POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF GENOTOXIC 2-AMINOFLUOREN-9-ONE AT MERCURY ELECTRODES

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Dedicated to Prof. Ing. Karel Štulík, DrSc. on the occasion of his 70th birthday.

Electrochemical behavior of genotoxic 2-aminofluoren-9-one (2-AFN) was investigated by DC tast polarography (DCTP) and differential pulse polarography (DPP), both at a classical dropping mercury electrode (DME), and by DC voltammetry (DCV), differential pulse voltammetry (DPV), and adsorptive stripping differential pulse voltammetry (AdSDPV), all at a miniaturized hanging mercury drop minielectrode (HMDmE), in buffered aqueous-methanolic solutions. Optimum conditions were found for the determination of 2-AFN by DCTP at DME in the concentration range from 1×10^{-6} to 1×10^{-4} mol l^{-1} (with a limit of quantification (L_Q) of 5×10^{-7} mol l^{-1}), by DPP at DME (from 1×10^{-7} to 1×10^{-4} mol l^{-1}; $L_Q \approx 1 \times 10^{-7}$ mol l^{-1}), by DCV and DPV at HMDmE (both from 1×10^{-7} to 1×10^{-4} mol l^{-1}; L_Qs $\approx 2 \times 10^{-7}$ and 1×10^{-7} mol l^{-1} for DCV and DPV, respectively), and by AdSDPV at HMDmE (from 2×10^{-9} to 1×10^{-7} mol l^{-1}; $L_Q \approx 4 \times 10^{-9}$ mol l^{-1}). Practical applicability of the developed methods was verified on the direct determination of 2-AFN in model samples of drinking and river water in nanomolar to micromolar concentrations.

Keywords: Analytical methods; Electrochemistry; Polarography; Voltammetry; Mercury electrodes; 2-Aminofluoren-9-one; Spiked water samples.

Oxo and/or amino functional group containing derivatives of polycyclic aromatic hydrocarbons (PAHs) are well-known environmental chemical carcinogens and/or mutagens frequently contaminating water, soil, and sediments[1,2]. Therefore, the need of continuous monitoring of such environmental pollutants should be raised to the highest priority[2]. Oxygenated

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PAHs (OPAHs) are mainly emitted in combustion processes[1]. However, they are also produced by heterogeneous reactions of particulate-associated PAHs with ozone[3] or as metabolites of PAHs in bacterial and fungal degradation[4–6]. In comparison to OPAHs, amino derivatives of PAHs (APAHs) are mainly of anthropogenic origin, because they are very useful chemicals from an industrial point of view[2]. Therefore, they can be found in a variety of workplaces and also in the industrial effluents. APAHs have been further identified in crude oil and oil distillation products, as well as in cigarette smoke and river water[7]. Furthermore, APAHs are metabolites of their parent nitrated PAHs (NPAHs) (refs[8,9]) and can be formed in small amounts by photoreduction of NPAHs in the atmosphere containing low concentration of oxygen and high concentration of hydrogen donors, e.g., in wood smoke[10].

The studied compound – 2-aminofluoren-9-one (2-AFN; Fig. 1) – is a biologically active and genotoxic substance[11], which belongs to the group of amino derivatives of the OPAHs [12]. Its occurrence in the environment is associated mainly with processing and purification of the natural gas in gas plants (ground water samples taken from these sites often contain a great variety of aromatic and heterocyclic compounds[13] and their metabolites[14–16]). 2-AFN was identified using liquid chromatography–mass spectrometry (LC-MS) in ground waters sampled in the surrounding of these facilities[17]. In another study[18], ground water obtained from sediments collected near an oil refinery discharge was toxic to *Lumbriculus variegatus* following exposure to UV light[19], while organisms exposed to the same ground water, but without subsequent UV treatment, showed no toxic effect. Phototoxic fractions analyzed by gas chromatography–mass spectrometry (GC-MS) revealed, among others, the presence of 2-AFN. Dorie et al.[20] also identified 2-AFN using GC-MS in HPLC fractions of heavy duty diesel exhaust particle extracts.

In living organisms, 2-AFN was shown to be one of the metabolites of 2-nitrofluorene[21–24], since it was identified using LC-MS in urine from rats treated with 2-nitrofluorene[25,26]. The genotoxic effect of 2-AFN as a metabolite was investigated *in vitro* using ^{32}P-postlabeled DNA adducts and the formation of a complex DNA–2-AFN was confirmed[11]. Under specific conditions, the presence of such adduct can then result in the formation of mutations in the DNA structure and lead to irreversible DNA damage[27].

Although several analytical techniques were used for the identification of 2-AFN [17–20], the sensitive method for its determination, to the best of our knowledge, has not yet been reported. Taking into account that 2-AFN contains two electrochemically active functional groups – cathodically reducible oxo group and anodically oxidizable amino group – bonded to the

aromatic rings of fluorene, modern polarographic and voltammetric techniques can be utilized for its sensitive determination[22,28–30].

In this study, mercury electrodes, which are believed to be the most suitable working electrodes for the determination of polarographically reducible substances because of their easily renewable and atomically smooth surface and wide cathodic potential window[29,31–34], were used. Gary and Day[35], the only ones who previously studied the polarographic reduction of 2-AFN, have found that in a buffered water–acetone mixture at pH values of the used buffer from 1.3 to 12.0, 2-AFN is reduced in a single two-electron step throughout the acid and neutral range, and that a separation of corresponding polarographic wave into two waves was not observed until the pH was raised to about 11. At pH 12.0, the waves were well separated into two one-electron steps[35]. Such polarographic behavior probably represents the electrochemical reduction of the oxo group to the secondary alcoholic group, as it is known from the polarographic behavior of unsubstituted fluoren-9-one[36–38].

The aim of this study was to find optimum conditions for the determination of trace amounts of 2-AFN using modern polarographic and voltammetric methods, namely DC tast polarography (DCTP) and differential pulse polarography (DPP) at a classical dropping mercury electrode (DME), DC voltammetry (DCV), differential pulse voltammetry (DPV), and adsorptive stripping differential pulse voltammetry (AdSDPV), all at a miniaturized hanging mercury drop minielectrode (HMDmE), and to verify their practical applicability on model samples of drinking and river water. All the used electroanalytical techniques are well described in monographs[39–41]. Great sensitivity, especially of DPP, DPV, and AdSDPV [42], and a relatively low price of instrumentation, in comparison with LC-MS or GC-MS instrumentation, are the most important advantages of these methods[43,44].

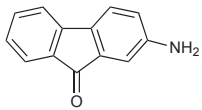

FIG. 1
Structural formula of 2-aminofluoren-9-one

EXPERIMENTAL

Reagents

A stock solution of 2-aminofluoren-9-one (2-AFN; 98%, Sigma–Aldrich, Prague, Czech Republic) in methanol (c = 1 × 10^{-3} mol l^{-1}) was prepared by dissolving 0.0488 g of the pure

substance in 250 ml of methanol (MeOH; 99.9%, p.a. purity, Merck, Darmstadt, Germany). A UV-Vis spectrophotometric study demonstrated that the methanolic stock solution is stable for at least three months[45]. Dilute solutions were prepared by dilution of the stock solution with MeOH. Britton–Robinson (BR) buffers were prepared in a usual way[28]; boric acid, phosphoric acid (85%), acetic acid (99%), and sodium hydroxide, all of p.a. purity, were supplied by Lachema, Brno, Czech Republic. An acetate buffer of a concentration of 0.2 mol l^{-1} and pH 4.0 was prepared by dissolving the calculated amount of sodium acetate trihydrate (p.a. purity, Lachema, Brno, Czech Republic) in the solution of 0.2 mol l^{-1} acetic acid. Deionized water produced by a Milli-Q Plus system (Millipore, Billerica, MA, USA) was used. All the solutions were stored in glass vessels in the dark at laboratory temperature.

Apparatus

All electrochemical measurements were carried out using an Eco-Tribo electrochemical analyzer driven by Polar Pro 5.1 software (all 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 – platinum wire auxiliary electrode (type PPE), silver|silver chloride reference electrode (type RAE 113, 1 mol l^{-1} KCl) (both from Monokrystaly, Turnov, Czech Republic) and an appropriate working mercury electrode.

For DCTP and DPP, 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 81 cm (mercury drop lifetime was 3.4 s at this height, measured in 0.1 mol l^{-1} KCl at zero potential, and the flow rate of mercury through the capillary was 2.22 mg s^{-1}); the scan rate 4 mV s^{-1} was used. For DPP, the pulse amplitude –50 mV and the pulse width 100 ms (with current sampling for the last 20 ms) were used.

For DCV, DPV, and AdSDPV, miniaturized HMDmE of the UMμE type (Polaro-Sensors, Prague, Czech Republic) was used as the working electrode – the valve opening time was 450 ms, the mercury drop surface was 1.42 mm^2, and the flow rate of mercury through the capillary was 5.92 mg s^{-1}; the scan rate 20 mV s^{-1} was used. The pulse amplitude –50 mV and the pulse width 100 ms (with current sampling for the last 20 ms) were used in DPV and AdSDPV.

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

Procedures

The general procedure to obtain polarograms or voltammograms was as follows: An appropriate amount of 2-AFN stock solution in MeOH was measured into a voltammetric cell, MeOH was added, if necessary, to the total volume 1.0 ml and the solution was filled up to 10.0 ml with BR buffer of 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 bubbler containing a MeOH–deionized water mixture in the same ratio as in the measured solution (1:9); deionized water alone was used in the bubbler when model water samples were measured.

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 AdSDPV were evaluated from the straight lines connecting the minima before and after the peak. The DPP and DPV peak areas (Q_p) were integrated using a straight line connecting the minima before and after the peak as a baseline. The parameters of calibration curves (i.e., slope, intercept, correlation coefficient, and confidence intervals) and other mathematical and statistical quantities (all for significance level α = 0.05; ref.[46]) were calculated using software Origin Pro 8.0 (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[47].

Model Samples

The drinking water from the public water pipeline in the building of Faculty of Science of Charles University in Prague, Prague, Czech Republic and the river water from the Labe river in Nymburk, Czech Republic (both used without further pretreatment or purification), spiked with an appropriate amount of 2-AFN stock solution, were used for model samples. The procedure for the polarographic or voltammetric determination of 2-AFN in the model samples was as follows: 9.0 ml of a model water sample, spiked with an appropriate amount of 2-AFN, were filled up to 10.0 ml with 0.2 mol l^{-1} acetate buffer pH 4.0 (acetate buffer was used instead of BR buffer pH 4.0 for simplification) and, after deaeration with nitrogen, DP polarograms at DME or DC, DP, or AdSDP voltammograms at HMDmE were recorded.

RESULTS AND DISCUSSION

DC Tast Polarography and Differential Pulse Polarography at Dropping Mercury Electrode

The influence of pH on polarographic behavior of 2-AFN was investigated using DCTP and DPP in solutions of MeOH–BR buffer (1:9). It can be seen in Fig. 2a that 2-AFN gave one well-developed cathodic wave in the whole investigated pH* (pH of the MeOH–BR buffer (1:9) medium) range from 2.0 to 12.7 (the wave is partly split at the pH* values higher than 3.2); the half-wave potential ($E_{1/2}$) of this first wave varied with pH according to the relationship $E_{1/2}$ [mV] = –56.2 pH* – 421.7 (correlation coefficient, R = –0.9951). At a pH* higher than 7.3, the second polarographic wave was observed, with $E_{1/2} \approx$ –1300 mV. However, the second wave was not suitable for analytical purposes because of its lower I_{lim} and deformed shape. The basic concept of the mechanism of polarographic reduction of 2-AFN at DME was presented in the introduction of this paper. Nevertheless, this

early study[35] published 50 years ago deserves to be extended and, therefore, the detailed investigation of the reduction mechanism of 2-AFN at mercury electrodes is in progress now.

The highest and best-developed first wave was obtained in the medium of MeOH–BR buffer pH 4.0 (1:9) (pH* 4.1), in which linear calibration dependences were obtained in the whole investigated concentration range from 1×10^{-6} to 1×10^{-4} mol l^{-1} of 2-AFN. The repeatability of the determination of 2-AFN ($c = 1 \times 10^{-4}$ mol l^{-1}) using DCTP at DME, expressed in the term of the relative standard deviation (RSD), was 0.35% ($n = 10$). The parameters of the calibration curves are summarized in Table I.

In good agreement with polarographic behavior of 2-AFN in DCTP at DME, 2-AFN gave one well-developed cathodic DPP peak at DME in the pH* range from 2.0 to 12.7 (Fig. 2b), which shifted towards more negative potentials with increasing pH. The peak potential (E_p) of this peak varied with

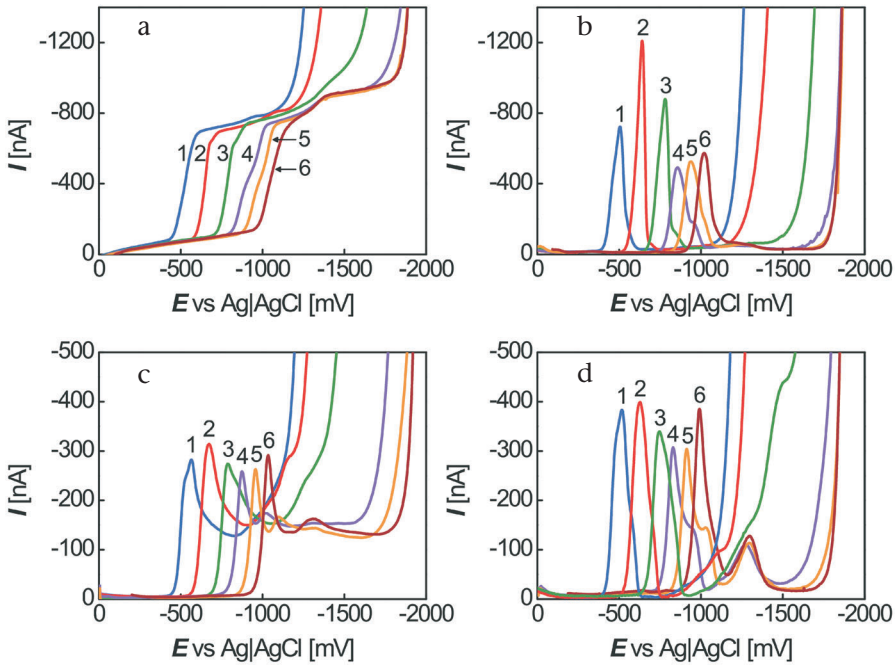

FIG. 2

DCT polarograms at DME (a), DP polarograms at DME (b), DC voltammograms at HMDmE (c), and DP voltammograms at HMDmE (d) of 2-AFN ($c = 1 \times 10^{-4}$ mol l^{-1}) recorded in the MeOH–BR buffer (1:9) medium; the BR buffer pH: 2.0 (1), 4.0 (2), 6.0 (3), 8.0 (4), 10.0 (5), and 12.0 (6)

TABLE I
Parameters of the calibration straight lines for the determination of 2-AFN using various polarographic and voltammetric techniques

Technique	Medium	Concentration mol l^{-1}	Slope[a] mA mol^{-1} l	Intercept[a] nA	R	L_Q mol l^{-1}
DCTP at DME	MeOH–BR buffer pH 4.0 (1:9)	2×10^{-5} – 1×10^{-4}	–5.61 ± 0.10	–19.1 ± 6.9[b]	–0.9993	–
		1×10^{-6} – 1×10^{-5}	–5.34 ± 0.13	–0.85 ± 0.80[b]	–0.9985	5×10^{-7}
DPP at DME	MeOH–BR buffer pH 4.0 (1:9)	2×10^{-5} – 1×10^{-4}	–[c,d]	–[c,d]	–[c,d]	–
		2×10^{-6} – 1×10^{-5}	–19.36 ± 0.35	4.9 ± 2.3[b]	–0.9994	–
		1×10^{-7} – 1×10^{-6}	–14.46 ± 0.25	–0.43 ± 0.15[b]	–0.9993	1×10^{-7}
DCV at HMDmE	MeOH–BR buffer pH 4.0 (1:9)	2×10^{-5} – 1×10^{-4}	–1.723 ± 0.042	–123.1 ± 2.8	–0.9988	–
		2×10^{-6} – 1×10^{-5}	–[c,e]	–[c,e]	–[c,e]	–
		1×10^{-7} – 1×10^{-6}	–34.4 ± 1.1	–0.29 ± 0.65[b]	–0.9976	2×10^{-7}
DPV at HMDmE	MeOH–BR buffer pH 4.0 (1:9)	2×10^{-5} – 1×10^{-4}	–2.068 ± 0.061	–169.3 ± 4.0	–0.9983	–
		2×10^{-6} – 1×10^{-5}	–[c,f]	–[c,f]	–[c,f]	–
		1×10^{-7} – 1×10^{-6}	–47.3 ± 1.2	0.72 ± 0.75[b]	–0.9983	1×10^{-7}
AdSDPV at HMDmE	0.2 mol l^{-1} acetate buffer pH 4.0	2×10^{-8} – 1×10^{-7}	–898 ± 22	–0.9 ± 1.4[b]	–0.9989	–
		2×10^{-9} – 1×10^{-8}	–859 ± 26	–0.35 ± 0.86[b]	–0.9981	4×10^{-9}

[a] Intervals represent the lower and upper confidence limits ($\alpha = 0.05$); [b] intercepts are not statistically significantly different from zero at the significance level $\alpha = 0.05$; [c] concentration dependences with the non-linear trend expressed by formal polynomial equations: [d] I_p [nA] = (0.147 ± 0.014)c^2 [μmol^2 l^{-2}] + (–26.9 ± 1.7)c [μmol l^{-1}] + (101 ± 45), R = –0.9989; [e] I_p [nA] = (0.77 ± 0.12)c^2 [μmol^2 l^{-2}] + (–16.2 ± 1.4)c [μmol l^{-1}] + (–39.6 ± 3.7), R = –0.9986; [f] I_p [nA] = (1.54 ± 0.44)c^2 [μmol^2 l^{-2}] + (–26.2 ± 5.3)c [μmol l^{-1}] + (–44 ± 14), R = –0.9856. R, correlation coefficient; L_Q, limit of quantification (10σ; $\alpha = 0.05$).

pH according to the relationship E_p [mV] = –52.2 pH* – 410.2 (R = –0.9858). The second, and much lower, peak was observed at pH* from 10.0 to 12.7.

Analogously to DCTP investigation, the medium of MeOH–BR buffer pH 4.0 (1:9) was used for construction of calibration curves. The calibration curves were linear within the concentration range from 1×10^{-7} to 1×10^{-5} mol l^{-1} of 2-AFN and their parameters are summarized also in Table I. The calibration dependence for concentrations from 2×10^{-5} to 1×10^{-4} mol l^{-1} showed a non-linear polynomial trend (Fig. 3); the repeatability of the determination of 2-AFN at the highest concentration 1×10^{-4} mol l^{-1} was 0.38% (n = 10). On the other hand, the concentration dependence constructed using an integrated peak area (Q_p) was linear in this concentration range and could be described by formal linear equation Q_p [nC] = (–187.5 ± 1.4)c [μmol l^{-1}] + (–552 ± 95), R = –0.9999. Moreover, a linear shift in the peak potential towards more negative values with decreasing concentration was observed.

An explanation of this potential shift and non-linear concentration dependence on the peak current in the concentration range from 2×10^{-5} to 1×10^{-4} mol l^{-1} of 2-AFN can be seen in the electrode reaction and its mechanism. Probably, the reduction of 2-AFN involves two independent reduction steps at higher concentrations of 2-AFN. The first reduction step occurring at less negative potentials influences the symmetry of DPP peak (see Fig. 3) and, therefore, the I_p dependence on the concentration is non-linear, whereas the value of the peak area remains unaffected in this

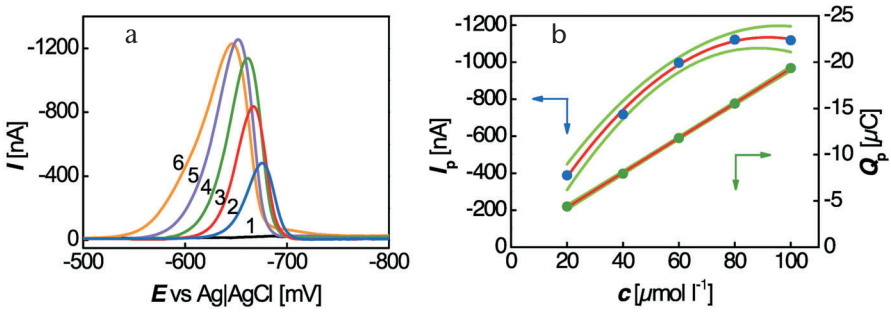

FIG. 3

a DP polarograms of 2-AFN recorded at DME in the MeOH–BR buffer pH 4.0 (1:9) medium. Concentrations of 2-AFN [mol l^{-1}]: 0 (1), 2×10^{-5} (2), 4×10^{-5} (3), 6×10^{-5} (4), 8×10^{-5} (5), and 1×10^{-4} (6). b The corresponding calibration curves constructed from the evaluated peak heights (blue points) and peak areas (green points); the confidence bands are constructed for α = 0.05 (n = 3)

case and the Q_p dependence on the concentration of 2-AFN is linear. For analytical purposes, the calibration dependence for lower concentrations of 2-AFN was also evaluated using the Q_p values. In the concentration range from 2×10^{-6} to 1×10^{-5} mol l^{-1} of 2-AFN, the dependence can be expressed by formal linear equation Q_p [nC] = (–234.6 ± 7.3)c [μmol l^{-1}] + (–313 ± 48), R = –0.9981. For the lowest investigated concentration range, the dependence of the Q_p values on the concentration of 2-AFN showed no simply describable trend.

The limits of quantification (L_Qs) attained for 2-AFN in DPP at DME using the I_p and Q_p values were 1×10^{-7} and 5×10^{-7} mol l^{-1}, respectively. Because of more complicated evaluation of DPP peak areas and higher L_Q reached, the classical evaluation of DPP responses from peak current seems to be more suitable for lower concentrations of 2-AFN.

DC Voltammetry and Differential Pulse Voltammetry at Hanging Mercury Drop Minielectrode

Electrochemical behavior of 2-AFN was further characterized using DCV and DPV at HMDmE. 2-AFN gave one well-developed cathodic DCV and DPV peak in the whole investigated pH* range from 2.0 to 12.7 (Figs 2c and 2d). The E_p value of these peaks varied with pH according to the relationships E_p [mV] = –46.6 pH* – 486.2 (R = –0.9985) and E_p [mV] = –46.9 pH* – 439.2 (R = 0.9983) for DCV and DPV, respectively. The second voltammetric peak (unsuitable for analytical purposes) was observed at pH* from 8.2 to 12.7. The calibration dependences evaluated from the first 2-AFN peak were measured, similarly as for polarographic techniques, in the mixed medium of MeOH–BR buffer pH 4.0 (1:9); the repeatabilities of the determination of 2-AFN at the highest concentration 1×10^{-4} mol l^{-1} were 0.62% (n = 10) and 1.0% (n = 10) for DCV and DPV, respectively.

For both DCV and DPV, the obtained calibration curves are linear within the concentration ranges from 1×10^{-7} to 1×10^{-6} mol l^{-1} and from 2×10^{-5} to 1×10^{-4} mol l^{-1} and their parameters are given in Table I. The calibration dependences for 2-AFN concentrations from 2×10^{-6} to 1×10^{-5} mol l^{-1} were not linear. This trend, similar to that observed in DPP at DME, is well seen in Fig. 4, where the whole concentration range, measured using DCV at HMDmE, is shown. An increased attention will also be paid to the investigation of this interesting phenomenon during our further research in this area.

As well as in the case of DPP at DME, the Q_p values can be used for construction of linear calibration curves of 2-AFN in DPV at HMDmE.

Such dependences can be expressed by formal linear equations Q_p [nC] = (–17.4 ± 1.0)c [μmol l^{-1}] + (–618 ± 35), R = –0.9981 (for 2 × 10^{-5}–1 × 10^{-4} mol l^{-1}) and Q_p [nC] = (–42.4 ± 2.3)c [μmol l^{-1}] + (–213 ± 16), R = –0.9940 (for 2 × 10^{-6}–1 × 10^{-5} mol l^{-1}). Unfortunately, the attained L_Q ≈ 1 × 10^{-6} mol l^{-1} is again higher in comparison with that obtained using the I_p values (L_Q ≈ 1 × 10^{-7} mol l^{-1}).

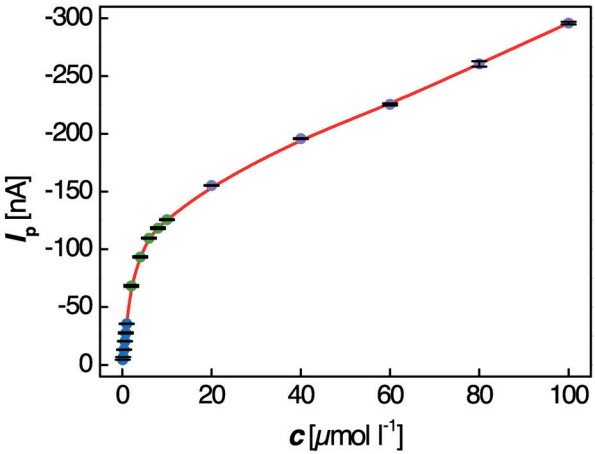

FIG. 4
Concentration dependence of the peak heights of 2-AFN obtained using DCV at HMDmE in the MeOH–BR buffer pH 4.0 (1:9) medium. The dependence is shown for the whole measured concentration range and is distinguished for particular concentration ranges from 1 × 10^{-7} to 1 × 10^{-6} mol l^{-1} (blue points), from 2 × 10^{-6} to 1 × 10^{-5} mol l^{-1} (green points), and from 2 × 10^{-5} to 1 × 10^{-4} mol l^{-1} (violet points) of 2-AFN. The error bars are constructed for α = 0.05 (n = 3)

Adsorptive Stripping Differential Pulse Voltammetry at Hanging Mercury Drop Minielectrode

For further decrease of the limit of quantification obtained for 2-AFN by DPV at HMDmE (L_Q ≈ 1 × 10^{-7}), a possible utilization of the adsorption of 2-AFN on the electrode surface was tested using AdSDPV at HMDmE. It has been previously found that the presence of methanol decreases the adsorption of the test substance on the surface of HMDmE (refs[29,42]). Therefore, the determinations of 2-AFN using AdSDPV were carried out in the absence of methanol in the supporting electrolyte.

The influence of pH on voltammetric behavior of 2-AFN (c = 1 × 10^{-7} mol l^{-1}) was investigated using AdSDPV at HMDmE in BR buffers of pH 2.0, 7.0, and 12.0 and in 0.2 mol l^{-1} acetate buffer pH 4.0. The accumulation po-

tential (E_{acc}) was changed from 200 mV at pH 2.0 to –400 mV at pH 12.0, always before the onset of corresponding voltammetric peak, and the accumulation time (t_{acc}) was 60 s. The best-developed voltammograms were obtained in 0.2 mol l^{-1} acetate buffer pH 4.0 at the optimum E_{acc} = 100 mV (Fig. 5a). Under these conditions, the optimum t_{acc} was chosen 120 s (Fig. 5b); peaks were well developed and longer accumulation times were not useful, probably because of concurrent adsorption of other substances present in very low concentrations in the supporting electrolyte.

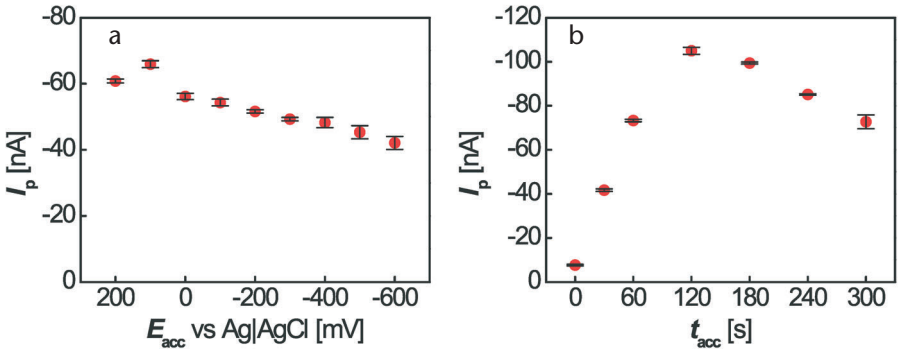

FIG. 5
Influence of (a) accumulation potential (AdSDPV with t_{acc} = 60 s) and (b) accumulation time (AdSDPV with E_{acc} = 100 mV) on the peak current of 2-AFN ($c = 1 \times 10^{-7}$ mol l^{-1}) at HMDmE in 0.2 mol l^{-1} acetate buffer pH 4.0. The error bars are constructed for $\alpha = 0.05$ ($n = 3$)

The calibration curves were constructed for the concentration range from 2×10^{-9} to 1×10^{-7} mol l^{-1}. The I_p value is proportional to the concentration of 2-AFN in the whole investigated concentration range (see Table I). AdSDP voltammograms corresponding to the lowest attainable concentration range are depicted in Fig. 6. Higher concentrations of 2-AFN were not measured due to the possibility of using DPV or DPP technique.

Direct Determination of 2-Aminofluoren-9-one in Drinking and River Water

The optimum conditions found above for polarographic and voltammetric determinations of 2-AFN using DPP at DME and DCV, DPV, and AdSDPV at HMDmE were used for direct determination of 2-AFN in model samples of drinking and river water. The BR buffer pH 4.0 was replaced by 0.2 mol l^{-1} acetate buffer pH 4.0 for simplification (9.0 ml of spiked drinking or river

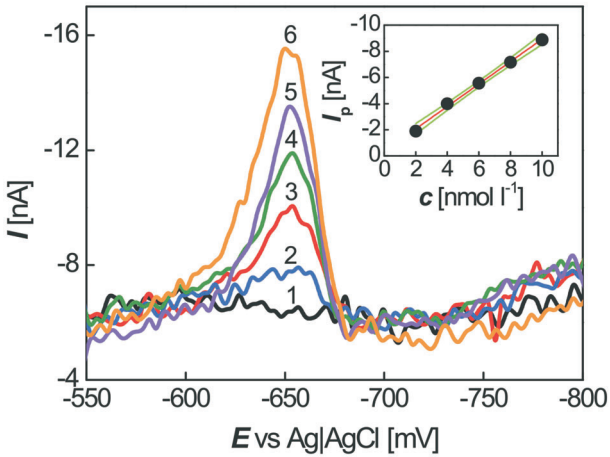

FIG. 6
AdSDP voltammograms of 2-AFN recorded in the lowest concentration range at HMDmE in 0.2 mol l^{-1} acetate buffer pH 4.0; t_{acc} = 120 s, E_{acc} = 100 mV. Concentrations of 2-AFN [mol l^{-1}]: 0 (1), 2 × 10^{-9} (2), 4 × 10^{-9} (3), 6 × 10^{-9} (4), 8 × 10^{-9} (5), and 1 × 10^{-8} (6). Inset: The corresponding calibration straight line; the confidence bands are constructed for α = 0.05 (n = 3).

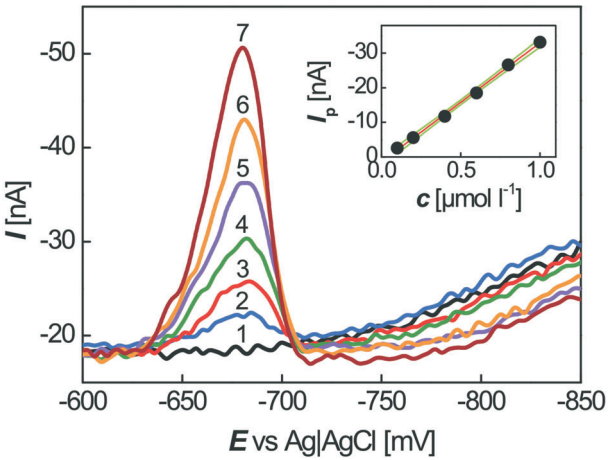

FIG. 7
DP voltammograms of 2-AFN recorded at HMDmE in the spiked river water–0.2 mol l^{-1} acetate buffer pH 4.0 (9:1) medium. Concentrations of 2-AFN in river water [mol l^{-1}]: 0 (1), 1 × 10^{-7} (2), 2 × 10^{-7} (3), 4 × 10^{-7} (4), 6 × 10^{-7} (5), 8 × 10^{-7} (6), and 1 × 10^{-6} (7). Inset: The corresponding calibration straight line; the confidence bands are constructed for α = 0.05 (n = 3).

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TABLE II

Parameters of the calibration straight lines for the determination of 2-AFN in model samples of water; measured polarografically or voltammetrically in the mixture of model water sample–0.2 mol l^{-1} acetate buffer pH 4.0 (9:1)

Technique	Matrix	Concentration mol l^{-1}	Slope[a] mA mol^{-1} l	Intercept[a,b] nA	R	L_Q mol l^{-1}
DPP at DME	drinking water	$2 \times 10^{-6} - 1 \times 10^{-5}$	–24.15 ± 0.96	2.29 ± 0.58	–0.9999	–
		$1 \times 10^{-7} - 1 \times 10^{-6}$	–20.84 ± 0.76	–1.24 ± 0.46	–0.9968	1×10^{-7}
	river water	$2 \times 10^{-6} - 1 \times 10^{-5}$	–23.23 ± 0.11	–0.64 ± 0.65	–0.9999	–
		$1 \times 10^{-7} - 1 \times 10^{-6}$	–20.23 ± 0.65	–1.08 ± 0.39	–0.9974	1×10^{-7}
DCV at HMDmE	drinking water	$2 \times 10^{-6} - 1 \times 10^{-5}$	–[c,d]	–[c,d]	–[c,d]	–
		$1 \times 10^{-7} - 1 \times 10^{-6}$	–36.04 ± 0.91	–1.67 ± 0.55	–0.9984	1×10^{-7}
	river water	$2 \times 10^{-6} - 1 \times 10^{-5}$	–[c,e]	–[c,e]	–[c,e]	–
		$1 \times 10^{-7} - 1 \times 10^{-6}$	–23.97 ± 0.74	0.84 ± 0.45	–0.9976	2×10^{-7}
DPV at HMDmE	drinking water	$2 \times 10^{-6} - 1 \times 10^{-5}$	–[c,f]	–[c,f]	–[c,f]	–
		$1 \times 10^{-7} - 1 \times 10^{-6}$	–49.3 ± 1.6	1.70 ± 0.99	–0.9973	8×10^{-8}
	river water	$2 \times 10^{-6} - 1 \times 10^{-5}$	–[c,g]	–[c,g]	–[c,g]	–
		$1 \times 10^{-7} - 1 \times 10^{-6}$	–34.37 ± 0.78	1.39 ± 0.47	–0.9987	2×10^{-7}
AdSDPV at HMDmE	drinking water	$2 \times 10^{-8} - 1 \times 10^{-7}$	–638 ± 33	–4.0 ± 2.2	–0.9847	–
		$2 \times 10^{-9} - 1 \times 10^{-8}$	–633 ± 31	–0.25 ± 0.21	–0.9951	4×10^{-9}
	river water	$2 \times 10^{-8} - 1 \times 10^{-7}$	–143.5 ± 8.8	–1.90 ± 0.59	–0.9825	2×10^{-8}

[a] Intervals represent the lower and upper confidence limits ($\alpha = 0.05$); [b] all intercepts are not statistically significantly different from zero at the significance level $\alpha = 0.05$; [c] concentration dependences with the non-linear trend expressed by formal polynomial equations: [d] I_p [nA] = (1.22 ± 0.40)c^2 [μmol^2 l^{-2}] + (–20.9 ± 4.8)c [μmol l^{-1}] + (–36 ± 13), R = –0.9821; [e] I_p [nA] = (0.54 ± 0.11)c^2 [μmol^2 l^{-2}] + (–12.6 ± 1.3)c [μmol l^{-1}] + (–31.2 ± 3.5), R = –0.9983; [f] I_p [nA] = (1.88 ± 0.59)c^2 [μmol^2 l^{-2}] + (–30.6 ± 7.2)c [μmol l^{-1}] + (–41 ± 19), R = –0.9778; [g] I_p [nA] = (0.92 ± 0.13)c^2 [μmol^2 l^{-2}] + (–18.7 ± 1.6)c [μmol l^{-1}] + (–39.0 ± 4.2), R = –0.9985. R, correlation coefficient; L_Q, limit of quantification (10σ; $\alpha = 0.05$).

water were filled up to 10.0 ml with 0.2 mol l^{-1} acetate buffer pH 4.0). All the parameters of measured calibration straight lines are summarized in Table II and, for the sake of an illustration, DP voltammograms of 2-AFN, obtained at HMDmE in the lowest measurable concentration range from 1×10^{-7} to 1×10^{-6} mol l^{-1} of 2-AFN spiked into the river water, are depicted in Fig. 7.

CONCLUSIONS

In this work, polarographic and voltammetric methods, based on the reduction of the aromatic oxo group at mercury electrodes, were developed for rapid and sensitive determination of the genotoxic environmental pollutant 2-aminofluoren-9-one (2-AFN). Electrochemical behavior of 2-AFN was studied in buffered aqueous-methanolic media (in the volume ratio of 9:1) in the pH range of the Britton–Robinson (BR) buffers used from 2.0 to 13.0. The optimum medium for its determination in micromolar and submicromolar concentrations using DC tast polarography (DCTP) and differential pulse polarography (DPP), both at a classical dropping mercury electrode (DME), and using DC voltammetry (DCV) and differential pulse voltammetry (DPV), both at a miniaturized hanging mercury drop minielectrode (HMDmE), was found to be methanol–BR buffer pH 4.0 (1:9). The obtained limits of quantification (L_Qs), all in the concentration order of 10^{-7} mol l^{-1} of 2-AFN, are comparable to those achieved using UV-Vis spectrophotometry (L_Qs $\approx 1 \times 10^{-7}$ mol l^{-1}; ref.[45]). An attempt to further increase the sensitivity of the determination using adsorptive stripping differential pulse voltammetry (AdSDPV) at HMDmE, under simplified optimum conditions (0.2 mol l^{-1} acetate buffer pH 4.0) with accumulation potential 100 mV and accumulation time 120 s, was successful, with the $L_Q \approx 4 \times 10^{-9}$ mol l^{-1} of 2-AFN.

The practical applicability of the newly developed methods (excluding DCTP at DME because of its lower sensitivity) was verified on model samples of drinking and river water, with L_Qs in the submicromolar and even nanomolar concentration range of 2-AFN. Extreme sensitivity of these methods can be further increased by their combination with a preliminary separation and preconcentration of the analyte using liquid–liquid or solid phase extraction[28], which is an object of our further investigations.

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REFERENCES

1. Bandowe B. A. M., Sobocká J., Wilcke W.: *Environ. Pollut.* **2011**, *159*, 539.
2. Vyskočil V., Barek J.: *Curr. Org. Chem.* **2011**, *15*, 3059.
3. Tsapakis M., Stephanou E. G.: *Environ. Sci. Technol.* **2007**, *41*, 8011.
4. Wischmann H., Steinhart H.: *Chemosphere* **1997**, *35*, 1681.
5. Sepic E., Bricelj M., Leskovsek H.: *J. Appl. Microbiol.* **1997**, *83*, 561.
6. Chupungars K., Rerngsamran P., Thaniyavarn S.: *Int. Biodeterior. Biodegrad.* **2009**, *63*, 93.
7. Čížek K., Barek J., Fischer J., Pecková K., Zima J.: *Electroanalysis* **2007**, *19*, 1295.
8. Moller L.: *Environ. Health Perspect.* **1994**, *102*, 139.
9. Pothuluri J. V., Sutherland J. B., Freeman J. P., Cerniglia C. E.: *Appl. Environ. Microbiol.* **1998**, *64*, 3106.
10. Feilberg A., Nielsen T.: *Environ. Sci. Technol.* **2001**, *35*, 108.
11. Zeisig M., Moller L.: *Carcinogenesis* **1995**, *16*, 1.
12. Strniste G. F., Nickols J. W., Okinaka R. T., Whaley T. W.: *Carcinogenesis* **1986**, *7*, 499.
13. Lang K. F., Eigen I.: *Top. Curr. Chem.* **1967**, *8*, 91.
14. Cerniglia C. E., White G. L., Heflich R. H.: *Arch. Microbiol.* **1985**, *143*, 105.
15. Guerin W. F., Jones G. E.: *Appl. Environ. Microbiol.* **1988**, *54*, 929.
16. Cerniglia C. E., Campbell W. L., Freeman J. P., Evans F. E.: *Appl. Environ. Microbiol.* **1989**, *55*, 2275.
17. Edler B., Zwiener C., Frimmel F. H.: *Fresenius J. Anal. Chem.* **1997**, *359*, 288.
18. Kosian P. A., Makynen E. A., Monson P. D., Mount D. R., Spacie A., Mekenyan O. G., Ankley G. T.: *Environ. Toxicol. Chem.* **1998**, *17*, 1021.
19. Ankley G. T., Mekenyan O. G., Kosian P. A., Makynen E. A., Mount D. R., Monson P. D., Call D. J.: *SAR QSAR Environ. Res.* **1996**, *5*, 177.
20. Dorie L. D., Bagley S. T., Leddy D. G., Johnson J. H.: *Environ. Sci. Technol.* **1987**, *21*, 757.
21. Vyskočil V., Labuda J., Barek J.: *Anal. Bioanal. Chem.* **2010**, *397*, 233.
22. Vyskočil V., Navrátil T., Polášková P., Barek J.: *Electroanalysis* **2010**, *22*, 2034.
23. Vyskočil V., Navrátil T., Daňhel A., Dědík J., Krejčová Z., Škvorová L., Tvrdíková J., Barek J.: *Electroanalysis* **2011**, *23*, 129.
24. Prchal V., Vyskočil V., Daňhel A., Barek J., Wang J.: *Chem. Listy* **2011**, *105*, 217.
25. Moller L., Gustafsson J. A.: *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 681.
26. Moller L., Corrie M., Midtvedt T., Rafter J., Gustafsson J. A.: *Carcinogenesis* **1988**, *9*, 823.
27. Heflich R. H., Neft R. E.: *Mutat. Res.-Rev. Genet. Toxicol.* **1994**, *318*, 73.
28. Vyskočil V., Barek J.: *Collect. Czech. Chem. Commun.* **2009**, *74*, 1675.
29. Vyskočil V., Barek J.: *Crit. Rev. Anal. Chem.* **2009**, *39*, 173.
30. Armalis S., Novikova N., Kubiliene E., Zima J., Barek J.: *Anal. Lett.* **2002**, *35*, 1551.
31. Deýlová D., Barek J., Vyskočil V.: *Collect. Czech. Chem. Commun.* **2009**, *74*, 1443.
32. Štulík K., Pacáková V., Podolák M.: *J. Chromatogr.* **1983**, *262*, 85.
33. Štulík K.: *Electroanalysis* **1992**, *4*, 829.
34. Štulík K., Amatore C., Holub K., Mareček V., Kutner W.: *Pure Appl. Chem.* **2000**, *72*, 1483.
35. Gary J. T., Day R. A.: *J. Electrochem. Soc.* **1960**, *107*, 616.

36. Ashworth M.: *Collect. Czech. Chem. Commun.* **1948**, *13*, 229.
37. Day R. A., Milliken S. R., Shults W. D.: *J. Am. Chem. Soc.* **1952**, *74*, 2741.
38. Vyskočil V., Polášková P., Bologa P., Barek J. in: *Sensing in Electroanalysis* (K. Vytřas, K. Kalcher and I. Švancara, Eds), Vol. 4, p. 91. University of Pardubice, Pardubice 2009.
39. Rajeshwar K., Ibanez J. G.: *Environmental Electrochemistry: Fundamentals and Applications in Pollution Sensors and Abatement.* Academic Press, London 1997.
40. Bard A. J., Faulkner L. R.: *Electrochemical Methods: Fundamentals and Applications*, 2nd ed. Wiley, New York 2001.
41. Wang J.: *Analytical Electrochemistry*, 3rd ed. John Wiley & Sons, Hoboken 2006.
42. Barek J., Pecková K., Vyskočil V.: *Curr. Anal. Chem.* **2008**, *4*, 242.
43. Barek J., Pecková K., Vyskočil V.: *Chem. Listy* **2009**, *103*, 889.
44. Vyskočil V., Daňhel A., Fischer J., Novotný V., Deýlová D., Musilová-Karaová J., Maixnerová L., Pecková K., Barek J.: *Chem. Listy* **2010**, *104*, 1181.
45. Hájková A.: *BSc Thesis*. Charles University in Prague, Prague 2010.
46. Miller J. N., Miller J. C.: *Statistics and Chemometrics for Analytical Chemistry*, 5th ed. Pearson Education, Harlow 2005.
47. Harvey D. in: *Modern Analytical Chemistry* (K. T. Kane, Ed.), p. 96. McGraw-Hill, Toronto 2000.