peak height is not directly proportional to either the polarization rate or the square root of this quantity, so that it can be assumed that this is a complex overall process controlled both by the diffusion of the test substance to the electrode surface and also by its adsorption on this surface.

Conditions are analogous in medium with pH 10.6 (Fig. 5). The electrode passivation is greater when the polarization direction is reversed in the region beyond the most negative peak. The anomalies observed in the region around  $-1.47$  V are attributed to the adsorption-desorption phenomena.

Only a single peak is present in medium with pH 13.3 (Fig. 6); the recording in the second and third cycle is completely identical with that in the first cycle. It can be assumed that the above-described electrode passivation is connected with processes occurring during the reduction of the triazene group. The fact that the potentials corresponding to the maxima of the cathodic and anodic peaks differ by less than 30

TABLE II

The effect of the pH on the differential pulse polarograms of 1-[4'(phenylazo)phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4}$  mol l<sup>-1</sup>) in mixed Britton-Robinson buffer-methanol medium (1 : 1)

pH <sup>a</sup>	$E_p^1$ mV	$I_p^1$ μA	$E_p^2$ mV	$I_p^2$ μA	$E_p^3$ mV	$I_p^3$ μA
1.0 <sup>a</sup>	-15	0.370	-470	0.125	-840	0.160
2.0 <sup>b</sup>	-60	0.405	-540	0.110	-900	0.215
2.6	-110	0.775	-545	0.095	-910	0.215
4.0	-220	0.735	-635	0.230	-990	0.205
4.8	-275	0.780	-750	0.285	-1 020	0.225
5.7	-340	0.820	-905	0.350	-1 035	0.375
6.9	-410	0.720	- <sup>c</sup>	- <sup>c</sup>	-1 090	0.780
8.0	-460	0.745	- <sup>c</sup>	- <sup>c</sup>	-1 150	0.925
8.7	-515	0.795	- <sup>c</sup>	- <sup>c</sup>	-1 185	0.930
9.8	-600	0.605	- <sup>c</sup>	- <sup>c</sup>	-1 250	0.850
10.2	-630	0.480	- <sup>c</sup>	- <sup>c</sup>	-1 270	0.775
11.1	-680	0.525	- <sup>c</sup>	- <sup>c</sup>	-1 265	0.135
12.1	-700	0.710	- <sup>c</sup>	- <sup>c</sup>	-1 285	0.050
13.4	-745	0.210	- <sup>d</sup>	- <sup>d</sup>	- <sup>d</sup>	- <sup>d</sup>

<sup>a</sup> Achieved using 0.1M-HClO<sub>4</sub>. <sup>b</sup> Achieved using 0.01M-HClO<sub>4</sub>. <sup>c</sup> The 2nd and 3rd peaks coalesce. <sup>d</sup> The more negative peak completely disappears.

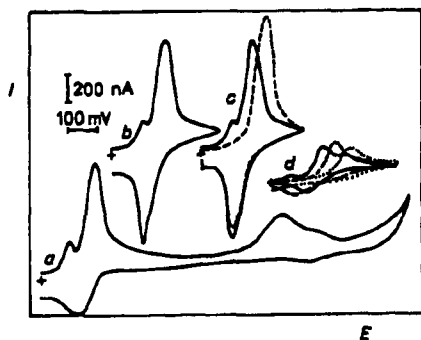


FIG. 4

Cyclic voltammogram of 1-[4'-(phenylazo)-phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ) in mixed Britton–Robinson buffer–methanol medium (1 : 1) at pH 2.5 and a polarization rate of  $50 \text{ mV s}^{-1}$  (a – c) and  $10 \text{ mV s}^{-1}$  (d). The first cycle is depicted as a solid line, the second as a dashed line and the third as a dotted line. The cross denotes the value of the current and potential zero for the individual curves

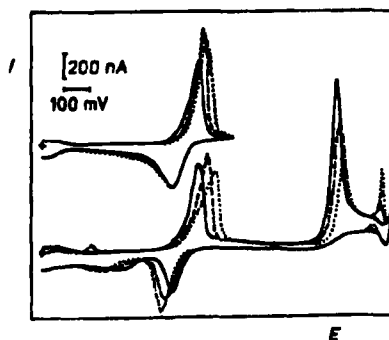


FIG. 5

Cyclic voltammogram of 1-[4'-(phenylazo)-phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ) in mixed Britton–Robinson buffer–methanol medium (1 : 1) at pH 10.6 and a polarization rate of  $50 \text{ mV s}^{-1}$ . The first cycle is depicted as a solid line, the second as a dashed line and the third as a dotted line. The cross denotes the values of the current and potential zero for the individual curves

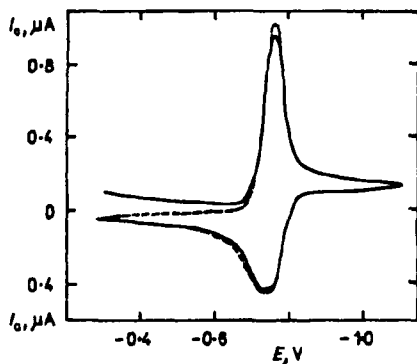


FIG. 6

The cyclic voltammogram of 1-[4'-(phenylazo)-phenyl]-3,3-dimethyltriazene ( $c = 1 \cdot 10^{-4} \text{ mol l}^{-1}$ ) in mixed Britton–Robinson buffer–methanol medium (1 : 1) at pH 13.3 and a polarization rate of  $50 \text{ mV s}^{-1}$ . The first cycle is depicted as a solid line and the second as a dashed line

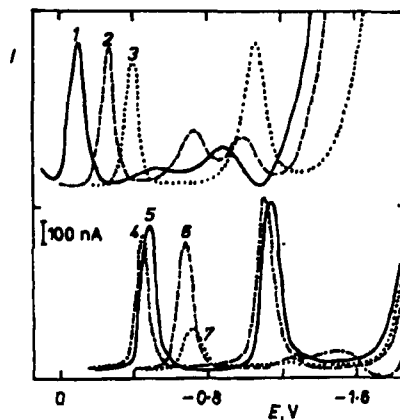


FIG. 7

Differential pulse polarograms of 1-[4'-(phenylazo)-phenyl]-3,3-dimethyltriazene in mixed Britton–Robinson buffer–methanol medium (1 : 1), pH: (1) 2.6, (2) 4.8, (3) 6.9, (4) 8.0, (5) 8.7, (6) 12.1, (7) 13.4

mV indicates that adsorption plays a substantial role in the overall process. This is also confirmed by the fact that the heights of the two peaks are not directly proportional to either the polarization rate or its square root.

*Differential pulse polarography.* It can be seen from Table II that the test substance yields 1 – 3 peaks in differential pulse polarography, in dependence on the pH value. The first peak, which corresponds to the reduction of the azo group, is shifted to more negative values with increasing pH and its height practically does not change in the range pH 3 – 9. The observed change in the height of this peak in the region pH 9 – 13 is apparently connected with the effect of the pH on the reversibility of the reduction of the azo group. The variation of the peak potential with the pH in the range pH 1 – 11 is given by the relationship  $E_p^1$  (mV) = 56 – 66.6 pH; at higher pH values, the slope of this dependence is decreased to about 25 mV pH<sup>-1</sup>. The third peak, corresponding to the reduction of the triazene group, is also shifted to more negative potentials with increasing pH. The variation of the potential of this peak in the range pH 1 – 11 is given by the relationship  $E_p^3$  (mV) = -799 – 44.3 pH. At higher pH values, the potential of this peak ceases to be dependent on the pH. The dependence of the height of the third peak on the pH passes through a sharp maximum at pH 8 – 10. The second peak,

TABLE III

Parameters of the calibration curves for the determination of 1-[4'-(phenylazo)phenyl]-3,3-dimethyltriazene in Britton–Robinson buffer–methanol medium (1 : 1) at pH 8.0<sup>a</sup>

Method	c mol l <sup>-1</sup>	Slope mA mol <sup>-1</sup> l	Intercept nA	r	L <sub>Q</sub> mol l <sup>-1</sup>
Tast <sup>b</sup>	(1 – 10) · 10 <sup>-5</sup>	2.32	3.5	0.9989	–
	(2 – 10) · 10 <sup>-6</sup>	2.43	-1.3	0.9974	1.2 · 10 <sup>-6</sup>
Tast <sup>c</sup>	(2 – 10) · 10 <sup>-5</sup>	5.53	-4.1	0.9994	–
	(2 – 10) · 10 <sup>-6</sup>	4.90	1.2	0.9985	0.9 · 10 <sup>-6</sup>
DPP <sup>b</sup>	(1 – 10) · 10 <sup>-5</sup>	7.51	18.0	0.9982	–
	(1 – 10) · 10 <sup>-6</sup>	7.87	2.1	0.9991	–
	(2 – 10) · 10 <sup>-7</sup>	8.43	0.2	0.9967	1.4 · 10 <sup>-7</sup>
DPP <sup>c</sup>	(1 – 10) · 10 <sup>-5</sup>	9.32	7.1	0.9998	–
	(1 – 10) · 10 <sup>-6</sup>	9.82	4.1	0.9991	–
	(2 – 10) · 10 <sup>-7</sup>	10.86	1.1	0.9975	1.2 · 10 <sup>-7</sup>

<sup>a</sup> r, Correlation coefficient; L<sub>Q</sub> determination limit. <sup>b</sup> Determined using the first wave or peak corresponding to the reduction of the azo group. <sup>c</sup> Determined using the second wave or peak corresponding to the reduction of the triazene group.

whose potential also shifts to more negative values with increasing pH, is present only in the region pH 1 – 6 and coalesces with the third peak at higher pH values (Fig. 7).

*Analytical Utilization of the Polarographic Reduction of 1-[4'(Phenylazo)phenyl]-3,3-dimethyltriazeno*

For analytical purposes, the highest, best developed and most easily evaluated peaks were obtained in a medium of Britton–Robinson buffer–methanol (1 : 1) at pH 8.0, where the reduction of both the azo group and the triazene group can be utilized. Under these conditions, the calibration curves are linear in the range  $1 \cdot 10^{-4}$  –  $2 \cdot 10^{-6}$  mol l<sup>-1</sup> for fast polarography and  $1 \cdot 10^{-4}$  –  $2 \cdot 10^{-7}$  mol l<sup>-1</sup> for differential pulse polarography. The parameters of these calibration curves are given in Table III.

#### REFERENCES

1. Burchenal J. H., Carter S. K.: *Cancer* (Philadelphia) **30**, 1636 (1972).
2. Schmidt F. A., Hutchinson D. J.: *Cancer Res.* **34**, 1917 (1974).
3. Matřka M., Zvěřina V., Marhold J.: *Cesk. Farm.* **24**, 262 (1975).
4. Iversen P. E. in: *Encyclopedia of the Electrochemistry of the Elements – Organic Section* (A. J. Bard and H. Lund, Eds), Vol. 13, Ch. 5, p. 212. Dekker, New York 1979.
5. Mejstřík V., Ságner Z., Držková L., Krampera F.: *Cesk. Farm.* **34**, 51 (1985).
6. Barek J., Toubar S., Zima J.: *Collect. Czech. Chem. Commun.* **56**, 2073 (1991).
7. Barek J., Mejstřík V., Toubar S., Zima J.: *Collect. Czech. Chem. Commun.* **57**, 1230 (1992).
8. Thomas F. G., Botto K. G. in: *The Chemistry of the Hydrazo, Azoxy and Azo Compounds* (S. Patai, Ed.), p. 443. Wiley, Chichester 1975.
9. Mairanovskii S. G., Stradyns J. P., Bezuglyi V. V.: *Polarografiya v organicheskoj khimii*, p. 218. Khimiya, Leningrad 1975.
10. Stradyns J., Glazer V. in: *Encyclopedia of the Electrochemistry of the Elements – Organic Section* (A. J. Bard and H. Lund, Eds), Vol. 13, p. 163. Dekker, New York 1978.
11. Zvěřina V., Chmátal V., Junková I., Diviš J., Matřka M.: *Chem. Prum.* **20**, 231 (1970).
12. Barek J., Švagrová I., Zima J.: *Collect. Czech. Chem. Commun.* **56**, 1434 (1991).
13. Štulík K., Pacáková V.: *J. Chromatogr.* **298**, 222 (1984).
14. Beyermann K.: *Organic Trace Analysis*, p. 45. Ellis Horwood, Chichester 1984.
15. Barek J., Haladová-Bláhová H., Zima J.: *Collect. Czech. Chem. Commun.* **54**, 1538 (1989).
16. Barek J., Pacáková V., Štulík K., Zima J.: *Talanta* **32**, 279 (1985).

Translated by M. Štulíková.