POLAROGRAPHIC AND VOLTAMMETRIC STUDY OF GENOTOXIC 2,7-DINITROFLUOREN-9-ONE AND ITS DETERMINATION USING MERCURY ELECTRODES

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Received August 28, 2009
Accepted November 27, 2009

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

Electrochemical behavior of genotoxic 2,7-dinitrofluoren-9-one was investigated by DC polarography and DC tast polarography, both at a dropping mercury electrode, and by cyclic voltammetry at a hanging mercury drop electrode, in buffered aqueous-methanolic solutions. The number of exchanged electrons was determined by constant-potential coulometry at a mercury pool electrode. A possible mechanism of the electrochemical reduction has been proposed. Optimal conditions were found for the determination of 2,7-dinitrofluoren-9-one by DC tast polarography in the concentration range from 2×10^{-6} to 1×10^{-5} mol l^{-1} and by differential pulse polarography (from 2×10^{-7} to 1×10^{-5} mol l^{-1}), both at dropping mercury electrode, by differential pulse voltammetry (from 2×10^{-8} to 1×10^{-5} mol l^{-1}) and by adsorptive stripping voltammetry (from 2×10^{-9} to 1×10^{-7} mol l^{-1}), both at hanging mercury drop electrode. Practical applicability of the developed methods was verified on the direct determination of 2,7-dinitrofluoren-9-one in drinking water in 10^{-8} mol l^{-1} concentration range, and in drinking and river water (both in 10^{-9} mol l^{-1} concentration range) using preliminary separation and preconcentration by solid-phase extraction.

Keywords: 2,7-Dinitrofluoren-9-one; Nitro compounds; Mercury electrodes; Polarography; Voltammetry; Potentiostatic coulometry; Reduction mechanism; Electroanalysis; Solid-phase extraction; Electroreduction.

Polycyclic aromatic hydrocarbons (PAHs) are well-known environmental chemical carcinogens[1] frequently contaminating water, soil and sediments. Their persistence is a result of their low water solubility, low reactivity and non-bioavailability[2]. Nitrated PAHs (NPAHs), which constitute a widely occurring class of environmental pollutants, can be either directly emitted

Collect. Czech. Chem. Commun. 2009, Vol. 74, Nos. 11–12, pp. 1675–1696

doi:10.1135/cccc2009108

from combustion sources such as diesel or gasoline engines[3,4], or formed from their parent PAHs by radical reactions initiated with atmospheric OH or NO_3 radicals[4]. Oxygenated PAHs (OPAHs) are mainly emitted in combustion processes. However, they are also produced by heterogeneous reactions of particulate associated PAHs with ozone[5] or as metabolites of PAHs in bacterial and fungal degradation[6–8]. Since it has been shown that mononitro and dinitro PAHs and/or OPAHs can be much more mutagenic and/or carcinogenic than their parent PAHs[9,10], the analysis of NPAHs or OPAHs in emission sources and in ambient air becomes important.

The studied compound, 2,7-dinitrofluoren-9-one (2,7-DNFN), belongs to the group of oxygenated NPAHs. Its presence in living environment is associated with oxidation and nitration of the parent PAH – fluorene[4]. The concentrations of 2,7-DNFN determined in extracts of exhaust particulates are 3.0 $\mu g\ g^{-1}$ and 8.6 $\mu g\ g^{-1}$ (ref.[11]). Although 2,7-DNFN is considered an environmental pollutant, this compound and its derivatives also have found applications in industry, since they are (or were) used as base materials for charge-transfer complexes in liquid-crystal display elements[12–15], for nonlinear optical materials in second-harmonic generation devices[16–18], for radiation-tolerant polymer films[19–21] or as a mediating system in electrochemical sensors[22,23].

2,7-DNFN is toxic in several bioassays and has a mutagenic potential[10,24–26]. That is why the need for extremely sensitive and selective methods for 2,7-DNFN (and NPAHs or OPAHs generally) determination is still growing. Current analytical methods of 2,7-DNFN determinations are focused on analysis of liquid samples using liquid chromatography with UV-Vis or MS detection[27] or using HPLC with UV-Vis spectrophotometric detection[28]. In contrast, the analysis of contaminated (or spiked) solid or water samples is neglected. Modern electrochemical analytical methods thus become suitable alternatives for these sample types[29–31]. The polarographic behavior of 2,7-DNFN at dropping mercury electrode has been studied in the sixties of the last century[32]. However, the exploitation of the reducibility of nitro groups in 2,7-DNFN for analytical purposes has been investigated only recently[28,33].

The aim of this study is to find optimal conditions for the determination of trace amounts of 2,7-DNFN using modern polarographic and voltammetric methods, namely direct current tast polarography (DCTP) and differential pulse polarography (DPP) at classical dropping mercury electrode (DME), differential pulse voltammetry (DPV) and adsorptive stripping voltammetry (AdSV), both at hanging mercury drop electrode (HMDE), and to verify their practical applicability on model samples of drinking and river water or on these samples preconcentrated by solid-phase extraction (SPE). 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 substance. Therefore, DC polarography (DCP) at DME, DCTP at DME, cyclic voltammetry (CV) at HMDE, and constant-potential coulometry (CPC) at a mercury pool electrode (MPE) were used to investigate the mechanism of electrochemical reduction of 2,7-DNFN at mercury electrodes.

All the used electroanalytical techniques are well described in monographs[34–36]. Great sensitivity, especially of DPP, DPV and AdSV, and a relatively low price of instrumentation, in comparison with LC-MS instrumentation, are most important advantages of these methods.

EXPERIMENTAL

Reagents

A stock solution of 2,7-dinitrofluoren-9-one (97%, Sigma–Aldrich, Prague, Czech Republic) in methanol ($c = 2 \times 10^{-4}$ mol l^{-1}) was prepared by dissolving 0.0270 g of the substance in 500 ml of methanol (99%, p.a. purity; Lachema, Brno, Czech Republic). A UV-Vis spectrophotometric study demonstrated that the stock solution is stable for at least one year[28]. The low concentration of 2,7-DNFN was used due to its limited solubility in methanol (MeOH). More dilute solutions were prepared by dilution of the stock solution; fresh dilute solutions were prepared every day. Britton–Robinson (BR) buffers were prepared in a 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 an appropriate amount of 0.2 M sodium hydroxide solution (all of p.a. purity; Lachema, Brno, Czech Republic). Other chemicals (hydrochloric acid, potassium chloride, all of p.a. purity) were supplied by Lachema, Brno, Czech Republic. Deionized water produced by a Milli Qplus system (Millipore, USA) was used. All solutions were kept in glass vessels in the dark at laboratory temperature.

Apparatus

DCP measurements were carried out using a PO 4 Polariter polarograph (Radiometer, Copenhagen, Denmark) with an integrated analog chart recorder. DCTP, DPP, DPV, AdSV, CV and CPC measurements were carried out using an Eco-Tribo electrochemical analyzer driven by PolarPro 5.1 software (all Polaro-Sensors, Prague, Czech Republic). The software worked under the operational system Microsoft Windows XP Professional (Microsoft Corporation, USA).

DCP measurements were carried out in a two-electrode system mercury|mercury(I) chloride reference electrode (saturated KCl solution) and with classical DME as working electrode. The mercury drop lifetime was 2.0 s, the height of the mercury reservoir was 81 cm (the flow rate of mercury through the capillary was 4.09 mg s^{-1}); the scan rate 200 mV min^{-1} was used. DCTP, DPP, DPV, AdSV, CV and CPC measurements were carried out in a three-electrode system – platinum electrode PPE as auxiliary electrode, silver|silver chloride reference electrode RAE 113 (1 M KCl) (both from Monokrystaly, Turnov, Czech Republic) and an appropriate working mercury electrode. For DCTP and DPP, classical DME was used as working electrode – the electronically controlled mercury drop lifetime was 1.0 s, the height of the mercury reservoir was 36 cm (mercury drop lifetime was 1.8 s at this height, measured in 0.1 M KCl at zero potential; the flow rate of mercury through the capillary was 6.96 mg s^{-1}); scan rate 4 mV s^{-1} was used. For DPV, AdSV and CV, a miniaturized HMDE of the UMμE type (Polaro-Sensors, Prague, Czech Republic), was used as working electrode; the valve opening time was 300 ms, the mercury drop surface was 0.0196 cm^2. The pulse amplitude –50 mV, pulse width 100 ms and scan rate 10 mV s^{-1} were used in DPV and AdSV. Unless stated otherwise, the scan rate 50 mV s^{-1} was used in CV. In CPC, MPE was used as working electrode with MPE surface 2.41 cm^2 and sampling rate 1.0 s.

pH was measured using the Conductivity & pH meter Jenway 4330 (Jenway, U.K.) with a combined glass electrode. For SPE techniques, LiChrolut columns RP-18 E (1000 mg, Merck, Darmstadt, Germany) were used.

Procedures

The general procedure to obtain polarograms/voltammograms was as follows. An appropriate amount of 2,7-DNFN stock solution in MeOH was measured into a polarographic vessel, MeOH was added to the total volume 5.0 ml and the solution was filled up to 10.0 ml with BR buffer of appropriate pH. For CPC, a modified polarographic vessel with large surface MPE was used and the measured solution was mechanically stirred during the analysis (20 min). A decrease in concentration of 2,7-DNFN during electrolysis at constant potential (corresponding to the limiting convection current of the first, second or third wave) was monitored by DPV at HMDE. The amount of 2,7-DNFN coulometrically reduced was calculated from this DPV peak decrease; the number of exchanged electrons (z) was calculated from the charge passed (Q) and the amount of substance reduced using Faraday constant $F = 96485.4$ C mol^{-1} (ref.[34]).

Before each polarographic, voltammetric and/or coulometric measurement, oxygen was removed from the measured solutions by bubbling with nitrogen (purity 4.0; Linde, Prague, Czech Republic) for 5 min. All curves were measured three times. The wave heights, i.e. limiting diffusion currents (I_{lim}), recorded using DCP and DCTP, were evaluated from the extrapolated linear portions of the currents. The peak heights (I_p) recorded using DPP, DPV and AdSV were evaluated from the straight lines connecting the minima before and after the peak. The parameters of calibration curves (slope, intercept, confidence intervals) and other mathematical and statistical quantities (for significance level $\alpha = 0.05$) were calculated using software OriginPro 7.5 (OriginLab Corporation, 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[37].

Model Samples

The drinking water from the public water line in the building of Faculty of Science of Charles University in Prague or the river water from Vltava river in Prague spiked with an appropriate amount of 2,7-DNFN stock solution was used for model samples. The procedure for DP voltammetric determination of 2,7-DNFN in model samples of drinking or river water after SPE was as follows. An SPE column was connected to a vacuum manifold and activated by washing with 3 ml of MeOH and 3 ml of deionized water. Afterwards, the model water sample spiked with different amounts of 2,7-DNFN was sucked through the column using volumetric flasks as sample reservoirs and polytetrafluoroethylene tubing for connecting the reservoirs and SPE columns. The adsorbed analyte was then eluted with 5.0 ml of MeOH, the solution was made up to 10.0 ml with BR buffer of pH 11.0 and, after deaeration with nitrogen, DP voltammograms were recorded. The recoveries were calculated from the ratio I_{pI}/I_{p0}, where I_{pI} is the peak height of the analyte after SPE and I_{p0} is the peak height of a reference solution prepared by addition of a standard solution of the studied analyte to the blank solution.

RESULTS AND DISCUSSION

Electrochemical Studies of 2,7-Dinitrofluoren-9-one at Mercury Electrodes – The Electrode Reduction Mechanism

Influence of pH on electrochemical behavior of 2,7-DNFN. The influence of pH on polarographic behavior of 2,7-DNFN was investigated using DCTP in a solution of MeOH–BR buffer (1:1). It can be seen in Figs 1, 2 and 3 that 2,7-DNFN gives two or three well-developed irreversible waves (Table I). At pH of the aqueous-methanolic solution (pH*) 2.7 (Fig. 1) and 4.0, three well-separated waves (*I*, *II* and *III*) can be observed. In the pH* range 4.9–8.2 it is possible to differentiate two waves (at pH* 4.9 the wave (*I*) is not

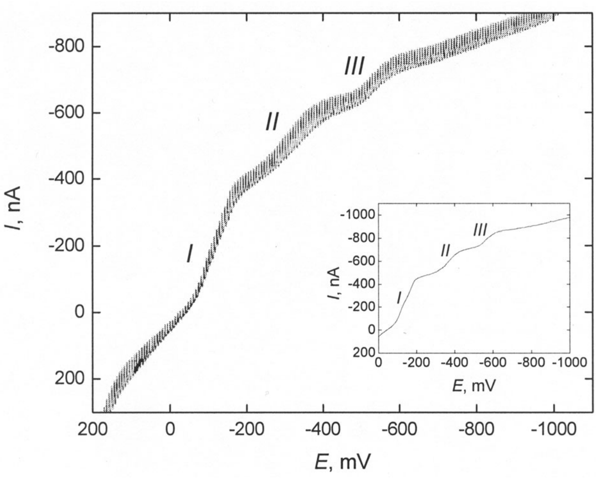


FIG. 1
DC polarogram of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at DME in MeOH–BR buffer of pH 2.0 (1:1). DCT polarogram of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at DME in MeOH–BR buffer of pH 2.0 (1:1) is in the inset. For waves description, see text

splitted while the next wave (*II* + *III*) is partly splitted but can be evaluated as one wave. At pH* 5.8 the wave (*I*) is not splitted; nor the wave (*II* + *III*). At pH* 7.0–8.2 (Fig. 2), the wave (*I*) is partly splitted but can be evaluated as

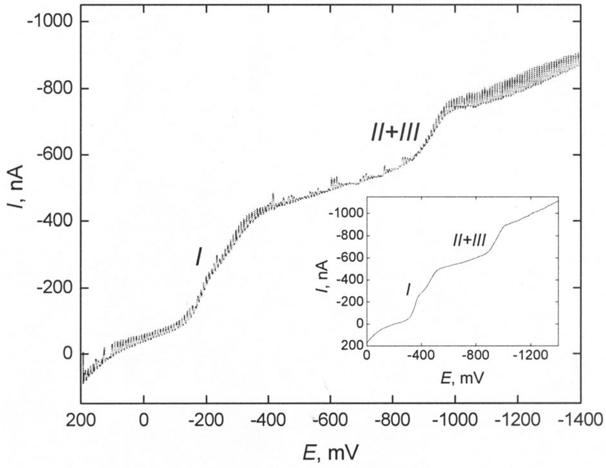


FIG. 2

DC polarogram of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at DME in MeOH–BR buffer of pH 6.0 (1:1). DCT polarogram of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at DME in MeOH–BR buffer of pH 6.0 (1:1) is in the inset. For waves description, see text

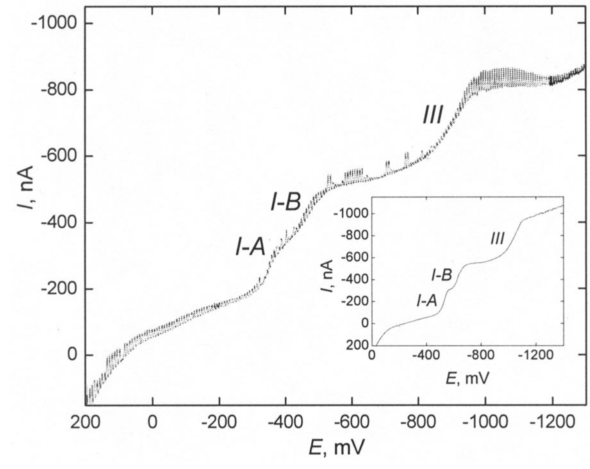


FIG. 3

DC polarogram of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at DME in MeOH–BR buffer of pH 11.0 (1:1). DCT polarogram of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at DME in MeOH–BR buffer of pH 11.0 (1:1) is in the inset. For waves description, see text

TABLE I
The effect of pH on DCT polarograms of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) at DME in MeOH–BR buffer (1:1)

pH of BR buffer	pH*	$E_{1/2}^{\mathrm{I}}$ mV	$I_{\mathrm{lim}}^{\mathrm{I}}$ nA	$E_{1/2}^{\mathrm{I\text{-}A}}$ mV	$I_{\mathrm{lim}}^{\mathrm{I\text{-}A}}$ nA	$E_{1/2}^{\mathrm{I\text{-}B}}$ mV	$I_{\mathrm{lim}}^{\mathrm{I\text{-}B}}$ nA	$E_{1/2}^{\mathrm{II}}$ mV	$I_{\mathrm{lim}}^{\mathrm{II}}$ nA	$E_{1/2}^{\mathrm{II+III}}$ mV	$I_{\mathrm{lim}}^{\mathrm{II+III}}$ nA	$E_{1/2}^{\mathrm{III}}$ mV	$I_{\mathrm{lim}}^{\mathrm{III}}$ nA
2.0	2.7	–170	–360	–[b]	–[b]	–[b]	–[b]	–400	–142	–[d]	–[d]	–600	–98
3.0	4.0	–220	–344	–[b]	–[b]	–[b]	–[b]	–490	–120	–[d]	–[d]	–670	–94
4.0	4.9	–260	–379	–[b]	–[b]	–[b]	–[b]	–[c]	–[c]	–670	–282	–[c]	–[c]
5.0	5.8	–300	–366	–[b]	–[b]	–[b]	–[b]	–[c]	–[c]	–790	–270	–[c]	–[c]
6.0	7.0	–360	–354	–[b]	–[b]	–[b]	–[b]	–[c]	–[c]	–[d]	–[d]	–880	–187
7.0	8.2	–410	–377	–[b]	–[b]	–[b]	–[b]	–[c]	–[c]	–[d]	–[d]	–940	–199
8.0	8.7	–[a]	–[a]	–370	–148	–520	–113	–[c]	–[c]	–[d]	–[d]	–950	–172
9.0	9.3	–[a]	–[a]	–410	–170	–560	–150	–[c]	–[c]	–[d]	–[d]	–980	–168
10.0	10.5	–[a]	–[a]	–450	–169	–600	–155	–[c]	–[c]	–[d]	–[d]	–1020	–169
11.0	11.2	–[a]	–[a]	–520	–193	–640	–191	–[c]	–[c]	–[d]	–[d]	–1020	–316
12.0	12.4	–[a]	–[a]	–530	–176	–640	–175	–[c]	–[c]	–[d]	–[d]	–1020	–318

pH*, pH of the BR buffer–MeOH (1:1) medium; $E_{1/2}$, half-wave potential; I_{lim}, limiting diffusion current. Superscripts and subscripts: *I*, first wave; *I-A*, first part of splitted first wave; *I-B*, second part of splitted first wave; *II*, second wave; *II + III*, merged second and third wave; *III*, third wave. [a] First wave (*I*) splitted into two separate waves (*I-A*) and (*I-B*); [b] splitting of first wave (*I*) not observed; [c] second (*II*) and/or third (*III*) wave not observed or merged into one wave (*II + III*); [d] merging of second (*II*) and third (*III*) wave not observed. Dashed lines separate different DCT polarographic behavior of 2,7-DNFN in acid, neutral and alkaline medium.

one wave; the wave (*II* + *III*) is not splitted. In the pH* range 8.7–12.4 (Fig. 3), three waves can be differentiated (*I-A*, *I-B* and *III*). It can be assumed that the reduction of 2,7-DNFN proceeds in two or three steps. These findings are well in agreement with DCP measurements, which were made to confirm the results of DCTP measurements and to provide confirmation of the DCP method used in ref.[32].

Determination of the exchanged-electron number in the electrode reduction of 2,7-DNFN. Using the Ilkovic equation we can estimate the number of exchanged electrons from limiting diffusion currents[36]. On the basis of an analogy with polarographic reduction of various dinitrofluorenes[32] and of the relative heights of the observed waves, we can assume that in acid medium (Fig. 1) 2,7-DNFN exchanges successively 8 electrons in the first wave (*I*), 4 electrons in the second wave (*II*) and 2 electrons in the third wave (*III*). In neutral medium (Fig. 2), we can assume that 2,7-DNFN exchanges successively 8 electrons in the first wave (*I*) (or 4 + 4 electrons in the case of partly splitted wave (*I*)) and 4 electrons in the second wave (*II* + *III*). In alkaline medium (Fig. 3), 2,7-DNFN exchanges successively 4 electrons in the first wave (*I-A*), 4 electrons in the second wave (*I-B*) and 6 electrons in the third wave (*III*). The ratio of limiting diffusion currents of individual waves corresponds to the presumed numbers of exchanged electrons in the corresponding steps.

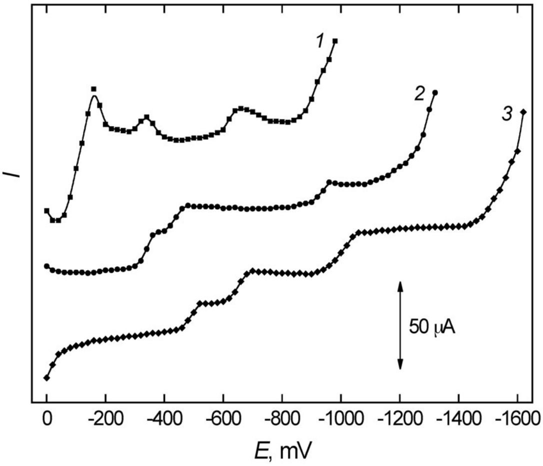


FIG. 4
Voltammograms of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded point by point at MPE in stirred MeOH–BR buffer (1:1) medium; resulting pH: 2.7 (*1*), 7.0 (*2*) and 11.2 (*3*). Potential step 20 mV

The number of exchanged electrons was further determined by CPC. Constant-potential electrolysis of 2,7-DNFN was carried out in a coulometric cell. Voltammetric curves of 2,7-DNFN at MPE (Fig. 4) were measured, point by point, in stirred acid (pH* 2.7), neutral (pH* 7.0) and alkaline (pH* 11.2) MeOH–BR buffer (1:1) media. The behavior of *I*–*E* curves at MPE is very similar to that of the obtained polarographic curves. Electrolysis potentials (E_E) were selected to correspond to the 2,7-DNFN limiting convection current of the first, second or third wave (Table II). The obtained numbers of exchanged electrons were rounded off to the nearest integers. In acid media 2,7-DNFN exchanges total 14 electrons, in neutral media 12 electrons and in alkaline medium 14 electrons.

Proposed mechanism of electrochemical reduction of 2,7-DNFN. Cyclic voltammetric measurements of 2,7-DNFN were used to obtain an overall view of electrochemical behavior of the tested substance. Three consecutive cathodic/anodic CV scans (at different switching potentials, E_S) of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) at HMDE in acid (pH* 2.7), neutral (pH* 7.0) and alkaline (pH* 11.2) medium of MeOH–BR buffer (1:1) were measured to observe reduction/oxidation behavior of 2,7-DNFN and its intermediates (Figs 5, 6 and 7).

TABLE II
Constant-potential coulometry of 2,7-DNFN at MPE in acid, neutral and alkaline media

Medium	E_E, mV	Q, mC	Δn, mol	z
BR buffer pH 2.0–MeOH (1:1) pH* 2.7[a]	–240	45.9	5.8×10^{-8}	8.2
	–440	62.1	5.5×10^{-8}	11.7
	–800	75.3	5.7×10^{-8}	13.7
BR buffer pH 6.0–MeOH (1:1) pH* 7.0[b]	–560	40.4	5.1×10^{-8}	8.2
	–1040	57.4	5.0×10^{-8}	11.9
BR buffer pH 11.0–MeOH (1:1) pH* 11.2[b]	–240	13.8	3.5×10^{-8}	4.1
	–740	25.9	3.4×10^{-8}	7.9
	–1160	50.2	3.8×10^{-8}	13.7

E_E, potential of electrolysis for CPC corresponding to the limiting current of the first, second or third wave (Fig. 4); Q, consumed charge; Δn, amount of electrochemically reduced substance; z, number of exchanged electrons; [a] the decrease of peak current evaluated from the second peak of 2,7-DNFN; [b] the decrease of peak current evaluated from the first peak of 2,7-DNFN.

Gary and Day[32], who studied the polarographic reduction of 2,7-DNFN in a buffered water–acetone mixture at pH 1.4, 4.3, 6.6, 7.7, 10.4 and 11.3 (pH values of the used buffer), found at pH 1.4 one eight-electron wave followed by a four-electron wave and a two-electron wave. At pH 4.3 they found one eight-electron wave followed by a four-electron wave and at pH 6.6 two consecutive four-electron waves followed by a two-electron wave. They determined coulometrically the number of exchanged electrons only in acid and neutral media, not in alkaline medium. Our findings in a mixture of water and MeOH (1:1) are different. On the basis of our results we have proposed a scheme of electrochemical reduction of 2,7-DNFN different in the acid medium (Scheme 1), in neutral medium (Scheme 2) and in alkaline medium (Scheme 3).

In the acid medium (see Fig. 5 and Scheme 1), first cathodic peak p_I corresponds to four-electron reduction of one nitro group to hydroxyamino group and second peak p_{II} corresponds to four-electron reduction of the other nitro group to hydroxyamino group in the presence of the previously formed hydroxyamino group (this reduction step corresponds to the first polarographic eight-electron wave (*I*) (Fig. 1), which splits above pH* 7.0 into two four-electron waves (*I-A*) and (*I-B*) (Fig. 2). The splitting of the first polarographic wave can be explained by the fact that the first nitro group is

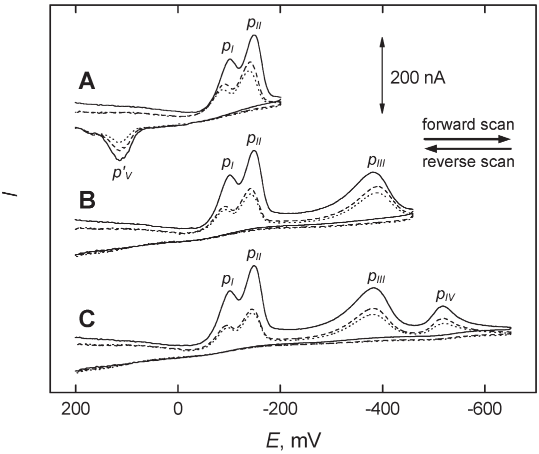
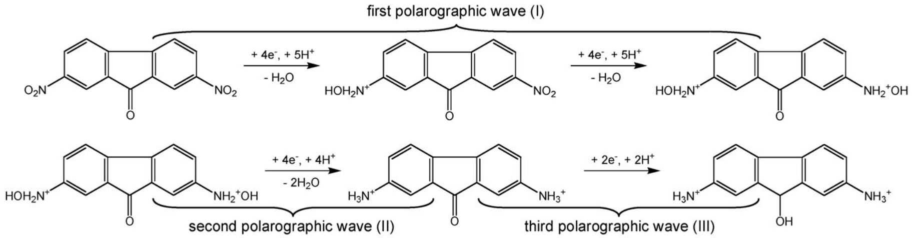
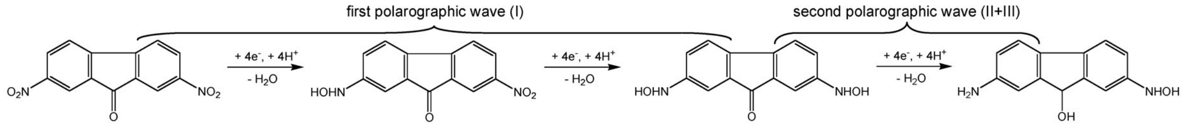


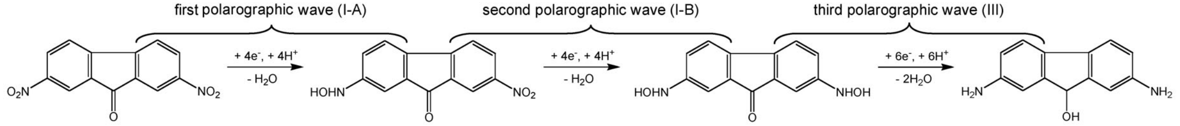
FIG. 5
Cyclic voltammograms of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at HMDE in the MeOH–BR buffer of pH 2.0 (1:1) medium at scan rate 50 mV s^{-1}. First E_S –200 mV (A), –460 mV (B) and –650 mV (C). Continuous line represents first scan, dashed line second scan and dotted line third scan. For peaks description, see text



SCHEME 1
Proposed electrochemical reduction of 2,7-DNFN in acid medium



SCHEME 2
Proposed electrochemical reduction of 2,7-DNFN in neutral medium



SCHEME 3
Proposed electrochemical reduction of 2,7-DNFN in alkaline medium

reduced in the presence of the second nitro group with a pronounced negative mesomeric (–M) effect, which decreases the electron density in the region of the first nitro group thus making its reduction easier. The second nitro group is reduced in the presence of protonated $–NH_2^+OH$ group (in acid media) or unprotonated –NHOH group (in neutral and alkaline media). The $–NH_2^+OH$ group with the positive mesomeric (+M) effect increases the electron density in the region of the second nitro group thus making its polarographic reduction more difficult. This assumption is in agreement with the observed fact that the splitting of the first eight-electron wave (*I*) is higher in the alkaline than in acid region. The protonated $–NH_2^+OH$ group withdraws electrons from the other nitro group thus making its reduction easier, i.e. shifts its half-wave potential closer to the first wave.

Gary and Day[32] suppose that the third cathodic peak p_{III} in acid media corresponds to simultaneous four-electron reduction of one hydroxyamino group to the amino group and the oxo group to the secondary alcoholic group. The fourth peak p_{IV} in acid media corresponds to the two-electron reduction of the second hydroxyamino group to amino group. Our findings are different in this case; while the first E_S is set immediately after both nitro groups are reduced to two hydroxyamino groups (Fig. 5A), the anodic peak p'_V appears in the first reverse scan; this peak corresponds to the well-known step of the hydroxyamino group oxidation to nitroso group[38]. This anodic peak is not observed while E_S is set after cathodic peaks p_{III} (Fig. 5B) or p_{IV} (Fig. 5C); these findings indicate that both the hydroxyamino groups are first reduced to amino groups (p_{III}) and the peak p_{IV} must thus correspond to a single two-electron reduction of the oxo group to secondary alcohol. The second and third CV scans in acid medium only copy the first CV scan with an observable decrease in the peaks height as the amount of 2,7-DNFN is irreversibly reduced. The linear dependence of peak currents (in the first cathodic scan) on the square root of the scan rate confirms the diffusion control of the observed processes.

In the neutral pH region (Fig. 6 and Scheme 2), cathodic peaks p_{VI} and p_{VIII} represent the same two four-electron $–NO_2$/–HNOH reductions as in acid medium. We suggest that the reduction process assumed by Gary and Day[32] for the acid medium is accurately valid for neutral medium in this case. It means that the peak p_{VII} involves four-electron simultaneous reduction of one hydroxyamino to amino group and of oxo group to secondary alcohol. Another cathodic peak at more negative potentials was not observed. The reduction process was thus stopped in a stable state, where one hydroxyamino group remains unreduced and the other hydroxyamino group was reduced to amino group. The presence of an unreduced hydroxyamino group is indicated by the reversible anodic peak p'_{IX}, which can be assigned to two-electron oxidation of the hydroxyamino group to nitroso group (–NO). The cathodic peak p_{IX} was observed in the second and third cathodic scan around –150 mV, which is in agreement with the well-known reversibility of the –NO/–NHOH system in neutral pH region[38]. Lund[39] has made reference to electrochemical reduction of an aromatic hydroxyamino group in the way that the –NHOH very rapidly loses water to form the easily reducible quinone mono- or diimine. This loss of water takes place faster in acid and alkaline solutions and somewhat more slowly in neutral pH region. This mechanism can elucidate a different electrochemical behavior of hydroxyamino groups in electrochemical reduction of 2,7-DNFN in neutral medium, in contrast to an acid and alkaline medium.

On the basis of second and third CV scan in neutral medium, a probable mechanism of subsequent redox steps has been suggested. As mentioned above, the first cathodic CV scan gives the peak of formation of 2,7-bis(hydroxyamino)fluoren-9-one at potential around –700 mV. While the first E_S is set at –700 mV (Fig. 6A), the subsequent anodic reverse scan indicates oxidation of hydroxyamino to nitroso group. At the moment when the unprotonated hydroxyamine form and nitroso form are present at HMDE surface together, a condensation reaction of hydroxyamino and nitroso

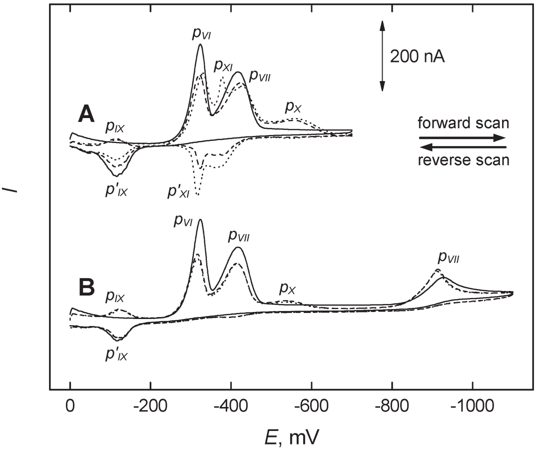


FIG. 6

Cyclic voltammograms of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at HMDE in the MeOH–BR buffer of pH 6.0 (1:1) medium at scan rate 50 mV s^{-1}. First E_S –700 mV (A) and –1100 mV (B). Continuous line represents first scan, dashed line second scan and dotted line third scan. For peaks description, see text groups can take place to give azoxy group[39]. The azoxy group is then reduced to hydrazo group in the second cathodic CV scan (peak p_X) and the formed hydrazo group is oxidized in the reversible oxidation to azo group (peak p'_{XI}), which is by return reduced back to hydrazo group (peak p_{XI}) in the third cathodic CV scan. In the third anodic scan in neutral medium, we can observe an increase in peak p'_{XI}. This fact indicates that the amount of hydrazo derivative is increasing partly by reduction of azoxy group (peak p_X) and partly by reduction of azo group (peak p_{XI})[39]. It is probable that both hydroxyamino groups take part in these reactions because, when the first E_S is set at –1100 mV (Fig. 6B), only reversible –NHOH/–NO redox pair (peaks p'_{IX}/p_{IX}) is clearly observed in the second and third CV scan.

In alkaline medium (Fig. 7 and Scheme 3), the reduction is similar to that in acid medium (peaks p_{XII} and p_{XIII} represent two four-electron reductions of nitro groups). Whereas the reduction from 2,7-bis(hydroxyamino)-fluoren-9-one to 2,7-diaminofluoren-9-ol proceeds in two consecutive steps in acid medium, simultaneous six-electron reduction is observed in alkaline medium. Peak p_{XIV} corresponds to this reduction step. The presumption that no hydroxyamino group is present after the last reduction step is supported by the absence of the reversible –NHOH/–NO system peak when first E_S is set at –1300 mV. While using first E_S –800 mV, a flicker of the presence of

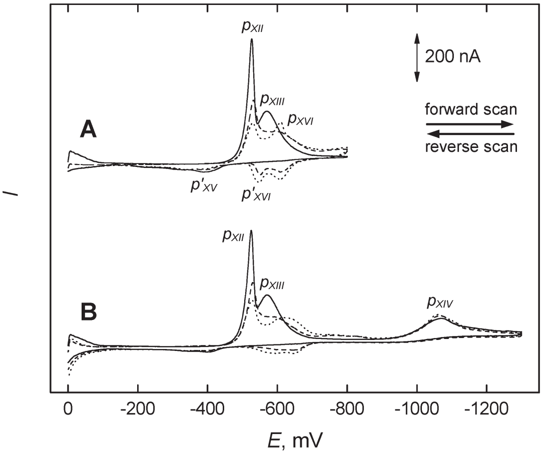


FIG. 7
Cyclic voltammograms of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at HMDE in the MeOH–BR buffer pH 11.0 (1:1) medium at scan rate 50 mV s^{-1}. First E_S –800 mV (A) and –1300 mV (B). Continuous line represents first scan, dashed line second scan and dotted line third scan. For peaks description, see text

the –NHOH/–NO system represents anodic peak p'_{XV}, and peaks p_{XVI} and p'_{XVI} represent the reversible redox azo/hydrazo system mentioned above.

Polarographic and Voltammetric Determination of 2,7-Dinitrofluoren-9-one at Mercury Electrodes

DC tast polarography at dropping mercury electrode. The influence of pH on DCT polarographic behavior of 2,7-DNFN was mentioned above. We have observed a different polarographic behavior of 2,7-DNFN in the acid, neutral and alkaline pH region; therefore, media with resulting pH 2.7, 7.0 and 11.2 were used for the construction of calibration curves in the concentration range from 2×10^{-6} to 1×10^{-5} mol l^{-1}. We have evaluated DCT polarograms for calibration curves in two ways. Firstly, we evaluated the limiting diffusion current of polarographic wave (I) or of the sum of the polarographic waves (I-A) and (I-B). Secondly, we evaluated the limiting diffusion current of the sum of all waves. The calibration curves parameters are summarized in Table III.

Differential pulse polarography at dropping mercury electrode. In good agreement with polarographic behavior of 2,7-DNFN in DCTP at DME, 2,7-DNFN gives four well-developed cathodic peaks at pH* 2.7 and 4.0 (the first and second peaks are not completely separated in the whole pH* region from 2.7 to 12.4, the third peak disappears at pH* from 4.9 to 12.4) in DPP at DME. Three peaks are observed at pH* from 4.9 to 12.4. Analogously to DCTP investigation, we used media with resulting pH 2.7, 7.0 and 11.2 for construction of calibration curves. The highest and best developed peak at the lowest concentrations of 2,7-DNFN was the first one, which was further evaluated (in the concentration range from 2×10^{-7} to 1×10^{-5} mol l^{-1}) to obtain data for calibration curves (see Table III).

Differential pulse voltammetry at hanging mercury drop electrode. It follows from DP voltammograms (Fig. 8) that 2,7-DNFN gives four well-developed cathodic peaks at pH* 2.7 and 4.0, three peaks being observed at pH* 4.9–12.4. The best developed first peak was obtained in the MeOH–BR buffer pH 11.0 (1:1) medium, which was further used for construction of calibration curves (Fig. 9) in concentration range from 2×10^{-8} to 1×10^{-5} mol l^{-1}. The parameters of calibration curves are summarized in Table III.

Adsorptive stripping voltammetry at hanging mercury drop electrode. The influence of pH on electrochemical behavior of 2,7-DNFN ($c = 2 \times 10^{-7}$ mol l^{-1}) using AdSV at HMDE was investigated in a mixture of MeOH and BR buffer of appropriate pH (2.0–12.0) in the volume ratio 1:9. A low volume of MeOH in the mixture was chosen due to the known competitive adsorption of MeOH at the surface of mercury electrode. The potential of accumulation was changed from 0 mV at pH* 2.2 to –400 mV at pH* 12.0, always before the onset of corresponding voltammetric peak. Best developed voltammograms were obtained in the MeOH–BR buffer pH 11.0 (1:9) medium. The time of accumulation (t_{acc}) was chosen 60 s (peaks were well developed, longer accumulation times were not useful). As optimal potential of accumulation (E_{acc}) was chosen –300 mV. The highest and best developed peak at the lowest concentrations of 2,7-DNFN was the first one (Fig. 10); first and second peaks merged at lowest concentrations. The calibration curves were constructed for the concentration range from 2×10^{-9} to 1×10^{-7} mol l^{-1}. Higher concentrations of 2,7-DNFN were not measured due to the possibility of using DPV or DPP technique (Table III).

TABLE III
Parameters of calibration straight lines for determination of 2,7-DNFN

Technique	Medium	Concentration mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	R	L_Q mol l^{-1}
DCTP at DME	MeOH–BR buffer (1:1), pH* 2.7	$(2–10) \times 10^{-6}$ [a]	–49.3	–8.4	–0.9960	1×10^{-6}
		$(2–10) \times 10^{-6}$ [b]	–109.8	–5.0	–0.9995	2×10^{-6}
	MeOH–BR buffer (1:1), pH* 7.0	$(2–10) \times 10^{-6}$ [a]	–35.6	–16.5	–0.9992	2×10^{-6}
		$(2–10) \times 10^{-6}$ [b]	–69.7	–17.7	–0.9996	1×10^{-6}
	MeOH–BR buffer (1:1), pH* 11.2	$(2–10) \times 10^{-6}$ [a]	–20.1	–4.8	–0.9999	1×10^{-6}
		$(2–10) \times 10^{-6}$ [b]	–64.3	–5.4	–0.9989	2×10^{-6}
DPP at DME	MeOH–BR buffer (1:1), pH* 2.7	$(2–10) \times 10^{-6}$ [c]	–53.7	–5.1	–0.9999	–
		$(2–10) \times 10^{-7}$ [c]	–59.6	–0.9	–0.9991	2×10^{-7}
	MeOH–BR buffer (1:1), pH* 7.0	$(2–10) \times 10^{-6}$ [c]	–49.8	–10.6	–0.9999	–
		$(2–10) \times 10^{-7}$ [c]	–66.4	–1.6	–0.9993	2×10^{-7}
	MeOH–BR buffer (1:1), pH* 11.2	$(2–10) \times 10^{-6}$ [c]	–45.1	–2.2	–0.9999	–
		$(2–10) \times 10^{-7}$ [c]	–46.0	–1.0	–0.9989	2×10^{-7}
DPV at HMDE	MeOH–BR buffer (1:1), pH* 11.2	$(2–10) \times 10^{-6}$ [c]	–37.3	–4.1	–0.9995	–
		$(2–10) \times 10^{-7}$ [c]	–33.1	–0.2	–0.9992	–
		$(2–10) \times 10^{-8}$ [c]	–29.4	–0.1	–0.9963	2×10^{-8}
AdSV at HMDE	MeOH–BR buffer (9:1), pH* 11.1	$(2–10) \times 10^{-8}$ [c]	–126.0	–0.1	–0.9987	–
		$(2–10) \times 10^{-9}$ [c]	–120.9	–0.1	–0.9943	4×10^{-9}

R, correlation coefficient; L_Q, limit of quantification; [a] limiting diffusion current evaluated from polarographic wave *I* or from the sum of polarographic waves *I-A* and *I-B*; [b] limiting diffusion current evaluated from the sum of all polarographic waves; [c] peak current evaluated from the first peak.

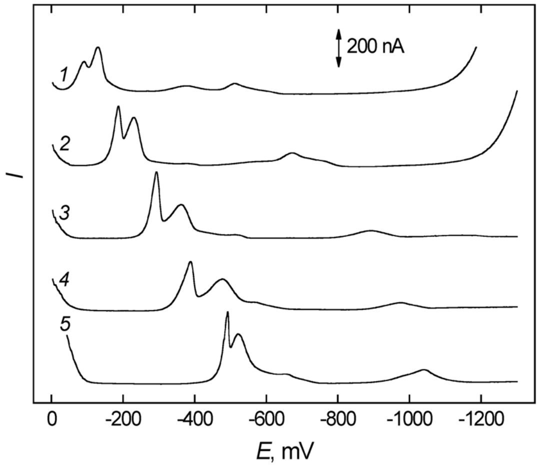


FIG. 8
DP voltammograms of 2,7-DNFN ($c = 1 \times 10^{-5}$ mol l^{-1}) recorded at HMDE in the MeOH–BR buffer (1:1) medium; resulting pH: 2.7 (*1*), 4.9 (*2*), 7.0 (*3*), 9.3 (*4*), 11.2 (*5*). Scan rate 10 mV s^{-1}

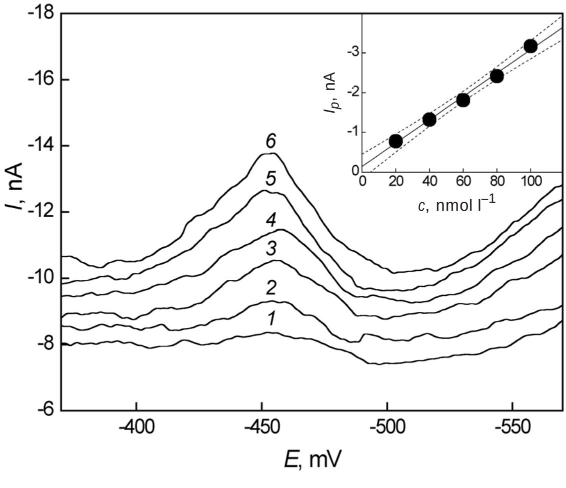


FIG. 9
DP voltammograms of 2,7-DNFN in the lowest concentration range recorded at HMDE in the MeOH–BR buffer pH 11.0 (1:1) medium; scan rate 10 mV s^{-1}. Concentrations of 2,7-DNFN (mol l^{-1}): 0 (*1*), 2×10^{-8} (*2*), 4×10^{-8} (*3*), 6×10^{-8} (*4*), 8×10^{-8} (*5*) and 1×10^{-7} (*6*). The corresponding calibration straight line (constructed using the first voltammetric peak) is in the inset. The confidence bands are constructed for $\alpha = 0.05$

Determination of 2,7-Dinitrofluoren-9-one in Drinking and River Water

The optimum conditions found above for DPV determination of 2,7-DNFN were used for direct determination of 2,7-DNFN in model samples of drinking water. The BR buffer pH 11.0 was replaced by 0.001 M NaOH for simplification (9.0 ml of spiked drinking water was filled up to 10.0 ml with 0.01 M NaOH). It was found that the calibration curve (Fig. 11) is linear (in the concentration range from 2×10^{-8} to 1×10^{-7} mol l^{-1}) and its parameters are presented in Table IV.

Furthermore, we tried to preconcentrate 2,7-DNFN using SPE. Recovery parameters were measured using samples of spiked deionized water and MeOH as eluent. Recoveries of SPE of 2,7-DNFN from 50 ml ($c = 1 \times 10^{-7}$ mol l^{-1}) and 500 ml ($c = 1 \times 10^{-8}$ mol l^{-1}) of deionized water were evaluated using DPV at HMDE. The values were 97% (about 10-fold preconcentration) and 95% (about 100-fold preconcentration), respectively. 2,7-DNFN was then extracted from 500 ml of spiked drinking water ($c = 1 \times 10^{-8}$ mol l^{-1}) with recovery 95% (extraction from 50 ml of drinking water was not tested due to the possibility of using direct determination in the identical concentration

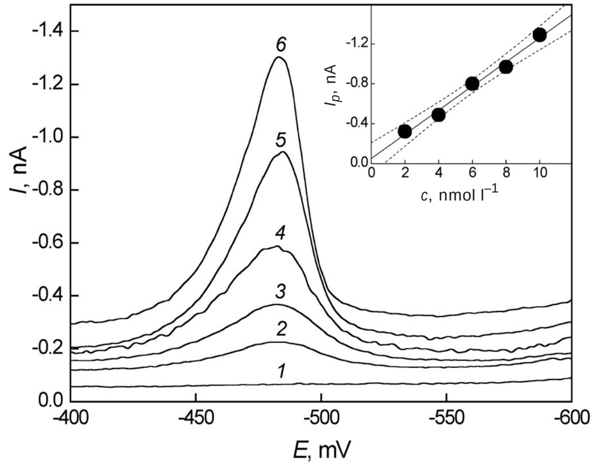


FIG. 10

AdS voltammograms of 2,7-DNFN in the lowest concentration range recorded at HMDE in the MeOH–BR buffer pH 11.0 (1:9) medium; t_{acc} = 60 s, E_{acc} = –300 mV, scan rate 10 mV s^{-1}. Concentrations of 2,7-DNFN (mol l^{-1}): 0 (*1*), 2×10^{-9} (*2*), 4×10^{-9} (*3*), 6×10^{-9} (*4*), 8×10^{-9} (*5*) and 1×10^{-8} (*6*). The corresponding calibration straight line (constructed using the first voltammetric peak) is in the inset. The confidence bands are constructed for $\alpha = 0.05$

TABLE IV
Parameters of calibration straight lines for determination of 2,7-DNFN in model samples of water

Technique	Matrix Medium	Concentration mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	R	L_Q mol l^{-1}
DPV at HMDE, SPE from 50 ml of deionized water	deionized water MeOH–BR buffer (9:1), pH* 11.2	$(2–10) \times 10^{-8\,a}$	–266.0	–0.1	–0.9997	1×10^{-8}
DPV at HMDE, SPE from 500 ml of deionized water	deionized water MeOH–BR buffer (9:1), pH* 11.2	$(2–10) \times 10^{-9\,a}$	–3059.2	–0.1	–0.9998	2×10^{-9}
DPV at HMDE, direct determination in drinking water	drinking water 0.001 M NaOH	$(2–10) \times 10^{-8\,a}$	–22.3	–0.1	–0.9995	2×10^{-8}
DPV at HMDE, SPE from 500 ml of drinking water	drinking water MeOH–BR buffer (9:1), pH* 11.2	$(2–10) \times 10^{-9\,a}$	–1816.4	–0.02	–0.9983	2×10^{-9}
DPV at HMDE, SPE from 50 ml of river water	river water MeOH–BR buffer (9:1), pH* 11.2	$(2–10) \times 10^{-8\,a}$	–182.0	–0.2	–0.9982	3×10^{-8}
DPV at HMDE, SPE from 500 ml of river water	river water MeOH–BR buffer (9:1), pH* 11.2	$(2–10) \times 10^{-9\,a}$	–2267.3	–0.2	–0.9966	4×10^{-9}

R, correlation coefficient; L_Q, limit of quantification; [a] first peak evaluated.

range), from 50 ml of spiked river water ($c = 1 \times 10^{-7}$ mol l^{-1}) with recovery 85% and from 500 ml of spiked river water ($c = 1 \times 10^{-8}$ mol l^{-1}) with recovery 82%. Parameters of extraction and parameters of the obtained regression dependences are presented in Table IV (the differences in digit places of slopes are due to the use of original values of concentrations of the orders 10^{-8} and 10^{-9} mol l^{-1} before 10-fold or 100-fold SPE preconcentration of 2,7-DNFN).

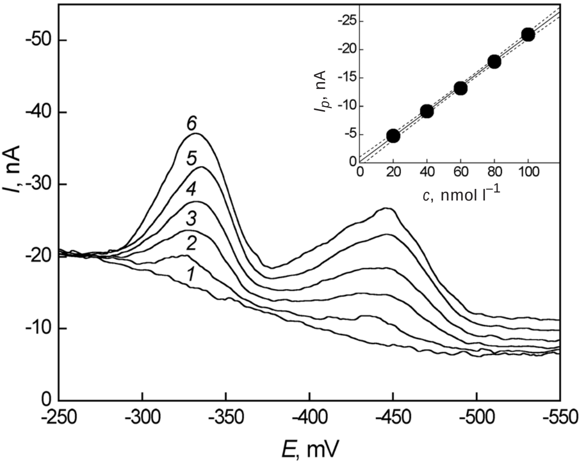


FIG. 11
DP voltammograms of 2,7-DNFN in model samples of drinking water recorded at HMDE in the 0.001 M NaOH medium; scan rate 10 mV s^{-1}. Concentrations of 2,7-DNFN (mol l^{-1}): 0 (*1*), 2×10^{-8} (*2*), 4×10^{-8} (*3*), 6×10^{-8} (*4*), 8×10^{-8} (*5*) and 1×10^{-7} (*6*). The corresponding calibration straight line (constructed using the first voltammetric peak) is in the inset. The confidence bands are constructed for $\alpha = 0.05$

CONCLUSIONS

Electrochemical reduction of the nitro and oxo groups of genotoxic 2,7-dinitrofluoren-9-one at mercury electrodes has been studied in buffered aqueous-methanolic solutions of pH 2–12. A possible mechanism of electrochemical reduction of 2,7-dinitrofluoren-9-one has been discussed and proposed. Furthermore, 2,7-dinitrofluoren-9-one has been determined using polarographic and voltammetric techniques at mercury electrodes. Nanomolar concentrations of this compound can be determined not only in deionized water but also in model matrix of drinking or river water. Thus the presented sensitivity of determination of 2,7-dinitrofluoren-9-one illustrates the usefulness of mercury electrodes in current analytical chemistry and confirms that mercury electrodes are still useful electrochemical sensors.

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (projects LC 06035, MSM 0021620857, and RP 14/63).

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