THE POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 1-(4'-CARBAMOYLPHENYL)-3,3-DIMETHYLTRIAZENE*

Jiří BAREK[a], Viktor MEJSTŘÍK[b], Saafa TOUBAR[c] and Jiří ZIMA[a]

[a] *UNESCO Laboratory of Environmental Electrochemistry, Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia*
[b] *Toxicological Department, Research Institute for Organic Synthesis, 533 51 Pardubice-Rybitvi, Czechoslovakia*
[c] *Department of Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Egypt*

Received August 2, 1991
Accepted October 6, 1991

A study was made of the polarographic behaviour of 1-(4'-carbamoylphenyl)-3,3-dimethyltriazene and optimum conditions were found for its determination by tast polarography and differential pulse polarography at a static mercury drop electrode and by fast scan differential pulse voltammetry at a hanging mercury drop electrode in the concentration range $1 . 10^{-4}$ to $2 . 10^{-7}$ mol l^{-1}. A further increase in the sensitivity can be achieved through adsorptive accumulation of the test substance on the surface of a hanging mercury drop, permitting the determination to be extended to the concentration range $1 . 10^{-7} - 2 . 10^{-9}$ mol l^{-1}.

Derivatives of 1-phenyl-3,3-dimethyltriazene substituted in the position 2,3 or 4 by a carboxylamide group belong among genotoxic substances that also exhibit carcinostatic activity[1,2]. They can be determined spectrophotometrically in the ultraviolet region and also through their protolysis followed by C-diazocoupling of the arendiazonium salt formed with N-ethyl-1-naphthylamine to form an azodye, which can be determined photometrically in the visible region[3]. DC polarography[3] has been used to determine these substances in the concentration range $1 . 10^{-3}$ to $1 . 10^{-4}$ mol l^{-1}, and a number of references to the polarographic behaviour of variously substituted derivatives of 1-phenyl-3,3-dimethyltriazene can be found in the paper[4], describing the determination of 1-(2'-carbamoylphenyl)-3,3-dimethyltriazene using modern polarographic and voltammetric techniques with a determination limit of about $3 . 10^{-9}$ mol l^{-1}.

This work describes the use of tast polarography and differential pulse polarography (DPP) at a static mercury drop electrode (SMDE) and fast scan differential pulse voltammetry (FS DPV) at a hanging mercury drop electrode (HMDE) to

---

* Part XX in the series Analysis of Chemical Carcinogens; Part XIX: Collect. Czech. Chem. Commun. *57*, 450 (1992).

determine the so-far unstudied compound 1-(4′-carbamoylphenyl)-3,3-dimethyltriazene. Work was carried out in a mixed water–methanol medium, ensuring sufficient solubility of the studied substance.

EXPERIMENTAL

Reagents

1-(4′-carbamoylphenyl)-3,3-dimethyltriazene was prepared by ammonolysis of 1-(4′-carboxymethylphenyl)-3,3-dimethyltriazene[2] and its purity was controlled by paper and thin-layer chromatography. The stock solution of this substance ($c = 1 \cdot 10^{-3}$ mol l^{-1}) was prepared by dissolving a precisely weighed amount of the substance in fresh redistilled methanol. More dilute solutions were prepared by precise dilution of the stock solution. It followed from a spectrophotometric study of the stability of these substances that solutions with a concentration of $1 \cdot 10^{-3}$ and $1 \cdot 10^{-4}$ mol l^{-1} are stable for at least one month in the dark and that a solution with a concentration of $1 \cdot 10^{-5}$ mol l^{-1} is stable for at least 14 days. Solutions with even lower concentrations were prepared fresh daily. The other chemicals were of p.a. purity (Lachema, Brno). Water was doubly distilled in a quartz apparatus. The methanol used was stored only in glass vessels as contact with polyethylene led to extraction of substances that unfavourably affected the determination of the lowest concentrations.

Apparatus

Experiments were carried out using a PA 4 polarographic analyzer with an XY 4106 recorder and SMDE 1 static mercury drop electrode (all from Laboratorní přístroje, Prague). The capillary had a diameter of 0·146 mm and the maximum drop size was used, obtained by opening the valve for a period of 160 ms. A saturated calomel reference electrode was used, to which all the potential values are related, along with a platinum wire auxiliary electrode. Where not stated otherwise, tast and DP polarography were carried out at a polarization rate of 5 mV s^{-1} and SMDE drop time of 1 s, with an FS DPV polarization rate of 20 mV s^{-1}, where the SMDE was connected as an HMDE. The pulse methods employed a modulation amplitude of -100 mV. The nitrogen used was purified by passing through a solution of chromium(II) ions in dilute hydrochloric acid (1 : 1) over zinc amalgam. A prebubbler containing a mixture of water and methanol in the same ratio as in the analyzed solution was placed prior to entrance of the nitrogen into the polarographic vessel. Coulometric determination of the number of exchanged electrons was carried out using the instrument described in the paper[5].

Procedure

The calibration curves were measured in triplicate and evaluated by the least squares linear regression method. The determination limit was calculated as ten-fold the standard deviation of the test analyte with a concentration corresponding to the lowest value on the appropriate calibration straight line[6]. The actual pH value of the Britton–Robinson buffer–methanol mixture (1 : 1) was measured with a glass electrode calibrated using acetate, borate and phosphate buffers in 50% methanol[7]. Coulometric determination of the number of exchanged electrons was carried out using a procedure analogous to that described in ref.[5].

RESULTS AND DISCUSSION

The Effect of the pH on the Polarographic and Voltammetric Behaviour of 1-(4'-*Carbamoylphenyl*)-3,3-*dimethyltriazene*

It can be seen from Fig. 1 that the tast polarography at a SMDE in Britton–Robinson buffer–methanol (1 : 1) of the test substance at pH < 8 yields a single wave whose parameters in dependence on the pH are given in Table I. The half-wave potential of this wave shifts to more negative values with increasing pH and this dependence has asymptotic character. The height of this wave is practically constant up to pH 7, rapidly decreases at higher pH values and, at pH < 10, this wave practically disappears. Simultaneously, at pH > 8 a new wave appears with a half-wave potential of about $-1{\cdot}8$ V, whose height corresponds to the first wave at pH < 7. However, this wave is difficult to utilize analytically, as it coincides with decomposition of the base electrolyte. In acidic medium, the wave height decreases slightly with time. For example, at pH 2·52, the wave height measured 20 and 30 min after preparation of the polarographed solution was equal to 95 and 90%, respectively, of the height of the wave measured immediately after bubbling the solution with nitrogen. This decrease is apparently connected with the protolytic splitting of the test substance[8] and is practically absent at pH > 4. On the other hand, this decrease is even more marked in a 0·1M-HCl–CH_3OH mixture (1 : 1) (see Fig. 2), and is accompanied by the formation of a new wave with a half-wave potential of about -275 mV, whose height also decreases with time. It can be assumed that this new

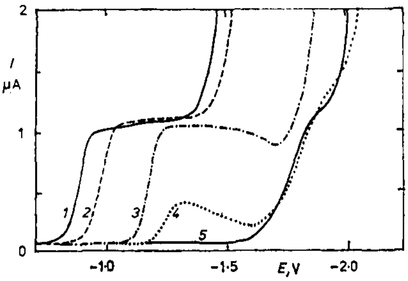


FIG. 1

Tast polarograms of 1-(4'-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 \,.\, 10^{-4}$ mol . l^{-1}) in Britton–Robinson buffer–methanol (1 : 1) medium, pH: 2·52 (*1*), 4·81 (*2*), 7·83 (*3*), 9·30 (*4*), and 10·45 (*5*)

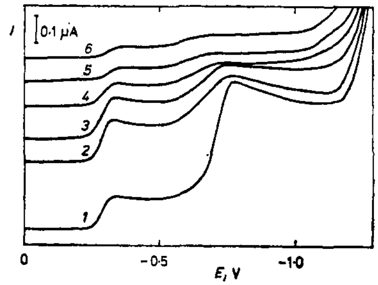
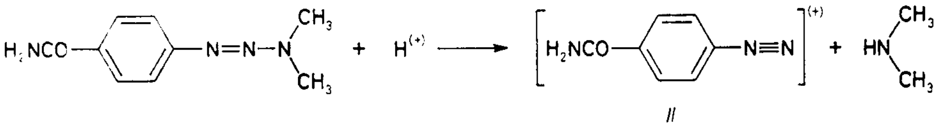
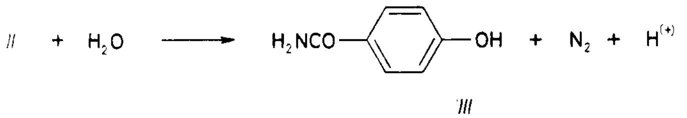


FIG. 2

Tast polarograms of 1-(4'-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) in mixed 0·1M HCl–CH_3OH (1 : 1) medium, pH 1·74, recorded: 10 (*1*), 20 (*2*), 40 (*3*), 60 (*4*), 90 (*5*), and 120 (*6*) minutes after preparing the solution wave corresponds to the arene diazonium compound (*II*), formed according to Eq. (*A*), which further decomposes according to Eq. (*B*) with formation of a polarographically inactive product (*III*).



(*A*)



(*B*)

It followed from logarithmic analysis that the electrode process is irreversible in the pH range from 3–8, as was also confirmed by cyclic voltammetry at an

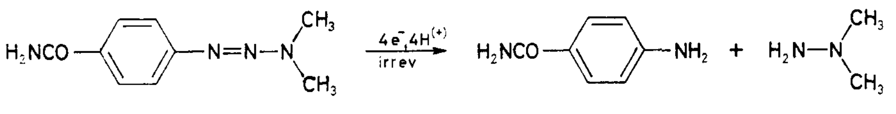
TABLE I

Effect of the pH on the polarographic behaviour of 1-(4′-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 \,.\, 10^{-4}$ mol l^{-1}) in Britton–Robinson buffer-methanol medium (1 : 1)

pH	$E_{1/2}$[a] mV	I_{lim}[a] μA	E_{p}[b] mV	I_{p}[b] μA
2·52	−885	0·96	−785	1·61
3·81	−970	1·00	−875	1·76
4·81	−1 025	1·00	−965	1·88
5·80	−1 100	1·00	−1 040	1·99
6·95	−1 150	0·98	−1 090	1·88
7·83	−1 175	1·00	−1 140	1·83
8·67	−1 235	0·51	−1 165	1·56
	−1 800[c]	1[c]	−1 775	0·80
9·30	−1 235	0·35	−1 175	0·82
	−1 800[c]	1[c]	−1 750	0·58
10·26	−1 235	0·08	−1 180	0·05
	−1 800[c]	1[c]	−1 735	0·64
10·90	−1 800[c]	1[c]	−1 730	0·59
11·95	−1 800[c]	1[c]	−1 730	0·53

[a] Measured by tast polarography at an SMDE; [b] measured by DPP at an SMDE; [c] the values given are only approximate because the more negative wave is difficult to evaluate as it coincides with the decomposition of the base electrolyte.

HMDE at a polarization rate of $5-50\,\mathrm{mV\,s^{-1}}$. At pH 5·8, the wave height of 1-(4'-carbamoylphenyl)-3,3-dimethyltriazene in DC polarography is directly proportional to the square root of the mercury reservoir height confirming that this is a diffusion-controlled process. It was found by constant-potential coulometry at a large-area mercury electrode that 4 electrons are exchanged. It can be assumed by analogy with the polarographic behaviour of unsubstituted 1-phenyl-3,3-dimethyltriazene[9] and its 2'-carboxylamido derivative[4] that the polarographic reduction of the test substance will occur according to Eq. (*C*).



(*C*)

The measured character of the $E_{1/2}$–pH dependence indicates that the substance adsorbed on the electrode surface undergoes preliminary protonation[10]. The observed decrease in the limiting current in the region around $-1{\cdot}5$ V at pH > 7 is apparently connected with a decrease in the rate of surface protonation as a consequence of desorption of the substance at a much more negative potential than that of the electrocapillary zero, where maximum adsorption of uncharged molecules of the test substance can be expected.

The effect of the pH on the peak position and height in DPP at the SMDE (see Table I) reflects the effect of the pH on the behaviour of the test substance in tast polarography. It can be seen from Fig. 3 that the more negative peak corresponds to a more irreversible process than the more positive peak.

Results obtained for FS DPV at the HMDE are analogous (see Table II).

Analytical Utilization of the Polarographic Reduction of 1-(4'-Carbamoylphenyl)-3,3-dimethyltriazene

From an analytical point of view, the highest and most easily measured curves were

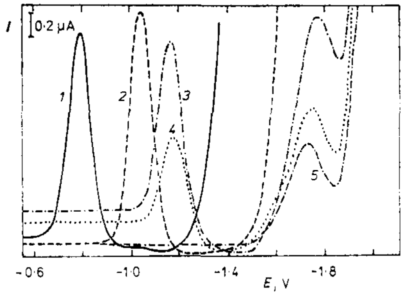


FIG. 3

Differential pulse polarograms of 1-(4'-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1\,.\,10^{-4}\,\mathrm{mol\,l^{-1}}$) in Britton–Robinson buffer–methanol (1 : 1) medium, pH: 2·52 (*1*), 5·80 (*2*), 8·97 (*3*), 9·30 (*4*), and 11·54 (*5*)

TABLE II

Effect of the pH on the FS DPV curves of 1-(4′-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 . 10^{-5}$ mol l^{-1}) in Britton–Robinson buffer–methanol medium (1 : 1)

pH	E_p mV	I_p nA	pH	E_p mV	I_p nA
2·52	−765	67	8·67[a]	−1 180	50
3·83	−930	75	9·30[a]	−1 190	32
4·81	−990	88	10·26[a]	—[b]	—
5·80	−1 045	90	10·90[a]	—[b]	—
6·95[a]	−1 110	93	11·95[a]	—[b]	—
7·83[a]	−1 160	75			

[a] A new peak appears (E_p = −1 560 mV; I_p = 80 nA), whose height and position do not change with the pH, but which is difficult to evaluate at pH < 10, as it coincides with the decomposition of the base electrolyte; [b] the more positive peak completely disappears at these pH values.

TABLE III

Parameters of the calibration straight lines and determination limit of 1-(4′-carbamoylphenyl)-3,3-dimethyltriazene found by various methods

Method	c mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correl. coef.	Determin. limit mol l^{-1}
Tast[a]	$(1-10) . 10^{-5}$	9·8	15	0·9993	—
	$(1-10) . 10^{-6}$	10·0	3	0·9981	$0·5 . 10^{-6}$
DPP[b]	$(1-10) . 10^{-5}$	19·8	10	0·9995	—
	$(1-10) . 10^{-6}$	18·9	−1	0·9990	—
DPP[c]	$(2-10) . 10^{-7}$	21·5	2	0·9981	$2 . 10^{-7}$
FS DPV[b]	$(1-10) . 10^{-6}$	9·0	5	0·9993	—
FS DPV[d]	$(2-10) . 10^{-7}$	34·1	−1	0·9987	$1·8 . 10^{-7}$
FS DPV[e]	$(2-10) . 10^{-8}$	151·0	0·2	0·9985	$3·2 . 10^{-8}$
FS DPV[f]	$(2-10) . 10^{-9}$	210·0	−0·05	0·9875	$3·9 . 10^{-9}$

[a] Britton–Robinson buffer–methanol (1 : 1) medium, pH 4·81; [b] Britton–Robinson buffer–methanol (1 : 1) medium, pH 5·80; [c] ten-fold diluted Britton–Robinson buffer–methanol (1 : 1) medium, pH 6·10; [d] ten-fold diluted Britton–Robinson buffer–methanol (99 : 1) medium, pH 5·26; [e] ten-fold diluted Britton–Robinson buffer–methanol (99 : 1) medium, pH 5·26 with 15 s adsorptive accumulation in stirred solution (recorded 10 s after termination of stirring); [f] hundred-fold diluted Britton–Robinson buffer–methanol (999 : 1), pH 4·00 after 60 s accumulation in stirred solution (recorded 10 s after termination of stirring).

obtained in medium with pH 4·81 for tast polarography and 5·80 for DPP. The calibration dependences are linear under these conditions in the range $1 . 10^{-4}$ to $1 . 10^{-6}$ mol l^{-1} for tast polarography and $1 . 10^{-4} - 2 . 10^{-7}$ mol l^{-1} for differential pulse polarography and their parameters are given in Table III together with the calculated determination limit. (The peak height in DPP was measured from the line connecting the minima on both sides.)

In FS DPV at the HMDE, a smoother curve is obtained for the base electrolyte when ten-fold diluted Britton–Robinson buffer is used. This is also true for DPP at the SMDE in the concentration range $(2-10) . 10^{-7}$ mol l^{-1}. The peak height increases with decreasing methanol concentration, probably as a consequence of an increase in the reversibility of the reduction as a result of greater velocity of the preliminary protonation. Consequently, it is preferable in the determination of lower concentrations of 1-(4′-carbamoylphenyl)-3,3-dimethyltriazene to employ the lowest possible methanol concentration in the voltamographed solution (1% or 0·1% – see Table III). In constructing the calibration curves, it is necessary to employ test substance solutions in a water–methanol mixture (9 : 1), which must be prepared fresh daily. It was found by a spectrophotometric study of the stability of a $1 . 10^{-4}$ mol l^{-1} solution of 1-(4′-carbamoylphenyl)-3,3-dimethyltriazene solution in this medium that the concentration of this substance after 24 and 48 h decreased by 1·5 and 3%, resp., and after 6 days by 6·2%.

A further increase in the sensitivity of the determination can be achieved through adsorptive accumulation of the test substance on the surface of a hanging mercury drop electrode. After 15 s accumulation in stirred solution, a measurable voltammogram was still obtained in the concentration range $(2-10) . 10^{-8}$ mol l^{-1} and, for 60 s accumulation, in the range $(2-10) . 10^{-9}$ mol l^{-1}. The parameters of these calibration curves are given together with the calculated determination limits in Table III.

REFERENCES

1. Lin Y. T., Loo T. L., Vadlamudi S., Goldin A.: J. Med. Chem. *15*, 201 (1972).
2. Matrka M., Rambousek V., Remeš M., Zvěřina V.: Cesk. Farm. *27*, 70 (1978).
3. Matrka M., Rambousek V., Držková L., Zvěřina V.: Cesk. Farm. *27*, 299 (1978).
4. Barek J., Toubar S., Zima J.: Collect. Czech. Chem. Commun. *56*, 2073 (1991).
5. Barek J., Bláhová-Haladová H., Zima J.: Collect. Czech. Chem. Commun. *54*, 1538 (1989).
6. Beyermann K.: *Organic Trace Analysis*, p. 45. Ellis Horwood, Chichester 1984.
7. Paabo M., Robinson R. A., Bates R. G.: J. Am. Chem. Soc. *87*, 415 (1965).
8. Matrka M., Rambousek V., Zvěřina V.: Cesk. Farm. *27*, 346 (1978).
9. Kazemifard G., Moatar F., Reisch J.: Acta Pharm. Jugosl. *28*, 151 (1978).
10. Mairanovskii S. G.: Talanta *12*, 1299 (1965).

Translated by M. Štulíková.