POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 1-NITROPYRENE

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Dedicated to Professor Karel Štulík on the occasion of his 60th birthday.

The polarographic behaviour of 1-nitropyrene was investigated by tast polarography, differential pulse polarography (both with a dropping mercury electrode), differential pulse voltammetry, and adsorptive stripping voltammetry (both with a hanging mercury drop electrode). Optimum conditions have been found for its determination by the given methods in the concentration ranges 2–100, 0.2–100, 0.1–10, and 0.001–0.01 μmol l^{-1}, respectively.

Key words: Polarography; Voltammetry; 1-Nitropyrene; Carcinogens, Electrochemistry.

Nitrated polycyclic aromatic hydrocarbons (NPAH) are a relatively new class of environmental carcinogens[1,2]. Nevertheless, there is an ever increasing demand for the determination of their trace concentrations. So far mostly chromatographic methods, such as GC-MS or HPLC with fluorimetric detection have been used for the purpose[3]. However, these methods are characterised by high investment and running costs. Because of easy polarographic reducibility of nitro group[4–6], modern electroanalytical methods, such as differential pulse polarography (DPP), differential pulse voltammetry (DPV), and adsorptive stripping voltammetry (AdSV) could be expected to satisfy high requirements for sensitivity of the determination. Nevertheless, the use of these methods for the determination of NPAH has not been properly investigated so far[7], even though they are much cheaper as far as investment and running costs are concerned and they present an independent alternative, very important from the practical point of view. Moreover, modern polarographic and voltammetric tech-

niques were shown to be sensitive enough for the determination of various types of chemical carcinogens[8]. Therefore, we have investigated the possibility to use differential pulse polarography, differential pulse voltammetry, and adsorptive stripping voltammetry for the determination of trace amounts of 1-nitropyrene as a typical example of a strong mutagen from the class of NPAH. 1-Nitropyrene is the major nitroarene observed in diesel engine exhaust. It is a powerful direct acting mutagen capable of generating active species binding to DNA, which is responsible for 20–27% of mutagenicity of a diesel engine exhaust particulate extract[9]. The polarographic reduction of 1-nitropyrene in buffers containing 55% of ethanol was investigated by Zahradník and Boček[10]. They confirmed that 1-nitropyrene is reduced similarly to most nitroaromatics in a diffusion controlled four-electron irreversible wave to a 1-hydroxylamino derivative. In acidic media the protonated form of this hydroxylamine is further reduced in a two-electron process to 1-aminopyrene. Krygowski *et al.*[11] investigated the mechanism of polarographic reduction of 1-nitropyrene in aprotic medium of dimethylformamide. However, the use of modern polarographic and voltammetric methods for the determination of submicromolar concentrations of 1-nitropyrene has not been investigated so far.

EXPERIMENTAL

Reagents

The stock solution of 1-nitropyrene (c = 1 mmol l^{-1}) was prepared by dissolving an accurately weighed amount of the pure substance (Sigma) in 100 ml of methanol. The purity of the substance was controlled by HPLC (ref.[12]). More dilute solutions were prepared by exact dilution of the stock solution with methanol. All the solutions were stored in the dark. It followed from a spectrophotometric study of stability of the stock solution[12] that the methanolic solution is stable for at least 90 days. Methanol was of analytical grade purity (Lachema, Brno, Czech Republic). Chemicals for the preparation of the Britton–Robinson buffers were obtained from Sigma. Britton–Robinson buffers were prepared in a usual way by mixing a 0.04 M solution of phosphoric acid, acetic acid and boric acid with an appropriate amount of 0.2 M sodium hydroxide. Deionised water was produced by Milli-Q_{plus} system, Millipore.

Apparatus

Tast and differential pulse polarograms were measured using a PA 3 polarographic analyser with an XY 4106 x–y recorder (both from Laboratorní přístroje, Prague). Measurements were carried out using a three-electrode arrangement with a platinum wire auxiliary electrode and saturated calomel reference electrode, to which all polarographic potential values are referred. The parameters of the classical dropping mercury electrode (DME) used in tast and

differential pulse polarography were as follows: mercury reservoir height h = 64 cm, flow rate m = 1.27 mg s^{-1}, drop time τ = 6.0 s (at an applied voltage of 0 V in 0.1 M KCl). If not stated otherwise, the DME was operated at polarisation rate 5 mV s^{-1}, controlled drop time 1 s, h = 64 cm and modulation amplitude in DPP –100 mV. DPV and AdSV measurements were carried out using a computer-driven potentiostat–galvanostat PGSTAT 10 with GPES 4.2 software (ECO-Chemie B.V., Utrecht, Netherlands) in combination with a Metrohm 663 VA voltammetric stand (Metrohm, Zürich, Switzerland). Initial potential was set at 0 V, modulation amplitude at 50 mV, and scanning was performed in negative direction at a rate of 10 mV s^{-1}. A three-electrode combination was used, consisting of a silver chloride reference electrode, to which all voltammetric potential values are referred, multimode mercury working electrode MME (Metrohm, Zürich, Switzerland) in the hanging mercury drop electrode mode, and a glassy-carbon rod as an auxiliary electrode. Oxygen was removed from analysed solutions with nitrogen by purge 8 min.

Procedures

The general procedure to obtain polarograms or voltammograms was as follows: A required amount of the stock solution of the test substance in methanol was placed in a 10 ml volumetric flask, an appropriate volume of methanol was added, and the solution was diluted to the volume with a Britton–Robinson buffer of required pH. (A different order of mixing the solutions resulted in precipitation of the test substance.) The calibration curves were measured in triplicate and evaluated by a least-squares linear regression method. The limit of determination was calculated as the ten-fold standard deviation from 7 analyte determinations at a concentration corresponding to the lowest point on the appropriate calibration straight line[13].

RESULTS AND DISCUSSION

Tast Polarography with a Dropping Mercury Electrode

It followed from preliminary experiments that in a medium containing 50 or 70% (v/v) of methanol, the height of the observed waves decreases with time, probably as a result of precipitation of 1-nitropyrene. Therefore, the influence of pH on tast polarographic behaviour of the substance was investigated in a mixed medium, Britton–Robinson buffer–methanol (1 : 9), the concentration of 1-nitropyrene being $1 \cdot 10^{-4}$ mol l^{-1} (see Figs 1 and 2). Under these conditions, the tast polarogram of the test substance exhibits a well developed irreversible wave in the whole investigated pH range the height of which is virtually pH independent. The fist wave obviously corresponds to the above mentioned four electron reduction of 1-nitropyrene to 1-hydroxylaminopyrene. The second, much lower and not well developed wave appears at pH 4–7. This wave probably corresponds to further two-electron reduction of the 1-hydroxylaminopyrene to 1-aminopyrene.

The highest and best developed waves were obtained in a mixed medium of Britton–Robinson buffer (pH 2 or 13)–methanol (1 : 9) (the resulting pH 4.1 or 12.4, respectively). The height of the wave is a linear function of

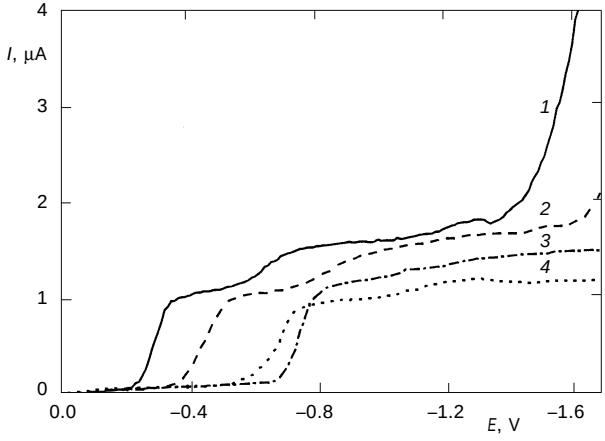

FIG. 1
Selected tast polarograms of 1-nitropyrene ($c = 1 \cdot 10^{-4}$ mol l^{-1}) in a Britton–Robinson buffer–methanol (1 : 9) mixture of pH: 4.1 (*1*), 5.9 (*2*), 8.2 (*3*), 10.2 (*4*)

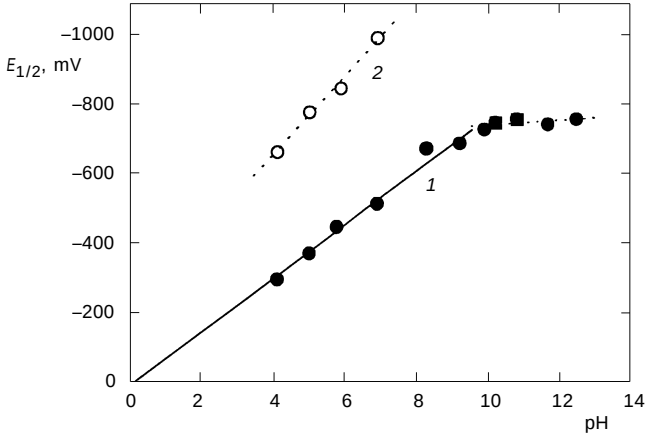

FIG. 2
The dependence of the half-wave potential ($E_{1/2}$) of 1-nitropyrene measured by tast polarography on the resulting pH of the mixture of Britton–Robinson buffer–methanol (1 : 9); *1* first wave, *2* second wave

1-nitropyrene concentration within the concentration range of 2–100 μmol l^{-1} (see Table I).

Differential Pulse Polarography with a Dropping Mercury Electrode

It can be seen from Fig. 3 that the effect of pH on the peak position (E_p) and height (I_p) of 1-nitropyrene reflects the effect of pH on the behaviour of the test substance in tast polarography. The highest, best developed and

TABLE I
Parameters of the calibration straight lines for the determination of 1-nitropyrene by tast polarography (TP), differential pulse polarography (DPP) and differential pulse voltammetry (DPV)

pH[a]	pH[b]	*c*, mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correlation coefficient *R*	L_Q[c] mol l^{-1}
TP with a DME in a mixed Britton–Robinson buffer–methanol (1 : 9) medium						
2.00	4.1	$(2–10) \cdot 10^{-5}$	9.72	–11.69	0.9993	–
2.00	4.1	$(2–10) \cdot 10^{-6}$	6.63	–11.83	0.9922	$3 \cdot 10^{-6}$
13.00	12.4	$(2–10) \cdot 10^{-5}$	9.40	–3.33	0.9950	–
13.00	12.4	$(2–10) \cdot 10^{-6}$	12.5	–1.82	0.9929	$3 \cdot 10^{-6}$
DPP with a DME in a mixed Britton–Robinson buffer–methanol (1 : 9) medium						
2.00	4.1	$(2–10) \cdot 10^{-5}$	10.87	56.82	0.9989	–
2.00	4.1	$(2–10) \cdot 10^{-6}$	12.50	–6.49	0.9916	–
2.00	4.1	$(2–10) \cdot 10^{-7}$	10.51	–0.96	0.9950	$4 \cdot 10^{-7}$
13.00	12.4	$(2–10) \cdot 10^{-5}$	9.29	23.07	0.9991	–
13.00	12.4	$(2–10) \cdot 10^{-6}$	11.37	–3.27	0.9937	–
13.00	12.4	$(2–10) \cdot 10^{-7}$	14.63	–0.79	0.9960	$2.5 \cdot 10^{-7}$
DPV with a HMDE in a mixed Britton–Robinson buffer–methanol (1 : 1) medium						
2.00	2.7	$(2–10) \cdot 10^{-6}$	1.01	–6.75	0.9990	–
2.00	2.7	$(2–10) \cdot 10^{-7}$	0.55	–0.07	0.9994	$0.8 \cdot 10^{-7}$
12.00	12.2	$(2–10) \cdot 10^{-6}$	26.99	–16.80	0.9982	–
12.00	12.2	$(0.8–10) \cdot 10^{-7}$	17.7	–0.07	0.9999	$6.5 \cdot 10^{-8}$

[a] Of the buffer; [b] of the mixed buffer; [c] limit of determination.

most easily evaluated peaks were again obtained in a mixed medium of Britton–Robinson buffer (pH 2 or 13)–methanol (1 : 9) (the resulting pH 4.1 or 12.4, respectively). The peak height was measured from the straight line

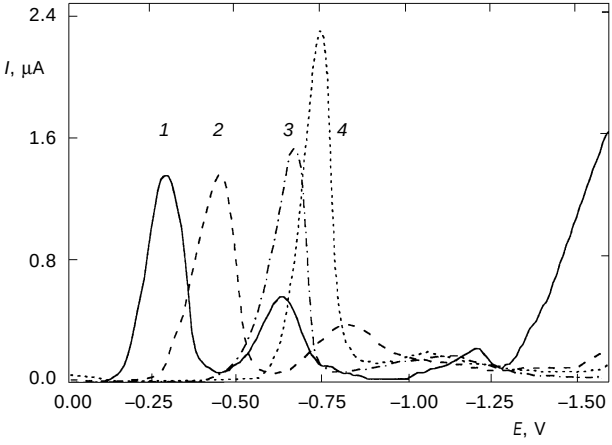

FIG. 3
Selected DP polarograms of 1-nitropyrene ($c = 1 \cdot 10^{-4}$ mol l^{-1}) in a Britton–Robinson buffer–methanol (1 : 9) mixture of pH: 4.1 (*1*), 5.9 (*2*), 8.2 (*3*), 10.2 (*4*)

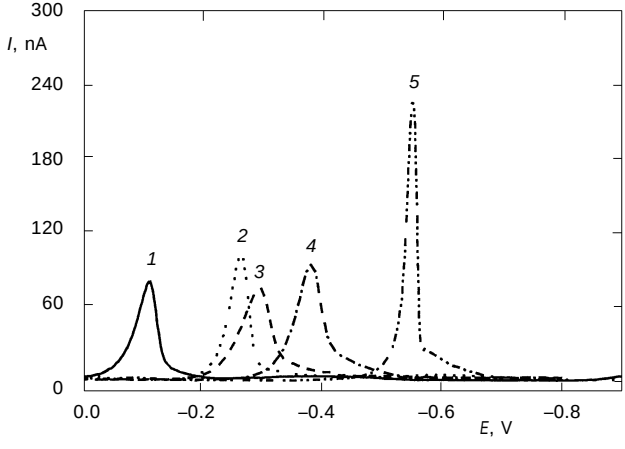

FIG. 4
Selected DP voltammograms of 1-nitropyrene at a HMDE ($c = 1 \cdot 10^{-5}$ mol l^{-1}) in a Britton–Robinson buffer–methanol (1 : 1) mixture of pH: 2.7 (*1*), 4.8 (*2*), 7.1 (*3*), 10.3 (*4*), 12.2 (*5*)

connecting the minima before and after the peak. The calibration curves are linear within the concentration range of 0.2–10 μmol l^{-1} and their parameters are given in Table I.

Differential Pulse Voltammetry with a Hanging Mercury Drop Electrode

The effect of pH on DP voltammograms of 1-nitropyrene in a Britton–Robinson buffer–methanol (1 : 1) mixture is documented in Fig. 4. The best developed peaks were obtained in a mixed medium Britton–Robinson buffer (pH 2 or 12)–methanol (1 : 1) (the resulting pH 2.7 or 12.2, respectively). The peak height was again measured from the straight line connecting the minima before and after the peak. The calibration curves are linear within the concentration range of 0.2–10 μmol l^{-1} and their parameters are given in Table I.

Adsorptive Stripping Voltammetry with a Hanging Mercury Drop Electrode

First, the influence of the accumulation potential in a Britton–Robinson buffer (pH 12.00)–methanol (1 : 1) (the resulting pH 12.2) was investigated. Concentration of 1-nitropyrene was $2 \cdot 10^{-7}$ mol l^{-1} and the accumulation time in a stirred solution was 60 s. Under these conditions, single peak at E_p = –0.63 V was observed, the height of which virtually did not depend on the

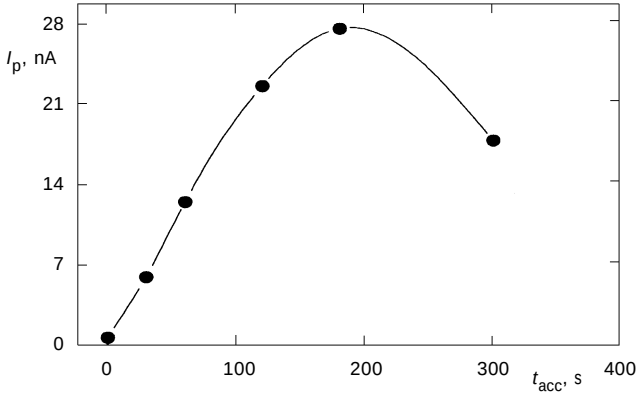

FIG. 5
The dependence of the peak height (I_p) of 1-nitropyrene ($c = 2 \cdot 10^{-7}$ mol l^{-1}) on the time of accumulation (t_{acc}) in a mixed Britton–Robinson buffer (pH 12.00)–methanol (1 : 1) medium (the resulting pH 12.2)

accumulation potential in the range from –0.1 to –0.4 V. The influence of the accumulation time on the peak height was investigated in a stirred solution of the above given composition at the accumulation potential –0.2 V. It can be seen from Fig. 5 that the optimum accumulation time is 180 s. However, better developed peaks were obtained[12] in a mixture of the ten-fold diluted Britton–Robinson buffer (pH 12.00) with methanol (9 : 1) (the resulting pH 12.2) for the concentration range $(2–10) \cdot 10^{-8}$ mol l^{-1} and in a mixture of the ten-fold diluted Britton–Robinson buffer (pH 2.00) with methanol (99 : 1) (the resulting pH 2.2) for the concentration range $(2–10) \cdot 10^{-9}$ mol l^{-1}. Parameters of the corresponding calibration curves are given in Table II.

TABLE II
Parameters of the calibration straight lines for the determination of 1-nitropyrene by adsorptive stripping voltammetry at a hanging mercury drop electrode

pH[a]	pH[b]	c, mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correlation coefficient R	L_Q[c] mol l^{-1}
12.00	12.2[d]	$(2–10) \cdot 10^{-8}$	$1.08 \cdot 10^2$	–1.11	0.9962	$1 \cdot 10^{-8}$
2.00	2.2[e]	$(2–10) \cdot 10^{-9}$	$2.15 \cdot 10^2$	0.05	0.9988	$1 \cdot 10^{-9}$

[a] Of the buffer; [b] of the mixed buffer; [c] limit o determination; [d] mixture of ten-fold diluted Britton–Robinson buffer with methanol (9 : 1), t_{acc} = 180 s, E_{acc} = –0.2 V; [e] mixture of ten-fold diluted Britton–Robinson buffer with methanol (99 : 1), t_{acc} = 600 s, E_{acc} = 0 V.

CONCLUSIONS

It has been shown that modern polarographic and voltammetric methods can be used for the determination of submicromolar concentrations of 1-nitropyrene. Limit of determination (LOD) of the newly developed methods is much lower than that of UV spectrophotometry or HPLC with UV spectrophotometric detection (LOD $\approx 1 \cdot 10^{-6}$ mol l^{-1}). The selectivity of newly developed methods can be further increased by their combination with a preliminary separation using liquid–liquid or solid phase extraction.

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