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Polarographic and voltammetric determination of trace amounts of 2-nitronaphthalene

Abstract The polarographic behaviour of 2-nitronaphthalene was investigated by DC tast polarography (DCTP) and differential pulse polarography (DPP), both at a dropping mercury electrode, and differential pulse voltammetry and adsorptive stripping voltammetry, both at a hanging mercury drop electrode. Optimum conditions have been found for the determination of 2-nitronaphthalene by the given methods in the concentration ranges of 2×10^{-6}–1×10^{-4}, 2×10^{-7}–1×10^{-4}, 1×10^{-8}–1×10^{-4} and 2×10^{-9}–1×10^{-8} M, respectively. Practical applicability of these techniques was demonstrated by the determination of 2-nitronaphthalene in drinking and river water after its preliminary separation and preconcentration using liquid–liquid and solid-phase extraction with limits of determination of 3×10^{-10} M (drinking water) and 3×10^{-9} M (river water).

Keywords DC tast polarography · Differential pulse polarography · Differential pulse voltammetry · Adsorptive stripping voltammetry · 2-Nitronaphthalene

Introduction

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are a relatively new class of environmental carcinogens [1]. Interest in them increased in 1978 when Jäger [2] and Pitts et al. [3] independently discovered that they are formed during incomplete combustion processes by reaction of polycyclic aromatic hydrocarbons (PAHs)

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with atmospheric nitrogen oxides. Since that time great efforts have been made to detect NPAHs in environmental matrices. 2-Nitronaphthalene (2-NN) as a typical representative of this class of compounds was found in diesel exhaust, ambient air, photocopier toners and carbon black. Many other NPAHs have been detected in cigarette smoke, soil sediments and foodstuffs [1, 4, 5]. The biological activity of these compounds is enabled as a result of their measurable solubilities in the aqueous phase. They have been found to be potent, direct-acting mutagens in the Ames tests; their mutagenicity is even higher than the mutagenicity of parent PAHs [6]; and some of them have been also found to be carcinogenic. The carcinogenicity of 2-NN has not been proven yet; however, 2-NN is a weak mutagen and one of its metabolites, 2-aminonaphthalene, is carcinogenic to humans [7].

So far, mostly chromatographic methods have been used for the determination of NPAHs. 2-NN was determined using HPLC with spectrophotometric [8, 9], fluorescence [9–12] or electrochemical [11, 13–17] detection. Another frequently used method is gas chromatography, which is also suitable for more complicated matrices. Various detectors have been employed, including the flame ionisation detector [20, 22], electron-capture detector [19–22], thermal energy analyzer [20, 21], thermoionic nitrogen/phosphorus detector [19, 22] and mass spectrometry [18–22]. There have also been some attempts to determine NPAHs by using thin-layer chromatography [2, 23], but the identification of compounds requires a more reliable method. Most chromatographic methods are characterised by high investment and running costs. On the other hand, modern electroanalytical methods, such as DC tast polarography (DCTP), differential pulse polarography (DPP), differential pulse voltammetry (DPV) and adsorptive stripping voltammetry (AdSV) represent independent and less-expensive alternatives. Because of the easy reducibility of the nitro group [24–26], the reduction of nitro compounds on the classical mercury dropping electrode (DME) has been studied almost from

the very beginning of polarography. The polarographic reduction of various nitronaphthalenes and their substituted derivatives in buffers containing 50% ethanol was investigated by Jubault and Peltier [27]. These authors confirmed that 2-NN is reduced similarly to most nitroaromatics in a diffusion-controlled, four-electron irreversible wave to the 2-hydroxylamino derivative. In acidic medium the protonated form of this hydroxylamine is further reduced in a two-electron process to 2-aminonaphthalene. There are several other reports dealing with the polarography of 2-NN in mixed water–methanol [28] or water–ethanol media [29, 30]. Krygowski et al. [31] investigated the mechanism of polarographic reduction in the aprotic medium of dimethylformamide. However, modern polarographic and voltammetric methods have not been used for the determination of 2-NN yet. Therefore, after other genotoxic NPAHs studied recently in our laboratory (1-nitronaphthalene, 1-nitropyrene, 9-nitroanthracene, 2-, 3- and 4-nitrobiphenyl, 2-nitrofluorene and 2,7-dinitrofluorene) [32–36] we turned our attention to 2-NN. Practical applicability of the newly developed methods in combination with solid-phase or liquid–liquid extraction was verified using model samples of drinking and river water.

Experimental

Reagents

The stock solution of 2-nitronaphthalene (CAS registry number 581-89-5; $c = 1\times10^{-3}$ M) was prepared by dissolving 0.0173 g of the substance (Fluka, Switzerland) in 100 mL of methanol. The purity of the substance was controlled by HPLC [37]. 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 the stability of the stock solution [38] that the solution in methanol is stable for at least 90 days. Methanol, lithium hydroxide and hexane were of analytical grade (Lachema, Brno, Czech Republic). Britton–Robinson buffers were prepared in the usual way (i.e. by mixing a solution of 0.04 M in phosphoric acid, 0.04 M in acetic acid and 0.04 M in boric acid with the appropriate amount of 0.2 M sodium hydroxide solution). Chemicals for the preparation of the Britton–Robinson buffers were obtained from Sigma. Deionised water was produced by Milli-Q$_{plus}$ system (Millipore, USA).

Apparatus

Measurements were carried out using a computer-driven EcoTriboPolarograph with PolarPro software, version 2.0 (both Polarosensors, Prague) in combination with a classical DME or a hanging mercury drop electrode (HMDE) UMμE (Polarosensors, Prague), a platinum

wire auxiliary electrode and silver/silver chloride (1 M KCl) reference electrode, to which all the potential values are referred. The parameters of the classical DME used in DC tast and DP polarography were as follows: mercury reservoir height (h) was 81 cm, the flow rate (m) was 0.48 mg s^{-1}, and the drop time (τ) was 5.8 s (at an applied voltage of 0 V in 0.1 M KCl). Work with the DME was carried out at a polarization rate of 4 mV s^{-1}, controlled drop time of 1 s, and modulation amplitude in DPP of −50 mV. For DPV and AdSV at HMDE, the maximum drop size attainable obtained by opening the valve for 100 ms, with a surface of 0.864 mm^2, a polarization rate of 20 mV s^{-1}, and the modulation amplitude of −50 mV were used. A rotary evaporator (Rotavapor R-114, Büchi, Flawil, Schwitzerland) was used. pH was measured using a conductivity and pH meter, Jenway 4330 (Jenway, England).

Procedures

The general procedure to obtain polarograms or voltammograms was as follows: the required amount of 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 system was diluted to volume with a Britton–Robinson buffer of the required pH. Oxygen was removed from the measured solutions by bubbling with nitrogen for 5 min. A pre-bubbler containing a water–methanol mixture in the same ratio as in the polarographed solution was placed prior to the polarographic vessel. The calibration curves were measured in triplicate and their statistical parameters (e.g. slope, intercept, limit of determination) were calculated according to methods reported by Oppenhelmer [39], Schwartz [40] and Ebel [41] by using statistic software Adstat version 2.0 (Trilobyte, Czech Republic).
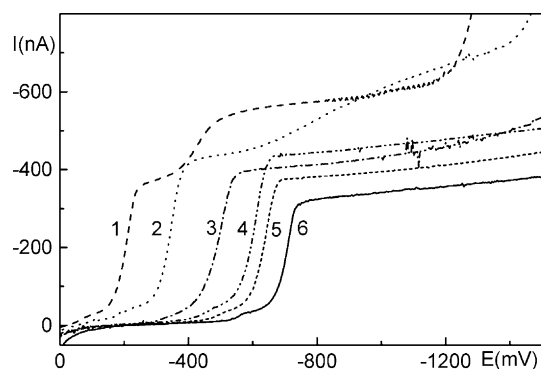
For the determination of 2-NN in drinking and river water samples after extraction with hexane the procedure was as follows: the model sample of drinking or river water containing an appropriate amount of added 2-NN was extracted with 10 mL of hexane, the organic phase was evaporated to dryness using a rotary evaporator, the residue was dissolved in 1 mL of methanol using sonication, 9 mL of 0.01 M NaOH was added and the DP voltammogram was recorded. The procedure for voltammetric determination of 2-nitronaphthalene in drinking or river water after solid-phase extraction was as follows: an SPE column LiChrolut RP-select B (catalog number K90154159, Merck, Darmstadt, Germany), which is an SPE column filled with 500 mg of RP-18 phases bonded on silicagel, was connected to a suction pump and activated by washing with 3 mL of methanol and 3 mL of deionised water. The sample of drinking or river water spiked with different amounts of 2-nitronaphthalene was then sucked through the column. Adsorbed 2-NN was then eluted with 1 mL of methanol, the solution was made-up to 10.00 mL with 0.01 M NaOH and the DP voltammogram was recorded. The

river water, used for extraction, was taken in the river Vltava in the centre of the city of Prague.

Results and discussion

Tast polarography and DPP at DME

The influence of pH on DC tast polarograms of 2-NN was investigated in a mixed Britton–Robinson buffer–methanol (1:1) medium and obtained polarograms are depicted in Fig. 1. Under these conditions the tast polarogram exhibits one, well-developed irreversible wave in the whole investigated pH range. This wave corresponds to the reduction of 2-NN to 2-hydroxylaminonaphthalene. The half-wave potential ($E_{1/2}$) of this first wave varies with the pH in the range 2.7–10.3 according to the relationship $E_{1/2}(V) = -0.062pH - 0.042$ ($R = -0.9976$). At pH 2.7–5.9, a second, much lower and poorly developed irreversible wave can be seen, obviously corresponding to the further reduction to 2-aminonaphthalene. This wave is not suited for analytical purposes. The highest and best developed waves were obtained in a mixed medium of Britton–Robinson buffer (pH 8.0)–methanol (1:1) (resulting pH of the mixture 8.8), over which calib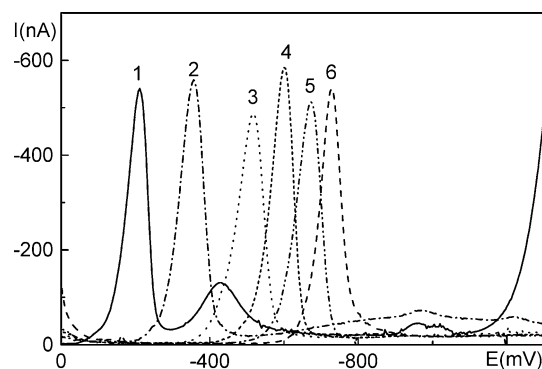ration dependence was measured. The height of the wave is a linear function of 2-NN concentration within the concentration range 1×10^{-6}–1×10^{-4} M (Table 1). However, the limit of determination (LOD) is not sufficient for environmental applications.

The electrochemical behaviour of 2-nitronaphthalene using DPP at DME was studied under the same conditions as above. It reflects its behaviour in DCTP: 2-NN gives in the pH range 2.7–12.2 one, well-developed peak, which shifts towards more negative potentials with increasing pH. At pH 2.7–5.9, a second, much lower and poorly developed peak appears. Obtained polarograms are depicted in Fig.2. The best-developed and most easily evaluated peak of 2-NN was again obtained in a mixed medium of Britton–Robinson buffer (pH 8.0)–methanol (1:1). The height of the peak was measured from the straight line connecting the minima before and after the peak. The calibration curves are linear within the concentration range of 2×10^{-7}–1×10^{-4} M and their parameters are given in Table 1.

Differential pulse voltammetry at HMDE

As in the previous case, the substance gives one or two (at pH 2.7–5.9) peaks. The highest peak was obtained in a mixed medium Britton–Robinson buffer (pH 12.0)–

Fig. 1 Selected DC tast polarograms of 2-nitronaphthalene ($c = 1\times10^{-4}$ M) in a Britton–Robinson buffer–methanol (1:1) mixture; resulting pH: 2.7 (*1*), 4.9 (*2*), 7.0(*3*), 8.8 (*4*), 10.3 (*5*) and 12.2 (*6*)

Fig. 2 Selected DP polarograms of 2-nitronaphthalene ($c = 1\times10^{-4}$ M) in a Britton–Robinson buffer–methanol (1:1) mixture, resulting pH: 2.7 (*1*), 4.9 (*2*), 7.0 (*3*), 8.8(*4*), 10.3 (*5*) and 12.2 (*6*)

Table 1 Optimum conditions and parameters of the calibration straight lines for the polarographic and voltammetric determination of 2-nitronaphthalene

[a]pH of the Britton–Robinson buffer or sodium hydroxide solution
[b]Resulting pH of the mixture
[c]Limit of determination
[d]Parameters for the mixed 0.01 M NaOH–methanol (1:1) medium

pH [a]	pH [b]	c(2-NN) (M)	Slope (mA M^{-1})	Intercept (nA)	Correlation coefficient	LOD[c] (M)
DCTP at DME in a mixed Britton–Robinson buffer–methanol (1:1) medium						
8.0	8.7	(2–10)×10^{-5}	4.0	−16.4	0.9901	–
8.0	8.7	(2–10)×10^{-6}	3.7	−1.7	0.9958	2×10^{-6}
DPP at DME in a mixed Britton–Robinson buffer–methanol (1:1) medium						
8.0	8.7	(2–10)×10^{-5}	5.0	4.5	0.9980	–
8.0	8.7	(2–10)×10^{-6}	4.8	0.4	0.9967	–
8.0	8.7	(2–10)×10^{-7}	4.3	0.8	0.9923	2×10^{-7}
DPV at HMDE in a mixed 0.01 M NaOH–methanol (9:1) medium						
12.0	12.2[d]	(2–10)×10^{-7}	16.2	−1.1	0.9938	3×10^{-7}
12.0	12.0	(2–10)×10^{-7}	40.2	0.1	0.9999	–
12.0	12.0	(2–10)×10^{-8}	41.9	0.1	0.9963	2×10^{-8}
AdSV at HMDE in 0.001 M LiOH, $t_{acc} = 120$ s, $E_{acc} = -400$ mV						
10.6	10.6	(2–10)×10^{-9}	247.6	0.1	0.9991	2×10^{-9}

methanol (1:1) (resulting pH of the mixture 12.2). To decrease the content of potential impurities in the supporting electrolyte, for the measurement of calibration curves, the buffer was replaced with 0.01 M NaOH. Calibration curves were at first measured in a medium containing 50% methanol; after that the content of methanol was decreased to 10%. The limit of determination in the medium containing less methanol is tenfold lower than for the medium with a higher content of methanol (Table 1). The calibration curves deviate from the linear course at concentrations higher than 5×10^{-5} M. This is presumably due to adsorption and following passivation of the electrode surface by the reduction products.
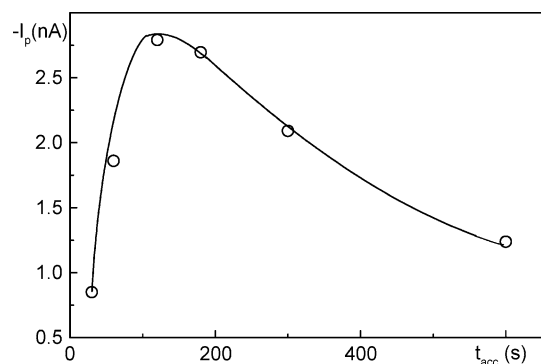
Adsorptive stripping voltammetry at HMDE

To lower the limit of detection obtained by DPV, we carried out the accumulation of the 2-NN on the electrode surface. Supporting electrolyte without methanol was used for the accumulation as a result of the presumed better adsorption of 2-NN at the electrode surface. Because the electrolyte baseline was complicated by peaks of accumulated impurities when using 0.01 M NaOH, 0.001 M LiOH was used as supporting electrolyte for further measurements. At first, the accumulation time was optimised (Fig. 3) and 120 s was chosen as an optimum accumulation time.

The influence of the accumulation potential on the peak height was then investigated. It did not differ significantly; however the best-developed and repeatable peaks were obtained at $E_{acc} = -400$ mV. The calibration curve measured under these conditions is linear in the concentration range 2×10^{-9}–1×10^{-8} M (Table 1) and voltammograms corresponding to this lowest attainable concentration range are depicted in Fig. 4.

Practical applications

For the determination of 2-NN in environmental samples, DP voltammetry was chosen, because it is suffi-

Fig. 3 Dependence of the height of the AdSV peak (I_p) of 2-nitronaphthalene ($c = 1\times10^{-7}$ M) on the time of accumulation. $E_{acc} = -300$ mV, supporting electrolyte 0.001 M LiOH
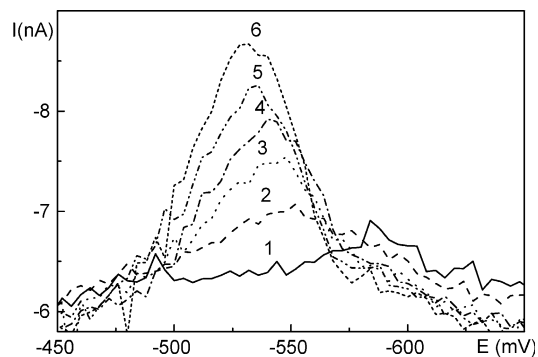
Fig. 4 Adsorptive stripping voltammograms of 2-nitronaphthalene (2-NN) in 0.001M LiOH, c(2-NN) = 0 M (*1*), 2×10^{-9} M (*2*), 4×10^{-9} M (*3*), 6×10^{-9} M (*4*), 8×10^{-9} M (*5*), 1×10^{-8} M (*6*), $E_{acc} = -400$ mV, $t_{acc} = 120$ s

ciently sensitive for the analysed compound and fast, so the adsorption of impurities contained in matrix does not influence the determination as in the case of AdSV. At first, we tried to determine 2-NN directly in the drinking and river water. For this purpose, the water was spiked with appropriate amounts of 2-NN; 9 mL of the sample was placed in a 10-mL volumetric flask and it was diluted to volume with 0.1 M NaOH. After deaeration, DP voltammograms were recorded immediately. As seen from Table 2, in which the parameters of calibration curves are given, the sensitivity of this direct determination is comparable with the previous DPV experiments carried out with deionised water. However, to lower the determinable amount of 2-NN, preconcentration using liquid–liquid or solid-phase extraction was necessary. For extraction with hexane, conditions specified in Table 3 were used.

The recovery was calculated for the highest concentration of 2-NN over the given concentration range from the ratio I_p/I_p^o, where I_p is the height of the peak of 2-NN after extraction with hexane and I_p^o is the height of peak of 2-NN in a reference solution prepared by the addition of standard solution of 2-NN to the blank solution. The recovery was found to be 90% (58%) for the extraction with 10 mL of hexane from 100 mL (1,000 mL) of the drinking water and it cannot be increased either by multiple extraction or by the increase of the solvent volume due to the loss of the compound during the evaporation. The recovery of the extraction–voltammetric determination at 1×10^{-8} mol L^{-1} of 2-NN in 100 mL river water was even lower (maximum about 40%) and the results were not repeatable, because in

Table 2 Parameters of the calibration straight lines for the direct DP voltammetric determination of 2-nitronaphthalene (2-NN) in drinking and river water

Matrix water	c(2-NN) (M)	Slope (mA M^{-1})	Intercept (nA)	Correlation coefficient	LOD (M)
Drinking	$(2–10)\times10^{-8}$	27.7	0.2	0.9988	2×10^{-8}
River	$(2–10)\times10^{-8}$	29.5	−0.1	0.9925	4×10^{-8}

Table 3 Parameters of extraction of 2-nitronaphthalene (2-NN) spiked with 10 mL of hexane from drinking water

V^a (L)	c(2-NN) (M)	Recovery (%)	Slope (nA M^{-1})	Intercept (nA)	Correlation coefficient	LOD (M)
0.1	$(2–10)\times10^{-9}$	90.5	0.3	0.1	0.9870	5×10^{-9}
1	$(4–10)\times10^{-10}$	58.3	2.0	−0.2	0.9977	4×10^{-10}

^{a}Volume of extracted water
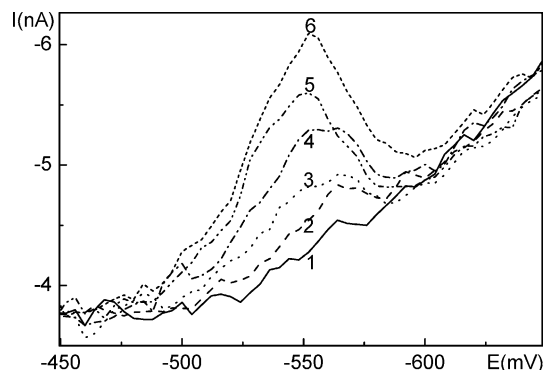
Fig. 5 Differential pulse voltammograms of 2-nitronaphthalene after solid-phase extraction from 1,000 mL of drinking water containing 0 M (*1*), 2×10^{-10} M (*2*), 4×10^{-10} M (*3*), 6×10^{-10} M (*4*), 8×10^{-10} M (*5*), 1×10^{-9} M (*6*) of the analyte

addition to the evaporation, interactions between the compound and impurities in the polluted river water affect the whole procedure. Therefore, calibration graphs were measured only for the determination of 2-nitronaphthalene in the drinking water (see the parameters in Table 3).

For the determination in river water and for the decrease of determination limits in drinking water, solid-phase extraction was used. At first, we applied the solid-phase extraction on the spiked samples of drinking water using the procedure described above. The recovery was slightly better than using liquid–liquid extraction; therefore calibration curves were measured. Their parameters are summarized in Table 4. DP voltammograms corresponding to the lowest attainable concentration range are depicted in Fig. 5.

The same approach was then applied on the spiked samples of highly polluted river water. It can be seen from Table 4 that the recovery is lower in the case of river water. A possible explanation is the presence of surface-active substances decreasing the height of DPV peak which are not completely removed by SPE.

Nevertheless, the dependence of the height of the DPV peak of 2-NN on its concentration in river water is again linear in the concentration range $(2–10)\times10^{-9}$ M. Attempts to lower the determination limit by passing 1,000 mL river water through the SPE column were not successful and resulted in an increase of the peaks of electrochemically active impurities on recorded voltammograms and in substantial decreases of the sample flow rate, and therefore unacceptable prolongation of analysis time.

Conclusions

It has been shown that mercury electrodes, namely classical dropping mercury electrode and hanging mercury drop electrode, in combination with modern polarographic and voltammetric techniques are suitable sensors for the determination of submicromolar and nanomolar concentrations of 2-nitronaphthalene. The most sensitive method is AdSV; the limit of detection reached by this method is 2×10^{-9} M. However, this method is not suitable for the determination of the compound in more complicated matrices due to passivation of the electrode surface by impurities. Therefore, DPV at HMDE was chosen for the determination of 2-nitronaphthalene in drinking and river water and it was verified that it is a useful method in connection with preliminary liquid–liquid or solid-phase extraction, the latter being more effective. Thus, it can be concluded that polarographic and voltammetric methods represent useful alternative tools for some specific determinations of 2-nitronaphthalene compared to more protracted chromatographic and spectrophotometric methods.

Table 4 Parameters of the calibration straight lines for the DPV determination of 2-nitronaphthalene (2-NN) in drinking and river water after solid-phase extraction

Matrix-water	V^a (L)	c(2-NN) (M)	Recovery (%)	Slope (nA M^{-1})	Intercept (nA)	Correlation coefficient	LOD (M)
Drinking	0.1	$(2–10)\times10^{-9}$	94.2	0.3	0.02	0.9984	3×10^{-9}
	1	$(2–10)\times10^{-10}$	71.4	1.4	−0.03	0.9983	3×10^{-10}
River	0.1	$(2–10)\times10^{-9}$	83.1	0.2	0.15	0.9963	3×10^{-9}

^{a}Volume of extracted water

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