POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF 6-NITROBENZIMIDAZOLE AND MECHANISM OF ITS ELECTROCHEMICAL REDUCTION

Dana DEYLOVÁ[1], Jiří BAREK[2,*] and Vlastimil VYSKOČIL[3]

UNESCO Laboratory of Environmental Electrochemistry, Department of Analytical Chemistry, Faculty of Science, Charles University, Hlavova 2030, 128 43 Prague 2, Czech Republic; e-mail: [1] d.deylova@centrum.cz, [2] barek@natur.cuni.cz, [3] vyskoci1@natur.cuni.cz

Received April 28, 2009
Accepted June 25, 2009
Published online November 29, 2009

Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of the 50th anniversary of the Nobel Prize for polarography.

Optimal conditions were found for the determination of 6-nitrobenzimidazole by tast polarography (at 1×10^{-4}–1×10^{-6} mol l^{-1}), by differential pulse polarography at dropping mercury electrode (at 1×10^{-4}–1×10^{-7} mol l^{-1}), and by differential pulse voltammetry at hanging mercury drop electrode (at 1×10^{-4}–1×10^{-8} mol l^{-1}). Practical applicability of the developed methods was verified on the determination of 6-nitrobenzimidazole in drinking water (at 10^{-8} mol l^{-1}). Coulometry at constant potential and cyclic voltammetry were used for elucidation of the mechanism of electrochemical reduction.

Keywords: 6-Nitrobenzimidazole; Tast polarography; Differential pulse polarography; Differential pulse voltammetry; Cyclic voltammetry; Constant potential coulometry; Electrochemistry.

6-Nitrobenzimidazole (6-NBIA; Fig. 1) ranks among genotoxic heterocyclic nitro compounds. As a proven carcinogen and mutagen[1], it can damage natural biological functions of living organisms. The occurrence of 6-NBIA in environment is associated with combustion of fossil fuels[2]. 6-NBIA was determined as a part of photographic processing solutions using DC and AC polarography[3] and its adsorptive and inhibitive properties have been studied in the frame of metal corrosion protection[4].

6-NBIA can be determined spectrophotometrically in the ultraviolet and visible region[5], by chromatographic techniques (high performance liquid chromatography[6], thin-layer chromatography[7]) and by potentiometric titration[8].

© 2009 Institute of Organic Chemistry and Biochemistry
doi:10.1135/cccc2009059

So far, modern polarographic and voltammetric methods (successfully used in the analysis of many environmental carcinogens[2,9]) were not applied to 6-NBIA. Therefore, the aim of this work was to find optimum conditions for the determination of this genotoxic compound using tast polarography and differential pulse polarography (DPP) at dropping mercury electrode (DME) and differential pulse voltammetry (DPV) at hanging mercury drop electrode (HMDE), and to verify its practical applicability in analysis of drinking water. The knowledge of the mechanism of electrochemical reaction can be useful both for optimization of conditions for electroanalytical determination and as a clue for further investigation of biological transformation of the compound. Therefore, cyclic voltammetry and constant potential coulometry were used to investigate the mechanism of electrochemical reduction of 6-NBIA at mercury electrodes.

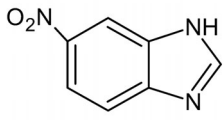

FIG. 1
Structural formula of 6-nitrobenzimidazole

EXPERIMENTAL

Reagents

6-Nitrobenzimidazole (98%) was supplied by Sigma Aldrich as nitrate. A 1×10^{-3} M stock solution was prepared by dissolving an exactly weighed amount of the compound in water. Its dilute solutions were prepared by dilution of the stock solution. The solutions were stored in refrigerator. A spectrophotometric study demonstrated that the stock solution is stable for at least three months[5]. The dilute solutions were prepared before use.

The Britton–Robinson (BR) buffer solutions 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, using analytical reagent grade chemicals obtained from Merck. Deionized water was produced by Milli-Qplus system (Millipore).

Apparatus

Polarographic and voltammetric measurements were carried out using an Eco-Tribo Polarograph driven by Polar Pro 2 software (all Polaro-Sensors, Prague, Czech Republic). All measurements were carried out in a three-electrode system with platinum electrode PPE (Monokrystaly, Turnov, Czech Republic) as an auxiliary electrode and silver|silver chloride electrode RAE 113 (1 M KCl, Monokrystaly, Turnov, Czech Republic) as a reference electrode. Dropping mercury electrode (DME) was used as a working electrode in polarographic mea-

surements. It had the following parameters: mercury reservoir height 49 cm, mercury drop time 1.30 s and mercury flow rate 6.61 mg s^{-1}. The electronically controlled drop time 1 s and scan rate 4 mV s^{-1} were used for tast polarography and DPP. A pen-type hanging mercury drop electrode UMμE (Polaro-Sensors, Prague, Czech Republic) was used as a working electrode in voltammetric measurements: valve opening time 100 ms, mercury drop surface 0.0137 cm^2. In DPV, scan rate 20 mV s^{-1}, pulse amplitude 50 mV and pulse width 80 ms were used.

In constant potential coulometry (CPC), a special glass vessel was used with mercury pool working electrode (S = 2.41 cm^2). pH of analyzed solutions was measured with a conductivity and pH-meter Jenway 4330 (Jenway, U.K.) with a combined glass electrode. All experiments were carried out at laboratory temperature.

Procedures

For polarographic and voltammetric measurements, an appropriate amount of 6-NBIA stock solution was introduced into a polarographic vessel and filled up to 10 ml with BR buffer of appropriate pH. Oxygen was removed from the measured solutions by bubbling with nitrogen for 5 min. All curves were measured three times. For voltammetric determination of 6-NBIA in model samples of drinking water, 9 ml of a sample containing (2–10) × 10^{-8} M 6-NBIA was filled up with BR buffer (pH 4) or 0.01 M NaOH to 10 ml and, after removal of oxygen, DP voltammogram was recorded.

In CPC, a DP voltammogram was recorded prior to CPC. Afterwards, CPC at a constant potential corresponding to the limiting currents of the first and second waves was carried out for 20 min, and a DP voltammogram was recorded again. The amount of coulometrically reduced 6-NBIA was calculated from the DPV peak decrease. The number of exchanged electrons was calculated from the charge passed and the amount of compound reduced.

The parameters of calibration curves (such as slope, intercept, limit of determination) were calculated with statistic software Adstat which uses confidence bands (α = 0.05) for calculation of the limit of determination (L_Q). It corresponds to the lowest signal for which the relative standard deviation is equal to 0.1 (ref.[10]).

RESULTS AND DISCUSSION

Polarographic and Voltammetric Determination of 6-Nitrobenzimidazole

Tast polarography at dropping mercury electrode. The influence of pH on polarographic behavior of 6-NBIA was investigated in a BR buffer. It can be seen in Fig. 2 that 6-NBIA gives one well-developed wave. At pH 2–4, a second wave (much lower and rather drawn-out) can be observed at a more negative potential. The probable assignment of these waves to individual electrochemical processes is discussed in the part on mechanism of this electrochemical reduction. With increasing pH, the half-wave potentials are shifted towards negative values, which can be explained by a preceding protonation of 6-NBIA. BR buffers of pH 2, 7, and 12 were used to measure

calibration curves (Fig. 3). The parameters of calibration straight lines are summarized in Table I.

Differential pulse polarography at dropping mercury electrode. It follows from DP polarograms that 6-NBIA gives two cathodic peaks at pH 2–5, the

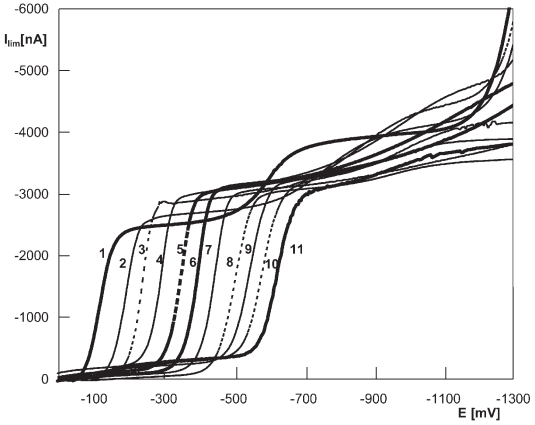

FIG. 2
Tast polarograms of 6-NBIA ($c = 1 \times 10^{-4}$ mol l^{-1}) measured at DME in BR buffer of pH: 2.0 (*1*), 3.0 (*2*), 4.0 (*3*), 5.0 (*4*), 6.0 (*5*), 7.0 (*6*), 8.0 (*7*), 9.0 (*8*), 10.0 (*9*), 11.0 (*10*), 12.0 (*11*)

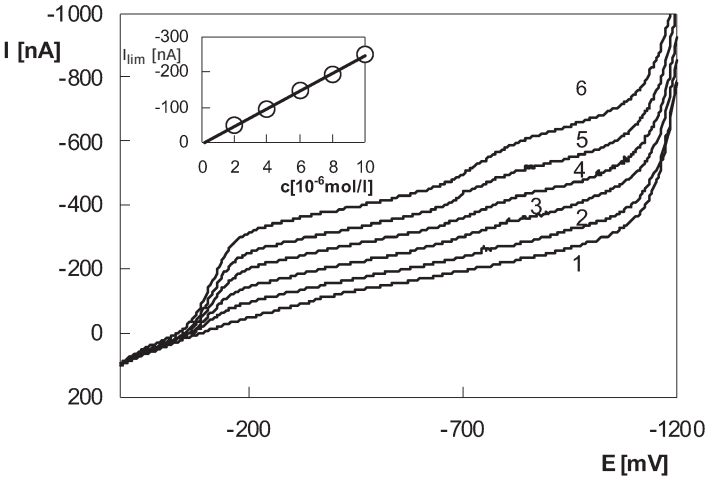

FIG. 3
Tast polarograms of 6-NBIA in BR buffer (pH 2) in the lowest concentration range. Concentration of 6-NBIA: 0 (*1*), 2 (*2*), 4 (*3*), 6 (*4*), 8 (*5*), 10 (*6*) × 10^{-6} mol l^{-1}

first one being much higher and better developed. (The second peak is ca. 500 mV more negative, much lower, it is not observable at lower concentrations and it is not analytically useful.) Only one peak is observed at pH 6–12 (Fig. 4). Similarly to tast polarography, we used solutions with the resulting pH 2, 7, and 12 to measure the calibration curves (Fig. 5). The parameters of calibration curves are summarized in Table I.

TABLE I
Parameters of calibration straight lines for polarographic and voltammetric determination of 6-NBIA

pH of BR buffer	Method	Concentration mol l^{-1}	Slope nA mol l^{-1}	Intercept nA	Correlation coefficient	L_Q mol l^{-1}
2.00	Tast	$(2–10) \times 10^{-5}$	2.58×10^7	–72.4	0.9973	–
	Tast	$(2–10) \times 10^{-6}$	2.50×10^7	–3.2	0.9993	2.1×10^{-6}
	DPP	$(2–10) \times 10^{-5}$	2.79×10^7	–37.7	0.9990	–
	DPP	$(2–10) \times 10^{-6}$	2.69×10^7	–2.4	0.9991	–
	DPP	$(2–10) \times 10^{-7}$	2.92×10^7	–6.6	0.9916	4.6×10^{-7}
7.00	Tast	$(2–10) \times 10^{-5}$	3.02×10^7	–79.1	0.9990	–
	Tast	$(2–10) \times 10^{-6}$	3.30×10^7	–17.3	0.9640	1.5×10^{-6}
	DPP	$(2–10) \times 10^{-5}$	4.18×10^7	–425.4	0.9929	–
	DPP	$(2–10) \times 10^{-6}$	4.86×10^7	–16.9	0.9992	–
	DPP	$(2–10) \times 10^{-7}$	4.45×10^7	4.0	0.9972	4.8×10^{-7}
12.00	Tast	$(2–10) \times 10^{-5}$	3.04×10^7	–13.7	0.9937	–
	Tast	$(2–10) \times 10^{-6}$	3.28×10^7	–7.5	0.9984	2.8×10^{-6}
	DPP	$(2–10) \times 10^{-5}$	4.03×10^7	–405.5	0.9955	–
	DPP	$(2–10) \times 10^{-6}$	5.04×10^7	–11.3	0.9992	–
	DPP	$(2–10) \times 10^{-7}$	6.44×10^7	–0.1	0.9964	4.5×10^{-7}
4.00	DPV	$(2–10) \times 10^{-5}$	1.70×10^7	–4.0	0.9976	–
	DPV	$(2–10) \times 10^{-6}$	2.30×10^7	–5.4	0.9996	–
	DPV	$(2–10) \times 10^{-7}$	1.90×10^7	–6.2	0.9906	–
	DPV	$(2–10) \times 10^{-8}$	3.60×10^7	–4.1	0.9859	2.7×10^{-8}

Differential pulse voltammetry at hanging mercury drop electrode. It follows from Fig. 6 that 6-NBIA gives two cathodic peaks at pH 2–5 and only one peak at pH 6–12. (The second at pH 2–5 is ca. 500 mV more negative, much lower, it is not observable at lower concentrations and it is not analytically useful.) The best developed peak was obtained in BR buffer (pH 4) medium,

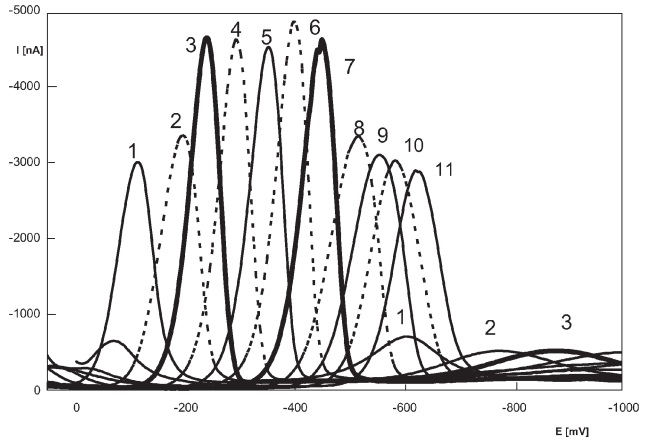

FIG. 4
Differential pulse polarograms of 6-NBIA ($c = 1 \times 10^{-4}$ mol l^{-1}) measured at DME in BR buffer of pH: 2.0 (*1*), 3.0 (*2*), 4.0 (*3*), 5.0 (*4*), 6.0 (*5*), 7.0 (*6*), 8.0 (*7*), 9.0 (*8*), 10.0 (*9*), 11.0 (*10*), 12.0 (*11*)

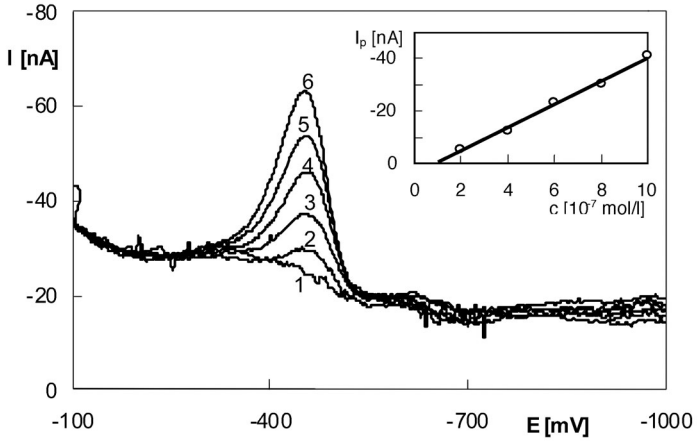

FIG. 5
Differential pulse polarograms of 6-NBIA at the lowest concentrations measured at DME in BR buffer (pH 7). Concentration of 6-NBIA: 0 (*1*), 2 (*2*), 4 (*3*), 6 (*4*), 8 (*5*), 10 (*6*) × 10^{-7} mol l^{-1}

which was used for measuring calibration curves (Fig. 7). The parameters of calibration curves are summarized in Table I.

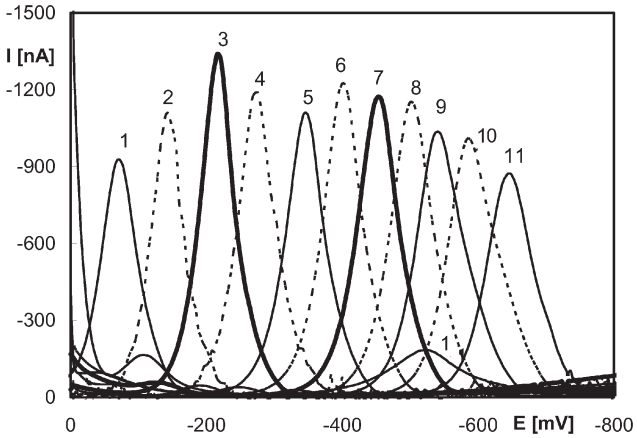

FIG. 6
Differential pulse voltammograms of 6-NBIA (c = 1 × 10^{-4} mol l^{-1}) measured at HMDE in BR buffer pH: 2.0 (*1*), 3.0 (*2*), 4.0 (*3*), 5.0 (*4*), 6.0 (*5*), 7.0 (*6*), 8.0 (*7*), 9.0 (*8*), 10.0 (*9*), 11.0 (*10*), 12.0 (*11*)

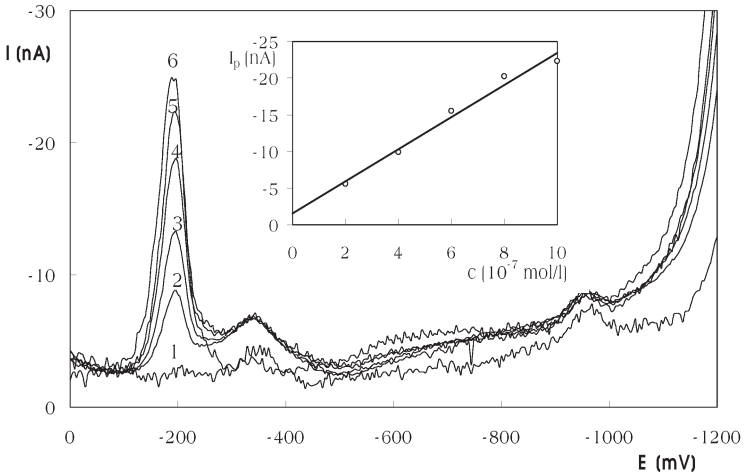

FIG. 7
Differential pulse voltammograms of 6-NBIA at lowest concentrations measured at HMDE in BR buffer pH 4. Concentration of 6-NBIA: 0 (*1*), 2 (*2*), 4 (*3*), 6 (*4*), 8 (*5*), 10 (*6*) × 10^{-7} mol l^{-1}

Voltammetric determination of 6-NBIA in drinking water. Practical applicability of the developed methods was tested on model samples of drinking water. Calibration straight line for BR buffer (pH 4) (Fig. 8) has a high inter-

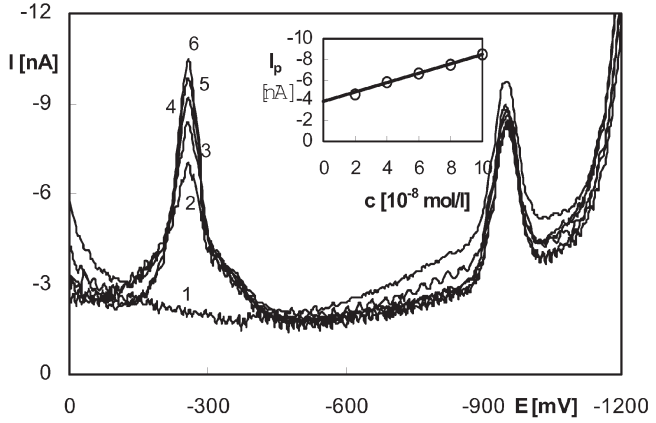

FIG. 8
Differential pulse voltammograms of 6-NBIA in drinking water at HMDE with BR buffer (pH 4) as a supporting electrolyte. Concentration of 6-NBIA in drinking water: 0 (*1*), 2 (*2*), 4 (*3*), 6 (*4*), 8 (*5*), 10 (*6*) × 10^{-8} mol l^{-1}

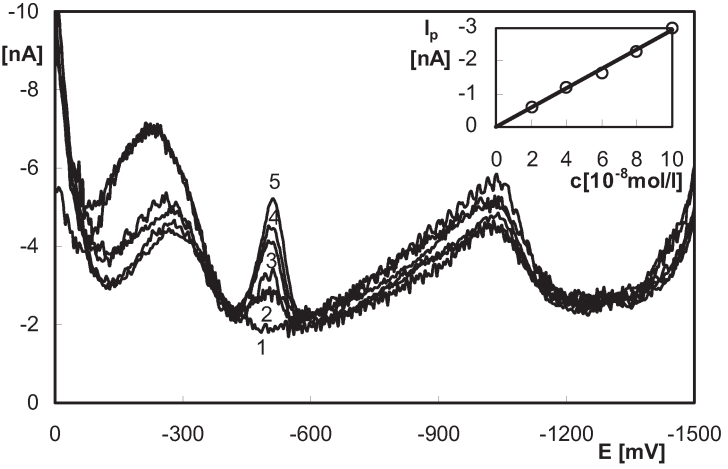

FIG. 9
Differential pulse voltammograms of 6-NBIA in drinking water at HMDE with 0.01 M NaOH as a supporting electrolyte. Concentration of 6-NBIA in drinking water: 0 (*1*), 2 (*2*), 4 (*3*), 6 (*4*), 8 (*5*), 10 (*6*) × 10^{-8} mol l^{-1}

cept complicating the application of standard addition method. Therefore, 0.01 M NaOH was used as a supporting electrolyte (Fig. 9). It was found that calibration curves are linear with negligible intercept. The method of standard addition should be used because the slope of calibration curve generally depends on the matrix.

Mechanism of Electrochemical Reduction of 6-Nitrobenzimidazole

We can assume[11–13] that the first tast polarographic wave corresponds to irreversible four-electron reduction of 6-NBIA to 6-(hydroxyamino)-benzimidazole and the second to irreversible two-electron reduction of 6-(hydroxyamino)benzimidazole to 6-aminobenzimidazole (Fig. 10). We have confirmed this assumption by cyclic voltammetry and CPC (see bellow).

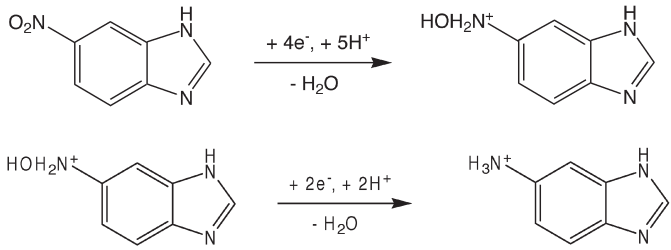

FIG. 10
Polarographic reduction of 6-NBIA. Eq. (*1*), the first wave; Eq. (*2*), the second wave

Cyclic voltammetry. Cyclic voltammograms of 6-NBIA ($c = 1 \times 10^{-4}$ mol l^{-1}) at HMDE were measured in BR buffer of pH 2, 7, and 12 at scan rates 50 mV s^{-1} (Figs 11–13). Peaks p_1, p_3 and p_5 in the first cathodic scan correspond to irreversible four-electron reduction of 6-NBIA to 6-(hydroxyamino)benzimidazole described in Fig. 10. Peak p_2 corresponds to irreversible two-electron reduction of 6-(hydroxyamino)benzimidazole to 6-aminobenzimidazole. Peaks p_4, $p_{4'}$ and p_6, $p_{6'}$ correspond to the two-electron reversible system 6-(hydroxyamino)benzimidazole/6-nitrosobenzimidazole (Fig. 14).

Constant potential coulometry. CPC at mercury pool electrode was used to confirm the number of electrons exchanged in the electrode reaction. Electrolysis of 6-NBIA was carried out in a coulometric cell described earlier[14]. The *I–E* curves at a mercury pool electrode were measured at pH 2, 7, and

12 in stirred solution. The constant potentials E_{C1} and E_{C2}, corresponding to the limiting currents of the first and second waves, respectively, at given pH were chosen for the electrolysis. It was confirmed (Table II) that the first wave corresponds to the exchange of four electrons and the second to the exchange of two electrons.

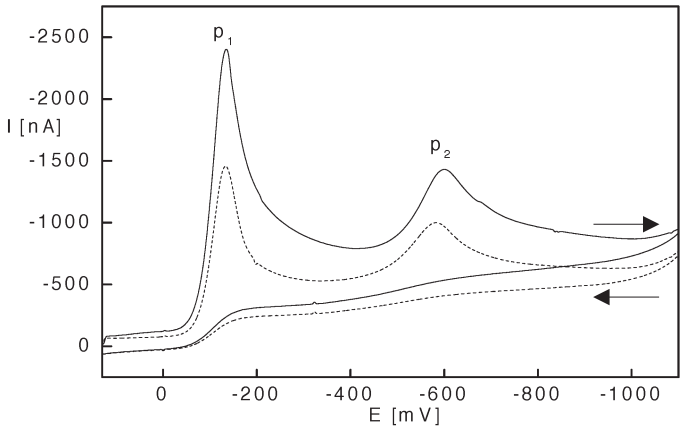

FIG. 11
Cyclic voltammograms of 6-NBIA ($c = 1 \times 10^{-4}$ mol l^{-1}) measured at HMDE in BR buffer (pH 2). Scan rate 50 mV s^{-1}. Full line, the first scan; dashed line, the second scan

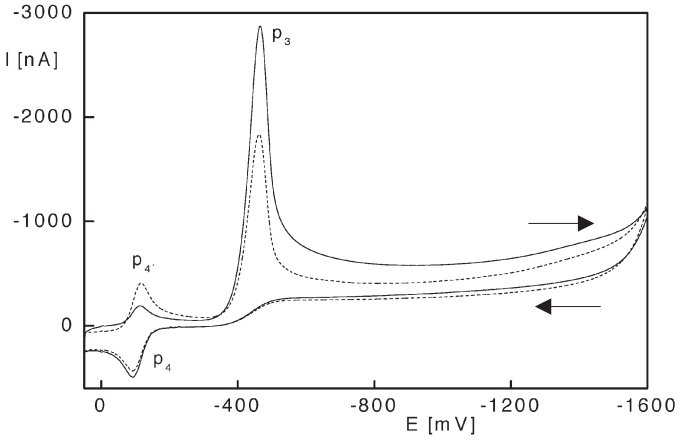

FIG. 12
Cyclic voltammograms of 6-NBIA ($c = 1 \times 10^{-4}$ mol l^{-1}) measured at HMDE in BR buffer (pH 7). Scan rate 50 mV s^{-1}. Full line, the first scan; dashed line, the second scan

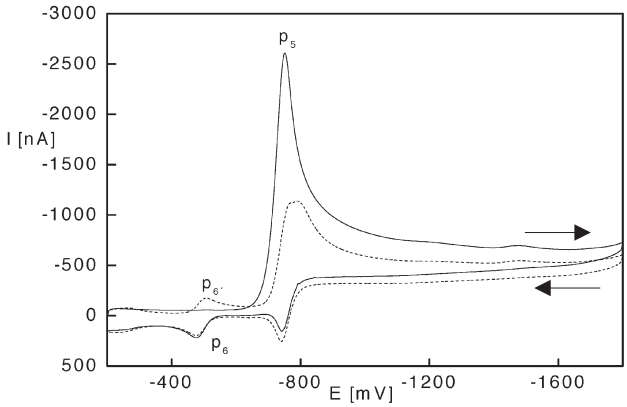

FIG. 13
Cyclic voltammograms of 6-NBIA (c = 1 × 10^{-4} mol l^{-1}) measured at HMDE in BR buffer (pH 12). Scan rate 50 mV s^{-1}. Full line, the first scan; dashed line, the second scan

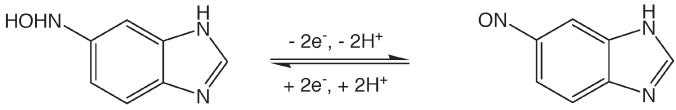

FIG. 14
Reversible electrochemical oxidation of 6-(hydroxyamino)benzimidazole to 6-nitrosobenzimidazole

TABLE II
Constant potential coulometry of 6-NBIA in BR buffer at different pH

Parameter	pH 2		pH 7	pH 12
	E_{C1} = –875 mV	E_{C2} = –1150 mV	E_{C1} = –1700 mV	E_{C1} = –1300 mV
Q, C	0.12	0.15	0.16	0.12
I_0, nA	418.1	92.0	230.2	184.5
I_1, nA	151.3	45.6	51.8	39.7
$n_{transform}$, mol l^{-1}	3.1×10^{-7}	2.5×10^{-7}	3.8×10^{-7}	3.9×10^{-7}
z	4.0	6.3	4.4	3.5

Q, consumed charge; I_0, DPP peak height before CPC; I_1, DPP peak height after CPC; n, amount of electrochemically reduced substance; z, number of exchanged electrons; E_{C1} and E_{C2}, constant potential for CPC corresponding to the limiting current of the first and second wave, respectively

CONCLUSIONS

It has been proved that modern polarographic and voltammetric techniques at mercury electrodes are suitable for the determination of submicromolar concentrations of genotoxic 6-nitrobenzimidazole. Taking into account the experimentally found value of molar absorption coefficient of 6-NBIA 2.43×10^4 l mol^{-1} cm^{-1} at 234 nm (ref.[5]), submicromolar concentrations are not amenable for UV spectrophotomeric determination. Practical applicability of the developed methods was demonstrated on determination of 6-NBIA in drinking water in the 10^{-8} mol l^{-1} concentration range. The attempt to increase the sensitivity of voltammetric determination by adsorptive accumulation was not successful. The possibility of a further increase in both sensitivity and selectivity of the determination using solid phase extraction for preliminary separation and preconcentration of 6-NBIA is under further investigation.

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (projects LC 06035, MSM 0021620857, and RP14/63).

REFERENCES

1. Rosenkranz H. S., Karol M. H.: *Mutat. Res.* **1999**, *431*, 81.
2. Barek J., Cvačka J., Muck A., Quaiserová V., Zima J.: *Electroanalysis* **2001**, *13*, 779.
3. Canterford D. R.: *J. Photogr. Sci.* **1978**, *26*, 65.
4. Popova A., Christov M., Raicheva S., Sokolova E.: *Corros. Sci.* **2004**, *46*, 1333.
5. Deylová D.: *M.Sc. Thesis*. Charles University, Prague 2008.
6. Rhemrev-Boom M. M.: *J. Photogr. Sci.* **1988**, *36*, 53.
7. Wang, Kung-Tsung, Iris S. Y., Lin, Alice L.: *J. Chin. Chem. Soc.* **1966**, *13*, 77.
8. Selig W., Lawrence L.: *Mikrochim. Acta* **1979**, *1*, 453.
9. Barek J., Cvačka J., Muck A., Quaiserová V., Zima J.: *Fresenius J. Anal. Chem.* **2001**, *369*, 556.
10. Meloun M., Militky J., Forina M.: *Chemometrics for Analytical Chemistry, PC-Aided Regression and Related Methods*, Vol. 2, pp. 1–175. Ellis Horwood, Chichester 1994.
11. Fry A. J. in: *Chemistry of Amino, Nitroso and Nitro Compounds and Their Derivatives* (S. Patai, Ed.), p. 319. Wiley, Chichester 1982.
12. Kemula W., Krygowski T. M. in: *Encyclopedia of the Electrochemistry of the Elements – Organic Section* (A. J. Bard and H. Lund, Eds), Vol. 13, p. 77. Dekker, New York 1979.
13. Kolthoff I. M., Elving P. J.: *Treatise on Analytical Chemistry*, Part II, Vol. 16, p. 188. Wiley, New York 1980.
14. Ludvík J., Nygard B.: *Electrochim. Acta* **1996**, *41*, 1661.