ADSORPTIVE STRIPPING VOLTAMMETRIC DETERMINATION OF 1,1-DIMETHYL-3-PHENYLTRIAZENE

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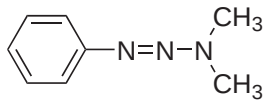
The optimum conditions were found for the determination of 1,1-dimethyl-3-phenyltriazene in the concentration range from 1×10^{-4} to 1×10^{-7} mol l^{-1} by differential pulse voltammetry at a hanging mercury drop electrode. The sensitivity of the determination can be improved by preliminary adsorptive accumulation of the substance on the surface of the hanging mercury drop. Differential pulse adsorptive stripping voltammetry can be used for the purpose in the concentration range from 1×10^{-6} to 1×10^{-9} mol l^{-1}. The determination limit is 1×10^{-9} mol l^{-1} for a deposition time of 10 min, the relative standard deviation being 5% (n = 10) for a concentration of 2×10^{-9} mol l^{-1}.

Keywords: Triazenes; Adsorptive stripping voltammetry; Differential pulse voltammetry; Carcinogens; Electrochemistry; Electroanalysis.

1,1-Dimethyl-3-phenyltriazene (DMPT) and its 2-, 3- and 4-substituted phenyl derivatives rank among genotoxic substances which act via an alkylation mechanism[1]. At the same time, these types of substances are considered as potential carcinostatics[2]. Therefore, an increasing demand for the determination of trace amounts of these substances can be observed.



They can be determined spectrophotometrically in the ultraviolet region[3] and also through their protolysis followed by azo-coupling of the arenediazonium salt formed with *N*-ethyl-1-naphthylamine to give an azo dye. This azo dye can be determined photometrically in the visible region[4].

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Direct current polarography has been used to study 1,1-dimethyl-3-phenyltriazene[5] and some of its derivatives[4]. More sensitive techniques, such as tast and differential-pulse polarography[6–8] and adsorptive stripping voltammetry[8–10], with determination limits of ca. 1×10^{-6}, 1×10^{-7} and down to 1×10^{-9} mol l^{-1}, respectively, have been used for the determination of variously substituted DMPT derivatives.

The polarographic behavior of 1,1-dimethyl-3-phenyltriazene, the mechanism of its polarographic reduction and the optimum conditions for its determination by differential pulse polarography at a static mercury drop electrode in the concentration range from 1×10^{-4} to 1×10^{-7} mol l^{-1} have been described[11].

In the present study, the possibility of increasing the sensitivity of the determination of DMPT using its adsorptive accumulation on the surface of a hanging mercury drop electrode was investigated.

EXPERIMENTAL

Reagents

The purity of the prepared DMPT[12] was checked by thin-layer chromatography, HPLC and by elemental analysis. A 1×10^{-3} mol l^{-1} stock standard solution of the investigated substance was prepared by dissolving an exactly weighed amount of the substance in methanol (analytical- reagent grade, Merck). The low-concentration solutions were prepared by dilution of the stock solution with methanol. The solutions were stored in a dark place. A spectrophotometrical study demonstrated that the stock standard solution was stable for at least two months. The dilute solutions (1×10^{-4} and 1×10^{-5} mol l^{-1}) were prepared every week and the most dilute solution (1×10^{-6} mol l^{-1}) was prepared every day.

The Britton–Robinson (BR) buffer solutions were prepared conventionally[13], using the chemicals of analytical-reagent grade, obtained from Merck. Deionized water, produced by an Ultra Clear basic SG Water apparatus (SG Wasseraufbereitung GmbH, Germany) was used.

Apparatus

Voltammetric measurements were carried out using a PA 4 polarographic analyzer interfaced with the multimode electrode stand model SMDE 1 (both from Laboratorní Přístroje, Praha, Czech Republic) composed of hanging mercury drop electrode (HMDE) as working electrode, platinum rod as auxiliary electrode and a saturated calomel reference electrode (SCE), to which all the potentials are related. The electrodes were kept in the triangular arrangement. The capillary employed had an internal diameter of 0.136 mm and the maximum drop size, obtained by opening the electromagnetic valve for 160 ms, was used. The measurements were performed at the sweep rate of 20 mV s^{-1}, with a pulse amplitude of –100 mV and a pulse interval of 0.2 s. The pH meter, model 744, equipped with a combined pH electrode and temperature sensor (all from Metrohm, Switzerland) was used for pH measurements.

Procedure

A mixed BR buffer–methanol medium was used as the supporting electrolyte. The actual pH of the BR buffer–methanol mixture (1:1) was measured with a combined pH electrode which was calibrated using potassium hydrogen phthalate and TRIS buffers in 50% (v/v) methanol[14]. pH of the BR buffer–methanol mixture containing 10% (v/v) of methanol was measured with a pH electrode calibrated using the buffers without added methanol[15].

A 10-ml aliquot of an appropriate supporting electrolyte solution was placed in a voltammetric cell and deaerated for 5 min with nitrogen. Prior to entering the voltammetric cell, nitrogen was passed successively through a solution of chromium(II) in dilute hydrochloric acid containing heavily amalgamated zinc granules, distilled water, molecular sieves and, finally, a solution having the same solvent and supporting electrolyte composition as the test solution.

After recording the baseline, the required amount of the DMPT solution was added. After a fresh mercury drop was formed, the voltammogram was recorded immediately or after accumulation for a chosen time in a stirred or quiescent solution. The accumulation period in a stirred solution was followed by a 15-s rest time allowing quiescence of the solution and uniform distribution of the deposited substance on the mercury drop.

The calibration curves were measured in triplicate and evaluated by the least-square linear regression method. The determination limit was calculated as ten times the standard deviation for ten determinations of the analyte at a concentration corresponding to the lowest point of the appropriate calibration graph[16]. The detection limit was calculated using the whole calibration line[17].

Electrocapillary measurements were performed by measuring the times of formation of mercury drops at a given potential. The drop time was determined by the time needed to spontaneously form 50 droplets of mercury in the solution from a capillary with an internal diameter of 0.045 mm, at a height of mercury reservoir of 45 cm.

All glassware was immersed in 20% (v/v) nitric acid and cleaned in ultrasonic bath for 1 h, in order to eliminate adsorption of the species of interest or impurities on the vessel walls, and rinsed carefully with deionized water before use.

All measurements were performed at laboratory temperature (20 ± 1 °C).

RESULTS AND DISCUSSION

To ensure the solubility of DMPT, the first experiments were carried out in a mixed BR buffer–methanol (1:1) medium as a supporting electrolyte. It can be seen in Fig. 1 that differential pulse voltammetry (DPV) of the investigated triazene yields a single peak within the investigated pH range of the supporting electrolyte. The potential of DPV peak, E_p, shifts towards negative values with increasing pH of the basic electrolyte, producing an asymptotic E_p–pH dependence. The obtained dependence can be approximated by two straight lines: the first, in the pH range of the supporting electrolyte from 4.0 to 6.5, has a slope of 60.25 mV per pH unit, whereas the slope of the other line is 18.33 mV per pH unit (pH 7.5–10.5). On the basis of the fact and previous polarographic and constant-potential coulometric investi-

gations[11], it can be concluded that the observed voltammetric peak corresponds to an irreversible four-electron reduction of the triazene group accompanied by a transfer of different numbers of protons in acid and alkaline media.

The observed decrease in the current beyond the DPV peaks in the region around –1.4 V at pH > 7.0 is associated with the decrease in the rate of surface protonation as a consequence of desorption of the investigated substance at potentials much more negative than the potential of electrocapillary maximum, where maximum adsorption of the uncharged substance is expected[18].

From the analytical point of view, the most suitable supporting electrolyte is a mixture of BR buffer and methanol with pH 6.50, where well defined and easily evaluated DPV curves, with the highest currents for the given concentration, were obtained. Using the buffers containing 50% (v/v) methanol, linear calibration dependences for DPV determinations of the investigated substances were obtained in the concentration range from 1×10^{-6} to 1×10^{-4} mol l^{-1}.

The sensitivity of the DP voltammetric determination of DMPT is increased by fivefold dilution of BR buffer and decreasing the methanol percentage to 10% (v/v), while the pH values of the outgoing solutions were kept unchanged. Thus, the concentration of the impurities present in the

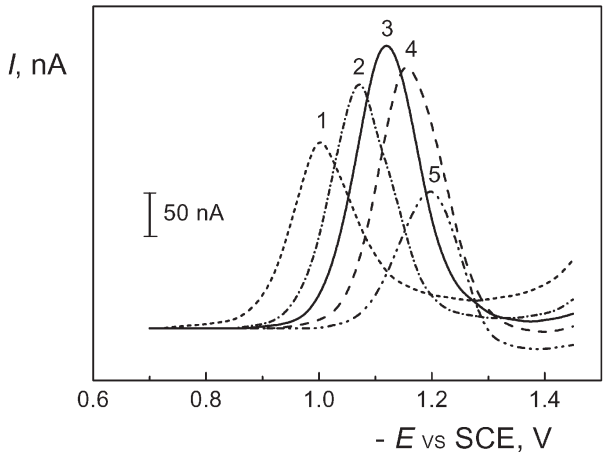

FIG. 1
Differential pulse voltammograms of DMPT (5×10^{-5} mol l^{-1}) in mixed BR buffer–methanol (1:1) medium at pH: 4.98 (1), 5.90 (2), 6.50 (3), 7.01 (4), 8.70 (5)

supporting electrolyte decreases, which leads to a smoother voltammetric curve of the supporting electrolyte and an increase in the sensitivity of determination. A decrease in methanol content in measured solutions leads to a shift of the DPV peak potentials to more positive values. The reversibility of the electrode process was also increased, which was reflected in increasing DPV peak currents and, thus, in increasing sensitivity of determination. The use of the last mentioned medium as supporting electrolyte enabled DPV determinations in the concentration range from 1×10^{-7} to 1×10^{-5} mol l^{-1}.

A further increase in the sensitivity of the determination could be achieved by adsorptive accumulation of the test substance on the surface of hanging mercury drop electrode. To determine whether the DMPT imparted surface-active properties to mercury, which would make the adsorptive stripping technique applicable, accumulation studies were carried out by electrocapillary measurements as well as by the use of the described procedure[19]. As shown in Figs 2 and 3, the adsorptive stripping method is suitable for DMPT determination.

In order to optimize the conditions required for the differential pulse adsorptive stripping voltammetry (DPAdSV), the following parameters were examined: pH of the supporting electrolyte, deposition potential, deposi-

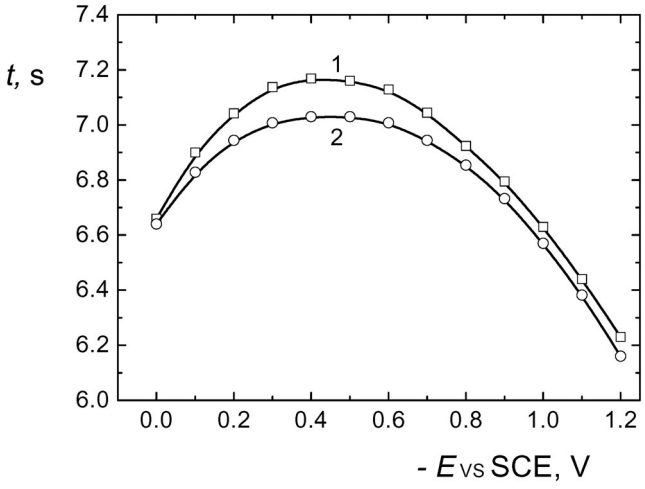

FIG. 2
Electrocapillary curves of 1 the fivefold-diluted BR buffer–methanol (9:1) at pH 6.50 (supporting electrolyte) and 2 DMPT (1×10^{-4} mol l^{-1}) in the supporting electrolyte

tion time, scan rate, modulation amplitude, mercury drop size and temperature.

As shown in Fig. 4, the peak current was found to vary with pH of the supporting electrolyte. The sharp maximum in that dependence, in the pH range of 6.0–7.0, is probably associated with the presence of the unprotonated species of the test substance, resulting in its higher adsorption on the surface of the hanging mercury drop. The supporting electrolyte with pH 6.50 was found to be the most suitable for DPAdSV measurements.

After an investigation covering the range from 0.0 to –1.2 V vs SCE, the optimum deposition potential providing the best peak shapes and the highest sensitivity was found to be –0.400 V. The deposition potential was also found on the basis of electrocapillary curves in Fig. 2, from which it can be seen that the maximum adsorption of the investigated substance and, consequently, the maximum decrease of Hg surface tension (represented as dropping time) was found at –0.400 V vs SCE.

The effect of the accumulation time on the peak height for 1×10^{-6} and 1×10^{-7} mol l^{-1} solutions of DMPT is shown in Figs 3 and 5, respectively. Generally, the peak height increases with the accumulation time, but the observed non-linear dependences suggest saturation of the electrode surface. It should be noted that for lower concentrations of the substance of

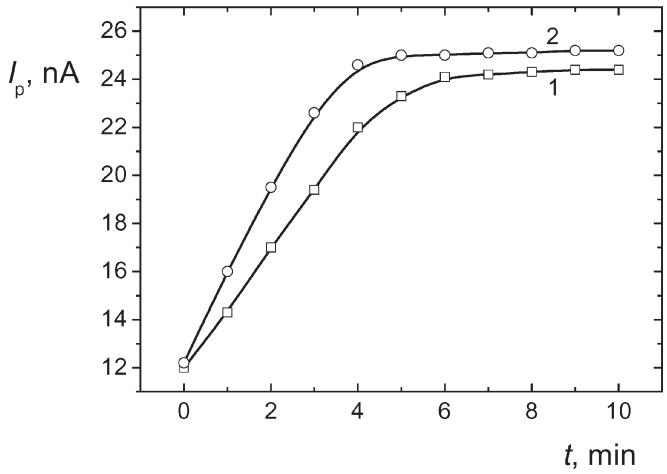

FIG. 3

Effect of the accumulation time on the peak current of differential pulse adsorptive stripping voltammograms of DMPT (1×10^{-6} mol l^{-1}) in tenfold-diluted BR buffer–methanol (99.5:0.5) solution at pH 6.50. Accumulation potential –0.400 V; 1 unstirred and 2 stirred solution

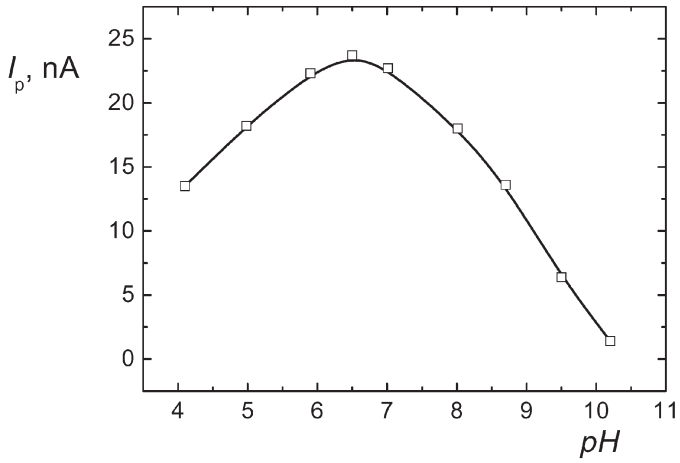

Fig. 4
The influence of pH of the supporting electrolyte on the DPAdSV peak current of DMPT (1×10^{-6} mol l^{-1}) in tenfold-diluted BR buffer–methanol (99.5:0.5) medium. Accumulation potential –0.400 V; 5-min accumulation in the unstirred solution

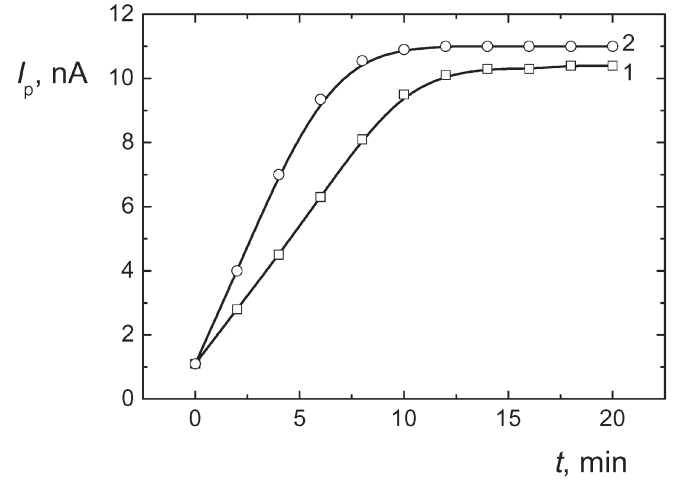

Fig. 5
Effect of the accumulation time on the peak current of differential pulse adsorptive stripping voltammograms of DMPT (1×10^{-7} mol l^{-1}) in 25-fold-diluted BR buffer–methanol (99.9:0.1) medium at pH 6.50. Accumulation potential –0.400 V; 1 unstirred and 2 stirred solution

interest, the optimum accumulation time is longer. On this basis, 5-min (for higher concentrations) or 10-min (for lower concentrations) deposition times were adopted.

The observed fact that the peak current is directly proportional to the scan rate within the range of 5–200 mV s^{-1} indicates that the adsorbed form of the test substance undergoes an electrode reduction process[9]. On the other hand, the peak became wider with increasing scan rate. For these reasons, a scan rate of 20 mV s^{-1} was chosen as a compromise value for all measurements.

The maximum size of a mercury drop (obtained by opening electromagnetic valve for a period of 160 ms) and modulation amplitude –100 mV were employed in order to reach a greater sensitivity of determination. Under those conditions, almost a perfect peak shape is maintained.

A temperature range of 10–40 °C was investigated. Between 10 and 25 °C, the peak current increased nearly linearly with temperature, but decreased at higher temperatures. It is obvious that with temperature increasing above 25 °C, the desorption rate of the tested substance increased, because the energy of thermal motion of DMPT molecules was higher than the bond energy between DMPT molecules and mercury surface. Hence, it can be concluded that the investigated DMPT accumulation on mercury surface is the mater of van der Waals adsorption. For practical reasons, the laboratory temperature (20 ± 1 °C) was chosen as an operation temperature.

Five-minute accumulation in an unstirred tenfold-diluted BR buffer–methanol (99.5:0.5) solution permits the DPAdSV determination of DMPT in the concentration range of (1–10) × 10^{-7} mol l^{-1}. The DPAdSV measurements in this medium were more sensitive by 30% than when the fivefold-diluted buffer containing 10% (v/v) methanol was used.

By using the 25-fold-diluted BR buffer and by decreasing the methanol content in the measured solution to 0.1% (v/v), it was possible to obtain a linear concentration dependence for the DPAdSV peak in the concentration range of (1–10) × 10^{-8} mol l^{-1} with a deposition time of 10 min in unstirred solutions.

Further dilution of the BR buffer (50-fold) and 10-min adsorptive accumulation in the stirred solution enabled linear calibration graph to be obtained within a concentration range of (1–10) × 10^{-9} mol l^{-1} of DMPT. The corresponding differential pulse adsorptive stripping voltammograms are shown in Fig. 6. The relative standard deviation was calculated from ten determinations of the analyte at a concentration of 2 × 10^{-9} mol l^{-1} to be 5%, giving the 1 × 10^{-9} mol l^{-1} as the limit of determination[16]. The limit of detection of DMPT was determined from the linear calibration graph[17] in the

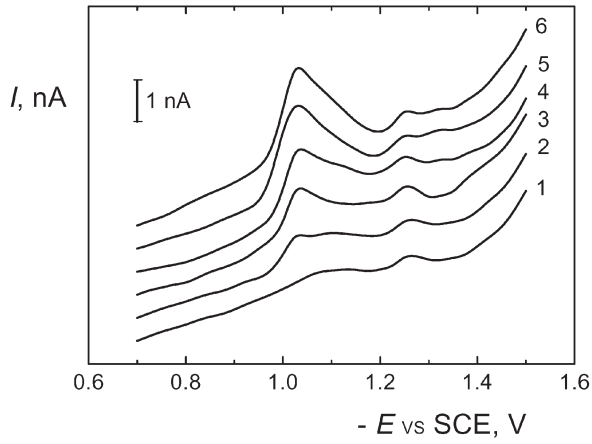

Fig. 6
Differential pulse adsorptive stripping voltammograms of DMPT in 50-fold-diluted BR buffer–methanol (99.9:0.1) medium at pH 6.50. Accumulation potential –0.400 V; 10-min accumulation in the stirred solution. Concentration (in mol l^{-1}): 0 (1), 2×10^{-9} (2), 4×10^{-9} (3), 6×10^{-9} (4), 8×10^{-9} (5), 10×10^{-9} (6)

TABLE I
Parameters of the calibration graphs for 1,1-dimethyl-3-phenyltriazene with the peak potential (E_p), the 95% confidence limits for the slope (A) and intercept (B), the deviation of the experimental points from the calculated straight line ($S_{I,C}$), the correlation coefficient (r) and the limit of determination (LOD). ($S(A)$, standard deviation of slope; S(B), standard deviation of intercept; t, value of Student's distribution coefficient for a confidence interval of 95%; (n – 2), degrees of freedom)

Method	C mol l^{-1}	E_p V	$A \pm t_{(n-2)}S(A)$ mA l mol^{-1}	$B \pm t_{(n-2)}S(B)$ nA	$S_{I,C}$ nA	r	LOD mol l^{-1}
DPV[a]	$(1–10) \times 10^{-5}$	–1.125	7.2 ± 0.3	12 ± 8	8.5	0.9990	
DPV[a]	$(1–10) \times 10^{-6}$	–1.115	7.9 ± 0.4	2 ± 2	1	0.9994	8×10^{-7}
DPV[b]	$(1–10) \times 10^{-6}$	–1.035	10.4 ± 0.8	6 ± 5	4	0.9984	
DPV[b]	$(1–10) \times 10^{-7}$	–1.030	11.3 ± 0.9	0.2 ± 0.3	0.2	0.9948	3×10^{-7}
DPAdSV[c]	$(1–10) \times 10^{-7}$	–1.030	18.6 ± 1.2	0.3 ± 0.2	0.2	0.9952	8×10^{-8}
DPAdSV[d]	$(1–10) \times 10^{-7}$	–1.015	24.1 ± 1.6	1.1 ± 0.9	0.9	0.9970	5×10^{-8}
DPAdSV[e]	$(1–10) \times 10^{-8}$	–1.020	101 ± 4	–0.1 ± 0.4	0.2	0.9980	1×10^{-8}
DPAdSV[f]	$(1–10) \times 10^{-9}$	–1.025	212 ± 5	0.4 ± 0.4	0.1	0.9989	1×10^{-9}

[a] BR buffer–methanol (1:1), pH 6.50. [b] Fivefold-diluted BR buffer–methanol (9:1), pH 6.50. [c] The same conditions as in [b] but after 5-min accumulation in the unstirred solution. [d] Tenfold-diluted BR buffer–methanol (99.5:0.5), pH 6.50, after 5-min accumulation in the unstirred solution. [e] Twentyfivefold-diluted BR buffer–methanol (99.9:0.1), pH 6.50, after 10-min accumulation in the unstirred solution. [f] Fiftyfold-diluted BR buffer–methanol (99.9:0.1), pH 6.50, after 10-min accumulation in the stirred solution.

lowest concentration range and its value is 0.5×10^{-9} mol l^{-1} (0.075 μg l^{-1}, i.e. aproximately 0.075 ppb in water samples).

The parameters of the calibration straight lines with the values of DPV peak potential (E_p) corresponding to the highest point of the appropriate concentration range and the calculated limits of determination (LOD) for the above-described voltammetric measurements are given in Table I.

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