POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 1-(2'-CARBAMOYLPHENYL)-3,3-DIMETHYLTRIAZENE*

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A study was carried out of the polarographic behaviour of the genotoxic substance 1-(2'-carbamoylphenyl)-3,3-dimethyltriazene and optimum conditions were found for its determination by tast polarography or 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 $1 \cdot 10^{-4}$—$2 \cdot 10^{-7}$ mol l^{-1}. The sensitivity of the determination can be further improved through adsorptive accumulation of the test substance on the surface of the hanging mercury drop electrode; five-minute accumulation in unstirred solution permits determination in the concentration range $(2-10) \cdot 10^{-8}$ mol l^{-1} and two-minute accumulation in stirred solution allows determination in the range $(2-10) \cdot 10^{-9}$ mol l^{-1}.

The derivatives of 1-phenyl-3,3-dimethyltriazene are genotoxic substances that are assumed to act through an alkylation mechanism[1]. In addition, this type of substance exhibits carcinostatic action[2,3]; in this connection, detailed studies have been carried out of its acute toxicity[4], metabolism[5] and protolytic splitting mechanism[6]. These substances can be determined by direct spectrophotometry in the ultraviolet region[7-9] or spectrophotometry in the visible region after the protolysis of triazene and subsequent coupling of the diazonium compound formed with N-ethyl-1-naphthylamine[9]. Although triazenes can be readily reduced electrochemically, very little attention has so far been devoted to their polarographic determination. The DC polarographic behaviour has been studied for triazene compounds derived from guanidine[10] and thiourea[11], along with the behaviour of 3'-hydroxy-1,3-diphenyltriazene[12] and a number of 4'-substituted derivatives of 1-phenyl-3-phenyl triazene[13-15] in aqueous alcoholic medium. In addition, the reduction of the latter substance in dimethylformamide[16] and of substances of the Y—C_6H_4—NH—N=N—$(CH_2)_n$—X type (where X = F, Cl, Br; Y = CN, $COOC_2H_5$, CH_3CO and $n = 2-5$) in mixed acetonitrile–water medium[17] and the electrochemical oxidation of some triazenes in acetonitrile medium[18] have been studied. DC polarography has

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also been used to study 1-phenyl-3,3-dimethyltriazene[19], its 4'-Cl, 4'-Br and 4'-COOH derivatives[20] and its 2', 3', and 4'-$COOCH_3$ or $CONH_2$ derivatives[9] and to study the kinetics of the protolysis of the latter substances[6]. The use of these techniques is limited to concentrations above $1 . 10^{-6}$ mol l^{-1}. In contrast, differential pulse polarography has been used to determine 1-(4'-carbamoyl-5'-imidazol)-3,3-dimethyltriazene, azahypoxanthine, 1,2,3-benzotriazin-4(3*H*)-one and 1-(2'-carbamoylphenyl)-3,3-dimethyltriazene[21] down to concentrations of $1 . 10^{-7}$ mol l^{-1}.

An attempt was made to improve the sensitivity of the determination of 1-(2'-carbamoylphenyl)-3,3-dimethyltriazene in this work by studying its determination by tast or differential pulse polarography (DPP) at a static mercury drop electrode (SMDE) and by fast scan differential pulse voltammetry (FSDPV) at a hanging mercury drop electrode (HMDE).

EXPERIMENTAL

Reagents

1-(2'-carbamoylphenyl)-3,3-dimethyltriazene was prepared in the Toxicological Department of the Research Institute for Organic Synthesis in Pardubice-Rybitví[22]. The stock solution of this substance in methanol ($c = 1 . 10^{-3}$ mol l^{-1}) was prepared by dissolving a precisely weighed amount of the substance in freshly redistilled solvent. It followed from a spectrophotometric study of the stability of the solutions that the stock solution ($c = 1 . 10^{-3}$ mol l^{-1}) must be prepared freshly once a month, while more dilute solutions ($c = 1 . 10^{-4}$ mol l^{-1}) must be prepared once a week, and even more dilute solutions ($c = 1 . 10^{-5}$ and $1 . 10^{-6}$ mol l^{-1}) should be 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 using a PA 4 polarographic analyzer with an XY 4106 *x–y* recorder and static mercury drop electrode SMDE 1 (all from Laboratorní přístroje, Prague). The capillary employed had a diameter of 0·146 mm and the maximum drop size attainable was employed, obtained by opening the valve for 160 ms. A saturated calomel electrode was used and all the potential values are related to this reference electrode; the auxiliary electrode was a platinum wire. Where not stated otherwise, tast polarography and DPP were carried out using a polarization rate of 5 mV s^{-1} and drop time of the SMDE of 1 s, FSDPV was carried out using a polarization rate of 20 mV s^{-1} with the SMDE connected as an HMDE. A modulation amplitude of $\Delta E = -100$ mV was employed in the pulse techniques. Prior to entering the polarographic vessel, nitrogen was passed through a prebubbler containing a mixture of water and methanol in the same ratio as in the analyzed solution. The coulometric and spectrophotometric measurements were carried out using the instrumentation described in an earlier communication[23].

Procedure

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 at a concentration corresponding to the lowest point on the given calibration curve. Measurements of the pH of the aqueous methanol solutions, coulometric determination of the number of electrons exchanged and spectrophotometric and polarographic study of the coulometric reduction were all carried out using the procedures described previously[23,24].

RESULTS AND DISCUSSION

The Effect of the pH *on the Polarographic and Voltammetric Behaviour of* 1-(2-*Carbamoylphenyl*)-3,3-*dimethyltriazene*

It can be seen from Fig. 1 that tast polarography of the test substance at the SMDE in Britton–Robinson buffer–methanol medium (1 : 1) yields two waves. The half-wave potential of the more positive wave shifts to more negative values with increasing pH, but this dependence is asymptotic rather than linear. At pH > 6, the height of this wave decreases and, above pH > 10, completely disappears. Simultaneously, a new wave appears at pH > 6; in acidic medium, this wave is apparently obscured

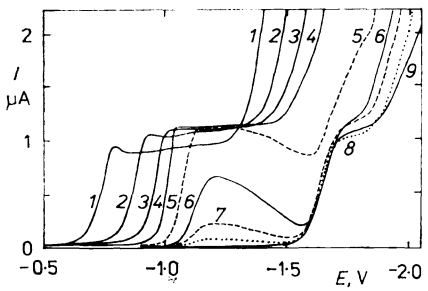


FIG. 1

Tast polarograms of 1-(2′-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 . 10^{-4}$ mol l^{-1}) in mixed Britton–Robinson buffer–methanol (1 : 1) medium. pH: *1* 2·83; *2* 3·84; *3* 4·72; *4* 5·73; *5* 6·87; *6* 7·91; *7* 8·68; *8* 9·59; *9* 11·33

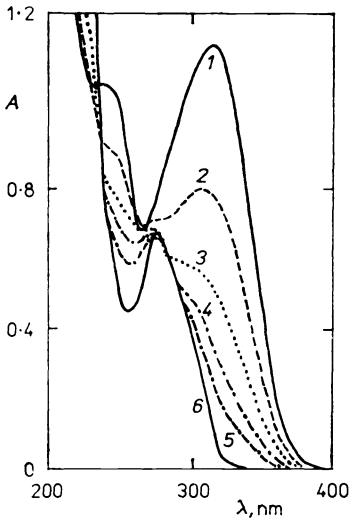
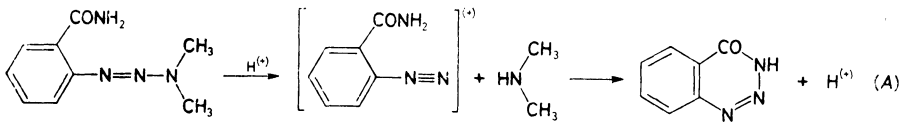


FIG. 2

UV spectra of 1-(2′-carbamoyphenyl)-3,3-dimethyltriazene ($c = 1 . 10^{-4}$ mol l^{-1}) in mixed 0·1M-HCl–methanol (1 : 1) medium, pH 1·74, measured at various times (t) after preparation of the solution. t(min): *1* 0; *2* 2; *3* 4; *4* 6; *5* 8; *6* 30

by the decomposition of the base electrolyte; its height and position are pH-independent. The observed decrease in the height of the more positive wave at pH < 4 is apparently connected with protolytic decomposition of the test substance[6]. At pH 2·83, the height of the wave measured after 30 or 60 min corresponded to 85 and 75%, respectively, of that of the original wave, measured at time $t = 0$ after ten-minute removal of oxygen from the polarographed solution. At pH 3·84, the height of the wave decreased to 96 or 93% of the original value, respectively, while at pH 4·72 and 5·73 the wave height did not change within experimental error. In 0·1M-HCl–methanol (1 : 1) at pH 1·74, marked changes occur in time in both the UV spectra of the test substance (see Fig. 2) and also in the tast polarograms (see Fig. 3). It can be seen from Fig. 2 that protolysis leads to disappearance of the peak in the region around 316 nm, assigned to the triazo group, while the protolysis product with an absorption maximum at about 276 nm is stable, with no change in the spectrum between 30 and 120 min after preparation of the solution. It can be seen from Fig. 3 that this stable protolysis product is also polarographically active, with a half-wave potential that is more positive than that of the initial triazene, as can be seen from the shift in the half-wave potential of the more negative wave in Fig. 3 with time to more positive potentials. The height of this more negative wave corresponds to about 40% of the height of the wave of the test substance at pH 4·72. The lower wave with a half-wave potential of about −300 mV apparently corresponds to the intermediate in this protolytically initiated reaction and disappears at times longer than 30 min. It can be assumed that this intermediate is the diazonium compound (*II*) and that the final product is 1,2,3-benzotriazin-4(3*H*)-one (*III*), formed in the cyclization reaction (see scheme (*A*)).



I *II* *III*

The fact that the height of the wave of the protolysis product corresponds to only 40% of the height of the wave of the test substance at pH 4·72 indicates that the cyclization reaction is not quantitative, for example because of the decomposition of the temporarily formed diazonium salt with the formation of elemental nitrogen. (It can be assumed that the 1,2,3-benzotriazin-4(3*H*)-one formed will exchange the same number of electrons and that its diffusion coefficient will be similar to that of 1-(2′-carbamoylphenyl)-3,3-dimethyltriazene).

The observed atypical tast polarograms in the pH region 7–9 are apparently connected with the presence of methanol and its effect on the preliminary protonization reaction. The fact that the slope of the $E_{1/2}$ vs pH dependence for the first wave

does not correspond to the relationship $\Delta E_{1/2}/\Delta pH = RT/\alpha n_a F$ indicates[25] that a substance adsorbed on the electrode surface undergoes the preliminary protonation reaction. As the methanol content increases, the rate of this preliminary reaction decreases both because of the decreased protonation rate constant and also because of the decreased adsorbance of the test triazene. The observed decrease in the current at pH 7–9 in the region between −1·2 and −1·5 V is apparently connected with a decrease in the surface concentration of the adsorbed triazene, resulting in a substantial decrease in the rate of the surface protonation reaction. This assumption is in agreement with the observed effect of the methanol content on the shape of the tast polarographic curves in neutral medium (Fig. 4). The fact that the observed changes are analogous at various depolarizer concentrations indicates that this phenomenon cannot be a result solely of exhaustion of the proton supply in the immediate vicinity of the electrode as a result of the faster electrode reaction at more negative potentials. The wave height in classical DC polarography at a DME is linearly dependent on the square root of the reservoir height at pH 4·72 and 12·22 and passes through the origin, indicating that the limiting current is diffusion-controlled under the given conditions.

Figure 5 depicts the effect of the pH on the DP polarograms at the SMDE. It can be seen that the first peak corresponds to a far more irreversible process than the first peak (assuming that both correspond to exchange of the same number of electrons). The much lower peak at pH 2·83 at a potential of about −0·95 V probably corresponds to the observed increase in the tast polarographic current for the wave

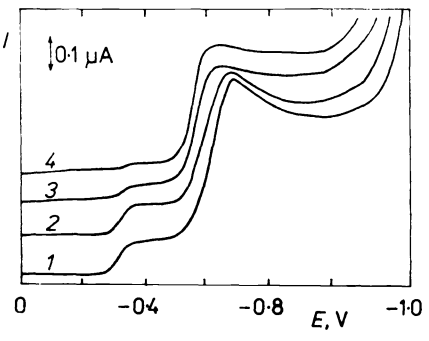


FIG. 3

Tast polarograms of 1-(2′-carbamoylphenyl)-3,3-dimethyltriazene ($c = 1 . 10^{-4}$ mol l^{-1}) in mixed 0·1M-HCl–methanol (1 : 1) medium, pH 1·74, measured at various times t after preparation of the solution. t(min): *1* 5; *2* 15; *3* 30; *4* 45

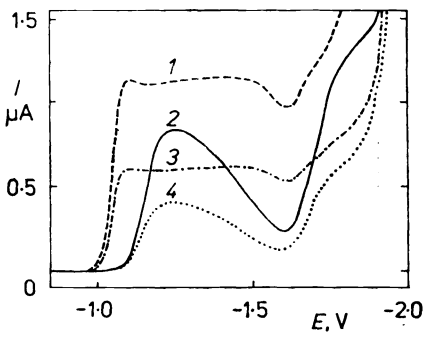


FIG. 4

Tast polarograms of 1-(2′-carbamoylphenyl)-3,3-dimethyltriazene at concentrations of $1 . 10^{-4}$ (*1*, *2*) and $5 . 10^{-5}$ (*3*, *4*) mol l^{-1} in 10% (v/v) methanol medium at pH 7·20 (*1*, *3*) and 50% (v/v) methanol at pH 7·85 (*2*, *4*)

in this region after a temporary decrease in height and not to the reduction of a further electrochemically active group in the test substance molecule, i.e. the carboxylamide groups, as suggested in ref.[21].

Figure 6 documents the effect of the pH on the FSDPV voltammograms at the HMDE; it can be seen that the pH has the same type of effect on the peak position and height as in tast polarography or DPP. It also follows from the FSDP voltammograms that the more negative peak corresponds to a more irreversible process than the more positive peak.

It has been demonstrated by cyclic voltammetry at the HMDE that this is an irreversible process (absence of an anodic peak), where the cyclic voltammogram consists of a single cathodic peak (E_p = −0·945 V) at pH 5·03 and polarization rate of 5 mV s^{-1}, two cathodic peaks at pH 8·6 (E_p = −1·125 and −1·660 V) and once again a single peak at pH 11·33 (E_p = −1·650 V). The peak height is directly proportional to the square root of the polarization rate in the range 5 − 50 mV s^{-1}; together with the shift of E_p to more negative potential, this dependence confirms the diffusion control of the studied processes and their irreversible character.

Constant-potential coulometry at a large-area mercury electrode indicated that exchange of 4 electrons occurs both at pH 12·31 with an applied potential of −1 850 mV and at pH 5·05 with an applied potential of −1 150 mV.

It can be assumed on the basis of the above observations and on the basis of analogy with the polarographic behaviour of unsubstituted 1-phenyl-3,3-dimethyl-

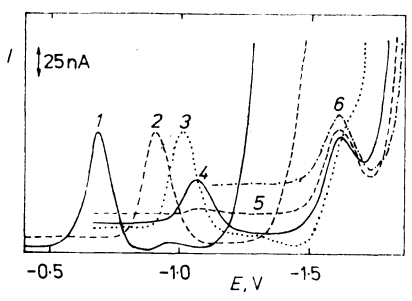


FIG. 5

DP polarograms of 1-(2′-carbamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-5} mol l^{-1}) in mixed Britton–Robinson buffer – methanol (1 : 1) medium. pH: *1* 2·83; *2* 4·72; *3* 6·87; *4* 7·91; *5* 9·59; *6* 11·53

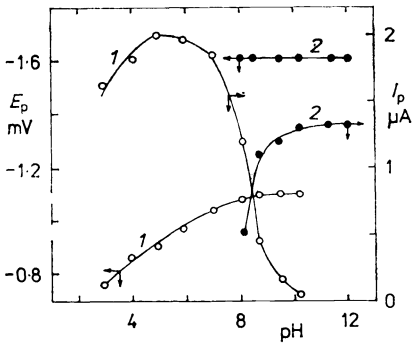
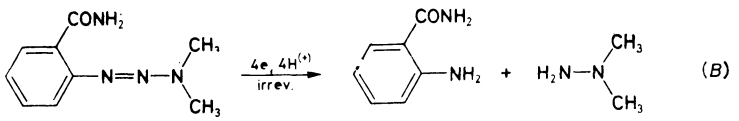


FIG. 6

The effect of the pH on the position (E_p) and height (I_p) of the first (*1*) and second (*2*) peak of 1-(2′-cabamoylphenyl)-3,3-dimethyltriazene (c = 1 . 10^{-4} mol l^{-1}) for FSDPV at the HMDE in Britton–Robinson buffer – methanol (1 : 1) medium

triazene[19] that the test substance is reduced according to scheme (*B*) at both pH values.



(*B*)

It can be assumed that the first reduction step yields the radical anion, which is immediately stabilized in acidic medium by accepting a readily available proton, resulting in the dependence of the half-wave potential on the pH. In contrast, in alkaline medium where there is a low concentration of free hydroxonium ions, this anion radical is stabilized by a proton that is removed from a water molecule so that the half-wave potential of the test substance is pH-independent in this region.

Analytical Utilization of the Polarographic Reduction of 1-(2'-*Carbamoylphenyl*)-3,3-*dimethyltriazene*

For analytical purposes, the optimum medium was found to be Britton–Robinson buffer – methanol (1 : 1) at pH 4·72, in which the test substance is sufficiently stable and the polarographic waves or peaks are well developed and readily evaluated. Linear calibration curves were obtained for the tast polarographic determination at the SMDE in the concentration range $2 \cdot 10^{-7}$ to $1 \cdot 10^{-4}$ mol l^{-1}.

It is preferable to carry out DPP at the SMDE in the concentration range $(2-10) \cdot 10^{-7}$ mol l^{-1} in a mixture of five-fold diluted Britton–Robinson buffer – methanol (9 : 1) with pH 4·13. As the concentration of the aqueous Britton–Robinson buffer decreases, the peak heights corresponding to impurities in the chemicals employed for its preparation also decrease, leading to a smoother base electrolyte curve. A decrease in the methanol content in the polarographed solution leads to a shift in the peak potential to more positive values (the differences in the E_p value in 50% (v/v) methanol and 10% (v/v) methanol at constant pH 4·1 is almost 70 mV) and increases the reversibility of the reduction, reflected in an increase in the peak height and thus also in the sensitivity of the determination.

Similarly, it is also preferable to employ five-fold diluted Britton–Robinson buffer – methanol (9 : 1) medium for FSDPV at the HMDE in the concentration range $(2-10) \cdot 10^{-8}$ mol l^{-1} for the above reasons.

A further increase in the sensitivity of the determination can be attained through adsorptive accumulation of the test substance on the surface of a hanging mercury drop electrode. The following dependence of the peak height I_p on the time t between formation of the hanging drop and recording of the voltammogram was found in five-fold diluted Britton–Robinson buffer – methanol medium (9 : 1) at pH 4·13 and an analyte concentration of $10 \cdot 10^{-8}$ mol l^{-1}.

t, s:	10	60	120	300
I_p, nA	6·0	8·7	11·8	15·5

Thus, five-minute accumulation in unstirred solution leads to roughly a three-fold increase in the sensitivity.

The increase in the peak height as a result of adsorptive accumulation can be further increased by decreasing the methanol content in the voltammetric solution, connected both with competitive adsorption of methanol on the HMDE surface and also with its effect on the solubility of the test substance. Two-minute adsorptive accumulation in stirred solution in medium containing only 1% (v/v) methanol leads to approximately a five-fold increase in the sensitivity compared with five-minute accumulation in unstirred solution containing 10% (v/v) methanol. The determination can then be carried out in the concentration range $(2-10) \cdot 10^{-9}$ mol l^{-1}.

Table I gives the parameters of the calibration curves and the calculated determination limits for all the above-described polarographic and voltammetric techniques.

TABLE I

Parameters of the calibration straight lines and the determination limits for 1-(2′-carbamoylphenyl)-3,3-dimethyltriazene (*I*) determined by various methods

Method	$c(I)$ mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correl. coeff.	Determin. limit mol l^{-1}
TAST/SMDE[a]	$(1-10) \cdot 10^{-5}$	10·9	27	0·9993	—
	$(1-10) \cdot 10^{-6}$	12·8	−4	0·9991	—
	$(2-10) \cdot 10^{-7}$	15·2	0·5	0·9975	$2·2 \cdot 10^{-7}$
DPP/SMDE[a]	$(1-10) \cdot 10^{-5}$	18·7	1	0·9998	—
	$(1-10) \cdot 10^{-6}$	19·0	2	0·9997	—
DPP/SMDE[b]	$(2-10) \cdot 10^{-7}$	42·6	−3	0·9995	$0·9 \cdot 10^{-7}$
FSDPV/HMDE[a]	$(1-10) \cdot 10^{-5}$	9·7	−25	0·9995	—
	$(1-10) \cdot 10^{-6}$	10·1	−4	0·9989	—
FSDPV/HMDE[b]	$(1-10) \cdot 10^{-7}$	51·0	4	0·9931	$0·8 \cdot 10^{-7}$
FSDPV/HMDE[c]	$(2-10) \cdot 10^{-8}$	155·0	0·8	0·9811	$1·1 \cdot 10^{-8}$
FSDPV/HMDE[d]	$(2-10) \cdot 10^{-9}$	751·0	0·05	0·9795	$3·2 \cdot 10^{-9}$

[a] Britton–Robinson buffer – methanol (1 : 1) medium, pH 4·72; [b] five-fold diluted Britton–Robinson buffer – methanol (9 : 1) medium, pH 4·13; [c] five-fold diluted Britton–Robinson buffer – – methanol (9 : 1) medium, pH 4·13, with five-minute accumulation in unstirred solution; [d] five-fold diluted Britton–Robinson buffer – methanol (99 : 1) medium, pH 5·05, with two-minute accumulation in stirred solution.

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