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SPECIAL ISSUE

Polarographic and voltammetric determination of genotoxic 4-nitroindane at mercury electrodes

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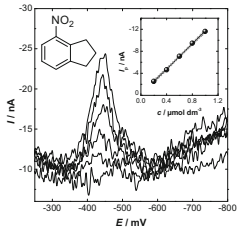
Abstract Determination and electrochemical investigation of genotoxic nitrated polycyclic aromatic hydrocarbon 4-nitroindane (4-NI) were carried out using direct current tast polarography (DCTP) and differential pulse polarography (DPP) at a classical dropping mercury electrode (DME) and using direct current voltammetry (DCV), differential pulse voltammetry (DPV), and cyclic voltammetry (CV) at a hanging mercury drop minielectrode (HMDmE). The following optimal media for the determination of 4-NI were found: methanol—Britton-Robinson (BR) buffer of pH 12.0 (1:1) for DCTP and DPP, methanol—BR buffer of pH 9.0 (1:9) for DCV, and methanol—BR buffer of pH 8.0 (1:9) for DPV. For CV investigation, mixtures of methanol—BR buffer of pH 3.0, 8.0, and 12.0 (1:1) were used. The limits of quantification were: 0.65 μmol dm^{-3} (DCTP at the DME), 0.10 μmol dm^{-3} (DPP at the DME), 0.075 μmol dm^{-3} (DCV at the HMDmE), and 0.070 μmol dm^{-3} (DPV at the HMDmE). An attempt to increase the determination sensitivity using adsorptive accumulation of 4-NI on the HMDmE surface was not successful. The newly developed polarographic and voltammetric methods were successfully applied for the determination of 4-NI in model samples of drinking and river water.

Graphical abstract



Keywords Electrochemistry · Sensors · Ecology · Environmental analysis · Nitrated polycyclic aromatic hydrocarbons · Drinking and river water

Introduction

Many organic compounds entering the environment naturally or as a result of human activities have dangerous genotoxic or ecotoxic properties. They can damage the environment or pose a threat to human population [1]. This causes increasing demand for methods suitable for the determination of trace amounts of dangerous compounds in complex environmental or biological matrices. Many of these compounds contain electrochemically easily reducible nitro group, e.g., nitrated polycyclic aromatic hydrocarbons (NPAHs), which allows us to use modern polarographic and/or voltammetric techniques for this purpose [2–5].

NPAHs are products of incomplete combustion processes in gasoline and diesel engines or products released from aluminum production, crude oil refineries, and natural combustion processes [6–9]. NPAHs can be either directly

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emitted from combustion sources [10] or formed from their parent polycyclic aromatic hydrocarbons (PAHs) by atmospheric OH or NO_3 radical-initiated reactions [11, 12]. It has been shown that NPAHs can be many times more mutagenic or carcinogenic than parent PAHs [13, 14], so the analysis of these dangerous pollutants becomes important for modern environmental analytical chemistry.

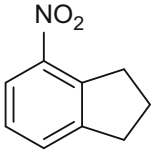
4-Nitroindane (4-NI) (Scheme 1), which is structurally similar to NPAHs (even though it has not fully aromatic structure), is a carcinogenic compound formed by the above-mentioned processes from its precursor—hydrocarbon indane [15] (a component of petrol). Mildly inhibitory effects of indane on microbial methanogenesis (i.e., methane production—an anaerobic respiration pathway of metabolism, using, for example, CO_2 as an alternative electron acceptor) were proved [16]. It has been documented that the unresolved complex mixture (UCM—important environmental toxicants) has effect on mussels *Mytilus edulis* which accumulate these toxicants rapidly and have a significant narcotic toxic response [15]. To demonstrate this fact, Booth et al. [15] used two-dimensional gas chromatography connected with time-of-flight mass spectrometry, and they compared the harmful influence of a commercial complex mixture of alkylaromatics on the reducing feeding rate of mussel with the influence of the UCM obtained in their research.

Other studies, employing gas chromatography connected with mass spectrometry, deal with identification of the main sources of nitrated and oxygenated PAHs in large cities [17, 18]. Although these techniques are frequently used for identification and determination of NPAHs, less expensive polarographic and voltammetric techniques play a significant role in the nanomolar range detection of these ecologically important compounds [5, 19–21]. Moreover, sensitivity of voltammetric methods can be often increased by their combination with preliminary separation and preconcentration steps [22, 23].

Since classical polarography was invented and developed for analytical purposes by Heyrovský [24], mercury became the most reliable and suitable material for voltammetry of electrochemically reducible compounds [19, 25–27]. Indisputable attributes of liquid mercury, such as a wide potential window at negative potentials and a renewable atomically smooth surface (each mercury drop represents a freshly renewed surface), predestine it for wide-ranged applications [19, 25, 28–31]. A classical dropping mercury electrode (DME) is nowadays mostly employed for mechanistic studies, especially in the field of organic compounds [19, 25]. A hanging mercury drop electrode (HMDE) represents an electrode with much lower consumption of mercury as compared with the DME [19, 25]. The whole voltammetric curve can be measured at one mercury drop, and the combination of the HMDE with adsorptive stripping voltammetry (AdSV) [4] enables determination of nanomolar concentrations of, for instance, genotoxic NPAHs [5].

The aim of this study was to find optimal conditions to achieve the lowest limits of quantification ($L_Q s$) for the determination of 4-NI using direct current tast polarography (DCTP) and differential pulse polarography (DPP) at a DME and using direct current voltammetry (DCV), differential pulse voltammetry (DPV), and differential pulse adsorptive stripping voltammetry (DPAdSV) at a hanging mercury drop minielectrode (HMDmE). Moreover, for investigation of the electrochemical behavior of 4-NI, CV at the HMDmE was used. The aim of this work was also to apply the newly developed polarographic and voltammetric methods for the determination of 4-NI in model samples of drinking and river water.

Scheme 1



Results and discussion

Polarographic behavior and determination of 4-NI at the DME

The influence of pH on the polarographic behavior of 4-NI was investigated using DCTP and DPP at the DME in 1:1 (*v*/*v*) mixtures of methanol and Britton–Robinson (BR) buffer of pH ranging from 2.4 to 13.0. It can be seen in Fig. 1a that 4-NI gave one well-developed irreversible polarographic wave, corresponding to the well-known four-electron reduction of the nitro group to the hydroxylamino group [5], in the whole investigated pH* (pH of the methanolic-aqueous medium) range from 2.8 to 13.0. Its half-wave potential ($E_{½}$) varied with pH in the pH* range from 2.8 to 9.6 (Fig. 1b) according to the Eq. (1). The absolute value of the slope is very close to the theoretical value 59.2 mV per pH unit required for a four-electron/four-proton transfer. On the other hand, in the pH* range from 10.7 to 13.0, the $E_{½}$ values were pH-independent.

$$E_{1/2}/\mathrm{mV} = -60.7 \times \mathrm{pH}^* - 101.2 \left(r^2 = 0.9913\right) \qquad (1)$$

Other strongly irreversible and badly developed wave (Fig. 1a), corresponding to the pH-dependent two-electron reduction of the previously formed hydroxylamino group to

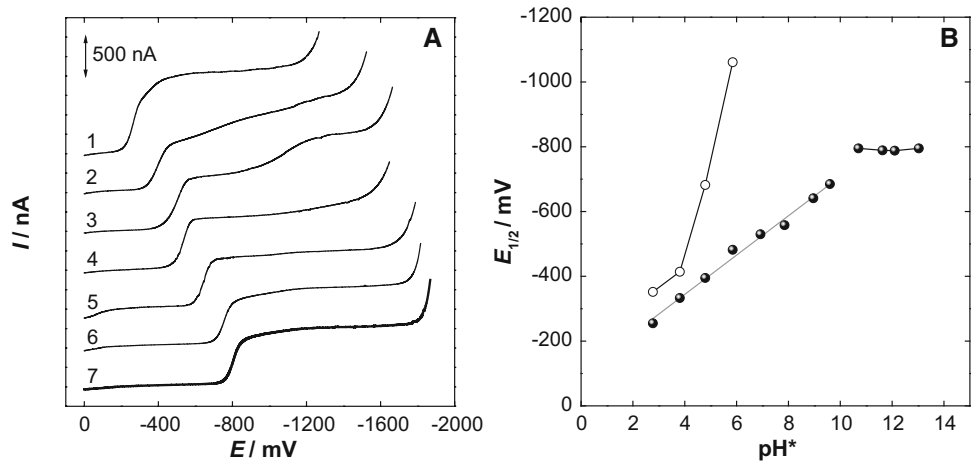


Fig. 1 **a** DCT polarograms of 4-NI (c = 100 μmol dm^{-3}) recorded at the DME in the methanol—BR buffer (1:1) medium at the polarization rate of 4 mV s^{-1}, with the initial potential of 0 mV; BR buffer pH: 2.4 (1), 4.0 (2), 5.0 (3), 6.0 (4), 8.0 (5), 10.0 (6), and 12.0 (7); the polarogram recorded under optimum conditions for the determination of 4-NI is in *bold*. **b** Attached corresponding dependences of the half-wave potential ($E_{½}$) of the first (*full points*) and second (*empty points*) polarographic wave on the resulting pH of the methanol—BR buffer (1:1) medium (pH*); the $E_{½}$ versus pH* dependence of the first polarographic wave is fitted to a linear function in the pH* range from 2.8 to 9.6 [Eq. (1)]

Table 1 Parameters of the calibration straight lines (including standard deviations) for the determination of 4-NI using various polarographic and voltammetric techniques

Technique	Medium	Concentration/ μmol dm^{-3}	Slope/mA dm^3 mol^{-1}	Intercept[a]/ nA	r^2	RSD/ %	L_Q/ μmol dm^{-3}
DCTP at the DME	Methanol—BR buffer of pH 12.0 (1:1)	10–100	−6.49 ± 0.14	21.1 ± 8.4	0.9982	–	–
		2–10	−4.32 ± 0.23	−1.1 ± 1.5	0.9917	3.0	0.65
DPP at the DME	Methanol—BR buffer of pH 12.0 (1:1)	10–100	−6.68 ± 0.14	16.2 ± 8.3	0.9983	–	–
		1–10	−4.72 ± 0.24	−0.8 ± 1.5	0.9984	–	–
		0.2–1	−3.01 ± 0.18	−0.24 ± 0.12	0.9983	3.9	0.10
DCV at the HMDmE	Methanol—BR buffer of pH 9.0 (1:9)	10–100	[b, c]	[b, c]	[b, c]	–	–
		1–10	−15.25 ± 0.70	−1.0 ± 4.6	0.9937	–	–
		0.2–1	−13.23 ± 0.40	−0.07 ± 0.26	0.9973	3.4	0.075
DPV at the HMDmE	Methanol—BR buffer of pH 8.0 (1:9)	10–100	[b, d]	[b, d]	[b, d]	–	–
		1–10	−16.26 ± 0.52	−2.3 ± 3.2	0.9959	–	–
		0.2–1	−18.90 ± 0.43	−0.34 ± 0.28	0.9985	3.5	0.070

r^2 coefficient of determination; RSD repeatability of the analyte determination at its lowest measured concentration (n = 10); L_Q limit of quantification (10σ; α = 0.05)

[a] All intercepts are not statistically significantly different from zero at the significance level α = 0.05

[b] Concentration dependences with a nonlinear trend expressed by the formal polynomial equations

[c] I_p (nA) = (0.0455 ± 0.0055) × c^2 (μmol^2 dm^{-6}) + (−15.46 ± 0.61) × c (μmol dm^{-3}) + (−5 ± 13), r^2 = 0.9995

[d] I_p (nA) = (0.0220 ± 0.0058) × c^2 (μmol^2 dm^{-6}) + (−14.15 ± 0.65) × c (μmol dm^{-3}) + (−24 ± 14), r^2 = 0.9995

the amino group [5], was obtained at more negative potentials in the pH* range from 2.8 to 5.9, but it is not suitable for analytical purposes.

The highest and best developed first wave was obtained in the methanol—BR buffer of pH 12.0 (1:1) medium (pH* 12.1) in which linear calibration dependences were obtained in the whole investigated concentration range from 2 to 100 μmol dm^{-3} of 4-NI. Their parameters are summarized in Table 1.

In good agreement with the polarographic behavior of 4-NI in DCTP at the DME, 4-NI gave one well-developed irreversible DPP peak at the DME in the pH* range from 2.8 to 13.0, which shifted towards more negative potentials with the increasing pH in the pH* range from 2.8 to 9.6 [its

peak potential (E_p) varied with pH according to the Eq. (2)]; however, in the pH* range from 10.7 to 13.0, the E_p values were pH-independent (data not shown). The second and much lower pH-dependent peak was observed at pH* from 2.8 to 6.9.

$$E_p/\text{mV} = -60.7 \times \text{pH}^* - 110.8\left(r^2 = 0.9740\right) \quad (2)$$

Analogously to DCTP investigation, the medium of methanol—BR buffer of pH 12.0 (1:1) was used for construction of calibration curves. The calibration curves were linear within the concentration range from 0.2 to 100 μmol dm^{-3} of 4-NI, and their parameters are summarized in Table 1.

Voltammetric behavior and determination of 4-NI at the HMDmE

Electrochemical behavior of 4-NI was further characterized using DCV and DPV at the HMDmE in 1:1 (*v*/*v*) mixtures of methanol and BR buffer of pH ranging from 2.0 to 13.0. 4-NI gave one well-developed cathodic DCV and DPV peak in the whole investigated pH* range from 2.5 to 12.9 (Fig. 2a). The E_p value of these peaks varied with pH in the pH* range from 2.5 to 11.4 according to the Eq. (3) (Fig. 2b) and Eq. (4) (data not shown) for DCV and DPV, respectively. In the strongly alkaline media, the E_p values were pH-independent.

$$E_p/\text{mV} = -62.2 \times \text{pH}^* - 114.0\left(r^2 = 0.9932\right) \quad (3)$$

$$E_p/\text{mV} = -59.2 \times \text{pH}^* - 95.5\left(r^2 = 0.9931\right) \quad (4)$$

The second DCV and DPV peak (unsuitable for analytical purposes) was observed at more negative potentials in the pH* range from 2.5 to 6.9. Such an electrochemical behavior is in good agreement with the above-described polarographic results.

The highest and best developed first peaks were obtained in methanol—BR buffer of pH 9.0 (1:1) and methanol—BR buffer of pH 8.0 (1:1) for DCV and DPV, respectively. The influence of methanol content in the supporting electrolyte on the height and shape of DPV peaks of 4-NI was investigated. The methanol contents of 50, 40, 30, 20, and 10 % were used, and, as it can be seen in Fig. 3, the highest peak was gained when the lowest amount of methanol was used. Therefore, the 1:9 (*v*/*v*) mixtures of methanol and BR buffer of pH 9.0 and 8.0, respectively, were used in the following DCV and DPV measurements.

For both DCV and DPV, the obtained calibration curves were linear within the concentration range from 0.2 to 10 μmol dm^{-3}, and their parameters are given in Table 1. The calibration dependences for the 4-NI concentrations from 10 to 100 μmol dm^{-3} were not linear, exhibiting a nonlinear polynomial trend (polynomial regression of the second degree was used to fit these dependences). The $L_Q s$ reached using DCV and DPV at the HMDmE are slightly lower than those attained using polarographic techniques at the DME.

A further increase in the sensitivity of the determination could be achieved by adsorptive accumulation of the test substance on the HMDmE surface. Since the presence of methanol inhibits the adsorption of the analyte on the electrode surface [4], the determination of 4-NI using DPAdSV was carried out in the absence of methanol in the supporting electrolytes whose pH varied over the whole pH scale. Potentials of accumulation were tested from

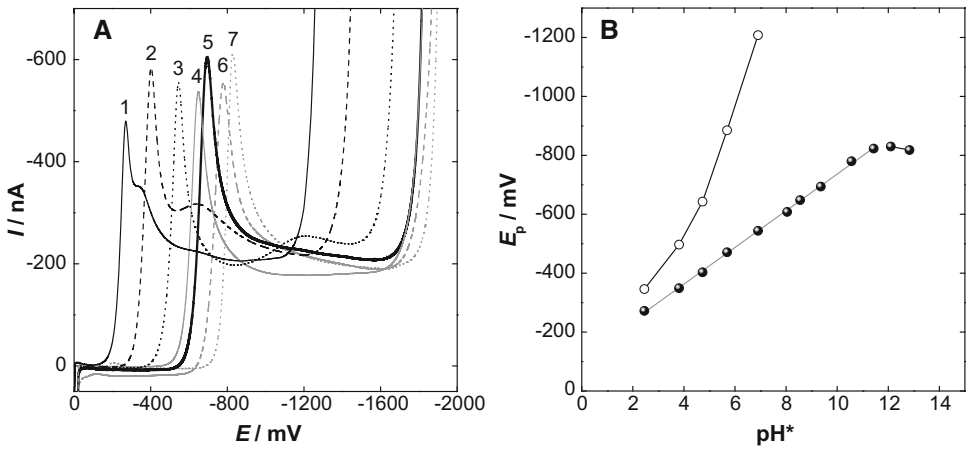


Fig. 2 **a** DC voltammograms of 4-NI (c = 100 μmol dm^{-3}) recorded at the HMDmE in the methanol—BR buffer (1:1) medium at the polarization rate of 20 mV s^{-1}, with the initial potential of 0 mV; BR buffer pH: 2.0 (1), 4.0 (2), 6.0 (3), 8.0 (4), 9.0 (5), 10.0 (6), and 12.0 (7); the voltammogram recorded under optimum conditions for the determination of 4-NI is in *bold*. **b** Attached corresponding dependences of the peak potential (E_p) of the first (*full points*) and second (*empty points*) voltammetric peak wave on the resulting pH of the methanol—BR buffer (1:1) medium (pH*); the E_p versus pH* dependence of the first voltammetric peak is fitted to a linear function in the pH* range from 2.5 to 11.4 [Eq. (3)]

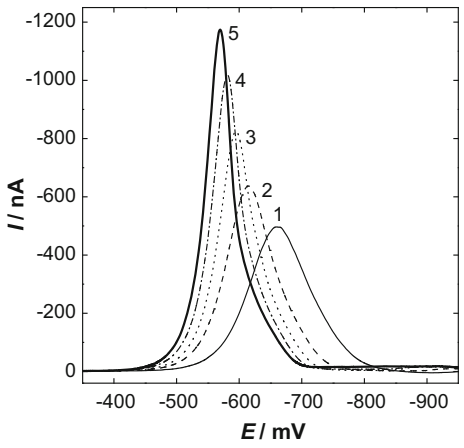


Fig. 3 DP voltammograms of 4-NI ($c = 100$ μmol dm^{-3}) recorded at the HMDmE in the methanol—BR buffer of pH 8.0 medium at the polarization rate of 20 mV s^{-1}, with the initial potential of 0 mV; methanol—BR buffer volume ratio: 5:5 (1), 4:6 (2), 3:7 (3), 2:8 (4), and 1:9 (5); the voltammogram recorded under optimum conditions for the determination of 4-NI is in *bold*

−100 mV to the 4-NI peak potential, with times of accumulation varying from 0 to 5 min.

Although the successful application of AdSV for the determination of structurally similar 1-nitrobenzene was previously published [32], this approach was unsuccessful for 4-NI ($c = 0.2$ μmol dm^{-3}), as its original DPV response did not significantly increase under any accumulation conditions examined. This fact is probably connected with the not fully aromatic structure of 4-NI which can alter the adsorption properties described for 1-nitrobenzene.

UV–Vis spectrophotometric determination of 4-NI

A UV–Vis spectrophotometric method for the determination of 4-NI in methanol was developed and used for comparison with the newly developed polarographic and voltammetric methods. By UV–Vis spectrophotometric measurements in the concentration range from 2 to 100 μmol dm^{-3} of 4-NI, the calibration straight line [Eq. (5)] was constructed from absorbance values evaluated at the wavelength of the absorption maximum (i.e., at 274 nm; optical path length of 0.1 cm; molar absorption coefficient of 7.3×10^4 dm^3 mol^{-1} cm^{-1}). The repeatability of the 4-NI determination [expressed in terms of the relative standard deviation (RSD) of absorbance values], evaluated from ten subsequent measurements ($n = 10$) at the lowest measured concentration (i.e., 2 μmol dm^{-3}), was 1.2 %, and the L_Q was 0.37 μmol dm^{-3}.

$$A = (7.316 \pm 0.050) \times c\left(\text{mmol dm}^{-3}\right)\left(r^2 = 0.9992\right) \quad (5)$$

The L_Q*s* reached using DCTP and DPP at the DME (Table 1) are comparable to that attained using UV–Vis spectrophotometry, and the L_Q*s* reached using DCV and DPV at the HMDmE are almost one order of magnitude lower.

Polarographic and voltammetric determination of 4-NI in drinking and river water

The optimal conditions found above for the polarographic and voltammetric determinations of 4-NI were used for its direct determination in model samples of drinking and river water. DPP at the DME and DPV at the HMDmE were selected for this purpose as suitable representatives of the newly developed methods. 9.0 cm^3 of spiked drinking or river water were filled up to 10.0 cm^3 with BR buffer of pH 12.0 (for DPP at the DME) or 8.0 (for DPV at the HMDmE). All parameters of the measured calibration straight lines are summarized in Table 2. For the sake of an illustration, DP voltammograms of 4-NI (obtained at the HMDmE in the lowest measurable concentration range from 0.2 to 1 μmol dm^{-3} of 4-NI spiked into drinking or river water) are depicted in Fig. 4.

CV investigation of 4-NI at the HMDmE

CV was used to verify the electrochemical reaction mechanisms of 4-NI formulated on the basis of the above-described polarographic results. Measurements were performed at the HMDmE, with different scan rates used (10, 20, 50, 100, 200, 500, and 1000 mV s^{-1}), in media with different pH: acidic [methanol—BR buffer of pH 3.0 (1:1)], neutral [methanol—BR buffer of pH 8.0 (1:1)], and alkaline [methanol—BR buffer of pH 12.0 (1:1)]. The concentration of 4-NI was 100 μmol dm^{-3}.

CV of 4-NI in the acidic medium gave two irreversible cathodic voltammetric peaks (p_1 and p_2) in the forward scan (Fig. 5a). The p_1 peak corresponds to the four-electron reduction of the nitro group to the hydroxylamino group [Eq. (6)]; the p_2 peak then corresponds to the two-electron reduction of the previously formed hydroxylamino group to the amino group [Eq. (7)] [5]. In the reverse scan, a hint of the reversible anodic voltammetric peak (p_3) can be seen (with the cathodic counter-peak p_3'), corresponding to the two-electron oxidation of the hydroxylamino group to the nitroso group [Eq. (8)]. This indicates that the second reaction (forming the p_2 peak) is much slower than the first one (forming the p_1 peak), as not all 4-hydroxylaminoindane is transformed to 4-aminoindane at the first switching potential of −1200 mV and can be oxidized in the reverse scan. Moreover, when the first switching potential was set to −550 mV (i.e., to the potential at which only 4-hydroxylaminoindane should be formed), the couple of the p_3/p_3' peaks exhibited higher heights in subsequent CV scans (Fig. 5a).

Table 2 Parameters of the calibration straight lines (including standard deviations) for the determination of 4-NI in model samples of drinking and river water

Technique	Medium	Concentration[a]/ μmol dm^{-3}	Slope/mA dm^3 mol^{-1}	Intercept[b]/ nA	r^2	RSD/ %	L_Q/ μmol dm^{-3}
DPP at the DME	Drinking water—BR buffer of pH 12.0 (9:1)	1–10	−7.24 ± 0.14	−2.9 ± 1.2	0.9988	–	–
		0.2–1	−7.62 ± 0.19	−0.31 ± 0.13	0.9981	5.8	0.12
	River water—BR buffer of pH 12.0 (9:1)	1–10	−8.81 ± 0.17	1.8 ± 2.3	0.9996	–	–
		0.2–1	−7.48 ± 0.26	−0.41 ± 0.17	0.9965	7.8	0.18
DPV at the HMDmE	Drinking water—BR buffer of pH 8.0 (9:1)	1–10	−13.52 ± 0.65	−0.2 ± 1.1	0.9898	–	–
		0.2–1	−10.12 ± 0.16	−0.16 ± 0.11	0.9992	7.5	0.15
	River water—BR buffer of pH 8.0 (9:1)	1–10	−7.23 ± 0.23	−2.9 ± 2.3	0.9988	–	–
		0.2–1	−11.48 ± 0.18	−0.18 ± 0.12	0.9993	13.5	0.27

r^2 coefficient of determination; RSD repeatability of the analyte determination at its lowest measured concentration ($n = 10$); L_Q limit of quantification (10σ; $\alpha = 0.05$)

[a] Concentration of 4-NI in drinking or river water

[b] All intercepts are not statistically significantly different from zero at the significance level $\alpha = 0.05$

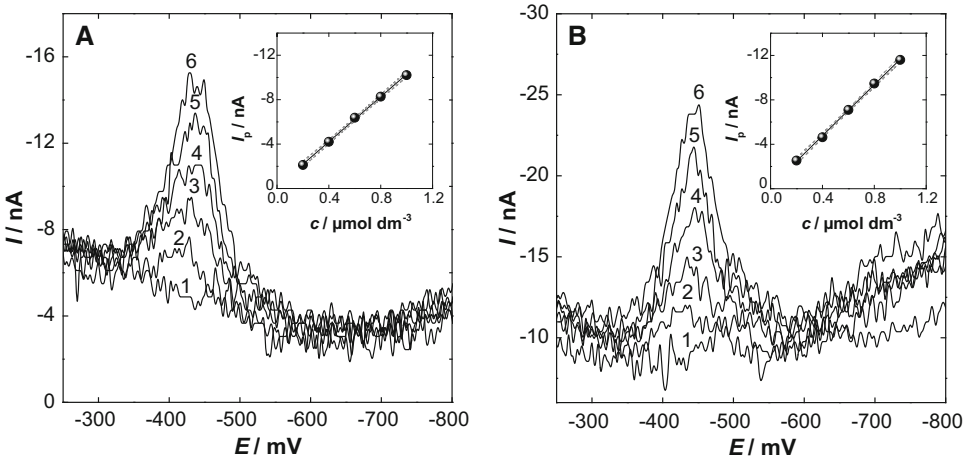


Fig. 4 DP voltammograms of 4-NI recorded at the HMDmE in the spiked drinking water—BR buffer of pH 8.0 (9:1) mixture (**a**) and in the spiked river water—BR buffer of pH 8.0 (9:1) mixture (**b**) at the polarization rate of 20 mV s^{-1}, with the initial potential of 0 mV; concentration of 4-NI in waters (μmol dm^{-3}): 0 (1), 0.2 (2), 0.4 (3), 0.6 (4), 0.8 (5), and 1.0 (6). *Insets* corresponding calibration curves; the confidence bands are constructed for $\alpha = 0.05$ ($n = 3$)

$$\mathrm{Ar{-}NO_2} + 4e^- + 4\mathrm{H^+} \rightarrow \mathrm{Ar{-}NHOH} + \mathrm{H_2O}(\text{peak } p_1) \quad (6)$$

$$\mathrm{Ar{-}NHOH} + 2e^- + 2\mathrm{H^+} \rightarrow \mathrm{Ar{-}NH_2} + \mathrm{H_2O}(\text{peak } p_2) \quad (7)$$

$$\mathrm{Ar{-}NHOH} \leftrightarrow \mathrm{Ar{-}NO} + 2e^- + 2\mathrm{H^+}\,(\text{peaks } p_3 \text{ and } p_3') \quad (8)$$

In the neutral (Fig. 5b) and alkaline (data not shown) media, only one irreversible cathodic voltammetric peak (p_1) can be observed in the forward scan, corresponding to the reaction described by the Eq. (6) [5]. Thus, the final reaction product is 4-hydroxylaminoindane which is consequently reoxidized in the reverse scan to 4-nitrosoindane (peak p_3) according to the Eq. (8).

To determine whether the reduction of 4-NI is limited by the diffusion or the adsorption of the analyte, cyclic voltammograms of 100 μmol dm^{-3} 4-NI were measured at scan rates (v) varying from 10 to 1000 mV s^{-1} in the 1:1 (v/v) mixtures of methanol—BR buffer of pH 3.0, 8.0, and 12.0, representing acidic, neutral, and alkaline media, respectively. A not fully diffusion-controlled process (controlled by the contribution of both diffusion and adsorption) was indicated in all the media tested for all the scan rates used. The slopes of plotting log I_p versus log v were 0.66, 0.70, and 0.81 for acidic, neutral, and alkaline media, respectively (values of 0.5 and 1.0 represent an electrochemical processes fully controlled by diffusion and adsorption, respectively [33]).

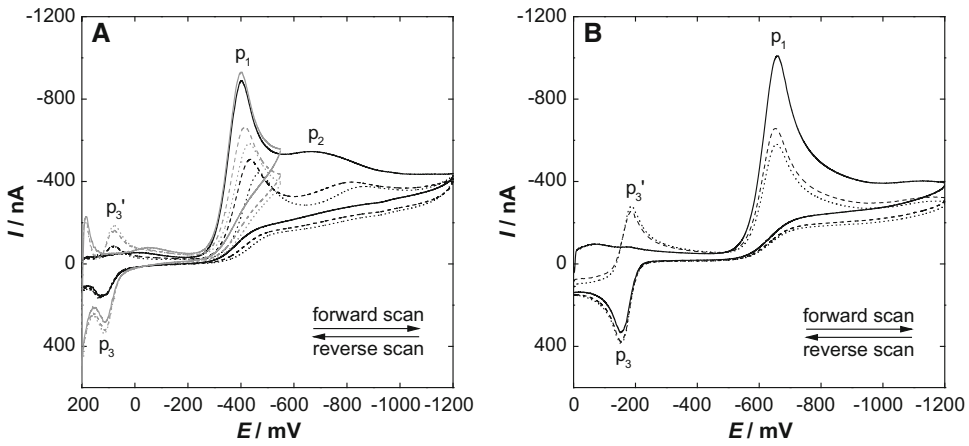


Fig. 5 Cyclic voltammograms of 4-NI ($c = 100$ μmol dm^{-3}) recorded at the HMDmE in the methanol—BR buffer of pH 3.0 (1:1) medium (**a**) and in the methanol—BR buffer of pH 8.0 (1:1) medium (**b**) at the scan rate of 50 mV s^{-1}; 1st scan (*solid line*), 2nd scan (*dashed line*), and 3rd scan (*dotted line*); for **a** the potential ranges from +200 to −1200 mV (*black line*) and from +200 mV to −550 mV (*gray line*); for **b** the potential range from 0 to −1200 mV (*black line*); for peak descriptions, see text

Conclusion

Electrochemical behavior of 4-NI was studied, and optimal conditions for its determination were found in this work. The determination of 4-NI was carried out using polarographic and voltammetric techniques which are shown to be simple and less time-consuming. Most of the techniques were used to determine 4-NI in the concentration range from 0.2 to 100 μmol dm^{-3}, with the $L_Q s$ achieved at submicromolar levels, which are well comparable to the one achieved using UV–Vis spectrophotometry (L_Q of 0.37 μmol dm^{-3}). The attempt to further increase the determination sensitivity using adsorptive accumulation of 4-NI on the HMDmE surface was not successful. CV at the HMDmE proved that the rate of the electrode reaction during the electrochemical reduction of 4-NI is controlled by the contribution of both diffusion and adsorption, and the reaction is irreversible. Verification of the applicability of the newly developed methods for the determination of 4-NI was successfully carried out under optimized conditions in model water samples, with the obtained $L_Q s$ of 0.12 and 0.18 μmol dm^{-3} (using DPP at the DME) and 0.15 and 0.27 μmol dm^{-3} (using DPV at the HMDmE) for drinking and river water, respectively.

Experimental

Reagents

A 1.0 mmol dm^{-3} stock solution of 4-NI (99 %, Sigma-Aldrich, St. Louis, MO, USA) was prepared in methanol (p.a. purity, Lachema, Brno, Czech Republic). A UV–Vis spectrophotometric study demonstrated that the methanolic stock solution is stable for at least 2 months [34]. More dilute solutions were prepared by dilution of the stock solution with methanol. BR buffers were prepared in a usual way [22]: boric acid (99.5 %), phosphoric acid (84–87 %), acetic acid (99 %), and sodium hydroxide (98.5 %), all of p.a. purity, were supplied by Lach-Ner, Neratovice, Czech Republic. EDTA disodium salt was supplied by Lachema in p.a. purity. Deionized water produced by a Milli-Q Plus system (Millipore, Billerica, MA, USA) was used. All the chemicals were used without further purification, and their solutions were maintained in glass vessels in the dark at laboratory temperature.

Apparatus

All electrochemical measurements were carried out using the Eco-Tribo electrochemical analyzer driven by the Polar Pro 5.1 software (both Polaro-Sensors, Prague, Czech Republic). The software worked under the operational system Microsoft Windows XP Professional (Microsoft Corporation, Redmond, WA, USA). The measurements were carried out in a three-electrode system: a platinum electrode (type PPE) as an auxiliary electrode, a silver|silver chloride electrode (type RAE 113, 1 mol dm^{-3} KCl) as a reference electrode (both Monokrystaly, Turnov, Czech Republic), and an appropriate mercury electrode as a working electrode.

For polarographic techniques, the classical DME was used as the working electrode: the electronically controlled mercury drop lifetime was 1.0 s, and the height of the mercury reservoir was 64 cm (mercury drop lifetime was 4.3 s at this height, measured in 0.1 mol dm^{-3} KCl at zero potential, and the flow rate of mercury through the capillary was 1.93 mg s^{-1}). The scan rate of 4 mV s^{-1} was

used. For DPP, the pulse amplitude of −50 mV and the pulse width of 100 ms (with current sampling for the last 20 ms) were used.

For voltammetric techniques, the miniaturized HMDmE of the UMμE type (Polaro-Sensors, Prague, Czech Republic) was used as the working electrode: the valve opening time was 400 ms, the mercury drop surface was 1.61 mm^2, and the flow rate of mercury through the capillary was 6.50 mg s^{-1}. The scan rate of 20 mV s^{-1} was used for DCV, DPV, and DPAdSV, while in CV, the scan rates varied from 10 to 1000 mV s^{-1}. The pulse amplitude of −50 mV and the pulse width of 100 ms (with current sampling for the last 20 ms) were used in DPV and DPAdSV.

The pH was measured using the pH meter Jenway 3510 with a combined glass electrode (type 924 005) (both Jenway, Chelmsford, UK).

UV–Vis spectra were measured in absorption quartz cuvettes (the optical path length of 0.1 cm, Hellma, Müllheim, Germany) versus methanol with the Agilent 8453 UV–Vis spectrophotometer driven by the UV–Visible ChemStation 9.01 software (both Agilent Technologies, Santa Clara, CA, USA).

Procedures

The general procedure to obtain polarograms or voltammograms was as follows: an appropriate amount of the 4-NI stock solution in methanol was measured into a voltammetric cell, a required amount of methanol was added, if necessary, and the solution was filled up to 10.0 cm^3 with BR buffer of an appropriate pH. Before each polarographic and/or voltammetric measurement, oxygen was removed from the measured solutions by bubbling with nitrogen (purity 4.0, Linde, Prague, Czech Republic) for 5 min. Before entering the voltammetric cell, nitrogen was first passed through a pre-bubbler containing a methanol—deionized water mixture in the same ratio as in the measured solution; deionized water alone was used in the pre-bubbler when model water samples were measured.

Unless stated otherwise, all the curves were measured three times, and all the measurements were carried out at laboratory temperature. The wave heights, i.e., limiting diffusion currents (I_{lim}), recorded using DCTP were evaluated from the extrapolated linear portions of the currents. The DCV peak height (I_p) was evaluated from the extrapolated linear portion of the voltammogram before the onset of the peak. The peak heights (represented by the same abbreviation I_p) recorded using DPP, DPV, and DPAdSV were evaluated from the straight lines connecting the minima before and after the peak. The parameters of calibration curves (i.e., slope, intercept, coefficient of determination (r^2), and confidence intervals) and other mathematical and statistical quantities (all for the significance level $\alpha = 0.05$ [35]) were calculated using the Origin Pro 8.0 software (OriginLab Corporation, Northampton, MA, USA). The limit of quantification (L_Q) was calculated as the analyte concentration corresponding to a tenfold standard deviation of the respective response from ten consecutive determinations at the lowest measurable concentration [36].

Model samples

Drinking water from the public water pipeline in the building of the Faculty of Science, Charles University in Prague, Prague, Czech Republic, and river water from the Botič stream in Prague, Czech Republic (filtered through the filter paper), both spiked with an appropriate amount of the 4-NI stock solution, were used for model samples. Solid EDTA disodium salt was added (0.5 g per 1 dm^3 of water) for masking of cations present in solution [37]. The procedure for the polarographic or voltammetric determination of 4-NI in model samples was as follows: 9.0 cm^3 of drinking or river water, spiked with an appropriate amount of 4-NI, were filled up to 10.0 cm^3 with BR buffer of an optimal pH, and, after deaeration with nitrogen, DP polarograms at the DME or DP voltammograms at the HMDmE were recorded.

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