6. APPENDIX I

VOLTAMMETRIC DETERMINATION OF 6-NITROBENZIMIDAZOLE IN THE PRESENCE OF SURFACTANTS

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Dedicated to the Dr. Michael Heyrovský on the occasion of his 80th birthday.

Determination of 6-nitrobenzimidazole by differential pulse voltammetry at a hanging mercury drop electrode, a polished silver solid amalgam electrode and a mercury meniscus modified silver solid amalgam electrode was studied in the presence of the surfactants Triton X-100, cetyltrimethylammonium bromide and sodium dodecyl sulfate. It was found that only cetyltrimethylammonium bromide at polished silver solid amalgam electrode increases the voltammetric signal. This fact was used for the determination of 6-nitrobenzimidazole in the concentration range from 1×10^{-7} to 1×10^{-4} mol l^{-1} by differential pulse voltammetry at polished silver solid amalgam electrode in the presence of cetyltrimethylammonium bromide (concentration 1×10^{-4} mol l^{-1}).

Keywords: 6-Nitrobenzimidazole; Triton X-100; Cetyltrimethylammonium bromide; Sodium dodecyl sulfate; Differential pulse voltammetry; Hanging mercury drop electrode; Polished silver solid amalgam electrode; Mercury meniscus modified silver solid amalgam electrode.

6-Nitrobenzimidazole (6-NBIA; Fig. 1) belongs to the group of genotoxic nitrated heterocyclic aromatic compounds. It can damage natural biological functions of living organisms. 6-NBIA was determined as a part of photographic processing solutions using DC and AC polarography[1] and its adsorptive and inhibitive properties have been studied in the area of metal corrosion protection[2]. It is proven carcinogen and mutagen[3]. The occur-

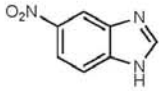

FIG. 1
Structural formula of 6-nitrobenzimidazole

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rence of 6-NBIA in the environment is connected with fossil fuels combustion[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].

The easy electrochemical reduction of nitro groups at the aromatic or heteroaromatic ring, whose mechanism is discussed in ref.[9], permits very sensitive determinations of a number of genotoxic and ecotoxic nitro compounds using modern voltammetric techniques such as differential pulse voltammetry (DPV) at a hanging mercury drop electrode (HMDE)[10] and adsorptive stripping voltammetry (AdSV) at HMDE [11]. Nevertheless, due to increasing fears of liquid mercury toxicity during the last decades[12], which has resulted in somewhat unreasonable "mercury phobia"[13], attention is paid to the development of solid electrodes (e.g., solid amalgam electrodes[14,15], solid composite electrodes[16], carbon paste electrodes[17] or boron-doped diamond film electrodes[18]), which can also be used for determination of various nitro compounds. From this point of view, working electrodes based on silver solid amalgam (p-AgSAE)[19] and modified by a mercury meniscus (m-AgSAE) represent a suitable non-toxic alternative to the traditional mercury electrodes. A good mechanical stability, simple handling and regeneration including an electrochemical pretreatment of the electrode surface are among the main advantages of these electrodes. The wide field of analytical applications of these electrodes has been reviewed[14,20]. The purpose of this work was to investigate how the presence of surfactants influences an electroanalytical response of 6-NBIA at HMDE, p-AgSAE and m-AgSAE and, possibly, to utilize any signal enhancement to increase the sensitivity of the determination.

According to the ionic character of the molecules dissolved in water, ionic surfactants are classified as anionactive, cationactive and ampholytic. Anionactive ions have a negative charge in aqueous solution, cationactive ions have a positive charge and the charge of ampholitic ions depends on the pH of the solution. Non-ionic surfactants do not have a charge in the molecule and their solubility in water is given by the presence of a hydrophilic group[21].

Good solubility, high conductivity, low toxicity and good chemical and electroanalytical stability of ionic surfactants make these substances useful for many electroanalytical applications. The presence of surfactants influences the parameters of measurement which often leads to the design of new procedures and methods for the determination. Traditional applica-

tion of the surfactants in electrochemistry is the suppression of maxima on polarographic curves[22]. They may change the shape of the polarographic wave and the reversibility of the electrode reaction which is reflected in the change of DPV peak height. The character of changes depends on the structure and concentration of the surfactant. By suitable choice, the limiting diffusion current might be increased, although under some circumstances, the decrease may also occur.

For this work, three surfactants were selected: Cationactive cetyltrimethylammonium bromide (CTMAB) (Fig. 2A), non-ionic Triton X-100 (Fig. 2B) and anionactive sodium dodecyl sulfate (SDS) (Fig. 2C). These surfactants might be used as modifiers for a variety of paste electrodes[23] and electroanalytical sensors[24]. Therefore, we have investigated their influence on voltammetric determination of genotoxic nitro compounds using 6-NBIA as a model substance.

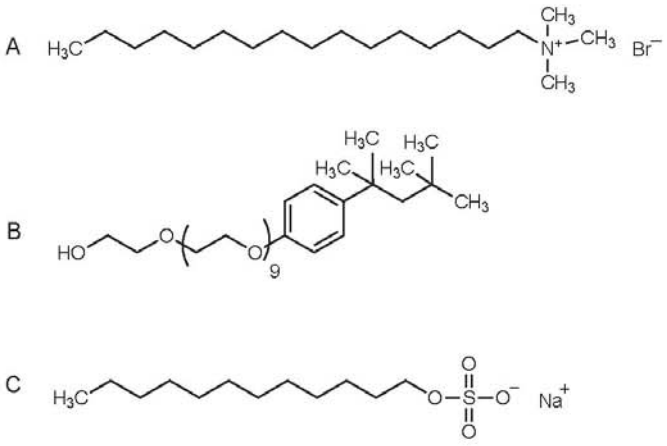

FIG. 2
Structural formulae of cetyltrimethylammonium bromide (A) Triton X-100 (B) and sodium dodecyl sulfate (C)

EXPERIMENTAL

Reagents

6-NBIA (98%, CAS Registry Number 94-52-0) was supplied by Sigma–Aldrich, Prague, Czech Republic in the form of nitrate. 1×10^{-3} mol l^{-1} stock solution was prepared by dissolving an exactly weighed amount of the substance in deionized water. A spectrophotometric study demonstrated that the stock solution is stable for at least three months[5]. The stock solution

of surfactants – CTMAB (98%, Sigma–Aldrich), Triton X-100 (laboratory grade, Sigma–Aldrich) and SDS (98%, Sigma–Aldrich) – were prepared in deionized water at the concentration 1×10^{-2} mol l^{-1}. The solutions were stored in refrigerator. Diluted solutions were prepared freshly every day by exact dilution of the stock solution.

The Britton–Robinson buffer solutions (BR-buffer) were prepared in a usual way by mixing a 0.04 mol l^{-1} solution of phosphoric acid, acetic acid and boric acid with an appropriate volume of 0.2 mol l^{-1} sodium hydroxide, using analytical-reagent grade chemicals obtained from Merck, Prague, Czech Republic. Deionized water was produced by Milli-Q Plus system, Millipore, Billerica, USA.

Apparatus

Voltammetric measurements were carried out using Eco-Tribo Polarograph driven by Polar Pro 2.0 software (all Polaro-Sensors, Prague, Czech Republic). The software worked under the operational system Microsoft Windows 98 Plus (Microsoft Corporation, Redmond, USA). All measurements were carried out in a three-electrode system using platinum electrode PPE as an auxiliary electrode and silver|silver chloride electrode RAE 113 (3 mol l^{-1} KCl) (both Monokrystaly, Turnov, Czech Republic) as a reference electrode. The following working electrodes were used: (i) Pen type hanging mercury drop electrode UMμE (Polaro-Sensors), the valve opening time 100 ms, the mercury drop surface 1.37 mm^2, (ii) laboratory-made p-AgSAE (the disc diameter 2.64 mm) prepared according ref.[19] and (iii) laboratory-made m-AgSAE (the disc diameter 2.64 mm) prepared according the same ref.[19]. The scan rate, 20 mV s^{-1}, the pulse amplitude, –50 mV, and the pulse width, 100 ms, with current sampling for last 20 ms were used for DPV. The p-AgSAE consisted of a drawn-out glass tube, whose tip was packed with a fine silver powder (2.0–3.5 μm, 99.9%, Sigma–Aldrich), amalgamated by liquid mercury (99.999%, Polarografie, Prague, Czech Republic) and connected to an electric contact. The m-AgSAE was prepared from p-AgSAE by immersing this electrode into a small volume of liquid mercury and agitating for 15 s (ref.[14]). The m-AgSAE could be used for several weeks, only its covering by mercury was usually repeated every week.

The solution pH was measured by Conductivity & pH meter 4330 (Jenway, Chelmsford, UK) with a combined glass electrode (type 924 005) of the same producer.

Procedures

Before starting the work with p-AgSAE and m-AgSAE, as well as after electrodes passivation or every break in the voltammetric measurements longer than 1 h, the electrochemical activation was carried out in 0.2 mol l^{-1} KCl (Lachema, Brno, Czech Republic) at –2200 mV under stirring for 300 s followed by rinsing with deionized water.

Regeneration of p-AgSAE and m-AgSAE lasting about 30 s preceded each measurement. It was based on application of 300 polarizing cycles (switching the electrode potential from $E_{1,reg}$ to $E_{2,reg}$ for 50 ms). For p-AgSAE, $E_{1,reg}$ = 0 mV and $E_{2,reg}$ = –1200 mV, and for m-AgSAE, $E_{1,reg}$ = –100 mV and $E_{2,reg}$ = –900 mV. Under these conditions, eventual oxides of mercury or silver are reduced and adsorbed molecules are desorbed[25]. The appropriate values of the potential and time of the regeneration were inset in the software of the computer-controlled instrument used and the regeneration of electrodes was thus carried out automatically.

The general voltammetric procedure was as follows: An appropriate volume of the stock solutions of 6-NBIA and selected surfactant was diluted with BR-buffer of the appropriate pH

in a 10.0 ml volumetric flask and transferred into the voltammetric cell. Oxygen was removed by bubbling with nitrogen (purity class 4.0, Linde, Prague, Czech Republic) for 5 min and the voltammogram at HMDE, p-AgSAE or m-AgSAE was recorded.

All curves were measured 3 times and all the measurements were carried out at laboratory temperature. DPV peaks were evaluated from the straight line connecting the minima before and after the peak. The parameters of calibration curves (such as slope, intercept, correlation coefficient (R) and limit of quantification (L_Q)) were calculated with statistic software Adstat 2.0 (TriloByte, Pardubice, Czech Republic)[26].

RESULTS AND DISCUSSION

As optimal pH for DPV measurement, pH 4.0 was selected for HMDE (on the basis of the ref.[27]) and pH 8.0 for p-AgSAE and m-AgSAE (on the basis of the ref.[28]).

The next step was to find out if surfactants have any influence on the DPV peak of 6-NBIA (Figs 3, 4 and 5). The concentration of studied surfactants varied from zero to 1.0 mmol l^{-1}. At all three electrodes, the decrease of the signal (or non-significant change in voltammetric signal) of 6-NBIA with addition of SDS and Triton X-100 was observed. Different behavior was observed only with CTMAB at p-AgSAE and m-AgSAE (at lower concentrations of CTMAB), where the signal increased (it decreased at HMDE). The maximum enhancement of DPV signal of 6-NBIA was higher using p-AgSAE (increase by about 130%) in comparison with m-AgSAE (increase by about 50%). Therefore, the combination of p-AgSAE and CTMAB was selected for further investigation.

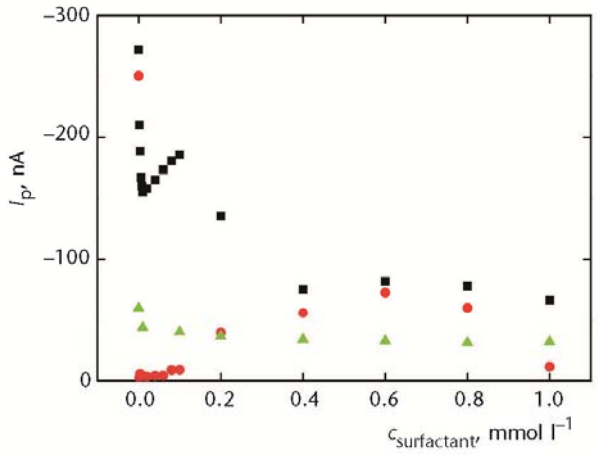

FIG. 3
The dependence of the DPV peak height of 6-NBIA (c = 1 × 10^{-5} mol l^{-1}) measured at HMDE in BR-buffer pH 4.0 on the surfactant concentration (SDS (■), Triton X-100 (●) and CTMAB (▲))

The addition of CTMAB can effectively enhance the signals of 6-NBIA at p-AgSAE even for a trace amount of CTMAB. With the increase of CTMAB concentration, the peak current initially increases and then remains constant up to 0.8 mmol l^{-1} of CTMAB. CTMAB might form a monolayer in this concentration range and hence there is an increase in the signal[29]. Above 0.8 mmol l^{-1}, addition of CTMAB surfactant results in the shift of peak potential of 6-NBIA towards more negative potential with decrease in

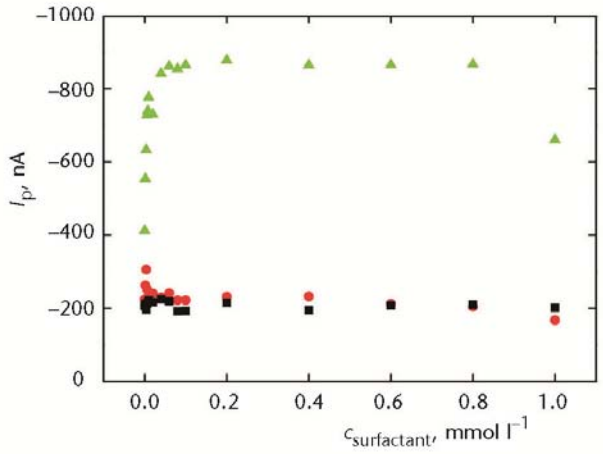

FIG. 4
The dependence of the DPV peak height of 6-NBIA (c = 1 × 10^{-5} mol l^{-1}) measured at p-AgSAE in BR-buffer pH 8.0 on the surfactant concentration (SDS (■), Triton X-100 (●) and CTMAB (▲))

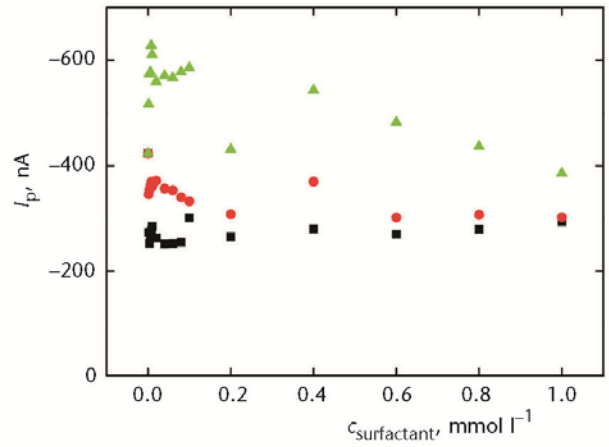

FIG. 5
The dependence of the DPV peak height of 6-NBIA (c = 1 × 10^{-5} mol l^{-1}) measured at m-AgSAE in BR-buffer pH 8.0 on the surfactant concentration (SDS (■), Triton X-100 (●) and CTMAB (▲))

peak current. This may be attributed to the direct adsorption of the surfactant at the p-AgSAE surface. Decrease in the availability of electroactive sites due to solubilization of the substrate may also contribute to this effect[30]. The results showed that CTMAB exhibited different types of adsorptive behavior at p-AgSAE at different CTMAB concentrations.

The concentration 1×10^{-4} mol l^{-1} of CTMAB was chosen as optimal for the measurement of calibration curves at p-AgSAE. Calibration curves were measured in the concentration range of 6-NBIA from 2×10^{-7} to 1×10^{-4} mol l^{-1} with and without addition of CTMAB ($c = 1 \times 10^{-4}$ mol l^{-1}) (Fig. 6). The peaks in the presence of the surfactant are up to four times higher than peaks without the surfactant. This trend can be seen in the whole concentration range. Parameters of calibration curves are summarized in Table I,

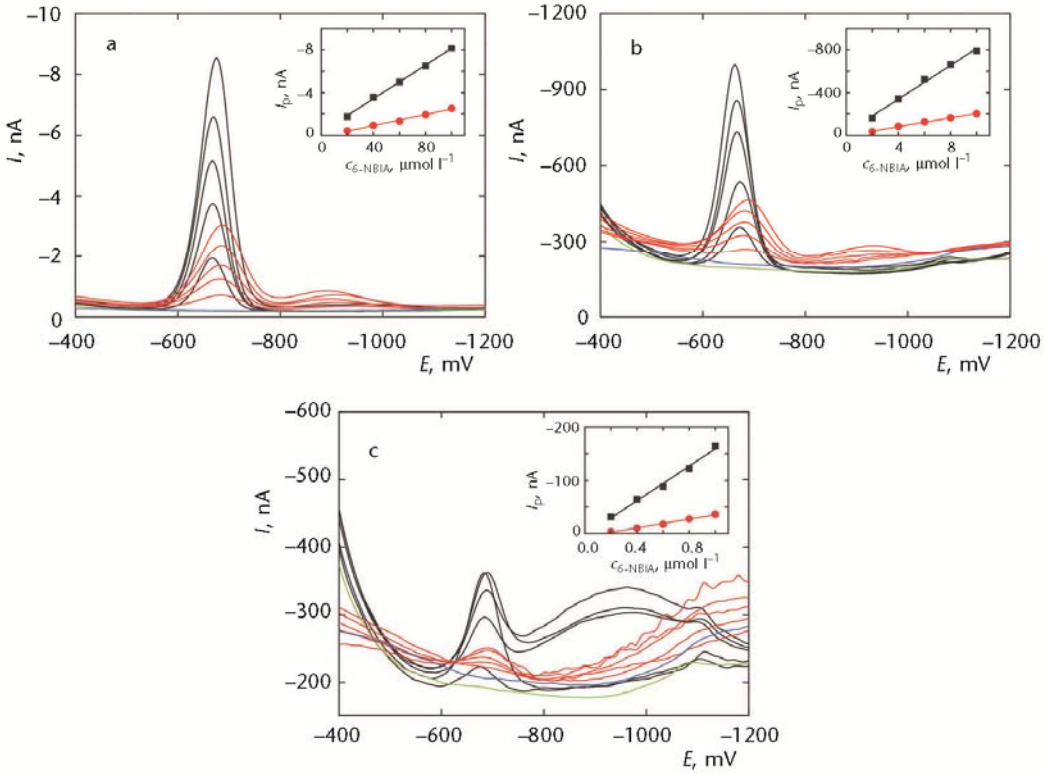

FIG. 6
Calibration curves for DPV determination of 6-NBIA ($c_{6\text{-NBIA}}$ = (2–10) × 10^{-5} mol l^{-1} (a), (2–10) × 10^{-6} mol l^{-1} (b) and (2–10) × 10^{-7} mol l^{-1} (c)) with CTMAB ($c_{CTMAB} = 1 \times 10^{-4}$ mol l^{-1}; black lines) and without CTMAB (red lines) in BR-buffer pH 8.0 at p-AgSAE (green and blue lines represent the supporting electrolytes with and without CTMAB, respectively)

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from which the linearity in the whole concentration range is obvious, as well as a decrease of the L_Q of 6-NBIA and a higher slope of calibration dependences in the presence of CTMAB.

TABLE I
Parameters of the calibration dependences for DPV of 6-NBIA at p-AgSAE with and without CTMAB in BR-buffer pH 8.0

$c_{6\text{-NBIA}}$ mol l^{-1}	c_{CTMAB} mol l^{-1}	Slope nA mol^{-1} l	Intercept nA	R	L_Q mol l^{-1}
$(2–10) \times 10^{-5}$	0	-2.60×10^7	132.6	–0.9971	–
	1×10^{-4}	-7.87×10^7	–280.5	–0.9992	–
$(2–10) \times 10^{-6}$	0	-2.90×10^7	3.1	–0.9981	–
	1×10^{-4}	-7.91×10^7	–22.6	–0.9964	–
$(2–10) \times 10^{-7}$	0	-4.18×10^6	5.9	–0.9969	4.3×10^{-7}
	1×10^{-4}	-1.62×10^7	2.9	–0.9953	2.5×10^{-7}

CONCLUSIONS

It has been proven that the addition of SDS and Triton X-100 does not significantly influence the DPV determination of 6-NBIA at HMDE, p-AgSAE and m-AgSAE. However, the addition of CTMAB significantly increases the height of DPV peak of 6-NBIA at p-AgSAE resulting in lower limit of quantification. The mechanism of this enhancement is not wholly clear at the moment and it will require further investigation. Most probably, cetyltrimethylammonium cation is adsorbed at the surface of negatively charged p-AgSAE. Silver obviously plays a certain role in this process[31] which can explain that no or low enhancement is observable with HMDE or m-AgSAE. Long cetyl chain then enables the adsorption of low polar 6-NBIA at the electrode surface thus increasing the height of DPV peak. Under the optimal conditions, the limit of quantification for DPV of 6-NBIA at p-AgSAE decreased about twice by addition of CTMAB in comparison with measurement in the absence of the surfactant with keeping good accuracy.

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7. APPENDIX II

Full Paper **ELECTROANALYSIS**

Voltammetric Determination of 4-Nitrophenol and 5-Nitrobenzimidazole Using Different Types of Silver Solid Amalgam Electrodes – A Comparative Study

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Abstract
The voltammetric behavior of two genotoxic nitro compounds (4-nitrophenol and 5-nitrobenzimidazole) has been investigated using direct current voltammetry (DCV) and differential pulse voltammetry (DPV) at a polished silver solid amalgam electrode (p-AgSAE), a mercury meniscus modified silver solid amalgam electrode (m-AgSAE), and a mercury film modified silver solid amalgam electrode (MF-AgSAE). The optimum conditions have been evaluated for their determination in Britton-Robinson buffer solutions. The limit of quantification (L_Q) for 5-nitrobenzimidazole at p-AgSAE was 0.77 μmol L^{-1} (DCV) and 0.47 μmol L^{-1} (DPV), at m-AgSAE it was 0.32 μmol L^{-1} (DCV) and 0.16 μmol L^{-1} (DPV), and at MF-AgSAE it was 0.97 μmol L^{-1} (DCV) and 0.70 μmol L^{-1} (DPV). For 4-nitrophenol at p-AgSAE, L_Q was 0.37 μmol L^{-1} (DCV) and 0.32 μmol L^{-1} (DPV), at m-AgSAE it was 0.14 μmol L^{-1} (DCV) and 0.1 μmol L^{-1} (DPV), and at MF-AgSAE, it was 0.87 μmol L^{-1} (DCV) and 0.37 μmol L^{-1} (DPV). Thorough comparative studies have shown that m-AgSAE is the best sensor for voltammetric determination of the two model genotoxic compounds because it gives the lowest L_Q, is easier to prepare, and its surface can be easily renewed both chemically (by new amalgamation) and/or electrochemically (by imposition of cleaning pulses). The practical applicability of the newly developed methods was verified on model samples of drinking water.

Keywords: Voltammetry, Polished silver solid amalgam electrode, Mercury meniscus modified silver solid amalgam electrode, Mercury film modified silver solid amalgam electrode, 4-Nitrophenol, 5-Nitrobenzimidazole

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1 Introduction

The environment is increasingly polluted with various genotoxic nitro compounds [1], one of them is 5-nitrobenzimidazole (5-NBIA) (see Figure 1). It can damage natural biological functions of living organisms. The occurrence of 5-NBIA (which is a potential carcinogen and mutagen [2]) in the environment is expected in connection with fossil fuels combustion [3]. 5-NBIA was polarographically determined as a part of photographic processing solutions [4]. Its electrochemical behavior has been studied in connection with metal corrosion protection [5]. Nitrophenols from pesticide degradation products, car exhausts, and industrial wastes are listed as priority pollutants by the United States Environmental Protection Agency [6,7]. Pesticides based on simple nitrophenols are generally not allowed today but some of them are still used as growth stimulators in agriculture [8]. They are potential carcinogens, teratogens, and mutagens [9]. Because of their toxicity and vast scale distribution in the environment, their determination has become one of the important goals of environmental analysis. Therefore, 4-nitrophenol (4-NP) (see Figure 1) was selected as another test substance.

The easy electrochemical reduction of nitro groups at the aromatic or heterocyclic ring, whose mechanism is discussed in [10], permits very sensitive determinations of a number of genotoxic and ecotoxic nitro compounds using modern voltammetric techniques such as differential pulse voltammetry (DPV) at a hanging mercury drop electrode (HMDE) [11] and adsorptive stripping voltammetry (AdSV) at HMDE [12]. Nevertheless, due to increasing fears of liquid mercury toxicity during the last decades [13], which has resulted in sometimes unreasonable "mercury phobia" [14], attention is paid to the development of solid electrodes (e.g., solid amalgam electrodes [15,16], amalgam paste electrodes [17,18], solid composite electrodes [19], solid amalgam composite electrodes [20,21], carbon paste electrodes [22], or boron-doped diamond film electrodes [23]), which can also be used to determine various nitro compounds. From this point of view, working electrodes based on silver solid amalgam (p-AgSAE) [24] and modified by a mercury meniscus (m-AgSAE) [25] or mercury film (MF-AgSAE) [26] repre-

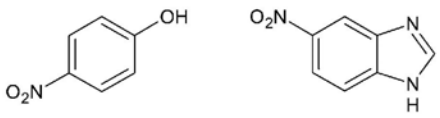

Fig. 1. Structural formulae of investigated compounds (4-nitrophenol and 5-nitrobenzimidazole).

sent a suitable nontoxic alternative to the traditional mercury electrodes. Good mechanical stability, simple handling and regeneration including an electrochemical pretreatment of the electrode surface are among the main advantages of these electrodes. The wide field of analytical applications of these electrodes has been reviewed [15].

This work aims at developing direct current voltammetric (DCV) and DPV methods for the determination of trace amounts of genotoxic 4-NP and 5-NBIA using p-AgSAE, m-AgSAE, and MF-AgSAE and comparing the results at these electrodes. Recently developed MF-AgSAE was for the first time used for voltammetric determination of electrochemically reducible nitro compounds. Measurements with p-AgSAE and m-AgSAE were performed for the sake of comparison, because each of these electrodes is a limiting case of MF-AgSAE. The p-AgSAE is a liquid mercury free nontoxic amalgam electrode and a substrate for MF-AgSAE preparation and m-AgSAE is well proven and reliable sensor for voltammetric determination of reducible analytes. Furthermore, practical applicability of the newly developed methods was verified through direct determination of the studied compounds in drinking water.

2 Experimental

2.1 Chemicals and Reagents

Stock solutions of 5-nitrobenzimidazole ($c = 1 \times 10^{-3}$ mol L^{-1}; CAS Number: 94-52-0) and 4-nitrophenol ($c = 1 \times 10^{-3}$ mol L^{-1}; CAS Number: 100-02-7), both obtained from Sigma-Aldrich, Prague, Czech Republic, were prepared by dissolving the appropriate substances in 100.0 mL of deionized water. UV-vis spectrophotometric studies have demonstrated that the stock solutions are stable for at least one year [27,28]. The reagents (phosphoric acid, acetic acid, boric acid, sodium hydroxide, mercuric chloride, and potassium iodide, all of p.a. purity grade) were supplied by Lachema (Brno, Czech Republic). Britton-Robinson (BR) buffers were prepared in a usual way [29]. Deionized water was produced by Milli-Q_{plus} system (Millipore, Billerica, USA). All chemicals were used without further purification and all solutions were maintained in glass vessels in the dark at laboratory temperature.

2.2 Apparatus

Voltammetric measurements were performed using an Eco-Tribo Polarograph driven by PolarPro 2.0 software (both Polaro-Sensors, Prague, Czech Republic). The software worked under the operational system Microsoft Windows 98 plus (Microsoft Corporation, Redmond, USA). The voltammetric measurements were carried out in a three-electrode system – a platinum auxiliary electrode PPE, a Ag|AgCl reference electrode RAE 113 (1 mol L^{-1} KCl) (both Monokrystaly, Turnov, Czech Republic) and laboratory-made p-AgSAE, m-AgSAE, MF-AgSAE (disc diameter, 2.64 mm) as working electrodes.

A scan rate of 20 mV s^{-1} was used for both DCV and DPV, a pulse amplitude of −50 mV and a pulse width of 100 ms with current sampling for the last 20 ms were used in DPV. The p-AgSAE consisted of a drawn-out glass tube, whose tip was packed with a fine silver powder (2–3.5 μm, 99.9%, Sigma-Aldrich, Prague, Czech Republic), amalgamated by liquid mercury (99.999%, Polarografie, Prague, Czech Republic) and connected to an electric contact [24]. It was mechanically polished every day always before starting the work. The m-AgSAE was prepared as described in our previous papers [24,30,31] from p-AgSAE by immersing this electrode into a small volume of liquid mercury and agitating for 15 s. The m-AgSAE could be used for several weeks, only its covering by mercury was usually repeated every week. The MF-AgSAE was prepared as described in our paper [26] using a special device for the preparation of mercury film of exactly defined thickness at a solid electrode, which consists of a plastic tube filled with 0.2 mL of 0.01 mol L^{-1} $HgCl_2$ and 1 mol L^{-1} KI, containing liquid mercury at the bottom with carbon contact. The MF-AgSAE must be prepared every day because the lifetime of 1–2 μm mercury film is about 2 hours [26].

The solution pH was measured by pH Meter 3510 (Jenway, Chelmsford, UK) with a combined glass electrode (type 924 005) of the same producer.

2.3 Procedures

Before starting the work, as well as after electrode passivation or after every break in the voltammetric measurements longer than 1 hour, the electrochemical activation of all three types of electrodes used was carried out in 0.2 mol L^{-1} KCl at −2200 mV under stirring for 300 s followed by rinsing with deionized water. The general voltammetric procedure was as follows: An appropriate volume of the stock solution was diluted by BR buffer of the appropriate pH in a 10.0 mL volumetric flask and transferred into the voltammetric cell. Oxygen was removed by bubbling with nitrogen (purity class 4.0; Linde, Prague, Czech Republic) for 5 min and the voltammogram at p-AgSAE, m-AgSAE, or MF-AgSAE was recorded.

Regeneration of the electrodes lasting about 30 s preceded each measurement. It was based on application of

300 polarizing cycles (switching the electrode potential from $E_{1,reg}$ to $E_{2,reg}$ for 50 ms). $E_{1,reg}$ was about 50 to 100 mV more negative than the potential of the anodic dissolution of the electrode, $E_{2,reg}$ was about 50 to 100 mV more positive than the potential of the hydrogen evolution in the given supporting electrolyte. Under these conditions, eventual oxides of mercury or silver are reduced and adsorbed molecules are desorbed [25]. The appropriate values of the potential and the regeneration time were inset in the software of the computer-controlled instrument used and the regeneration of electrodes was thus carried out automatically.

All curves were measured 3 times and all the measurements were carried out at laboratory temperature. The DCV peak height (I_p) was evaluated from the extrapolated linear portion of the voltammogram before the onset of the peak. DPV peaks were evaluated from the straight line connecting the minima before and after the peak. The parameters of calibration curves (such as slope, intercept, correlation coefficient (R), and limit of quantification (L_Q)) were calculated with statistic software Adstat (TriloByte, Pardubice, Czech Republic) [32].

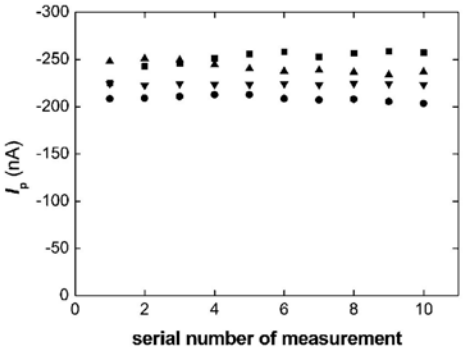

Fig. 2. Repeatability of DPV determination of 4-NP ($c = 1\times10^{-5}$ mol L^{-1}) at MF-AgSAE in BR buffer pH 6.0 ($E_{1,reg} = -100$ mV, $E_{2,reg} = -600$ mV) with deposition times of films of 3600 s (■), 1800 s (●), 900 s (▲), and 300 s (▼).

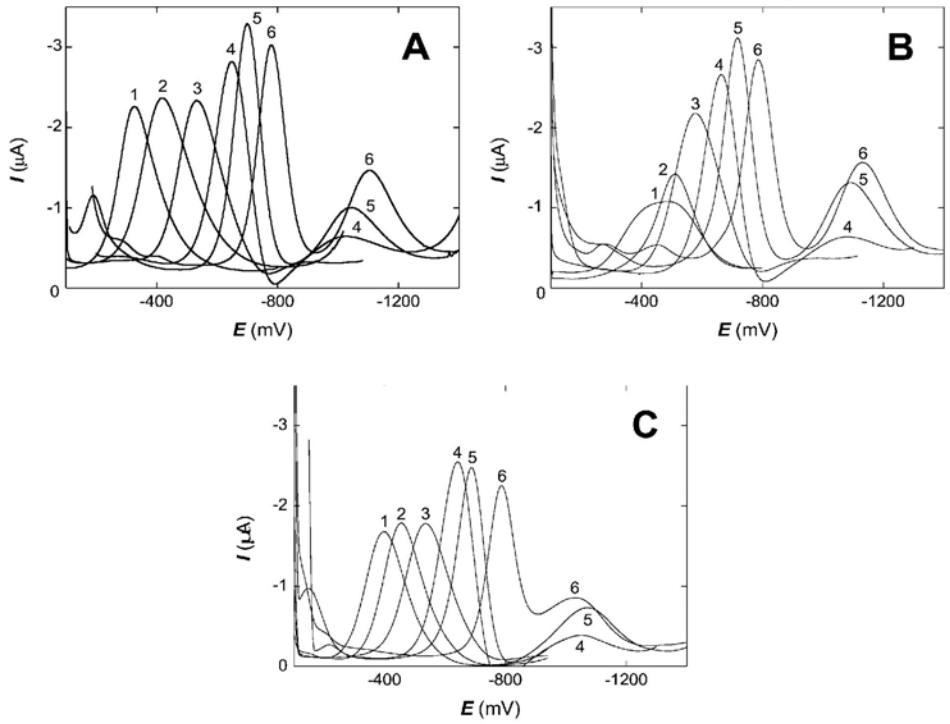

Fig. 3. DP voltammograms of 5-NBIA ($c = 1\times10^{-4}$ mol L^{-1}) at p-AgSAE (A), m-AgSAE (B), and MF-AgSAE (C) in BR buffer of pH: 2.0 (1), 4.0 (2), 6.0 (3), 8.0 (4), 10.0 (5), and 12.0 (6).

Table 1. Optimum conditions for DCV and DPV determination of 4-NP and 5-NBIA in BR buffer at p-AgSAE, m-AgSAE, and MF-AgSAE.

Substance	pH	p-AgSAE		m-AgSAE		MF-AgSAE	
		$E_{1,reg}$ (mV)	$E_{2,reg}$ (mV)	$E_{1,reg}$ (mV)	$E_{2,reg}$ (mV)	$E_{1,reg}$ (mV)	$E_{2,reg}$ (mV)
4-NP	6.0 [a]	0 [a]	−1200 [a]	−100 [a]	−900 [a]	−100	−600
5-NBIA	8.0	−100	−600	−100	−600	−100	−600

[a] Experimental conditions taken from [28].

2.4 Model Samples

Drinking water from the public water pipeline in the building of Faculty of Science of the Charles University in Prague spiked with appropriate amounts of stock solutions of test compounds was used as a model sample. The procedure for the DPV determination of 4-NP and 5-NBIA in the model samples was as follows: 9.0 mL of a model water sample were diluted to 10.0 mL with 0.01 mol L^{-1} sodium hydroxide and, after deaeration with nitrogen, DP voltammograms were recorded. Based on our previous results [27], BR buffer was substituted by 0.01 mol L^{-1} sodium hydroxide for the direct determination of 4-NP and 5-NBIA in drinking water.

3 Results and Discussion

3.1 Optimization of the Preparation of MF-AgSAE

At first, an optimum mercury film deposition time was investigated. Repeatability of determination of test substances at MF-AgSAE was very good at a film deposited for 300 s to 3600 s (Figure 2). Shorter times of film deposition were not tested because then the deposited film was not compact over the whole electrode surface [26]. The relative standard deviation (*RSD*) of DPV determination of 4-NP ($c = 1 \times 10^{-5}$ mol L^{-1}) in BR buffer pH 6.0 at MF-AgSAE was 0.3 %, 2.5 %, 1.4 %, and 2.0 % for film deposition times of 300 s, 900 s, 1800 s, and 3600 s, respectively

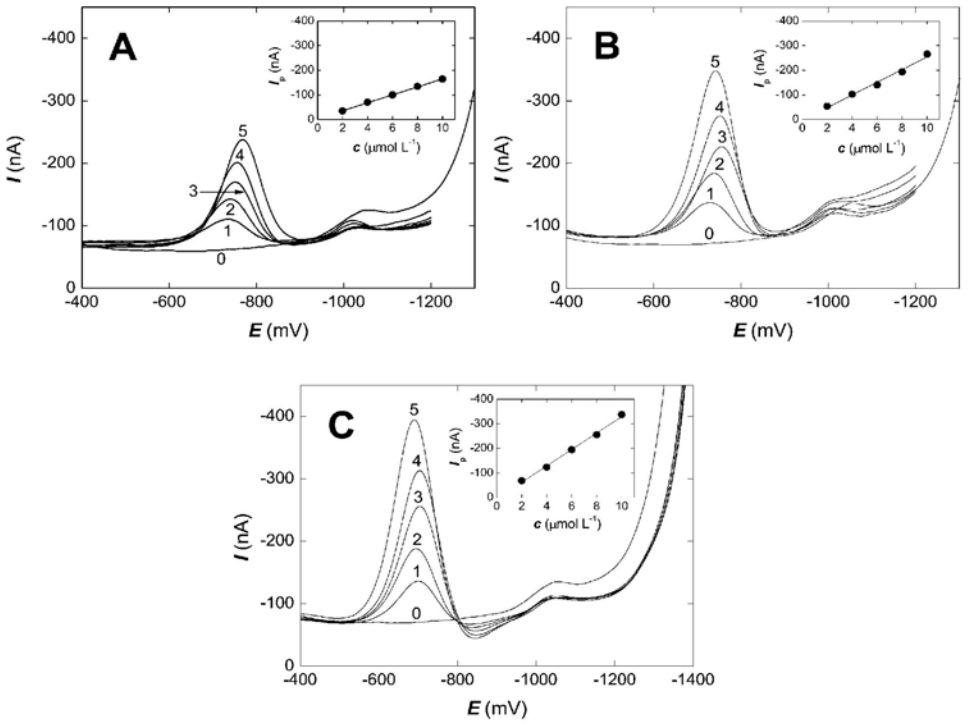

Fig. 4. DP voltammograms of 4-NP at p-AgSAE (A), m-AgSAE (B), and MF-AgSAE (C) in BR buffer of pH 6.0; $c_{4\text{-NP}} = 0$ (0), 2 (1), 4 (2), 6 (3), 8 (4), and 10 (5) μmol L^{-1}. Insets: Corresponding calibration straight lines.

(with $E_{1,reg} = -100$ mV and $E_{2,reg} = -600$ mV). It follows from Figure 2 that results with film deposited for different times are comparable. However, films deposited for shorter times are not as stable as films deposited for longer times. The thickness of the freshly prepared film deposited for 3600 s was (11.4 ± 4.2) μm. After two hours of work with the prepared electrode, the thickness decreased to (1.6 ± 0.6) μm. (The film thickness was calculated from the charge passed during its dissolution [26]). Deterioration of the deposited film was obvious from the shift of the peak potential of the tested substance close to peak potential at plain p-AgSAE (not covered with mercury meniscus or film). Therefore, a mercury film deposition time of 3600 s was used for all following measurements.

3.2 Voltammetric Behavior of the Test Substances

The influence of pH on DCV and DPV curves (see Figure 3) of 1×10^{-4} mol L^{-1} 5-NBIA at p-AgSAE, m-AgSAE, and MF-AgSAE was investigated in BR buffer with pH values from 2.0 to 12.0. 5-NBIA yielded one cathodic peak over the whole pH range and, with the growing pH, a second peak appeared at a more negative potential at pH from 7.0 to 12.0. The first peak corresponds to four-electron reduction of the nitro group to a hydroxylamino group and the second peak corresponds to two-electron reduction of the hydroxylamino group to an amino group [27,33]. 4-NP gave rise only to one irreversible voltammetric peak in acidic, neutral, and alkaline media, corresponding to the four-electron reduction of the nitro group to the hydroxylamino group [28]. The peak potential (E_p) values of all the observed peaks shifted towards more negative potentials with increasing pH.

Table 2. Parameters of the calibration straight lines for the determination of 4-NP and 5-NBIA using DCV and DPV at p-AgSAE, m-AgSAE, and MF-AgSAE.

Electrode	Substance	Method	Concentration (mol L^{-1})	Slope (nA mol^{-1} L)	Intercept (nA)	R [a]	L_Q [b] (mol L^{-1})
p-AgSAE	4-NP	DCV	$(2–10) \times 10^{-5}$	-9.45×10^{6}	−103.2 [c]	−0.9873	–
			$(2–10) \times 10^{-6}$	-8.03×10^{6}	−17.4	−0.9898	–
			$(2–10) \times 10^{-7}$	-1.17×10^{7}	1.1	−0.9987	3.7×10^{-7}
		DPV	$(2–10) \times 10^{-5}$	-8.63×10^{6}	−69.4 [c]	−0.9913	–
			$(2–10) \times 10^{-6}$	-1.62×10^{7}	−3.0 [c]	−0.9996	–
			$(2–10) \times 10^{-7}$	-2.19×10^{7}	1.7	−0.9990	3.2×10^{-7}
	5-NBIA	DCV	$(2–10) \times 10^{-5}$	-1.60×10^{7}	−253.4 [c]	−0.9733	–
			$(2–10) \times 10^{-6}$	-2.77×10^{7}	8.9	−0.9997	–
			$(2–10) \times 10^{-7}$	-1.95×10^{7}	0.5 [c]	−0.9904	7.7×10^{-7}
		DPV	$(2–10) \times 10^{-5}$	-1.93×10^{7}	115.4 [c]	−0.9978	–
			$(2–10) \times 10^{-6}$	-1.69×10^{7}	−8.4 [c]	−0.9757	–
			$(2–10) \times 10^{-7}$	-2.09×10^{7}	1.2 [c]	−0.9969	4.7×10^{-7}
m-AgSAE	4-NP	DCV	$(2–10) \times 10^{-5}$	-1.31×10^{7}	−122.0 [c]	−0.9955	–
			$(2–10) \times 10^{-6}$	-2.06×10^{7}	−10.2 [c]	−0.9722	–
			$(2–10) \times 10^{-7}$	-1.25×10^{7}	−1.3 [c]	−0.9636	1.4×10^{-7}
		DPV	$(2–10) \times 10^{-5}$	-1.20×10^{7}	−120.7 [c]	−0.9893	–
			$(2–10) \times 10^{-6}$	-2.58×10^{7}	3.8 [c]	−0.9931	–
			$(2–10) \times 10^{-7}$	-2.68×10^{7}	−1.3	−0.9997	1.0×10^{-7}
	5-NBIA	DCV	$(2–10) \times 10^{-5}$	-2.56×10^{7}	121.4	−0.9999	–
			$(2–10) \times 10^{-6}$	-3.08×10^{7}	−19.6	−0.9996	–
			$(2–10) \times 10^{-7}$	-4.94×10^{7}	5.6	−0.9993	3.2×10^{-7}
		DPV	$(2–10) \times 10^{-5}$	-3.88×10^{7}	−67.5 [c]	−0.9987	–
			$(2–10) \times 10^{-6}$	-4.41×10^{7}	12.4 [c]	−0.9993	–
			$(2–10) \times 10^{-7}$	-4.91×10^{7}	−0.8 [c]	−0.9930	1.6×10^{-7}
MF-AgSAE [d]	4-NP	DCV	$(2–10) \times 10^{-5}$	-2.39×10^{7}	−108.3 [c]	−0.9989	–
			$(2–10) \times 10^{-6}$	-2.92×10^{7}	11,7 [c]	−0,9988	–
			$(2–10) \times 10^{-7}$	-3.02×10^{7}	3.6 [c]	−0.9899	8.7×10^{-7}
		DPV	$(2–10) \times 10^{-5}$	-2.58×10^{7}	−204.7 [c]	−0.9932	–
			$(2–10) \times 10^{-6}$	-3.35×10^{7}	5.3 [c]	−0.9978	–
			$(2–10) \times 10^{-7}$	-3.47×10^{7}	3.1	−0.9986	3.7×10^{-7}
	5-NBIA	DCV	$(2–10) \times 10^{-5}$	-4.52×10^{7}	397.0 [c]	−0.9826	–
			$(2–10) \times 10^{-6}$	-1.94×10^{7}	441.3	−0.9902	–
			$(2–10) \times 10^{-7}$	-4.48×10^{7}	9.3	−0.9897	9.7×10^{-7}
		DPV	$(2–10) \times 10^{-5}$	-1.80×10^{7}	−269.3	−0.9933	–
			$(2–10) \times 10^{-6}$	-6.10×10^{7}	54.2	−0.9992	–
			$(2–10) \times 10^{-7}$	-4.71×10^{7}	9.3	−0.9954	7.0×10^{-7}

[a] Correlation coefficient. [b] Limit of quantification (10σ; $\alpha = 0.05$). [c] The intercept is not statistically significantly different from zero at a significance level $\alpha = 0.05$. [d] Deposition time of mercury film was 3600 s.

This shift can be explained by a preliminary protonation of the test substances leading to a decrease in the electron density at nitro groups and resulting in easier electron acceptance at low pH values.

The best developed and highest voltammetric peaks were obtained under conditions summarized in Table 1. Repeated measurements can cause pronounced passivation of the electrodes, probably by the electrode reaction products, resulting in decreasing peak heights and their shift towards more negative potentials. The optimum regeneration potentials ($E_{1,reg}$ and $E_{2,reg}$) thus had to be found for each analyte (see Table 1). The regeneration was carried out in the same supporting electrolyte as that used for the analyte determination. The procedure was based on potential switching (300 polarizing cycles) between two limiting potentials. For example for 5-NBIA at MF-AgSAE in BR buffer pH 8.0, the regeneration step applied prior to each voltammetric measurement led not only to improvement of the determination repeatability (*RSD* of DPV determinations of 5-NBIA at a concentration of 1×10^{-5} mol L^{-1} decreased from 7.5% to 0.3%), but also to the stabilization and enhancement of DPV responses of the studied analyte.

3.3 Voltammetric Determination of the Test Substances

Under the optimum conditions given in Table 1, DCV and DPV calibration curves were measured over concentration ranges $(2–10)\times10^{-7}$, $(2–10)\times10^{-6}$, and $(2–10)\times10^{-5}$ mol L^{-1} for 4-NP and 5-NBIA at all three tested electrodes (see Figure 4).

It can be seen that MF-AgSAE gives the highest peaks with the highest sensitivity of determinations (slopes of calibration curves). However, the repeatability of measurements (mainly at lower concentrations) is worse than for p-AgSAE or m-AgSAE, which results in higher L_Qs obtained for MF-AgSAE. Parameters of the calibration straight lines for the determination of 4-NP and 5-NBIA are summarized in Table 2. The sensitivity slightly differs between individual concentration orders. This behavior, typical for voltammetry at solid electrodes [21,30], is usually caused by the passivation of the electrode surface by the electrode reaction products or by analyte adsorption. Nevertheless, within the individual concentration orders, the concentration dependences obtained are linear. It can be seen that lowest L_Qs can be achieved using m-AgSAE.

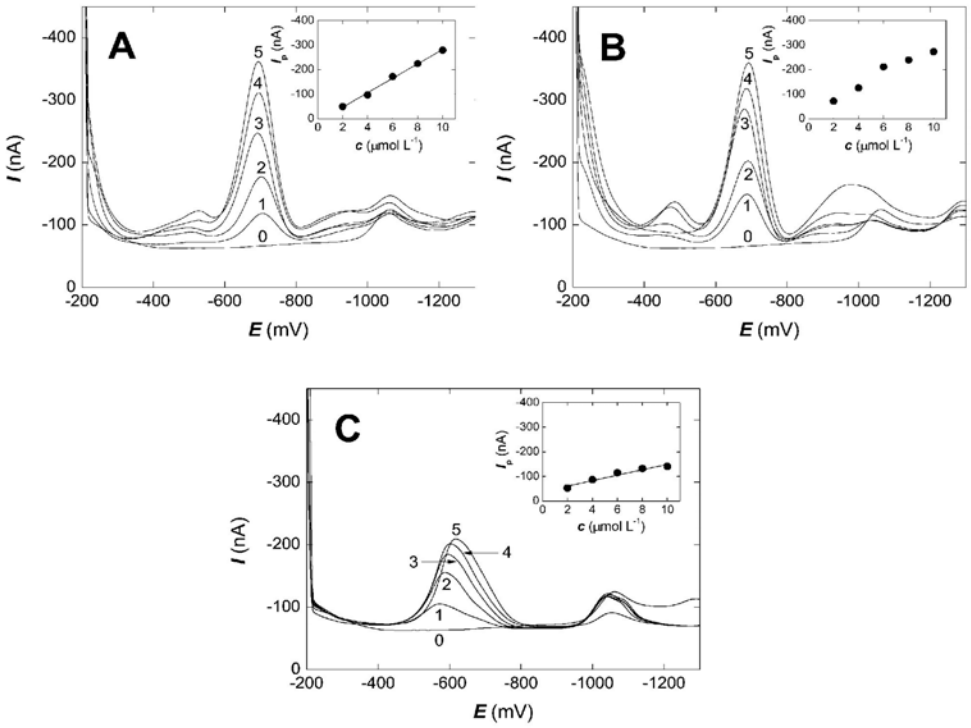

Fig. 5. DP voltammograms of 5-NBIA at p-AgSAE (A), m-AgSAE (B), and MF-AgSAE (C) in spiked drinking water −0.01 mol L^{-1} NaOH (9:1); $c_{5\text{-NBIA}}$ = 0 (0), 2 (1), 4 (2), 6 (3), 8 (4), and 10 (5) μmol L^{-1}. Insets: Corresponding calibration straight lines.

3.4 Voltammetric Determination of the Test Substances in Drinking Water

In order to verify practical applicability of the developed DCV and DPV methods, the determination of 4-NP and 5-NBIA was carried out in model samples of drinking water in a submicromolar concentration range under optimum conditions. Calibration curves were measured in a mixture of 9.0 mL of a spiked model water sample and 1.0 mL of a 0.01 mol L^{-1} sodium hydroxide. DP voltammograms 5-NBIA in spiked drinking water in the concentration range of 2–10 μmol L^{-1} are depicted in Figure 5 for p-AgSAE, m-AgSAE, and MF-AgSAE. The parameters of the calibration curves obtained are summarized in Table 3. These results confirm the possible application of all tested electrodes for the determination of micromolar concentrations of substances under investigation in drinking water.

4 Conclusions

It has been demonstrated that a polished silver solid amalgam electrode (p-AgSAE) and this electrode covered by a mercury meniscus (m-AgSAE) or by a mercury film (MF-AgSAE) represent suitable nontoxic alternatives to the traditional mercury electrodes. These electrodes, combined with direct current voltammetry or differential pulse voltammetry, are suitable sensors for the determination of submicromolar concentrations of 4-nitrophenol and 5-nitrobenzimidazole. They provide stable and reproducible responses during long-time measurements. The applicability of all tested electrodes for differential pulse voltammetric determination of 4-nitrophenol and 5-nitrobenzimidazole in model samples of drinking water has also been verified. The lowest limits of quantification can be achieved with m-AgSAE. Therefore, this electrode should be preferred for practical applications. The possible utilization of these electrodes for determinations of the above analytes in flowing systems (flow injection analysis and high performance liquid chromatography with amperometric detection) is under investigation.

Table 3. Parameters of the calibration straight lines for the determination of 4-NP and 5-NBIA in model samples of drinking water using DCV and DPV at p-AgSAE, m-AgSAE, and MF-AgSAE.

Electrode	Substance	Method	Concentration (mol L^{-1})	Slope (nA mol^{-1} L)	Intercept (nA)	R [a]	L_Q [b] (mol L^{-1})
p-AgSAE	4-NP	DCV	$(2–10)\times10^{-5}$	-9.35×10^{6}	31.3 [c]	−0.9924	–
			$(2–10)\times10^{-6}$	-9.68×10^{6}	−1.0 [c]	−0.9932	4.1×10^{-6}
		DPV	$(2–10)\times10^{-5}$	-9.54×10^{6}	92.4 [c]	−0.9861	–
			$(2–10)\times10^{-6}$	-9.76×10^{6}	6.3 [c]	−0.9975	3.8×10^{-6}
	5-NBIA	DCV	$(2–10)\times10^{-5}$	-2.27×10^{7}	−156.1 [c]	−0.9970	–
			$(2–10)\times10^{-6}$	-2.86×10^{7}	−23.3 [c]	−0.9955	–
			$(2–10)\times10^{-7}$	-6.14×10^{7}	−0.2 [c]	−0.9833	9.7×10^{-7}
		DPV	$(2–10)\times10^{-5}$	-2.01×10^{7}	−222.0 [c]	−0.9940	–
			$(2–10)\times10^{-6}$	-2.94×10^{7}	12.0 [c]	−0.9982	–
			$(2–10)\times10^{-7}$	-3.97×10^{7}	1.3 [c]	−0.9972	4.3×10^{-7}
m-AgSAE	4-NP	DCV	$(2–10)\times10^{-5}$	-2.32×10^{7}	94.6 [c]	−0.9637	–
			$(2–10)\times10^{-6}$	-1.88×10^{7}	17.7 [c]	−0.9884	–
			$(2–10)\times10^{-7}$	-3.64×10^{7}	−1.3 [c]	−0.9842	9.2×10^{-7}
		DPV	$(2–10)\times10^{-5}$	-2.04×10^{7}	52.5 [c]	−0.9667	–
			$(2–10)\times10^{-6}$	-2.33×10^{7}	4.2 [c]	−0.9826	–
			$(2–10)\times10^{-7}$	-3.43×10^{7}	4.0 [c]	−0.9882	8.4×10^{-7}
	5-NBIA	DCV	$(2–10)\times10^{-5}$	-2.86×10^{7}	−42.9 [c]	−0.9991	–
			$(2–10)\times10^{-6}$	-2.71×10^{7}	−48.4	−0.9967	–
			$(2–10)\times10^{-7}$	-3.75×10^{7}	−1.0 [c]	−0.9984	2.7×10^{-7}
		DPV	$(2–10)\times10^{-5}$	-2.37×10^{7}	−191.8 [c]	−0.9935	–
			$(2–10)\times10^{-6}$	-2.56×10^{7}	−29.2 [c]	−0.9805	–
			$(2–10)\times10^{-7}$	-3.14×10^{7}	1.1 [c]	−0.9963	2.5×10^{-7}
MF-AgSAE [d]	4-NP	DCV	$(2–10)\times10^{-5}$	-1.94×10^{7}	−242.6 [c]	−0.9801	–
			$(2–10)\times10^{-6}$	-1.87×10^{7}	10.4 [c]	−0.9877	4.7×10^{-6}
		DPV	$(2–10)\times10^{-5}$	-1.80×10^{7}	−250.4 [c]	−0.9496	–
			$(2–10)\times10^{-6}$	-1.61×10^{7}	6.4	−0.9998	1.4×10^{-6}
	5-NBIA	DCV	$(2–10)\times10^{-5}$	-2.54×10^{7}	−204.6 [c]	−0.9971	–
			$(2–10)\times10^{-6}$	-2.71×10^{7}	−48.4	−0.9967	–
			$(2–10)\times10^{-7}$	-4.77×10^{7}	−1.2 [c]	−0.9879	9.3×10^{-7}
		DPV	$(2–10)\times10^{-5}$	-2.37×10^{7}	−191.8 [c]	−0.9995	–
			$(2–10)\times10^{-6}$	-5.11×10^{7}	−38.5	−0.9731	–
			$(2–10)\times10^{-7}$	-4.41×10^{7}	2.6 [c]	−0.9961	7.6×10^{-7}

[a] Correlation coefficient. [b] Limit of quantification (10σ; $\alpha = 0.05$). [c] The intercept is not statistically significantly different from zero at a significance level of $\alpha = 0.05$. [d] Deposition time of mercury film was 3600 s.

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8. APPENDIX III

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Voltammetric determination of 2-amino-6-nitrobenzothiazole at two different silver amalgam electrodes

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Dedicated to the Memory of Professor Jaroslav Heyrovský on the Occasion of the 90th Anniversary of the Invention of Polarography.

Keywords:
Voltammetry
Polished silver solid amalgam electrode
Mercury meniscus modified silver solid amalgam electrode
2-Amino-6-nitrobenzothiazole
Drinking and river water samples

ABSTRACT

Voltammetric behavior of genotoxic 2-amino-6-nitrobenzothiazole (ANBT) has been investigated using direct current voltammetry (DCV) and differential pulse voltammetry (DPV) at a polished silver solid amalgam electrode (p-AgSAE) and at a mercury meniscus modified silver solid amalgam electrode (m-AgSAE). The optimum conditions have been found for its determination in the concentration range of 0.2–100 $\mu mol\,l^{-1}$ in a 9:1 (v/v) mixture of aqueous Britton–Robinson buffer solution (pH 10.0) and methanol, with the limits of quantification ($L_Q s$) 0.10 $\mu mol\,l^{-1}$ (DCV at p-AgSAE), 0.19 $\mu mol\,l^{-1}$ (DPV at p-AgSAE), 0.17 $\mu mol\,l^{-1}$ (DCV at m-AgSAE) and 0.12 $\mu mol\,l^{-1}$ (DPV at m-AgSAE). An attempt at increasing the sensitivity using adsorptive stripping DCV or DPV at both p-AgSAE and m-AgSAE was not successful. Practical applicability of the newly developed methods was verified on direct determination of ANBT in spiked samples of drinking and river water, with $L_Q s \sim 10^{-7}\ mol\,l^{-1}$.

1. Introduction

2-Amino-6-nitrobenzothiazole (ANBT, see Fig. 1) is most often used as a base in dyes production by diazotation [1]. Colors of thus prepared dyes are mainly red and violet [2]. This substance is a chromophore for the photoconductive measurements [3] and is also used as a high glass transition chromophore in nonlinear optical applications [4]. Although this substance is useful from the industrial point of view, ANBT has also been shown to exhibit both genotoxic and mutagenic properties [5]. Therefore, a monitoring of detrimental substances like ANBT in the environment is one of the most important tasks of modern analytical chemistry. Electrochemical methods are especially suitable for large scale environmental monitoring of electrochemically active pollutants (ANBT contains two electrochemically active functional groups suitable for this purpose – cathodically reducible nitro-group and anodically oxidizable amino-group) because they are inexpensive, highly sensitive and they present an independent alternative to so far prevalent spectrometric and separation techniques [6]. Moreover, this topic becomes important in the light of the fact that although there are several analytical techniques reviewed for the identification of ANBT [7,8], the sensitive method for its determination, to the best of our knowledge, has not yet been reported.

In the past, the electrochemical behavior of ANBT has been investigated using direct current polarography, oscillopolarography [9], cyclic voltammetry and coulometric techniques [10] at mercury electrodes. The first wave/peak of its electrochemical reduction has been shown to be corresponding to a four-electron reduction of the nitro-group to the hydroxyamino-group and the second wave/peak to the reduction of the previously formed hydroxyamino-group to the amino-group involving two-electron electrode reaction [10]. It is more than obvious that mercury-based working electrodes are suitable for possible polarographic and/or voltammetric determination of ANBT.

Nevertheless, due to increasing fears of liquid mercury toxicity during the last decades [11], which has resulted in the "mercurophobia" [12], a certain attention is paid to the development of working electrodes containing no (e.g., solid bismuth electrodes [13] or bismuth film-based electrodes [14]) or minimum amount of liquid mercury, usually transformed into non-toxic solid

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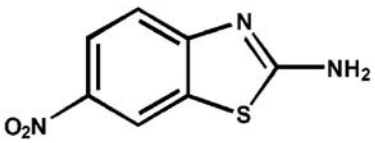


Fig. 1. Structural formula of 2-amino-6-nitrobenzothiazole (ANBT).

or paste amalgam during the preparation of the electrode (e.g., silver amalgam-based electrodes [15–17] such as silver solid amalgam electrodes [18,19], silver solid amalgam composite electrodes [20–22], silver solid amalgam paste electrodes with an organic pasting liquid [23], silver amalgam paste electrodes [24] or single crystal silver amalgam microelectrodes [25,26]); these electrodes can also be used to determine various organic nitro-compounds [27]. From this point of view, working electrodes based on silver solid amalgam (p-AgSAE) [28] and modified by a mercury meniscus (m-AgSAE) represent a suitable less-toxic alternative to the traditional mercury electrodes [29]. A good mechanical stability, simple handling and regeneration including an electrochemical pretreatment of the electrode surface are among the main advantages of these electrodes [30,31]. The wide field of analytical applications of these electrodes has been reviewed [15–17].

This work aims (i) at developing direct current voltammetric (DCV) and differential pulse voltammetric (DPV) methods (making the use of cathodic voltammetric responses provided by the nitro-group in ANBT) for the determination of trace amounts of genotoxic ANBT using p-AgSAE and m-AgSAE and (ii) at testing the possible utilization of the adsorptive stripping technique enabling the accumulation of the analyte at the electrode surface [32]. Furthermore, practical applicability of the newly developed methods was verified on the direct determinations of the studied substance in spiked samples of drinking and river water. Drinking and river water were used for the preparation of spiked samples because of the possible hazard of surface and ground waters contamination by ANBT during the industrial production of dyes and pigments [33].

2. Experimental

2.1. Reagents

2-Amino-6-nitrobenzothiazole (ANBT, ≥97%, CAS Registry Number: 6285-57-0) was supplied by Sigma–Aldrich, Prague, Czech Republic. A 1×10^{-3} mol l^{-1} stock solution was prepared by dissolving an exactly weighed amount of the substance in methanol (99.9%, Merck, Darmstadt, Germany). The stock solution was stored in refrigerator at 4 °C and the dilute solutions were prepared freshly before use.

The Britton–Robinson (BR) buffer solutions were prepared in a usual way, by mixing a solution containing 0.04 mol l^{-1} solution of phosphoric acid, acetic acid and boric acid with an appropriate amount of 0.2 mol l^{-1} sodium hydroxide (all p.a., Lachema, Brno, Czech Republic). Deionized water was produced by Milli-Q Plus system (Millipore, Billerica, MA, USA). All the chemicals were used without further purification and all the solutions (except the stock solution of ANBT) were maintained in glass vessels in dark at laboratory temperature.

2.2. Instrumentation

2.2.1. Apparatus

Voltammetric measurements were carried out using an Eco-Tribo Polarograph driven by Polar Pro 2.0 software (both Polaro–Sensors, Prague, Czech Republic). The software worked under the operational system Microsoft Windows 98 Plus (Microsoft Corporation, Redmond, WA, USA). All measurements were carried out in a three-electrode system using a platinum electrode (type PPE, Monokrystaly, Turnov, Czech Republic) as an auxiliary electrode and silver/silver chloride electrode (type RAE 113, 3 mol l^{-1} KCl, Monokrystaly, Turnov, Czech Republic) as a reference electrode. The polished silver solid amalgam electrode (p-AgSAE) and the mercury meniscus modified silver solid amalgam electrode (m-AgSAE) were used as working electrodes (the disc diameter 0.50 mm) [28]. A scan rate 20 mV s^{-1} was used for both DCV and DPV, a pulse amplitude −50 mV and a pulse width 100 ms, with current sampling for last 20 ms, were used in DPV.

The solution pH was measured by a Jenway digital pH meter (type 3510, Jenway, Chelmsford, UK) with a combined glass electrode (of the same producer) calibrated with standard aqueous buffers at laboratory temperature.

2.2.2. Working electrodes

The p-AgSAE consisted of a drawn-out glass tube, whose tip was packed with a fine silver powder (2–3.5 μm, 99.9+%, Aldrich, Munich, Germany), amalgamated by liquid mercury (triply distilled polarographic mercury, 99.999%, Polarografie, Prague, Czech Republic) and connected to an electric contact. The m-AgSAE was prepared from p-AgSAE by immersing this electrode into a small volume of liquid mercury and agitating for 15 s [15]. The m-AgSAE could be used for several weeks, only its covering by mercury was usually repeated every week.

2.3. Procedures

For voltammetric measurements, an appropriate amount of ANBT stock solution in methanol was measured into a voltammetric vessel, methanol was added, if necessarry, to a total volume of 1.0 ml and filled up to 10.0 ml with a BR buffer of appropriate pH. Oxygen was removed from the measured solutions by bubbling with nitrogen (purity class 4.0, Linde, Prague, Czech Republic) for 5 min. All curves were measured 3 times and all the measurements were carried out at laboratory temperature.

At the beginning, p-AgSAE was polished on the alumina with particle size 1.1 μm (Monokrystaly, Turnov, Czech Republic). The m-AgSAE was prepared by the modification of p-AgSAE by dipping on 15 s to the mercury. Before starting the work on p-AgSAE and m-AgSAE, as well as after electrodes passivation, or every a break in the voltammetric measurements longer than 1 h, the electrochemical activation of the electrodes was carried out in 0.2 mol l^{-1} KCl at −2200 mV, under stirring for 300 s, followed by rinsing with deionized water.

Regeneration of p-AgSAE and m-AgSAE lasting about 30 s preceded each measurement. It was based on application of 300 polarizing cycles (switching the electrode potential from $E_{1,reg}$ to $E_{2,reg}$ for 50 ms). The optimum value of $E_{1,reg}$ was about 50–100 mV more negative than the potential of the anodic dissolution of the electrode and of $E_{2,reg}$ was about 50–100 mV more positive than the potential of the hydrogen evolution in the given supporting electrolyte. Under the optimum conditions, $E_{1,reg}$ was selected −200 mV for p-AgSAE and −400 mV for m-AgSAE, $E_{2,reg}$ was selected −600 mV for p-AgSAE and −900 mV for m-AgSAE. Under these conditions, eventual oxides of mercury or silver are reduced and adsorbed molecules are desorbed [34]. The appropriate values of the potential and the time of regeneration were inset in the software of the computer-controlled instrument used and the regeneration of electrodes was thus carried out automatically.

The DCV peak height (I_p) was evaluated from the extrapolated linear portion of the voltammogram before the onset of the peak. DPV peaks were evaluated from the straight line connecting the minima before and after the peak. The parameters of calibration curves (such as slope, intercept, correlation coefficient, limit of

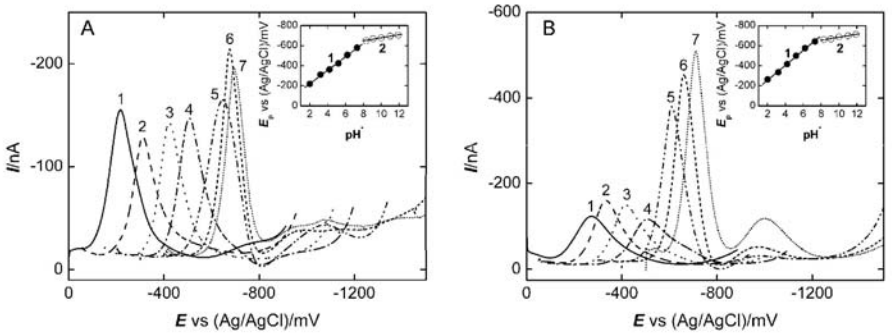


Fig. 2. DP voltammograms of ANBT ($c = 1 \times 10^{-4}$ mol l^{-1}) at p-AgSAE (A) in the BR buffer–methanol (9:1) medium; the BR buffer pH: 2.0 (1), 3.0 (2), 5.0 (3), 6.0 (4), 8.0 (5), 10.0 (6) and 11.0 (7); and at m-AgSAE (B) in the BR buffer–methanol (9:1) medium; the BR buffer pH: 2.0 (1), 3.0 (2), 4.0 (3), 5.0 (4), 9.0 (5), 10.0 (6) and 12.0 (7); polarization rate 20 mV s^{-1}. The corresponding dependences of the E_p values on pH* are given in the insets, depicted for the pH* ranges 2.0–7.3 (1) and 8.3–11.9 (2).

quantification) were calculated with statistic software Adstat 2.0 (TriloByte, Pardubice, Czech Republic) [35].

2.4. Spiked water samples

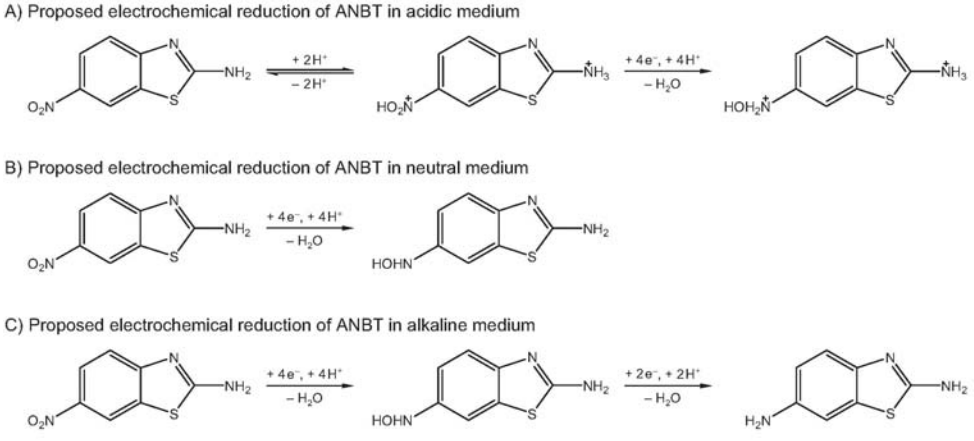
Drinking water from the public water pipeline in the building of Faculty of Science of the Charles University in Prague, Czech Republic and river water obtained from Vltava river (sampled at the locality Výtoň, Prague, Czech Republic), spiked with appropriate amount of ANBT stock solution, were used as samples. Both drinking and river water were used without further pretreatment or purification. The procedure for DCV or DPV determination of ANBT in the spiked samples was as follows: 9.0 ml of the spiked water sample were diluted to 10.0 ml with the BR buffer pH 10.0 and, after deaeration with nitrogen, DC or DP voltammograms at p-AgSAE or m-AgSAE were recorded.

3. Results and discussion

3.1. Voltammetric behavior of 2-amino-6-nitrobenzothiazole at silver amalgam electrodes

The influence of pH on DC and DP voltammetric behavior of 1×10^{-4} mol l^{-1} ANBT at p-AgSAE (see Fig. 2A for DPV) and m-AgSAE (see Fig. 2B for DPV) was investigated in the BR buffer–methanol (9:1) media in the range of pH 2.0–12.0. It can be seen from Fig. 2 that ANBT gives one well-developed cathodic DPV peak, corresponding to the four-electron reduction of the nitro-group to the hydroxyamino-group (Scheme 1), over the whole pH* (pH of the BR buffer–methanol (9:1) medium) region, the second peak can be observed at more negative potentials in the pH* range from 8.3 to 11.9 (for both p-AgSAE and m-AgSAE).

The peak potential (E_p) values of the first peak are shifted towards more negative potentials with increasing pH, which can be explained by a preceding protonation of the nitro-group in ANBT (Scheme 1A) leading to a decrease in the electron density at the nitro-group and resulting in easier electron acceptance at low pH values. The presence of the second peak at more negative potentials gives evidence about a change in the reduction mechanism. This change is also evident from the dependences of the E_p on pH*. Biphasic covering of the whole investigated pH range is notable, the intersection of the straight lines lies at about pH* 7.3–8.3 (Scheme 1B). Upon the DPV of ANBT at p-AgSAE, the E_p of this first peak varied with pH according to the relationships (see the inset in Fig. 2A): E_p vs. Ag/AgCl [mV] = −67.1 pH* − 86.4 (correlation coefficient (R) = −0.9982) in the pH* range 2.0–7.3 and E_p vs. Ag/AgCl [mV] = −15.1 pH* − 526.9 (R = −0.9897) in the pH* range



Scheme 1. Proposed mechanism of electrochemical reduction of ANBT.

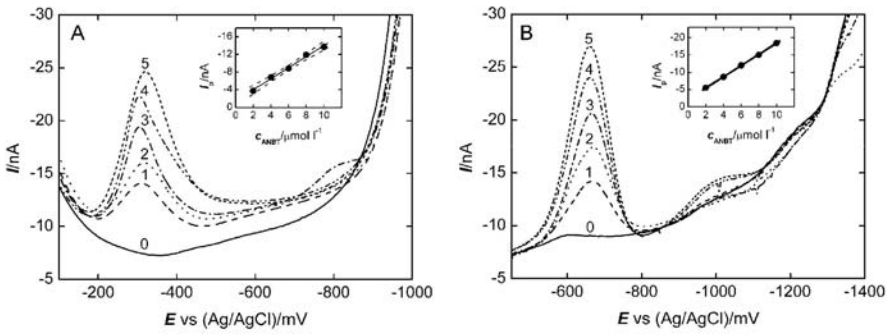


Fig. 3. DP voltammograms of ANBT (c_{ANBT} = 0 (0), 2 (1), 4 (2), 6 (3), 8 (4) and 10 (5) μmol l^{-1}) at p-AgSAE in the BR buffer pH 3.0–methanol (9:1) medium (A) and in the BR buffer pH 10.0–methanol (9:1) medium (B); regeneration potentials $E_{1,reg}$ = −200 mV, $E_{2,reg}$ = −600 mV; polarization rate 20 mV s^{-1}. The corresponding calibration straight lines are given in the insets; the confidence bands are constructed for α = 0.05 (n = 3).

8.3–11.9. Upon the DPV of ANBT at m-AgSAE, the E_p of the first peak varied with pH according to the relationships (see the inset in Fig. 2B): E_p vs. Ag/AgCl [mV] = −73.7 pH* − 110.2 (R = −0.9985) in the pH* range 2.0–7.3 and E_p vs. Ag/AgCl [mV] = −14.9 pH* − 538.5 (R = −0.9924) in the pH* range 8.3–11.9. From these relationships, we can conclude that the reduction of the nitro-group in ANBT is easier at p-AgSAE in the pH* range 2.0–7.3 (because of lower absolute value of the intercept at approximately the same values of the slope), whereas in the pH* range 8.3–11.9, the dependence of the E_p on pH* is almost identical at both p-AgSAE and m-AgSAE. The second voltammetric peak can probably be ascribed to the two-electron reduction of the previously formed hydroxyamino-group to the amino-group (Scheme 1C) [10], but this presumption will need further investigation to be confirmed.

The difference in voltammetric behavior of ANBT in acidic, neutral and alkaline media is also evident on the peak height (I_p) values, but less at p-AgSAE than at m-AgSAE (Fig. 2). Whereas the both DC and DP voltammetric I_p values of ANBT are comparable at m-AgSAE and p-AgSAE in the pH* range 2.0–7.3, the I_p values obtained in the pH* range 8.3–11.9 are about two times higher at m-AgSAE than those at p-AgSAE. This fact predicts a higher sensitivity for the determination of the analyte at m-AgSAE in alkaline media.

3.2. Voltammetric determination of 2-amino-6-nitrobenzothiazole

The highest and best developed DCV and DPV peaks were obtained in the BR buffer pH 10.0–methanol (9:1) medium. This medium was further used for measuring the calibration curves at both electrodes. Moreover, the media representing the acidic region were also tested for the determination of ANBT because of its voltammetric behavior different from that observed in the alkaline media. These media were BR buffer pH 3.0–methanol (9:1) and BR buffer pH 4.0–methanol (9:1) for DPV and DCV, respectively, at both electrodes. For the sake of an illustration, DP voltammograms of ANBT recorded at p-AgSAE in the concentration range 2–10 μmol l^{-1} in acidic (Fig. 3A) and alkaline (Fig. 3B) media are depicted in Fig. 3 for comparison; there is a well observable difference in sensitivities of the determination documented in this figure. The difference can also be seen in Table 1, where all the parameters of the calibration straight lines obtained are summarized. In acidic media, the concentration range 2–100 μmol l^{-1} of ANBT was only measurable, whereas in the BR buffer pH 10.0–methanol (9:1) medium, the concentration range measured was 0.2–100 μmol l^{-1} of ANBT. The limits of quantification (L_Qs) reached were also lower for the alkaline medium used. Upon the comparison of p-AgSAE and m-AgSAE, the L_Q values attained are comparable for both electrodes and both voltammetric techniques used. Nevertheless, the higher sensitivity of the ANBT determination was observed at m-AgSAE, as we supposed at the end of Section 3.1. Therefore, from this point of view, the m-AgSAE seems to be more suitable for the determination of submicromolar concentrations of ANBT.

Generally, the sensitivity slightly differs between individual concentration orders (see Table 1). This behavior, typical for voltammetry at solid electrodes, is usually caused by the passivation of the electrode surface by the electrode reaction products or by analyte adsorption [6]. Nevertheless, within the individual concentration orders, the concentration dependences obtained are linear.

A further increase in the sensitivity of the determination could be achieved by adsorptive accumulation of the test substance on the p-AgSAE or m-AgSAE surface [32]. Optimum conditions, found for the DC and DP voltammetric determination of ANBT (BR buffer pH 10.0), have been used for investigating the possible accumulations. Moreover, media representing acidic (BR buffer pH 3.0) and neutral (BR buffer pH 7.0) pH values were also tested to cover the whole pH range. Suitable potentials of accumulation (E_{acc}) were tested with time of accumulation (t_{acc}) varied from 0 to 5 min. Because methanol is also usually adsorbed on the electrode surface [32], it was not contained in the supporting electrolyte. However, it has been proved that ANBT did not significantly increase its voltammetric response in dependence on the t_{acc} under any conditions tested. This fact is probably caused by the chemical structure of ANBT. Adsorptive stripping methods, enabling successful accumulation of the analyte at the m-AgSAE surface, were previously published [31,36]. Nevertheless, these were cases of very specific molecules – low polar mononitro-derivatives of polycyclic aromatic hydrocarbons (2-nitrofluorene [31] and 3-nitrofluoranthene [36]).

3.3. Spiked samples of drinking and river water

In order to verify practical applicability of the developed DCV and DPV methods, the determination of ANBT was carried out in spiked samples of drinking and river water in a submicromolar concentration range under optimum conditions. Calibration curves were measured using a mixture of 9.0 ml of a spiked water sample and 1.0 ml of the BR buffer pH 10.0. DP voltammograms of ANBT at the p-AgSAE representing its direct determination in spiked drinking (Fig. 4A) and river (Fig. 4B) water, in the concentration range from 0.2 to 1.0 μmol l^{-1}, are depicted in Fig. 4 and DP

Table 1
Parameters of the calibration straight lines (including standard errors) for the determination of ANBT obtained using DCV and DPV at p-AgSAE and m-AgSAE.

Electrode	Method	Optimum medium	Concentration (μmol l^{-1})	Slope (nA l μmol^{-1})	Intercept (nA)	R^a	$L_Q{}^b$ (μmol l^{-1})
p-AgSAE	DCV	BR buffer pH 4.0–methanol (9:1)	20–100	−0.945 ± 0.066	−19.35 ± 0.95	−0.9928	–
			2–10	−1.215 ± 0.036	−13.12 ± 0.24	−0.9987	3.2
	DPV	BR buffer pH 3.0–methanol (9:1)	20–100	−0.825 ± 0.078	−25.6 ± 5.2	−0.9867	–
			2–10	−1.249 ± 0.064	−1.51 ± 0.14[c]	−0.9961	2.6
	DCV	BR buffer pH 10.0–methanol (9:1)	20–100	−1.238 ± 0.041	−14.0 ± 2.7	−0.9983	–
			2–10	−1.612 ± 0.021	−4.42 ± 0.14	−0.9997	–
			0.2–1	−4.922 ± 0.049	0.036 ± 0.032[c]	−0.9999	0.10
	DPV	BR buffer pH 10.0–methanol (9:1)	20–100	−1.646 ± 0.021	−4.1 ± 1.4[c]	−0.9998	–
			2–10	−1.617 ± 0.015	−2.223 ± 0.099	−0.9999	–
			0.2–1	−2.92 ± 0.21	−0.752 ± 0.014	−0.9999	0.19
m-AgSAE	DCV	BR buffer pH 4.0–methanol (9:1)	20–100	−1.445 ± 0.078	−10.2 ± 5.2[c]	−0.9957	–
			2–10	−1.206 ± 0.088	−1.53 ± 0.58[c]	−0.9921	–
			0.2–1	−1.421 ± 0.055	0.950 ± 0.043[c]	−0.9880	0.68
	DPV	BR buffer pH 3.0–methanol (9:1)	20–100	−0.946 ± 0.053	−42.2 ± 3.6	−0.9952	–
			2–10	−1.05 ± 0.16	−1.8 ± 1.0[c]	−0.9687	–
			0.2–1	−1.58 ± 0.11	0.324 ± 0.071[c]	−0.9932	0.45
	DCV	BR buffer pH 10.0–methanol (9:1)	20–100	−3.807 ± 0.030	−16.5 ± 1.9	−0.9908	–
			2–10	−4.611 ± 0.091	−4.7 ± 1.7	−0.9901	–
			0.2–1	−6.253 ± 0.099	0.4 ± 1.1	−0.9895	0.17
	DPV	BR buffer pH 10.0–methanol (9:1)	20–100	−2.884 ± 0.028	−22.6 ± 1.8	−0.9897	–
			2–10	−2.901 ± 0.076	−2.71 ± 0.51	−0.9910	–
			0.2–1	−2.51 ± 0.15	−0.41 ± 0.10[c]	−0.9944	0.12

[a] Correlation coefficient.
[b] Limit of quantification (10σ; $\alpha = 0.05$).
[c] Intercepts are not statistically significantly different from zero at a significance level $\alpha = 0.05$.

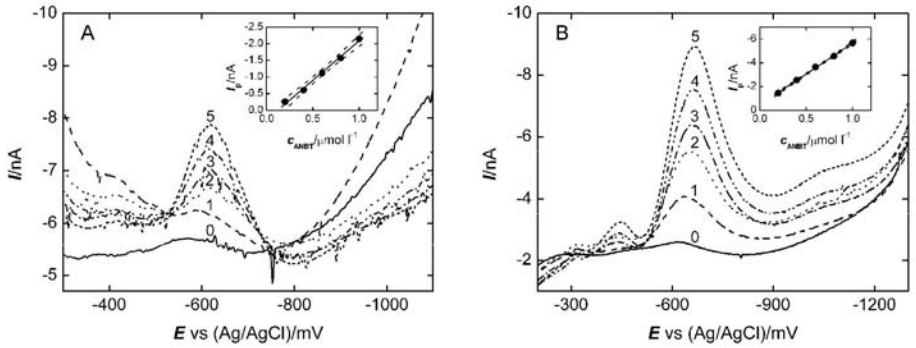


Fig. 4. DP voltammograms of ANBT at p-AgSAE in drinking (A) and river (B) water samples; measured in 9 ml of spiked drinking or river water diluted by BR buffer pH 10.0 to 10 ml (dilution factor 0.9); c_{ANBT} in waters: 0 (0), 0.2 (1), 0.4 (2), 0.6 (3), 0.8 (4) and 1.0 (5) μmol l^{-1}, regeneration potentials $E_{1,reg} = -200$ mV, $E_{2,reg} = -600$ mV; polarization rate 20 mV s^{-1}. The corresponding calibration straight lines are given in the insets; the confidence bands are constructed for $\alpha = 0.05$ ($n = 3$).

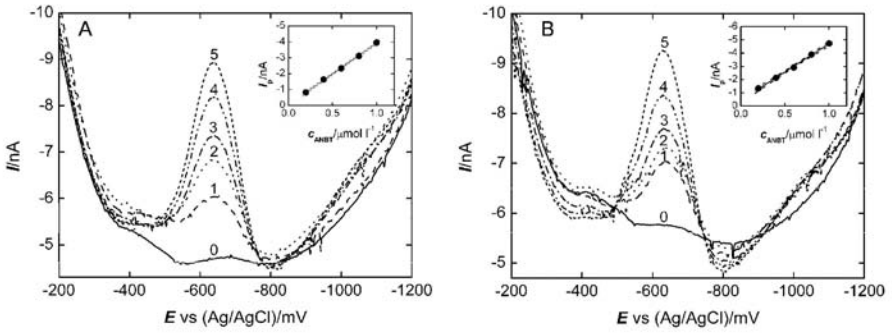


Fig. 5. DP voltammograms of ANBT at m-AgSAE in drinking (A) and river (B) water samples; measured in 9 ml of spiked drinking or river water diluted by BR buffer pH 10.0 to 10 ml (dilution factor 0.9); c_{ANBT} in waters: 0 (0), 0.2 (1), 0.4 (2), 0.6 (3), 0.8 (4) and 1.0 (5) μmol l^{-1}, regeneration potentials $E_{1,reg} = -400$ mV, $E_{2,reg} = -900$ mV; polarization rate 20 mV s^{-1}. The corresponding calibration straight lines are given in the insets; the confidence bands are constructed for $\alpha = 0.05$ ($n = 3$).

Table 2
Parameters of the calibration straight lines (including standard errors) for the determination of ANBT obtained using DCV and DPV at p-AgSAE and m-AgSAE in spiked samples of water, i.e., in the mixture of spiked water sample–BR buffer pH 10.0 (9:1).

Electrode	Method	Matrix	Concentration (μmol l^{-1})	Slope (nA l μmol^{-1})	Intercept (nA)	R^a	$L_Q{}^b$ (μmol l^{-1})
p-AgSAE	DCV	Drinking water	2–10	−1.000 ± 0.057	−1.20 ± 0.38[c]	−0.9951	–
			0.2–1	−2.769 ± 0.086	−1.037 ± 0.057	−0.9986	0.12
	DPV	Drinking water	2–10	−1.790 ± 0.047	0.37 ± 0.31[c]	−0.9990	–
			0.2–1	−2.37 ± 0.11	0.283 ± 0.072	−0.9969	0.13
	DCV	River water	2–10	−2.78 ± 0.22	3.1 ± 1.5[c]	−0.9907	–
			0.2–1	−4.441 ± 0.086	−0.876 ± 0.058	−0.9959	0.41
	DPV	River water	2–10	−2.85 ± 0.33	4.4 ± 2.2[c]	−0.9809	–
			0.2–1	−5.208 ± 0.085	−0.451 ± 0.056	−0.9996	0.11
m-AgSAE	DCV	Drinking water	2–10	−2.32 ± 0.13	1.52 ± 0.84[c]	−0.9961	–
			0.2–1	−4.56 ± 0.17	0.16 ± 0.12[c]	−0.9978	0.39
	DPV	Drinking water	2–10	−2.26 ± 0.15	1.52 ± 0.84[c]	−0.9933	–
			0.2–1	−3.877 ± 0.062	0.045 ± 0.041[c]	−0.9996	0.13
	DCV	River water	2–10	−1.944 ± 0.099	−1.62 ± 0.66[c]	−0.9969	–
			0.2–1	−4.85 ± 0.26	−0.17 ± 0.17[c]	−0.9994	0.20
	DPV	River water	2–10	−2.006 ± 0.052	−0.20 ± 0.34[c]	−0.9990	–
			0.2–1	−4.26 ± 0.10	−0.446 ± 0.069	−0.9991	0.12

[a] Correlation coefficient.
[b] Limit of quantification (10σ; $\alpha = 0.05$).
[c] Intercepts are not statistically significantly different from zero at a significance level $\alpha = 0.05$.

voltammograms of ANBT at the m-AgSAE in spiked drinking (Fig. 5A) and river (Fig. 5B) water, in the same concentration range, are depicted in Fig. 5. The parameters of the calibration curves obtained are summarized in Table 2. These results confirm the possible application of both tested electrodes for the determination of even submicromolar concentrations of substance under investigation in drinking and river waters.

4. Conclusions

It has been shown that modern voltammetric methods at two different silver amalgam working electrodes can be used for the determination of submicromolar concentrations of genotoxic 2-amino-6-nitrobenzothiazole (ANBT). In the optimum medium found (BR buffer pH 10.0–methanol (9:1)), the measurable concentration range was 0.2–100 μmol l^{-1} of ANBT for both direct current voltammetric (DCV) and differential pulse voltammetric (DPV) determination at the polished silver solid amalgam electrode (p-AgSAE) and at the mercury meniscus modified silver solid amalgam electrode (m-AgSAE). The attempt at increasing the sensitivity using adsorptive stripping DCV or DPV at both p-AgSAE and m-AgSAE was not successful. The applicability of both tested electrodes for DCV and DPV determination of ANBT in spiked samples of drinking and river water (in the concentration range from 0.2 to 10 μmol l^{-1} of ANBT) has also been verified, with the limits of quantification (L_Qs) in the concentration order of 10^{-7} mol l^{-1}. The parameters of the calibration straight lines obtained at p-AgSAE are well comparable with those obtained at m-AgSAE. Therefore, it can be concluded that both p-AgSAE and m-AgSAE can be successfully used for the determination of trace amounts of ANBT as suitable non-toxic and environmentally friendly alternatives to mercury electrodes.

Acknowledgments

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9. APPENDIX IV

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Bismuth film electrode at a silver solid amalgam substrate as a new tool for voltammetric determination of electrochemically reducible organic compounds

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ABSTRACT

New type of bismuth film electrode prepared by electrodeposition of bismuth film on a silver solid amalgam substrate (BiF–AgSAE) was tested as a sensor for voltammetric determination of electrochemically reducible organic substances using 2-amino-6-nitrobenzothiazole (ANBT) as a model analyte. Using the optimized conditions (a 9:1 (v/v) mixture of aqueous Britton–Robinson buffer solution (pH 10.0) and methanol), the limits of quantification are 0.16 μmol L^{-1} for direct current voltammetry (DCV) and 0.22 μmol L^{-1} for differential pulse voltammetry (DPV). The obtained calibration dependences are linear in the concentration range from 0.2 to 100 μmol L^{-1} and the practical applicability of the newly developed electrode for the direct determination of ANBT in tap and mineral water model samples was confirmed in the concentration range from 0.2 to 10 μmol L^{-1}.

1. Introduction

Mercury is obviously the best available electrode material for voltammetric determination of electrochemically reducible organic compounds because of easily renewable and atomically smooth surface and large cathodic potential window [1]. However, due to increasing fears of liquid mercury toxicity resulting in somewhat unsubstantiated "mercurophobia" [2], considerable attention is paid to the search for new electrode materials applicable for cathodic voltammetry. Boron-doped diamond film electrodes [3,4], various types of non-toxic solid [5–8], paste [9–11] or composite [12,13] silver amalgam electrodes and, quite recently, solid bismuth electrodes [14] or bismuth film electrodes [15] are typical examples of this approach. In the case of silver solid amalgam, our recent results showed that the mercury vapor pressure of such amalgam containing no liquid mercury is far lower than the mercury vapor pressure of liquid mercury (about two orders of signal magnitude) and comparable with mercury vapor pressure of dental amalgams [16]. A number of papers deal with the bismuth film deposited on a glassy carbon (BiF–GCE) or carbon paste (BiF–CPE) substrate [17–23]. However, silver solid amalgam, which was found useful for deposition of a uniform mercury film [24,25], was not tested as a substrate for bismuth film so far. Therefore, we have investigated the possibility of electrochemical preparation of uniform bismuth film at this substrate and practical application of thus-prepared bismuth film electrode at the silver solid amalgam substrate (BiF–AgSAE) for voltammetric determination of electrochemically reducible organic compounds.

We have used 2-amino-6-nitrobenzothiazole (ANBT, see Fig. 1) as a model substance. This compound (used, e.g., in the dye industry) is known for its genotoxic and mutagenic properties [26] and, thus, it is desirable to develop inexpensive and sensitive electroanalytical methods for its determination in the environment. It contains cathodically reducible nitro-group and its investigation using direct current polarography [27,28], oscillopolarography [27], cyclic voltammetry [28] and coulometry [28] at mercury electrodes and DC voltammetry (DCV) and differential pulse voltammetry (DPV) at a polished silver solid amalgam electrode (p-AgSAE) and a mercury meniscus modified silver solid amalgam electrode (m-AgSAE) [29] confirmed a four-electron reduction of the nitro-group to the hydroxyamino-group followed by its two-electron reduction to the amino-group. In addition, to testing the possibility to prepare and use the BiF–AgSAE, we have verified its application for the direct determination of the target compound in model samples of tap and mineral water. We have used these model matrices because of the potential hazard of surface and ground waters contamination by ANBT during the industrial production of dyes and pigments [30].

2. Experimental

2.1. Reagents

A 1×10^{-3} mol L^{-1} stock solution of 2-amino-6-nitrobenzothiazole (ANBT) was prepared by dissolving an exactly weighed

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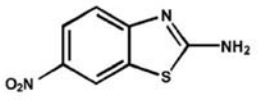

Fig. 1. Structural formula of 2-amino-6-nitrobenzothiazole (ANBT).

amount of the substance supplied by Sigma-Aldrich, Prague, Czech Republic, in methanol (99.9%, Merck, Darmstadt, Germany). The stock solution was stored in refrigerator at 4 °C and the dilute solutions were prepared freshly before use.

The Britton–Robinson (BR) buffer solutions were prepared in a usual way, by mixing a solution containing 0.04 mol L^{-1} solution of phosphoric acid (p.a. purity, Merck), acetic acid (p.a. purity, Panreac, Barcelona, Spain) and boric acid (p.a. purity, Lach-Ner, Neratovice, Czech Republic) with an appropriate amount of 0.2 mol L^{-1} sodium hydroxide (p.a. purity, Merck). Sodium acetate trihydrate (p.a. purity, Lach-Ner) and ethylenediaminetetraacetic acid (EDTA, p.a. purity, Panreac) were also used. Deionized water was produced by Milli-Q Plus system (Millipore, Billerica, MA, USA).

2.2. Instrumentation

2.2.1. Apparatus

Voltammetric measurements were carried out using a PalmSens electrochemical analyzer driven by PSTrace 2.1 software (both Palm Instruments, Houten, The Netherlands). The software worked under the operational system Microsoft Windows XP (Microsoft Corporation, Redmond, WA, USA). All measurements were carried out in a three-electrode system using platinum electrode (type PPE, Monokrystaly, Turnov, Czech Republic) as an auxiliary electrode and silver|silver chloride electrode (type RAE 113, 3 mol L^{-1} KCl, Monokrystaly) as a reference electrode. The BiF–AgSAE (the disc diameter 0.50 mm) was used as a working electrode. The scan rate 20 mV s^{-1} was used for both DCV and DPV, the pulse amplitude −50 mV and the pulse width 100 ms, with current sampling for the last 20 ms, were used in DPV.

The solution pH was measured by a Jenway digital pH meter (type 3510, Jenway, Chelmsford, UK) with a combined glass electrode (of the same producer) calibrated with standard aqueous buffers at laboratory temperature.

2.2.2. Preparation of bismuth film electrode on silver solid amalgam substrate (BiF–AgSAE)

The BiF–AgSAE was prepared *ex situ* by deposition of the bismuth film on the AgSAE at constant potential of −1.2 V (vs. Ag|AgCl) for selected time (t_{dep}) under stirring in 10 mL of plating solution (after deaeration of the solution for 5 min with pure nitrogen) containing 0.5 mL of bismuth standard solution (1000 mg L^{-1} Bi(III), Darmstadt, Merck, Germany) and 9.5 mL of 1.0 mol L^{-1} acetate buffer pH 4.75. The BiF–AgSAE was plated every day with new bismuth film because the lifetime of the electrode surface was relatively short, namely just a few hours. No mechanical, electrochemical or chemical cleaning or activation was performed, since such procedures damaged the bismuth film coating leading to less reproducible results.

The AgSAE used as a substrate consisted of a drawn-out glass tube, whose tip was packed with a fine silver powder (2–3.5 μm, 99.9%, Sigma-Aldrich, Prague, Czech Republic), amalgamated by liquid mercury (triply distilled polarographic mercury, 99.999%, Polarografie, Prague, Czech Republic), polished on the alumina with particle size 1.1 μm (Monokrystaly, Turnov, Czech Republic) and connected to an electric contact [5].

2.3. Procedures

An appropriate amount of ANBT stock solution in methanol was measured into a voltammetric cell, methanol was added, if necessary, to a total volume of 1.0 mL and filled up to 10.0 mL with a BR buffer of appropriate pH. Oxygen was removed from the measured solutions by bubbling with pure nitrogen for 5 min. Unless stated otherwise, all curves were measured three times, and all the measurements were carried out at laboratory temperature.

The DCV peak height (I_p) was evaluated from the extrapolated linear portion of the voltammogram before the onset of the peak. DPV peaks were evaluated from the straight line connecting the minima before and after the peak. The parameters of calibration curves (such as slope, intercept, correlation coefficient, limit of quantification) were calculated with statistic software Adstat 2.0 (TriloByte, Pardubice, Czech Republic) [31].

2.4. Model samples

The tap water from the public water pipeline in the building of National and Kapodistrian University, Athens, Greece, and natural mineral water (Spring Olympos, Katerini, Greece), spiked with appropriate amounts of ANBT stock solution, were used as model samples. Both tap and mineral water were either used without further pretreatment/purification or solid EDTA was added (1 g per 50 mL of water) for masking of cations present in solution. The procedure for DCV or DPV determination of ANBT in model samples was as follows: 9.0 mL of the model water sample were diluted to 10.0 mL with the BR buffer pH 10.0 and, after deaeration with nitrogen, DC or DP voltammograms at the BiF–AgSAE were recorded.

3. Results and discussion

3.1. Bismuth film deposition

At first, parameters for the deposition of the bismuth film on the AgSAE surface were optimized. The times of deposition (t_{dep}) 60, 120, 180, 240, 300 and 600 s were tested. The t_{dep}=300 s was selected as the optimum one because shorter times of deposition resulted in unstable bismuth films, leading to low repeatability of twenty consecutive DPV measurements of ANBT. At t_{dep}=600 s, the results were very similar to those obtained at 300 s.

The reproducibility of the bismuth film deposition was also investigated because the new film had to be prepared every day. The relative standard deviation (RSD) of DPV determination of ANBT ($c=1\times10^{-4}$ mol L^{-1}) in the BR buffer pH 10.0–methanol (9:1) medium (this medium was selected on the basis of initial experiments conducted at different pH values; see below) at ten different BiF–AgSAEs was 16% (Fig. 2A); this value was assumed to be satisfactory. Moreover, the repeatability of twenty consecutive DPV measurements of ANBT (also expressed as RSD), performed under the conditions mentioned above at a single freshly prepared bismuth film, was 4.5% (Fig. 2B), which also represents a satisfactory value for this type of electrode [19]. It can be seen in Fig. 2B that the I_p value decreased with the serial number of DPV scan performed. Nevertheless, such decrease usually stopped after about thirty to fifty DPV scans, resulting in RSD about 2% for another following twenty consecutive measurements.

3.2. Voltammetric behavior of 2-amino-6-nitrobenzothiazole at the BiF–AgSAE

The influence of pH on the DC and DP voltammetric behavior of 1×10^{-4} mol L^{-1} ANBT at the BiF–AgSAE was investigated in

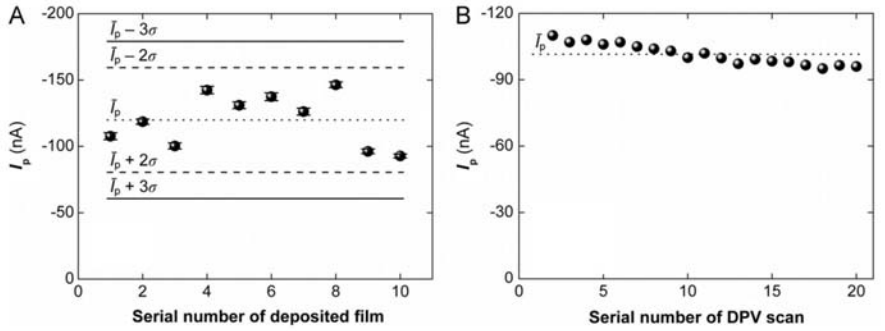

Fig. 2. (A) Reproducibility of the bismuth film deposition shown as the control chart (average value (dotted line), warning limits (dashed lines), control limits (solid lines)) of the DPV peak height of ANBT ($c = 1 \times 10^{-4}$ mol L^{-1}), recorded at ten different BiF–AgSAEs ($t_{dep} = 300$ s) in the BR buffer pH 10.0–methanol (9:1) medium; the error bars are constructed for $\alpha = 0.05$ ($n = 3$). (B) Repeatability of twenty consecutive DPV determinations of ANBT ($c = 1 \times 10^{-4}$ mol L^{-1}) at a single BiF–AgSAE ($t_{dep} = 300$ s) in the BR buffer pH 10.0–methanol (9:1) medium; the first measured value was discarded as an outlier; the average peak height is marked by the dotted line ($n = 19$).

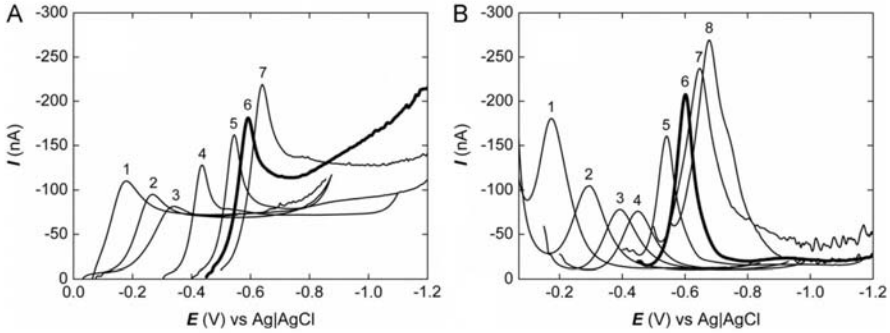

Fig. 3. DC (A) and DP (B) voltammograms of ANBT ($c = 1 \times 10^{-4}$ mol L^{-1}) at the BiF–AgSAE ($t_{dep} = 300$ s) in BR buffer–methanol (9:1) media; polarization rate 20 mV s^{-1}. The BR buffer pH values for A: 2.0 (1), 4.0 (2), 5.0 (3), 7.0 (4), 9.0 (5), 10.0 (6), 11.0 (7); and for B: 2.0 (1), 3.0 (2), 4.0 (3), 5.0 (4), 8.0 (5), 10.0 (6), 11.0 (7), 12.0 (8). The voltammograms recorded under optimum conditions for the determination of ANBT are in bold.

the BR buffer–methanol (9:1) media in the pH range of 2.0–12.0. Fig. 3 illustrates that ANBT gives one well-developed cathodic DCV or DPV peak, corresponding to the four-electron reduction of the nitro-group to the hydroxyamino-group, over the whole pH range explored. The second peak, which can be observed at more negative potentials at mercury [28] and silver solid amalgam [29] working electrodes and which corresponds to a two-electron reduction of the hydroxyamino-group to the amino-group, is not observable at the BiF–AgSAE.

The difference in voltammetric behavior of ANBT in acidic, neutral and alkaline media is evident from the peak shapes and peak height values (Fig. 3). The peak potential shifted towards more negative potentials with increasing pH, which can be explained by a preceding protonation of the nitro-group in ANBT, leading to a decrease in the electron density at the nitro-group and resulting in easier electron acceptance at low pH values [32].

3.3. Voltammetric determination of 2-amino-6-nitrobenzothiazole

The highest and best developed DCV and DPV peaks were obtained in the BR buffer pH 10.0–methanol (9:1) medium. This medium was then used for measuring the calibration curves in a concentration range from 0.2 to 100 μmol L^{-1} of ANBT; DP voltammograms of ANBT at the BiF–AgSAE in the concentration range 2–10 μmol L^{-1} and 0.2–1.0 μmol L^{-1} are depicted in Fig. 4A and B, respectively. The parameters of all the calibration straight lines are summarized in Table 1.

Generally, the measurement sensitivity slightly varies between the individual concentration orders (see Table 1). This behavior, typical for voltammetry at solid electrodes, is usually caused by the passivation of the working electrode surface by electrode reaction products or by adsorption of the analyte [33]. However, within the individual concentration orders, the concentration dependences obtained are linear. For the concentration range from 2 to 100 μmol L^{-1} of ANBT, the sensitivity of both DCV and DPV determinations achieved at the BiF–AgSAE is approximately two times higher, on average, than that at the bare p-AgSAE [29], whereas, for the concentration range from 0.2 to 1.0 μmol L^{-1} of ANBT, the determination sensitivity is approximately 1.6–1.7 times higher at the p-AgSAE [29]. These differences can probably be related to the different morphology of working electrode surfaces (p-AgSAE vs. BiF–AgSAE), which is under our current investigation. Nevertheless, the limits of quantification (L_Qs) reached at the BiF–AgSAE and p-AgSAE are comparable (see Table 1 and Ref. [29], respectively).

A further increase in the sensitivity of the determination could be achieved by adsorptive accumulation of the analyte on the surface of the working electrode [34]. The aqueous component of the optimum medium found for DCV and DPV determination of ANBT (BR buffer pH 10.0) has been used for investigating the possible accumulation.

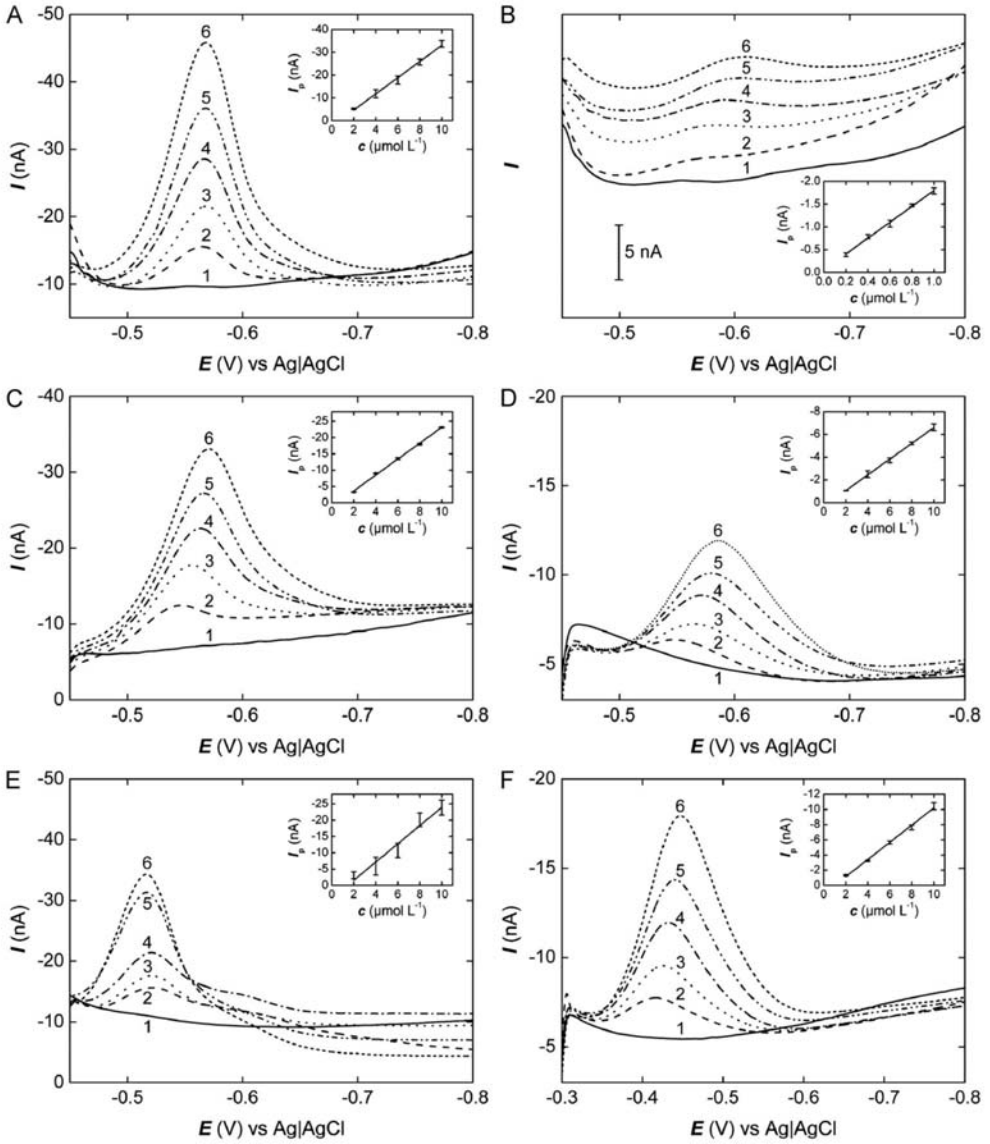

Fig. 4. (A and B) DP voltammograms of ANBT at the BiF–AgSAE (t_{dep} = 300 s) in the BR buffer pH 10.0–methanol (9:1) medium; concentrations of ANBT (μmol L^{-1}) for A: 0 (1), 2 (2), 4 (3), 6 (4), 8 (5), 10 (6); and for B: 0 (1), 0.2 (2), 0.4 (3), 0.6 (4), 0.8 (5), 1.0 (6). (C–F) DP voltammograms of ANBT at the BiF–AgSAE (t_{dep} = 300 s) in model samples of tap (C and D) and mineral (E and F) water; measured in 9 mL of spiked waters diluted by BR buffer pH 10.0 to 10 mL (dilution factor 0.9) without (C and E) and with the addition of EDTA (D and F) to tap or mineral water; concentrations of ANBT (μmol L^{-1}) in waters: 0 (1), 2 (2), 4 (3), 6 (4), 8 (5), 10 (6). Polarization rate 20 mV s^{-1}; the corresponding calibration straight lines are given in the insets; the error bars are constructed for α = 0.05 (n = 3).

Moreover, media representing acidic (BR buffer pH 3.0) and neutral (BR buffer pH 7.0) pH values have also been tested to cover the whole pH range. Because methanol is also usually adsorbed on the electrode surface [34], it was avoided from the supporting electrolyte. Suitable accumulation potentials were tested with the accumulation time varying from 0 to 5 min. Unfortunately, it has been found that the voltammetric peak of ANBT did not significantly increase with prolonging accumulation time under any conditions tested. Similar negative results have also been obtained for adsorptive accumulation of ANBT at the p-AgSAE and m-AgSAE [29].

Table 1
Parameters of the calibration straight lines (including standard deviations) for DCV and DPV determination of ANBT at the BiF–AgSAE in the BR buffer pH 10.0–methanol (9:1) medium.

Method	Concentration (μmol L^{-1})	Slope (nA L μmol^{-1})	Intercept (nA)	R	L_Q (μmol L^{-1})
DCV	20–100	-2.81 ± 0.11	14.6 ± 7.1[a]	−0.9978	–
	2–10	-2.74 ± 0.10	-1.25 ± 0.68[a]	−0.9979	–
	0.2–1	-3.029 ± 0.034	-0.186 ± 0.022	−0.9998	0.16
DPV	20–100	-3.21 ± 0.11	6.0 ± 7.5[a]	−0.9981	–
	2–10	-3.54 ± 0.13	2.43 ± 0.84[a]	−0.9974	–
	0.2–1	-1.739 ± 0.048	-0.058 ± 0.032[a]	−0.9984	0.22

R, correlation coefficient; L_Q, limit of quantification (10σ; $\alpha = 0.05$).
[a] Intercepts are not statistically significantly different from zero at the significance level $\alpha = 0.05$.

Table 2
Parameters of the calibration straight lines (including standard deviations) for DCV and DPV determination of ANBT at the BiF–AgSAE in model samples of water, i.e., in the mixture of spiked water sample–BR buffer pH 10.0 (9:1).

Water	Method	Concentration (μmol L^{-1})	Slope (nA L μmol^{-1})	Intercept (nA)	R	L_Q (μmol L^{-1})
Tap	DCV	2–10	-1.595 ± 0.049	1.46 ± 0.33	−0.9986	–
		0.2–1	-2.055 ± 0.086	0.130 ± 0.057[a]	−0.9974	0.45
	DPV	2–10	-2.440 ± 0.062	1.26 ± 0.41[a]	−0.9987	–
		0.2–1	-2.002 ± 0.063	0.236 ± 0.042	−0.9985	0.40
Tap with EDTA	DCV	2–10	-0.713 ± 0.081	0.447 ± 0.053	−0.9998	–
		0.2–1	-0.920 ± 0.017	0.166 ± 0.011	−0.9995	0.35
	DPV	2–10	-0.690 ± 0.012	0.321 ± 0.077	−0.9994	–
		0.2–1	-0.291 ± 0.016	0.058 ± 0.011	−0.9938	0.31
Mineral with EDTA	DCV	2–10	-0.528 ± 0.046	0.25 ± 0.31[a]	−0.9934	–
		0.2–1	-0.612 ± 0.077	-0.32 ± 0.12[a]	−0.9964	0.40
	DPV	2–10	-1.130 ± 0.042	1.12 ± 0.28[a]	−0.9972	–
		0.2–1	-0.638 ± 0.080	0.171 ± 0.053[a]	−0.9696	0.44

R, correlation coefficient; L_Q, limit of quantification (10σ; $\alpha = 0.05$).
[a] Intercepts are not statistically significantly different from zero at the significance level $\alpha = 0.05$.

3.4. Model samples of tap and mineral water

In order to verify practical applicability of the developed DCV and DPV methods, the determination of ANBT was carried out in model samples of tap and mineral water in a submicromolar concentration range under optimum conditions. Calibration curves were measured using a mixture of 9.0 mL of spiked model water sample and 1.0 mL of the BR buffer pH 10.0. DP voltammograms of ANBT at the BiF–AgSAE representing its direct determination in spiked tap (Fig. 4C and D) and mineral (Fig. 4E and F) water, in the concentration range 2–10 μmol L^{-1}, are depicted in Fig. 4.

During the preparation of model samples of waters, both tap and mineral water were either used without further pretreatment/purification or solid EDTA was added (1 g per 50 mL of water) for masking of cations (e.g., Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}) present in solution to eliminate their negative effect. The effect of the addition of EDTA on voltammetric determination of ANBT is demonstrated in Fig. 4D and F and Table 2. Upon the addition of EDTA to tap water, the sensitivity of determination decreased, however, the L_Qs attained are rather lower. Therefore, the addition of EDTA to samples containing the tap water contaminated with ANBT could be considered for individual cases. On the other hand, in the case of the addition of EDTA to mineral water, the better results were obtained. Although the sensitivity of the determination after the addition of EDTA also decreased, calibration dependences with better linearity were obtained and the repeatability of the determination was better as well. Thus, it is advisable to add EDTA always upon the determination of ANBT in water samples with higher content of mineral cations. DPV voltammograms corresponding to the lowest attainable concentration range are illustrated in Fig. 5 for both tap and mineral water model samples with the addition of EDTA. The obtained parameters of the calibration curves are summarized in Table 2. These results confirm the possible application of tested electrode for the determination of submicromolar concentrations of ANBT in both tap and mineral waters.

3.5. Possibilities of the newly introduced BiF–AgSAE

The area, in which bismuth film electrodes (BiFEs) find most applications, is trace analysis of metals by electrochemical stripping techniques [18]. However, determinations of low-molecular-weight organic compounds by adsorptive stripping analysis at the BiFEs have not been reported so far [19]. There are only sporadic reports on voltammetric or amperometric determinations of some genotoxic nitro-compounds (e.g., nitrobenzene [35], 2-nitrophenol [15,36], 4-nitrophenol [15] and 2,4-dinitrophenol [15]) and some pesticides (e.g., paraquat [37], thiamethoxam, acetamiprid, nitenpyram [38], imidacloprid [38,39] and clothianidin [39]). Therefore, the BiF–AgSAE presented in this paper represents valuable alternative to previously reported BiFEs, moreover, with one of the lowest L_Qs achieved in organic electroanalysis (this is probably thanks to the property of bismuth to form "fused alloys" with heavy metals, which is analogous to the amalgams that mercury forms [19,40]).

We suppose that the p-AgSAE substrate also plays important role in the observed benefits of BiF–AgSAE. In our recent comparative studies [41,42], the BiF–AgSAE has been confronted with BiFEs based on glassy carbon (BiF–GCE) and gold (BiF–AuE) substrates upon the voltammetric determination of 5-nitrobenzimidazole (a compound structurally very similar to ANBT). The L_Q

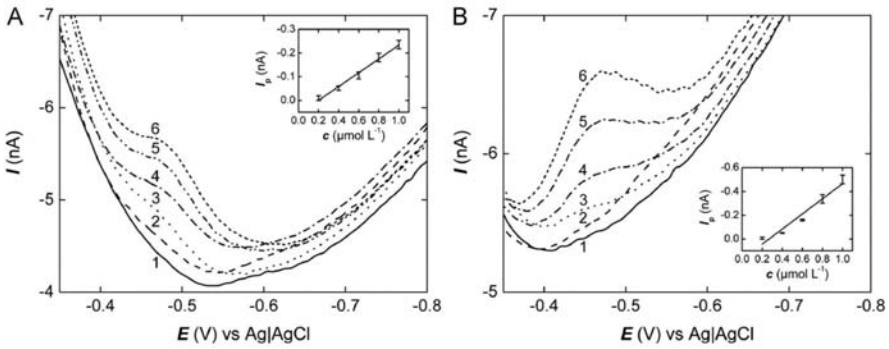

Fig. 5. DP voltammograms of ANBT at the BiF–AgSAE (t_{dep}=300 s) in model samples of tap (A) and mineral (B) water; measured in 9 mL of spiked waters diluted by BR buffer pH 10.0 to 10 mL (dilution factor 0.9) with the addition of EDTA to tap or mineral water; polarization rate 20 mV s^{-1}; concentrations of ANBT (μmol L^{-1}) in waters: 0 (1), 0.2 (2), 0.4 (3), 0.6 (4), 0.8 (5), 1.0 (6); the corresponding calibration straight lines are given in the insets; the error bars are constructed for α=0.05 (*n*=3).

in the concentration order of 10^{-8} mol L^{-1} was achieved for 5-nitrobenzimidazole at the BiF–AgSAE [41], whereas, at both BiF–GCE and BiF–AuE, the L_Qs were about two orders of magnitude higher [42]. In addition, the repeatabilities of twenty consecutive DPV measurements of 5-nitrobenzimidazole ($c=1\times10^{-4}$ mol L^{-1}) were 3%, > 5% and 1% at the BiF–AgSAE, BiF–GCE and BiF–AuE, respectively.

Simple mechanical renewal of the BiF–AgSAE surface, good reproducibility of measurements and elimination of problems connected with "electrode history" confirm practical usefulness of this electrode. Its major drawback is collective for all the BiFEs, i.e., its limited anodic range that prevents the use of this electrode for the determination or accumulation of species at more positive potentials [19].

4. Conclusions

It has been proved that newly developed bismuth film electrode on a silver solid amalgam support can be used as a working electrode for cathodic voltammetry. Both DC voltammetry (DCV) and differential pulse voltammetry (DPV) at the bismuth film modified silver solid amalgam electrode (BiF–AgSAE) can be used for the determination of submicromolar concentrations of genotoxic 2-amino-6-nitrobenzothiazole (ANBT). The sensitivity of this determination is ca. two times higher than that in the determination at the substrate electrode. The optimum time of the bismuth film deposition on the silver solid amalgam substrate (disc diameter 0.50 mm) was found to be 300 s. In the optimum medium found (BR buffer pH 10.0–methanol (9:1)), the measurable concentration range was 0.2–100 μmol L^{-1} of ANBT for both DCV (the limit of quantification, $L_Q\approx0.16$ μmol L^{-1}) and DPV ($L_Q\approx0.22$ μmol L^{-1}) determination at the BiF–AgSAE. The attempt to increase the sensitivity using adsorptive stripping DCV or DPV at the BiF–AgSAE was not successful.

The applicability of the tested electrode for DCV and DPV determination of ANBT in model samples of tap and mineral water (in the concentration range from 0.2 to 10 μmol L^{-1} of ANBT) has also been verified, with the L_Qs in the concentration order of 10^{-7} mol L^{-1}. Therefore, it can be concluded that the BiF–AgSAE can be successfully used for the determination of trace amounts of ANBT as suitable non-toxic and environmentally friendly alternative to electrodes containing metallic mercury.

Acknowledgments

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10. APPENDIX V

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A New Type of Large-Surface Bismuth Film Electrode on a Silver Solid Amalgam Substrate and its Application for the Voltammetric Determination of 5-Nitrobenzimidazole

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A novel type of large-surface bismuth film electrode prepared by electrochemical deposition of bismuth on a silver solid amalgam electrode (LSBiF-AgSAE) was developed. A silver solid amalgam substrate with a diameter of 2.64 mm was used to achieve larger surface of this electrode resulting in higher sensitivity in the voltammetric analysis. The larger electrode surface required longer time (i.e. 1800 s) for the electrochemical deposition of the bismuth film. The electrode surface of thus prepared electrode was characterized by atomic force microscopy (AFM) which confirmed that bismuth film was not grown uniformly over the supporting surface, but created three-dimensional higher formations which cover only a fraction of the surface. To verify practical application of the new LSBiF-AgSAE, voltammetric behavior of the genotoxic 5-nitrobenzimidazole (5-NBIA) has been investigated using direct current voltammetry (DCV) and differential pulse voltammetry (DPV) and optimum conditions

have been found for its determination in the concentration range from 0.2 to 1000 μmol L^{-1} in Britton–Robinson buffer solution (pH 7.0), with the limits of quantification (L_Qs) 0.37 μmol L^{-1} (DCV) and 0.07 μmol L^{-1} (DPV). An attempt to increase the sensitivity using adsorptive stripping DCV or DPV at the BiF-AgSAE was not successful. Practical applicability of the newly developed electrode was verified on direct determination of 5-NBIA in tap and mineral water model samples, with L_Qs ≈ 1×10^{-7} mol L^{-1}.

Keywords: Large-surface bismuth film electrode; Solid amalgam substrate; Atomic force microscopy; 5-Nitrobenzimidazole; Direct current voltammetry; Differential pulse voltammetry; Tap and mineral water.

1. INTRODUCTION

There is an ever increasing demand for sensitive, simple, fast and inexpensive methods for determination of biologically active organic compounds in various environmental matrices. For electrochemically reducible organic compounds, modern voltammetric methods at mercury electrodes can be successfully applied [1]. Although recent European Regulations (REACH, and Directive 2007/51/CE of 25th September 2007) do not prevent the use of mercury as electrode material, development of "greener" electrode materials is extremely relevant. Due to increasing fears of liquid mercury toxicity during the last decades [2, 3], which has resulted in not quite reasonable "mercurophobia" [4], great attention is paid to the development of working electrodes free of mercury, e.g., solid bismuth electrodes [5] or bismuth film-based electrodes [6]). Another possibility is to use electrodes containing minimum amount of liquid mercury, usually transformed into non-toxic solid or paste amalgam during the preparation of the electrode (e.g., silver amalgam-based electrodes [7-9] such as silver solid amalgam electrodes [10, 11], silver solid amalgam composite electrodes [12-14], silver solid amalgam paste electrodes with an organic pasting liquid [15, 16], silver amalgam paste electrodes [17] or single crystal silver amalgam microelectrodes [16, 18]). These electrodes were successfully used to determine various organic nitro-compounds [19].

Recently developed working electrodes based on silver solid amalgam substrate [20] modified by a deposited bismuth film (BiF-AgSAE) represent a suitable non-toxic alternative to traditional mercury electrodes [1]. Although many previously published papers deal with the bismuth film deposited on glassy carbon (BiF-GCE) or carbon paste (BiF-CPE) substrate [21-27], only our previous communication [28] described the application of bismuth film electrode on silver solid amalgam substrate for voltammetric determination of an organic nitro compound. However, the low surface area of this electrode with small diameter of electroactive part (0.5 mm) resulted in relatively low current signals. Therefore, in this paper a large-surface bismuth film electrode was fabricated on silver solid amalgam substrate resulting in enhanced sensitivity. Moreover, we have studied the morphology of the

bismuth film deposited on this substrate by AFM and we have found that it is different than the morphology on carbon substrates investigated earlier [29].

The environment is increasingly polluted with various genotoxic nitro compounds [30]. Therefore, we have selected 5-nitrobenzimidazole (NBIA, see Figure 1) as a typical model representative of electrochemically reducible nitrated heterocyclic compounds and a proven carcinogen and mutagen [31] for testing analytical performance of the new LSBiF-AgSAE. The occurrence of 5-NBIA in environment is connected with fossil fuels combustion [32]; it was polarographically determined as a part of photographic processing solutions [33] and its properties have been extensively studied in connection with its application in the area of metal corrosion protection [34]. Furthermore, the practical applicability of newly prepared LSBiF-AgSAE was verified on direct determinations of 5-NBIA in model samples of tap and mineral water because of the possible hazard of surface and ground waters contamination by this genotoxic compound after its application as anti-corrosive agent [34].

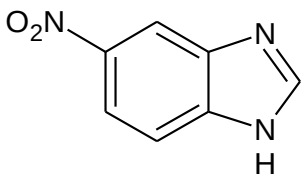

Figure 1. Structural formula of 5-nitrobenzimidazole

2. EXPERIMENTAL

2.1 Reagents

The stock solution of 5-NBIA (c = 1.0×10^{-3} mol L^{-1}; CAS Number: 94-52-0; Sigma-Aldrich, Prague, Czech Republic) was prepared by dissolving the appropriate weight of the substance in 100.0 mL of deionized water. UV-VIS spectrophotometric study has demonstrated that the stock solution is stable for at least one year [35] .

The Britton–Robinson (BR) buffer solutions were prepared in a usual way by mixing a solution containing 0.04 mol L^{-1} solution of phosphoric acid (p.a. purity, Merck), acetic acid (p.a. purity, Panreac, Barcelona, Spain) and boric acid (p.a. purity, Lach-Ner, Neratovice, Czech Republic) with the appropriate amount of 0.2 mol L^{-1} sodium hydroxide (p.a. purity, Merck) to achieve the desired pH. Sodium acetate trihydrate (p.a. purity, Lach-Ner) and ethylenediaminetetraacetic acid (EDTA, p.a. purity, Panreac) were further used. Deionized water was produced by Milli-Q Plus system (Millipore, Billerica, MA, USA).

All the chemicals were used without further purification and all the solutions were maintained in glass vessels in dark at laboratory temperature.

2.2. Instrumentation

2.2.1. Apparatus

Voltammetric measurements were carried out using a PalmSens electrochemical analyzer driven by PSTrace 2.1 software (both Palm Instruments, Houten, The Netherlands). The software worked under the operational system Microsoft Windows XP (Microsoft Corporation Redmond, WA, USA). All measurements were carried out in a three-electrode system using platinum electrode (type PPE, Monokrystaly, Turnov, Czech Republic) auxiliary electrode and silver|silver chloride reference electrode (type RAE 113, 3 mol L^{-1} KCl, Monokrystaly, Turnov, Czech Republic). The LSBiF-AgSAE (disc diameter, 2.64 mm) was used as a working electrode. Scan rate of 20 mV s^{-1} was used for both DCV and DPV, pulse amplitude –50 mV and pulse width 100 ms with current sampling for the last 20 ms, were used in DPV.

The solution pH was measured by a Jenway digital pH meter (type 3510, Jenway, Chelmsford, UK) with a combined glass electrode (of the same producer) calibrated with standard aqueous buffers at laboratory temperature.

2.2.2. Preparation of large surface bismuth film electrode on silver solid amalgam substrate)

The novel type of working electrode was prepared *ex situ* in a plating solution (after deaeration of the solution for 5 min with pure nitrogen) containing 0.5 mL bismuth standard solution (1000 mg L^{-1} Bi(III), Darmstadt, Merck, Germany) and 9.5 mL of 1.0 mol L^{-1} acetate buffer pH 4.75 by the deposition of the bismuth film on the AgSAE substrate at a constant potential of –1.2 V (vs Ag|AgCl) for selected time (t_{dep}) under stirred conditions. The disc diameter of AgSAE was 2.64 mm, i.e. five times larger than in our previous paper [28] and that is why we use the term large-surface bismuth film electrode at silver solid amalgam substrate (LSBiF-AgSAE).

The LSBiF-AgSAE had to be prepared every day because of the relatively short lifetime of the electrode (similar as in the case of mercury film [2]). No mechanical, electrochemical or chemical cleaning or activation can be performed, since such procedures damage the bismuth film formed. The substrate AgSAE consisted of a glass tube, whose tip was packed with a fine silver powder (2 – 3.5 μm, 99,9%, Sigma-Aldrich, Prague, Czech Republic), amalgamated by liquid mercury (triply distilled polarographic mercury, 99.999%, Polarografie, Prague, Czech Republic), polished on alumina with particle size 1.1 μm (Monokrystaly, Turnov, Czech Republic) and connected to an electric contact [20].

2.3. Morphology observation

Surface topography of the electrochemically deposited bismuth film of LSBiF-AgSAE was characterized by AFM (Multimode Nanoscope IIIa, Veeco, USA) in tapping mode. The Si cantilevers OTESPA (Veeco, USA) oscilating at ca. 300 kHz were used to minimize the tip interaction with examined surface. The surface imaging was performed with the rate 0.5 and 1.0 Hz, respectively.

Image noise was filtered off using the "low-pass" filtering software feature. The topographic image of electrode surface was evaluated by commercial Nanoscope III Particle and Bearing Analysis Software (Nanoscope Reference Manual, version 5.12r5).

2.4 Procedures

For voltammetric measurements during optimization procedure, an appropriate amount of 5-NBIA stock solution was filled up to 10.0 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 and all the measurements were carried out at laboratory temperature. The procedure for DCV or DPV determination of 5-NBIA in model samples was as follows: 9.0 mL of the model water sample were diluted to 10.0 mL with the BR buffer pH 7.0 and, after deaeration with nitrogen, DC or DP voltammograms at the LSBiF-AgSAE were recorded. The DCV peak height (I_p) was evaluated from the extrapolated linear portion of the voltammogram before the onset of the peak. DPV peaks were evaluated from the straight line connecting the minima before and after the peak. The parameters of calibration curves (such as slope, intercept, correlation coefficient, limit of quantification) were calculated with statistic software Adstat 2.0 (TriloByte, Pardubice, Czech Republic) [36].

2.5 Model samples

The tap water from the public water pipeline in the building of National and Kapodistrian University, Athens, Greece and natural mineral water (Spring Olympos, Katerini, Greece), spiked with appropriate amounts of 5-NBIA stock solution, were used as model samples. Both tap and mineral water were either used without further pretreatment/purification or solid EDTA was added (1 g per 50 mL of water) for masking of inerfering cations.

3. RESULTS AND DISCUSSION

3.1 Bismuth film deposition

Initially, the deposition time of the bismuth film was studied. Deposition times (t_{dep}) 300, 900, 1800 and 3600 s were tested. The t_{dep} = 1800 s was selected as the optimum one because at shorter times of deposition, lower repeatability of consecutive DPV determinations of 5-NBIA was observed. At t_{dep} = 3600 s, the results were very similar to those obtained at 1800 s. Compared with small-surface AgSAE substrate [28], where optimum deposition time was found to be 300 s, much longer time is recommended in the case of LSBiF-AgSAE.

Then, the reproducibility of the bismuth film deposition was investigated because it was necessary to prepare a new film every day. The % relative standard deviation (%*RSD*) of DPV determination of 5-NBIA ($c = 1.0\times10^{-4}$ mol L^{-1}) in BR buffer pH 7.0 (this medium was selected on the

base of established pH dependence) at ten different LSBiF-AgSAEs was 11.9% (Fig. 2A). The repeatability of twenty consecutive DPV measurements of 5-NBIA (also expressed as % *RSD*), performed under the conditions mentioned above at one freshly prepared LSBiF-AgSAE, was 2.8% (Fig. 2B). These % RSD values are satusfactory for this type of working electrode [23]. Nevertheless, it should be stressed that each calibration curve must be constructed using the same bismuth film as in proper determination and that the observed small decrease of signal at one film (Fig. 2B) can be connected with weak passivation of the working electrode.

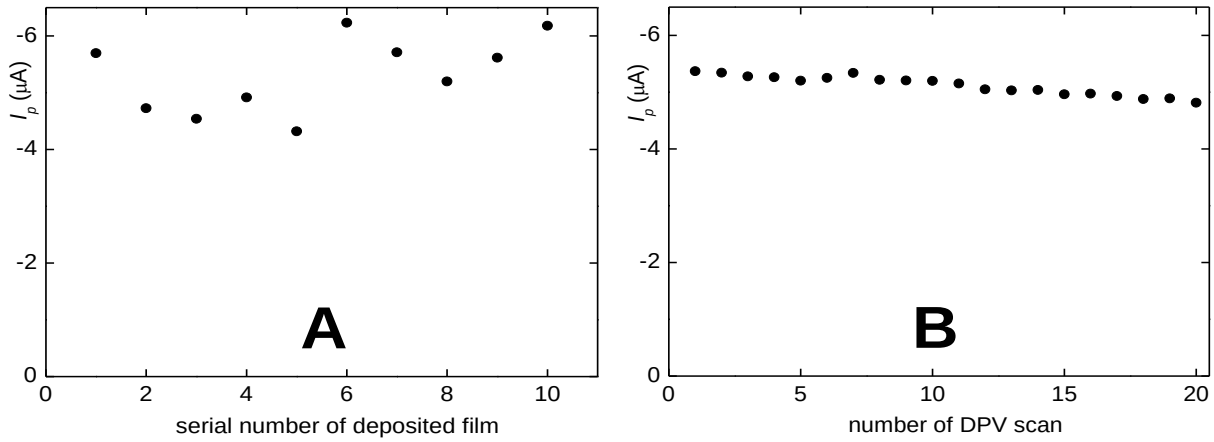

Figure 2. (A) Repeatability of DPV determination of 5-NBIA (c = 1×10^{-4} mol L^{-1}) at consecutively prepared LSBiF-AgSAEs in BR buffer pH 7.0 with deposition time 1800 s. **(B)** Repeatability (n=20) of DPV peaks of 5-NBIA (c = 1,0×10^{-4} mol L^{-1}) at one LSSBF-AgSAE in BR buffer pH 7.0 with deposition time 1800 s.

3.2 Investigation of the electrode morphology

The use of AFM allows us to observe the surface morphology of the modified surface. The surface of the electrode was prepared as described above (cleaning silver solid amalgam substrate with alumina and depositing bismuth film for 1800 s). The scanned area was 1000 × 1000 nm and several spots were investigated simultaneously. Fig. 3A and 3B clearly show that the bismuth film was not grown uniformly over the supporting surface, but created three-dimensional higher formations which covered only a fraction of the surface. Line analysis is shown in Figure 3C and 3D. The observed grains range in size from 30 to 130 nm and it is very likely that they nucleate at defects of the surface. The AFM picture of the substrate surface before the deposition shows formations with vertical dimension up to 20 nm. It is not possible to decide whether the growth of the three–dimensional grains starts from the amalgam substrate or from a bismuth oxide base underneath higher formations.

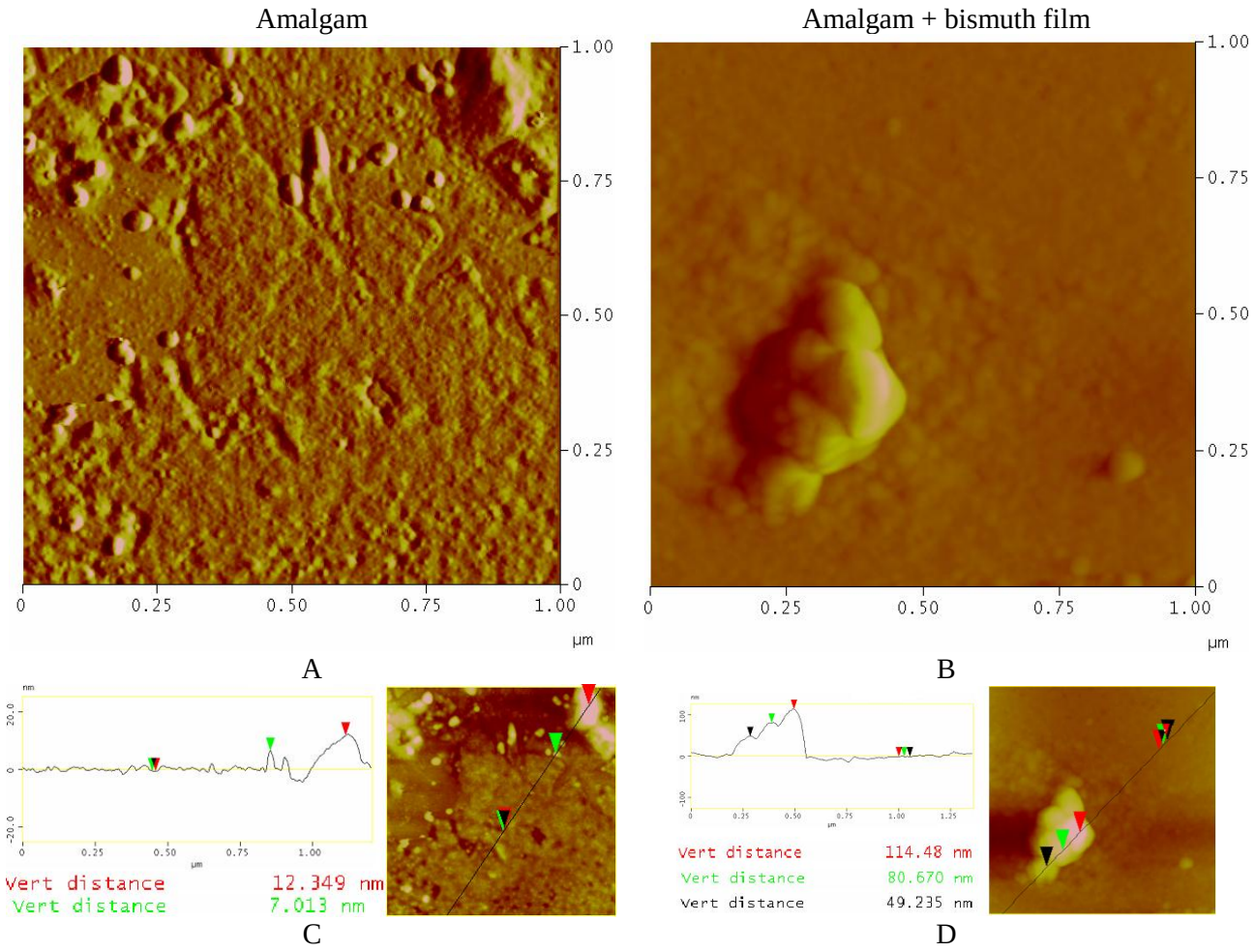

Figure 3. AFM images of tested electrode surface: **(A)** Topographic image of silver solid amalgam support (1×1 μm^2) without modification. **(B)** topographic image of the support (1×1 μm^2) modified by bismuth film. **(C)** line analysis of image A. **(D)** line analysis of image B.

3.2 Voltammetric behavior of 5-nitrobenzimidazole at the LSBiF-AgSAE

The influence of pH on DC and DP voltammograms of 1.0×10^{-4} mol L^{-1} 5-NBIA at LSBiF-AgSAE is depicted in Fig. 4. The differences in peak shapes, positions and peak heights in acidic, neutral and alkaline media are evident. It can be seen that the analyte gives one well-developed cathodic DCV or DPV peak, corresponding to the four-electron reduction of the nitro-group to the hydroxyamino-group, over the whole pH region. The second peak probably corresponding to a two-electron reduction of the hydroxyamino-group to the amino-group can be observed at more negative potentials at higher pHs (see dashed line 8 on Fig.4B). Different responses at pH 11.0 and 12.0 are probably connected with the formation of bismuth hydroxo-complexes at such high pH values. The observed peak potential shift towards more negative potentials with increasing pH can be

explained by a preceding protonation of the nitro-group leading to a decrease in the electron density at the nitro-group and thus to easier electron acceptance at lower pHs [19].

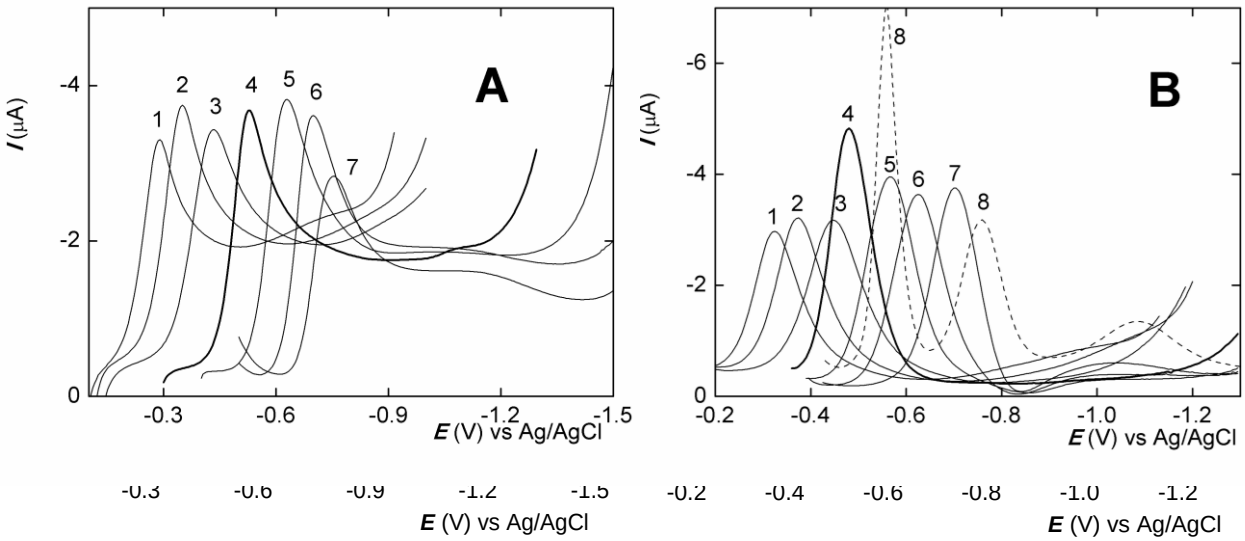

Figure 4. DC (A) and DP (B) voltammograms of 5-NBIA (c = 1.0×10^{-4} mol L^{-1}) at LSBiF-AgSAE (t_{dep} = 1800 s) in BR buffer. **(A)** pH 2.0 (1), 3.0 (2), 5.0 (3), 7.0 (4), 8.0 (5), 10.0 (6), 11.0 (7); **(B)** pH 2.0 (1), 4.0 (2), 6.0 (3), 7.0 (4), 9.0 (5), 9.0 (6), 10.0 (7), 12.0 (8). The voltammograms recorded under optimum conditions for the determination of 5-NBIA are in bold.

3.3 Voltammetric determination of 5-NBIA at LSBiF-AgSAE

The highest and best developed DCV and DPV peaks were obtained in the BR buffer pH 7.0 medium which was further used for plotting of calibration curves in the concentration range from 0.2 to 1000 μmol L^{-1} of 5-NBIA. For the sake of illustration, DP voltammograms of 5-NBIA at LSBiF-AgSAE in the concentration range 0.2–1.0 μmol L^{-1} are depicted in Fig 5. The parameters of all the calibration straight lines are summarized in Table 1. Sensitivity slightly differs between individual concentration orders (see Table 1), which is typical for voltammetry at solid electrodes [37]. Nevertheless, within the individual concentration orders, the obtained concentration dependences are linear. In accordance with our presumption, both the sensitivity and limit of quantification obtained with LSBiF-AgSAE are more favorable than those values obtained on bismuth film electrode with smaller surface [28]. Therefore, LSBiF-AgSAE should be preferred for trace environmental analysis.

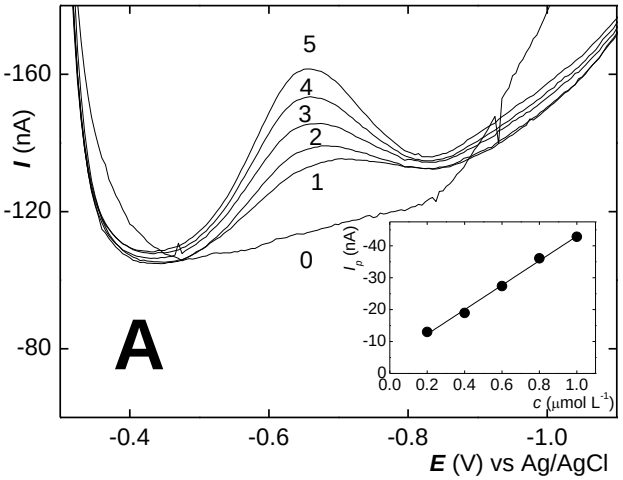

Figure 5. DP voltammograms of 5NBIA ($c_{5\text{-NBIA}}$ = 0 (0), 0.2 (1), 0.4 (2), 0.6 (3), 0.8 (4) and 1 (5) μmol L^{-1}) at LSBiF-AgSAE in BR-buffer of pH 7.0; the corresponding calibration straight line is in inset.

Unfortunately, an attempt to increase the sensitivity of the determination by adsorptive accumulation of the analyte [38] was not successful. The signal of 5-NBIA did not significantly increase at accumulation times up to 5 min neither at optimum conditions for DCV and DPV determination of 5-NBIA (BR buffer pH 7.0) nor at more acidic (BR buffer pH 3.0) or more alkaline (BR buffer pH 10.0) solutions and at different accumulation potentials.

Table 1. Parameters of the calibration straight lines (± standard errors) for DCV and DPV determination of 5-NBIA at the LSBiF-AgSAE in the BR buffer pH 7.0 medium.

Method	Concentration mol L^{-1}	Slope nA mol^{-1} L	Intercept nA	R	L_Q mol L^{-1}
DCV	$(2–10)\times10^{-5}$	$-3.70\times10^{7} \pm 0.89$	102 ± 59[a]	–0.9991	–
	$(2–10)\times10^{-6}$	$-3.13\times10^{7} \pm 1.05$	-35 ± 7	–0.9983	–
	$(2–10)\times10^{-7}$	$-5.00\times10^{7} \pm 1.23$	-9.4 ± 0.8	–0.9991	3.7×10^{-7}
DPV	$(2–10)\times10^{-5}$	$-3.48\times10^{7} \pm 0.59$	-83 ± 39[a]	–0.9996	–
	$(2–10)\times10^{-6}$	$-2.55\times10^{7} \pm 0.49$	6.2 ± 3.2[a]	–0.9995	–
	$(2–10)\times10^{-7}$	$-3.84\times10^{7} \pm 1.34$	-4.6 ± 0.9	–0.9982	–
	$(2–10)\times10^{-8}$	$-1.20\times10^{8} \pm 6.72$	-2.0 ± 0.5	–0.9953	6.8×10^{-8}

R, correlation coefficient; L_Q, limit of quantification (10σ; α = 0.05).
[a] Intercepts are not statistically significantly different from zero at the significance level α = 0.05.

3.4 Model samples of tap and mineral water

A practical application of the proposed new LSBiF-AgSAE was the determination of 5-NBIA in model samples of tap and mineral water at submicromolar concentration range under the optimum conditions using 9.0 mL of spiked model water sample and 1.0 mL of the BR buffer pH 7.0 (see Fig 6). Both tap and mineral water was used either directly or after addition of solid EDTA (1 g per 50 mL of water) to mask cations (e.g., Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}) and to eliminate their negative effect. Addition of EDTA resulted in higher and better shaped peaks in the case of tap water while in the case of mineral water the determination without EDTA addition was impossible. The effect of the addition of EDTA on voltammetric determination of 5-NBIA is demonstrated in Table 2.

Without addition of EDTA, both DCV and DPV determination is possible only when using a lower ratio of water sample to BR buffer, namely 1:1. DP voltammograms of 5-NBIA at LSBiF-AgSAE measured using a mixture of 5.0 mL of model water sample and 5.0 mL of the BR buffer pH 7.0 are depicted in Fig 6B for spiked tap water and Fig 6D for spiked mineral water. From Table 2 it could be concluded that measurement with a 9:1 ratio of model mineral water sample with EDTA to BR buffer pH 7.0 is preferable due to lower L_Q and higher sensitivity.

The obtained parameters of the calibration curves for all measurements are summarized in Table 2. These results confirm the applicability of LSBiF-AgSAE electrode for the determination of submicromolar concentrations of 5-NBIA (and possibly of many other electrochemically reducible organic pollutants) in both tap and mineral waters.

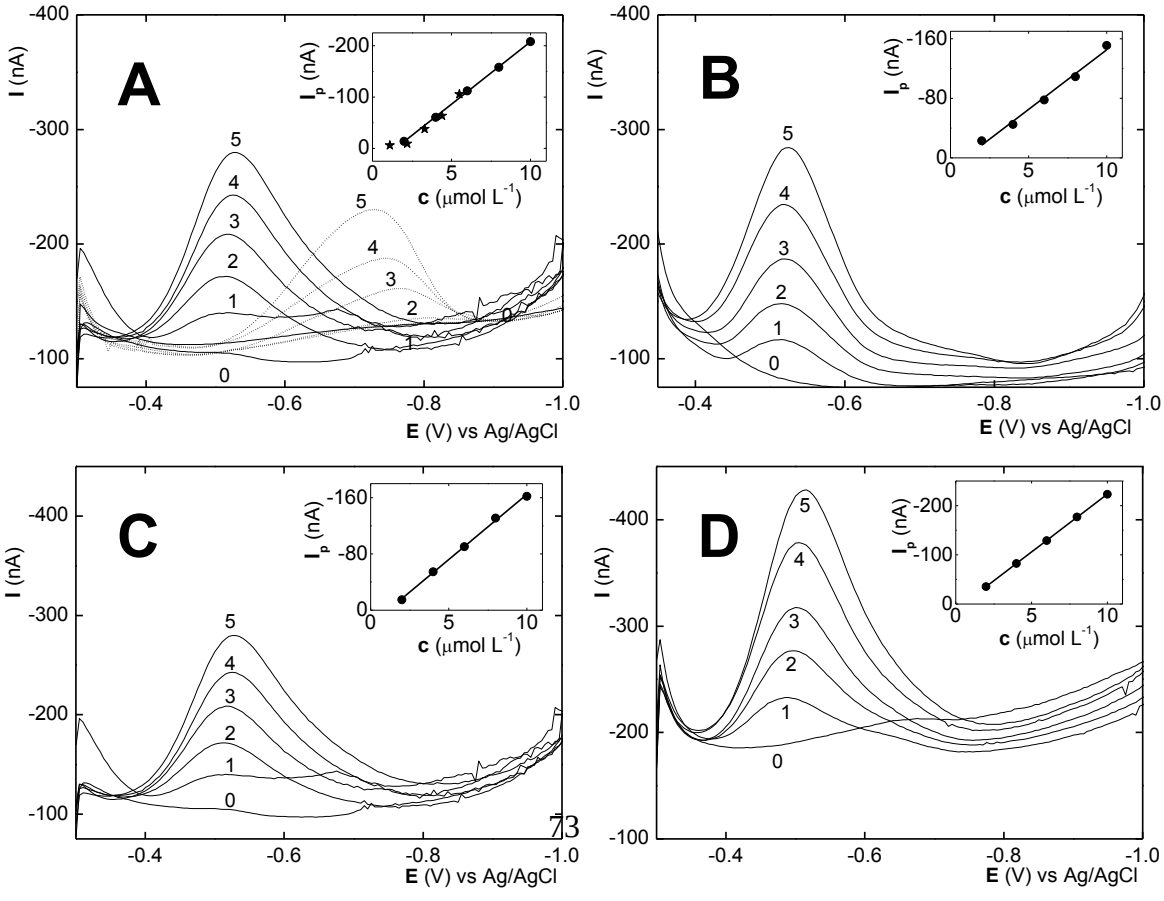

Figure 6. DP voltammograms of 5-NBIA at LSBiF-AgSAE. **(A)** Spiked tap water with addition of 1g of EDTA to 50 ml of water (full line) and without EDTA (dotted line). BR-buffer pH 7.0 – tap water (1:9). **(B)** Spiked tap water without addition of EDTA. BR-buffer of pH 7.0 : tap water (5:5). **(C)** Spiked mineral water with addition of EDTA (1g to 50 ml water); BR-buffer pH 7.0 : water (1:9). **(D)** Spiked mineral water with addition of 1g of EDTA to 50 ml of water), BR-buffer pH 7.0 : water (5:5). **(A-D)** c_{5NBIA} in tap or mineral water: 0 (0), 2.0 (1), 4.0 (2), 6.0 (3), 8.0 (4) and 10.0 (5) μmol L^{-1}, for **(A)** without EDTA (dotted line with stars) c_{5NBIA} in spiked tap water: 0 (0), 1.1 (1), 2.2 (2), 3.3 (3), 4.4 (4) and 5.5 (5) μmol L^{-1}; The corresponding calibration straight lines are always given inset.

Table 2. Parameters of the calibration straight lines (± standard errors) for DCV and DPV determination of 5-NBIA at the BiF-AgSAE in model samples of tap and mineral water (measurement in the of spiked water samples:BR buffer pH 7.0 (9:1 and 5:5).

Sample	Method	Concentration mol L^{-1}	Slope nA mol^{-1} L	Intercept nA	Correlation coefficient	L_Q mol L^{-1}
Tap water[b]	DCV	$(1.1–5.5)\times10^{-5}$	$-3.40\times10^{7} \pm 0.87$	6.70 ± 5.8[a]	–0.9990	–
		$(1.1–5.5)\times10^{-6}$	$-1.58\times10^{7} \pm 0.84$	–37.0 ± 5.6	–0.9957	–
		$(1.1–5.5)\times10^{-7}$	$-6.41\times10^{7} \pm 1.90$	4.5 ± 1.3	–0.9987	6.82×10^{-7}
	DPV	$(1.1–5.5)\times10^{-5}$	$-3.10\times10^{7} \pm 2.24$	–193 ± 149[a]	–0.9922	–
		$(1.1–5.5)\times10^{-6}$	$-1.60\times10^{7} \pm 0.98$	14.9 ± 6.5[a]	–0.9944	–
		$(1.1–5.5)\times10^{-7}$	$-5.13\times10^{7} \pm 2.04$	6.3 ± 1.4	–0.9976	9.76×10^{-7}
Tap water[c]	DCV	$(2–10)\times10^{-5}$	$-2.37\times10^{7} \pm 0.31$	61.5 ± 20.4[a]	–0.9997	–
		$(2–10)\times10^{-6}$	$-2.73\times10^{7} \pm 0.81$	27.5 ± 5.4	–0.9987	–
		$(2–10)\times10^{-7}$	$-2.81\times10^{7} \pm 1.25$	–5.1 ± 0.8	–0.9970	2.25×10^{-7}
	DPV	$(2–10)\times10^{-5}$	$-2.12\times10^{7} \pm 1.01$	133 ± 67[a]	–0.9966	–
		$(2–10)\times10^{-6}$	$-2.43\times10^{7} \pm 0.21$	35.4 ± 1.4	–0.9999	–
		$(2–10)\times10^{-7}$	$-1.72\times10^{7} \pm 0.84$	3.62 ± 0.55	–0.9965	6.58×10^{-7}
Mineral water[d]	DCV	$(1.1–5.5)\times10^{-5}$	$-2.92\times10^{7} \pm 0.56$	6 ± 37[a]	–0.9995	–
		$(1.1–5.5)\times10^{-6}$	$-3.21\times10^{7} \pm 0.64$	–27 ± 4	–0.9994	–
		$(1.1–5.5)\times10^{-7}$	$-8.43\times10^{7} \pm 3.92$	–3.0 ± 2.6[a]	–0.9968	7.82×10^{-7}
	DPV	$(1.1–5.5)\times10^{-5}$	$-2.62\times10^{7} \pm 0.26$	6 ± 17[a]	–0.9999	–
		$(1.1–5.5)\times10^{-6}$	$-2.35\times10^{7} \pm 0.07$	11.6 ± 0.5	–1.0000	–
		$(1.1–5.5)\times10^{-7}$	$-4.80\times10^{7} \pm 0.80$	2.8 ± 0.5	–0.9996	4.20×10^{-7}
Mineral water[e]	DCV	$(2–10)\times10^{-5}$	$-2.72\times10^{7} \pm 0.80$	123 ± 53[a]	–0.9987	–
		$(2–10)\times10^{-6}$	$-2.94\times10^{7} \pm 0.32$	–16.4 ± 2.1	–0.9998	–
		$(2–10)\times10^{-7}$	$-7.92\times10^{7} \pm 4.79$	–0.8 ± 3.2[a]	–0.9945	5.45×10^{-7}

	$(2–10)\times10^{-5}$	$-2.76\times10^{7} \pm 1.01$	14 ± 66[a]	–0.9980	–
DPV	$(2–10)\times10^{-6}$	$-1.85\times10^{7} \pm 0.43$	20.5 ± 2.9	–0.9992	–
	$(2–10)\times10^{-7}$	$-2.70\times10^{7} \pm 0.49$	3.8 ± 0.3	–0.9993	3.6×10^{-7}

R, correlation coefficient; L_Q, limit of quantification (10σ; $\alpha = 0.05$).
[a] Intercepts are not statistically significantly different from zero at the significance level $\alpha = 0.05$;
[b] 5:5 water:buffer ratio in measured solution;
[c] 9:1 water:buffer ratio in measured solution with adition of EDTA;
[d] 5:5 water:buffer ratio in measured solution with adition of EDTA;
[e] 9:1 water:buffer ratio in measured solution with adition of EDTA.

4. CONCLUSIONS

It has been shown that the newly developed LSBiF-AgSAE (disc diameter 2.64 mm, deposition time 1800 s) is a suitable sensor DCV and DPV determination of submicromolar concentrations of genotoxic 5-NBIA. We believe that this conclusion can be justifiably extrapolated to many other electrochemically reducible organic pollutants. The morphological study found non-uniform growth of bismuth film on the substrate surface.

In the optimum medium found (BR buffer pH 7.0), the measurable concentration range was 0.2–1000 μmol L^{-1} of 5-NBIA for both DCV (the limit of quantification, $L_Q \approx 0.37$ μmol L^{-1}) and DPV ($L_Q \approx 0.07$ μmol L^{-1}) at LSBiF-AgSAE. The attempt at increasing the sensitivity using adsorptive stripping DCV or DPV at the LSBiF-AgSAE was not successful.

The applicability of the tested electrode for DCV and DPV determination of 5-NBIA in model samples of tap and mineral water (in the concentration range from 0.2 to 1000 μmol L^{-1} of 5-NBIA) has also been verified, with the L_Qs in the concentration range 10^{-7} mol L^{-1}. Therefore, it can be concluded that LSBiF-AgSAE can be successfully used for the determination of trace amounts of 5-NBIA and possibly of other electrochemically reducible pollutants as a suitable non-toxic and environmentally friendly alternative to mercury electrodes.

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11. APPENDIX VI

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Voltammetric determination of 2-amino-6-nitrobenzothiazole and 5-nitrobenzimidazole using a silver solid amalgam electrode modified by a microcrystalline natural graphite–polystyrene composite film

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5-Nitrobenzimidazole

ABSTRACT

Voltammetric behavior of two genotoxic nitro compounds (2-amino-6-nitrobenzothiazole, ANBT, and 5-nitrobenzimidazole, 5-NBIA) has been investigated using direct current voltammetry (DCV) and differential pulse voltammetry (DPV) at a polished silver solid amalgam electrode modified by microcrystalline natural graphite–polystyrene composite transducer (shortly denoted as carbon film electrode – CFE). The optimum conditions have been found for their determination in the Britton–Robinson buffer solutions. The practical applicability of the newly developed methods was verified on model samples of drinking and river waters.

1. Introduction

The environment is increasingly polluted with various genotoxic nitro compounds [1]. The easy electrochemical reduction of nitro groups at the aromatic or heterocyclic ring, whose mechanism is discussed in [2], permits very sensitive determinations of a number of genotoxic and ecotoxic nitro compounds by means of differential pulse voltammetry (DPV) at a hanging mercury drop electrode (HMDE) [3] and adsorptive stripping voltammetry (AdSV) at HMDE [4]. However, due to increasing even thou not quite justified fears of liquid mercury toxicity [5] and somewhat unreasonable "mercury phobia" [6], new working electrodes are tested for these purposes, namely solid amalgam electrodes [7,8], solid composite electrodes [9], boron-doped diamond film electrodes [10], bismuth film electrodes [11], carbon paste electrodes [12], carbon film electrodes [13], etc. Cathodic voltammetry of nitro compounds on carbon paste electrode does not give sufficiently low limit of quantitation (L_Q) and required reproducibility because of strong signal of oxygen contained in the paste [14]. Therefore, in this paper we have focused on the use of working electrode based on silver amalgam covered by a film of natural graphite–polystyrene composite transducer which eliminates unfavorable influence of oxygen. This working electrode, shortly denoted as carbon film electrode (CFE) represent a possible, nontoxic alternative to the traditional mercury electrodes for measurement both oxidation and reduction processes [15]. CFEs are useful and promising alternative to disposable electrodes. They can be easily prepared (the surface of any solid working electrode is covered by carbon ink suspension and left to evaporate to dryness), simple mechanically renewed (by wiping off an old film with filter paper and forming a new one), and simply chemically modified. Possible electrochemical cleaning together with even more efficient total renewal of carbon film eliminate most problems connected with "electrode history", namely its passivation with products of electrochemical reactions and/or with adsorbable compounds in the sample.

The aim of this work was to develop direct current voltammetric (DCV) and differential pulse voltammetric (DPV) methods based

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on cathodic voltammetric responses provided by the nitro-group in selected genotoxic heterocyclic nitro compounds and to verify practical applicability of the newly developed methods on the direct determination of studied substances in model samples of drinking and river water.

The following two representatives of nitrated heterocyclic compounds were selected for this study: 5-nitrobenzimidazole (5-NBIA) and 2-amino-6-nitrobenzothiazole (ANBT) (see Fig. 1). 5-NBIA, which is proven carcinogen and mutagen [16], is formed during fossil fuels combustion [17]. It is used in photographic processing solutions [18] and as an anti-corrosion agent [19]. ANBT exhibits both genotoxic and mutagenic properties [20]. It is used in dyes production [21,22], in photoconductive measurements [23] and in nonlinear optical applications [24].

Because of ecotoxic and genotoxic properties of both compounds, their monitoring in the environment is of increasing importance. There are several methods for determination of ANBT [25,26]. However, to our best knowledge, only voltammetric methods recently developed in our UNESCO Laboratory of Environmental Electrochemistry [11,27] are sensitive enough for environmental applications. Electrochemical methods are especially suitable for large scale environmental monitoring of electrochemically active pollutants because they are inexpensive, extremely sensitive and they present an independent alternative to so far prevalent spectrometric and separation techniques [28]. ANBT contains two electrochemically active functional groups (cathodically reducible nitro-group and anodically oxidizable amino-group) and 5-NBIA contains electrochemically reducible nitro-group. Therefore, they are amenable to a voltammetric determination on a suitable working electrode. The drinking and river water were used as model samples because of the possible hazard of surface and ground waters contamination by ANBT during the industrial production of dyes and pigments [29] or by 5-NBIA in connection with its anticorrosive applications [19].

2. Experimental

2.1. Chemicals and reagents

A 1×10^{-3} mol L^{-1} stock solution of 5-NBIA (CAS Number: 94-52-0, Sigma–Aldrich, Prague, Czech Republic) was prepared by dissolving appropriate amount of the substance in 100.0 mL of deionized water. A 1×10^{-3} mol L^{-1} stock solution of ANBT (CAS Registry Number: 6285-57-0; 97%; Sigma–Aldrich, Prague, Czech Republic) was prepared by dissolving an exactly weighed amount of the substance in methanol (99.9%, Merck, Darmstadt, Germany). The stock solutions were stored in refrigerator at 4 °C and more dilute solutions were prepared freshly before use.

The Britton–Robinson (BR) buffers were prepared in a usual way, i.e., by mixing a solution of 0.04 mol L^{-1} in phosphoric acid, of 0.04 mol L^{-1} acetic acid, of 0.04 mol L^{-1} boric acid with the appropriate amount of 0.2 mol L^{-1} sodium hydroxide solution (all p.a. purity; Lachema, Brno, Czech Republic)

The carbon film was prepared from carbon powder (crystalline graphite 2 μm, CR 2, Maziva Týn, Czech Republic), 1,2-dichlorethane (Merck, Darmstadt, Germany) and polystyrene. Deionized water was produced by Milli-Q Plus system (Millipore, Billerica, MA, USA).

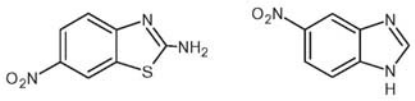

Fig. 1. Structural formulae of 2-amino-6-nitrobenzthiaoazole (ANBT) and 5-nitro-benz-imidazole (5-NBIA).

2.2. Apparatus

Voltammetric measurements were carried out using an Eco-Tribo Polarograph driven by Polar Pro 5.1 software (all Polaro-Sensors, Prague, Czech Republic). The software worked under the operational system Microsoft Windows XP (Microsoft Corporation). All measurements were carried out in a three-electrode system using a platinum auxiliary electrode PPE (Monokrystaly, Turnov, Czech Republic) and a silver/silver chloride reference electrode RAE 113 (3 mol L^{-1} KCl, Monokrystaly, Turnov, Czech Republic). As a working electrode, CFE prepared by deposition of carbon film at a polished silver solid amalgam electrode was used. Its preparation is described in Section 2.3.1. For both DCV and DPV, a scan rate of 20 mV s^{-1} was used and for DPV a pulse amplitude −50 mV (for reduction) or 50 mV (for oxidation of ANBT) and a pulse width 100 ms were used with current sampling for the last 20 ms.pH was measured by Conductivity & pH meter 4330 with a combined glass electrode type 924 005 (both Jenway, Chelmsford, UK).

2.3. Procedures

2.3.1. Preparation of carbon film electrode

The silver solid amalgam electrode (Eco-Trend Plus, Prague, CZ, disc diameter, 0.5 mm) was polished on the alumina with particle size 1.1 μm. The film was formed by immersing the electrode surface into conductive carbon ink (the active part of the electrode just touched the surface of the ink). Conductive carbon ink carbon was prepared by mixing 0.01 g of polystyrene, 0.09 g of carbon powder (crystalline graphite 2 μm, CR 2, Maziva Týn, Czech Republic) and 0.5 ml of 1,2-dichloroethane. Two minutes after immersing, 1,2-dichloroethane evaporates and the film, resp. the electrode is ready to use. When it is necessary to renew the old film (for example because of the passivation), it can be easily removed by wiping it off with a filter paper.

2.3.2. Voltammetric measurements

In the case of ANBT, an appropriate amount of stock solution in methanol was measured into a voltammetric vessel; methanol was added, if necessary, to a total volume of 1.0 mL and filled up to 10.0 mL with a BR buffer of appropriate pH. In the case of 5-NBIA, an appropriate volume of the stock solution was measured into voltammetric vessel and filled up to 10.0 mL with a BR buffer of appropriate pH. In the case of cathodic measurements, oxygen was removed by bubbling with nitrogen (purity class 4.0; Linde, Prague, Czech Republic) for 5 min. In the case of anodic measurements, removal of oxygen was not necessary.

Regeneration of electrodes was necessary in the case of reduction measurements because of the observed electrode passivation. The optimum regeneration potentials ($E_{1,reg}$ and $E_{2,reg}$) were found to be for both ANBT and 5-NBIA $E_{1,reg}$ = −400 mV and $E_{2,reg}$ = −900 mV. The regeneration was carried out in supporting electrolyte used for proper determination. The procedure was based on potential switching (300 polarizing cycles) between the two limiting potentials. For oxidation process the regeneration was not necessary. The appropriate values of potential and time of regeneration were inset in the software of the computer-controlled instrument used and the regeneration of electrodes was thus carried out automatically. For the oxidation measurements the regeneration was not necessary.

All curves were measured three times and all the measurements were carried out at laboratory temperature. The DCV peak height (I_p) was evaluated from the extrapolated linear portion of the voltammogram before the onset of the peak. DPV peaks were

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evaluated from the straight line connecting the minima before and after the peak. The parameters of calibration curves (such as slope, intercept, correlation coefficient, limit of quantification) were calculated with statistic software Adstat 2.0 (TriloByte, Pardubice, Czech Republic) [30].

The procedure for DCV or DPV determination of ANBT and 5-NBIA in model samples of drinking and river water was as follows: 9.0 ml of the model water sample were diluted to 10.0 ml with the BR buffer pH 4.0 for oxidation and reduction of ANBT and pH 7.0 for reduction of 5-NBIA, and after deaeration with nitrogen (used only for reductions), DC or DP voltammograms at CFE were recorded. Drinking water from the public water pipeline in the building of Faculty of Science of the Charles University in Prague, Czech Republic and river water obtained from Vltava river (sampled at the locality Výtoň, Prague, Czech Republic), spiked with appropriate amount of ANBT and 5-NBIA stock solution, were used as the model samples. Both drinking and river water were used without further pretreatment or purification.

3. Results and discussion

3.1. Voltammetric behavior of the tested substances

The influence of pH on DC and DP voltammetric behavior of 1×10^{-4} mol L^{-1} ANBT and 5-NBIA at CFE (Fig. 2) was investigated in the BR buffer–methanol (9:1) media for ANBT (oxidation and reduction) and in BR buffer only for 5-NBIA (reduction) in the range of pH 2.0–12.0. It can be seen from Fig. 2 that ANBT and 5-NBIA gives one well-developed cathodic DCV or DPV peak, corresponding to four-electron reduction of nitro-group to the hydroxyamino-group, over the whole pH region. ANBT gives one well-developed anodic DCV or DPV peak, corresponding to the oxidation of aromatic amino-group, over the whole pH region. The best developed and reproducible peaks were obtained at pH 4.0. Peaks were well developed at higher pH as well but strong passivation above pH 7 was observed especially for oxidation. Therefore pH 4.0 was selected because both the oxidation and reduction gives one well developed peak with optimal shape and height. For 5-NBIA pH 7 was selected as optimal.

The potential of the peaks of both ANBT and 5-NBIA shifted towards more negative potentials with increasing pH, which can be explained by a preceding protonation of the nitro-group, leading to a decrease in the electron density at the nitro-group and resulting in easier electron acceptance at low pH values.

3.2. Voltammetric determination of the tested substances

Repeated measurements can cause pronounced passivation of the electrode, probably by the electrodes reaction products, resulting in the decrease of peak heights and their shift toward more negative potentials. The optimum regeneration potentials ($E_{1,reg}$ and $E_{2,reg}$) thus had to be found for ANBT and 5-NBIA $E_{1,reg}$ = −400 mV and $E_{2,reg}$ = −900 mV. The regeneration was carried out in supporting electrolyte used for proper determination. The procedure was based on potential switching (300 polarizing cycles) between two limiting potentials. For oxidation process the regeneration was not necessary.

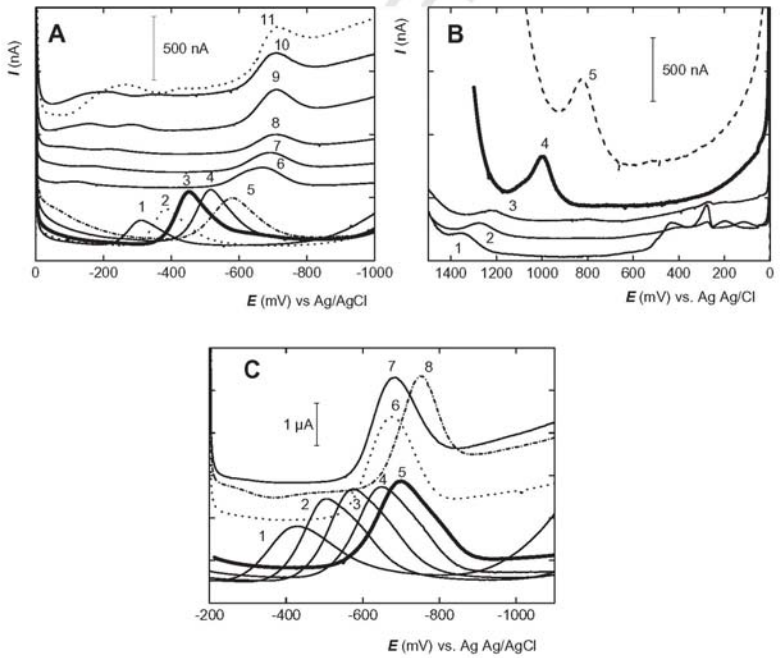

Fig. 2. DP voltammograms of ANBT ($c = 1 \times 10^{-5}$ mol L^{-1}) and 5-NBIA ($c = 1 \times 10^{-4}$ mol L^{-1}) at CFE at polarization rate 20 mV s^{-1}. (A) ANBT; cathodic region; methanol-BR buffer-(9:1); BR buffer pH: 2.0 (1), 3.0 (2), 4.0 (3), 5.0 (4), 6.0 (5), 7.0 (6), 8.0 (7), 9.0 (8), 10.0 (9), 11.0 (10), 12.0 (11). (B) ANBT; anodic region; methanol-BR buffer (9:1); BR buffer pH: 3.0 (1), 4.0 (2), 5.0 (3), 7.0 (4), 10.0 (5). (C) 5-NBIA; BR buffer pH 2.0 (1), 4.0 (2), 5.0 (3), 6.0 (4), 7.0 (5), 9.0 (6), 10 (7), 12 (8). The voltammograms recorded under optimum conditions for the determination of ANBT and 5-NBIA are in bold.

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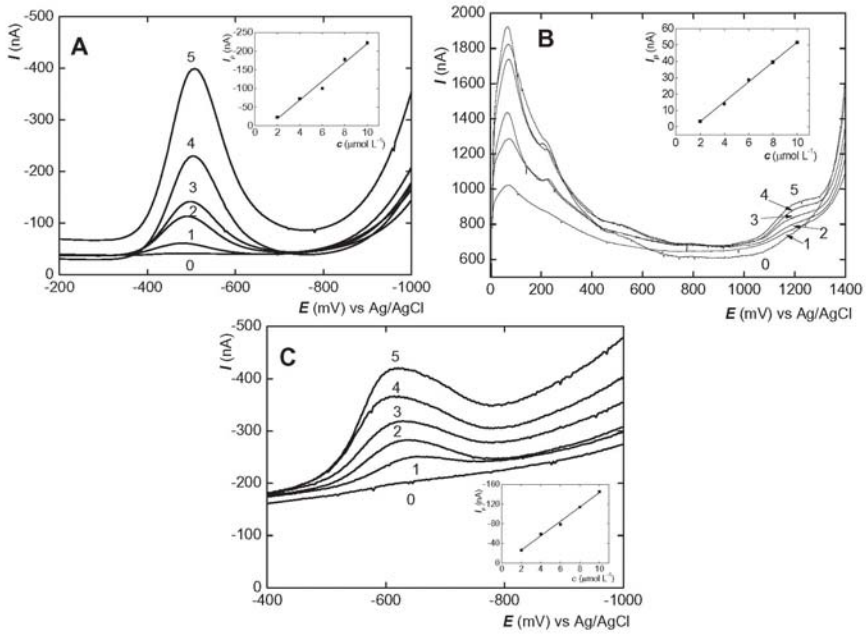

Fig. 3. DP voltammograms of ANBT(c = 0 (0), 2 (1), 4 (2), 6 (3), 8 (4) and 10 (5) μmol L^{-1}) and 5-NBIA (c = 0 (0), 2 (1), 4 (2), 6 (3), 8 (4) and 10 (5) μmol L^{-1}) at CFE at polarization rate 20 mV s^{-1}. (A) ABNT; cathodic region; methanol-BR buffer pH 4 (9:1); $E_{1,reg}$ = −400 mV, $E_{2,reg}$ = −900 mV. (B) ANBT; anodic region; methanol-BR buffer pH 4 (9:1); (C) 5-NBIA; BR buffer pH 7.0; $E_{1,reg}$ = −400 mV, $E_{2,reg}$ = −900 mV. The corresponding calibration straight lines are given the insets. The error bars are constructed for α = 0.05 (n = 3).

The highest and the best developed DCV and DPV peaks were obtained in the BR buffer pH 4.0-methanol (9:1) medium for ANBT and in BR buffer pH 7.0 for 5-NBIA. These media were further used for measuring of calibration curves in the concentration range from 0.2 to 100 μmol L^{-1} of ANBT and from 0.2 to 1000 μmol L^{-1} for 5-NBIA. For the sake of illustration, DP voltammograms of ANBT and 5-NBIA at CFE in the concentration range 2–10 μmol L^{-1} are depicted in Fig. 3A–C respectively. The parameters of all the calibration straight lines are summarized in Table 1.

Generally, the sensitivity slightly differs between individual concentration orders (see Table 1). This behavior, typical for voltammetry at solid electrodes, is usually caused by the passivation of the electrode surface by the electrode reaction products or by the analyte adsorption [28]. Nevertheless, within the individual concentration orders, the concentration dependences obtained are linear.

3.3. Differential pulse voltammetric determination of the tested substances in drinking and river water

In order to verify practical applicability of the developed DCV and DPV methods, the determination of ANBT and 5-NBIA was carried out in model samples of drinking and river water in a submicromolar concentration range under optimum conditions.

Table 1
Parameters of the calibration straight lines for the determination of ANBT and 5-NBIA using DCV and DPV at CFE in a Britton–Robinson buffer.

Substance	Process	pH BR buffer	Method	Concentration (mol L^{-1})	Slope (nA mol L^{-1})	Intercept (nA)	R	L_Q (mol L^{-1})
ANBT	Reduction	4	DCV	$(2–10) \times 10^{-6}$	$-(1.09 \pm 0.50) \times 10^7$	-3.2 ± 3.3	−0.9968	–
				$(2–10) \times 10^{-7}$	$-(1.83 \pm 0.14) \times 10^7$	3.34 ± 0.94	−0.9832	8.9×10^{-7}
			DPV	$(2–10) \times 10^{-6}$	$-(2.51 \pm 0.20) \times 10^7$	31 ± 13^a	−0.9908	–
				$(2–10) \times 10^{-7}$	$-(4.18 \pm 0.25) \times 10^7$	9.0 ± 1.7	−0.9990	7.7×10^{-7}
	Oxidation	4	DCV	$(2–10) \times 10^{-6}$	$-(4.09 \pm 0.40) \times 10^6$	-0.3 ± 2.6	−0.9954	5.1×10^{-6}
			DPV	$(2–10) \times 10^{-6}$	$-(2.51 \pm 0.16) \times 10^6$	-9.1 ± 1.1	−0.9989	4.0×10^{-6}
5-NBIA	Reduction	7	DCV	$(2–10) \times 10^{-5}$	$-(4.6 \pm 1.3) \times 10^7$	367 ± 88	−0.9987	–
				$(2–10) \times 10^{-6}$	$-(1.92 \pm 0.18) \times 10^7$	-3 ± 12^a	−0.9874	–
				$(2–10) \times 10^{-7}$	$-(4.54 \pm 0.49) \times 10^7$	8.6 ± 3.2	−0.9832	6.2×10^{-7}
			DPV	$(2–10) \times 10^{-5}$	$-(4.50 \pm 0.79) \times 10^7$	531 ± 52	−0.9995	–
				$(2–10) \times 10^{-6}$	$-(1.47 \pm 0.64) \times 10^7$	3.2 ± 4.3	−0.9971	–
				$(2–10) \times 10^{-7}$	$-(1.57 \pm 0.16) \times 10^7$	-0.9 ± 0.1	−0.9990	3.1×10^{-7}

R, correlation coefficient; L_Q, limit of quantification (10σ; α = 0.05).

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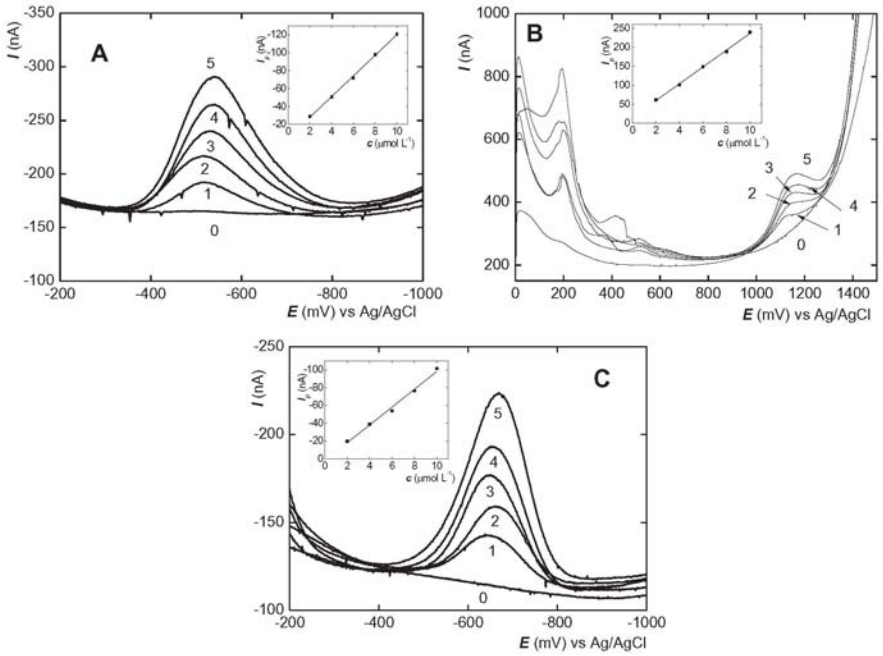

Fig. 4. DP voltammograms of ANBT (c = 0 (0), 2 (1), 4 (2), 6 (3), 8 (4) and 10 (5) μmol L^{-1}) and 5-NBIA (c = 0 (0), 2 (1), 4 (2), 6 (3), 8 (4) and 10 (5) μmol L^{-1}) in model samples of drinking water at CFE measured in 9 mL of spiked waters diluted by BR buffer pH 4.0 (for ANBT) and pH 7 (for 5-NBIA) to 10 mL; polarization rate 20 mV s^{-1}. (A) ABNT; cathodic region; $E_{1,reg}$ = −400 mV, $E_{2,reg}$ = −900 mV. (B) ANBT; anodic region; (C) 5-NBIA; $E_{1,reg}$ = −400 mV, $E_{2,reg}$ = −900 mV. The corresponding calibration straight lines are given the insets. The error bars are constructed for α = 0.05 (n = 3).

Table 2
Parameters of the calibration straight lines for the determination of ANBT and 5-NBIA using DCV and DPV at CFE in model samples of drinking and river waters.

Substance	Process	BR buffer pH	Water	Method	Concentration (mol L^{-1})	Slope (nA mol L^{-1})	Intercept (nA)	R	L_Q (mol L^{-1})
ANBT	Reduction	4	Drinking	DCV	$(2–10) \times 10^{-6}$	$-(1.62 \pm 0.44) \times 10^{7}$	5.9 ± 2.9	−0.9989	–
					$(2–10) \times 10^{-7}$	$-(3.50 \pm 0.15) \times 10^{7}$	4.16 ± 0.99	−0.9973	5.1×10^{-7}
				DPV	$(2–10) \times 10^{-6}$	$-(1.15 \pm 0.24) \times 10^{7}$	−4.8 ± 1.6	−0.9993	–
					$(2–10) \times 10^{-7}$	$-(2.36 \pm 0.74) \times 10^{7}$	−2.77 ± 0.49	−0.9985	1.7×10^{-7}
			River	DCV	$(2–10) \times 10^{-6}$	$-(1.60 \pm 0.33) \times 10^{7}$	4.1 ± 2.2	−0.9994	–
					$(2–10) \times 10^{-7}$	$-(2.61 \pm 0.40) \times 10^{7}$	1.52 ± 0.26	−0.9997	2.0×10^{-7}
				DPV	$(2–10) \times 10^{-6}$	$-(9.72 \pm 0.38) \times 10^{6}$	−15.8 ± 2.5	−0.9978	–
					$(2–10) \times 10^{-7}$	$-(8.03 \pm 0.22) \times 10^{6}$	−1.62 ± 0.15	−0.9989	5.0×10^{-7}
	Oxidation	4	Drinking	DCV	$(2–10) \times 10^{-6}$	$-(1.03 \pm 0.26) \times 10^{7}$	−10.7 ± 1.8	−0.9990	3.4×10^{-6}
				DPV	$(2–10) \times 10^{-6}$	$-(4.41 \pm 0.12) \times 10^{6}$	3.15 ± 0.77	−0.9990	1.7×10^{-6}
			River	DCV	$(2–10) \times 10^{-6}$	$-(6.10 \pm 0.18) \times 10^{6}$	2.0 ± 1.2	−0.9987	3.4×10^{-6}
				DPV	$(2–10) \times 10^{-6}$	$-(5.24 \pm 0.23) \times 10^{6}$	3.2 ± 1.5	−0.9972	2.4×10^{-6}
5-NBIA	Reduction	7	Drinking	DCV	$(2–10) \times 10^{-5}$	$-(1.32 \pm 0.58) \times 10^{7}$	−113 ± 38	−0.9971	–
					$(2–10) \times 10^{-6}$	$-(2.21 \pm 0.15) \times 10^{6}$	−10.2 ± 1.0	−0.9930	–
					$(2–10) \times 10^{-7}$	$-(1.29 \pm 0.73) \times 10^{7}$	−3.40 ± 0.48	−0.9953	2.5×10^{-7}
				DPV	$(2–10) \times 10^{-5}$	$-(2.25 \pm 0.12) \times 10^{6}$	−85 ± 8	−0.9957	–
					$(2–10) \times 10^{-6}$	$-(1.00 \pm 0.54) \times 10^{7}$	2 ± 3	−0.9957	–
					$(2–10) \times 10^{-7}$	$-(2.89 \pm 0.67) \times 10^{7}$	−2.35 ± 0.45	−0.9992	1.3×10^{-7}
			River	DCV	$(2–10) \times 10^{-5}$	$-(1.80 \pm 0.24) \times 10^{7}$	−75 ± 15	−0.9997	–
					$(2–10) \times 10^{-6}$	$-(1.16 \pm 0.30) \times 10^{7}$	−9.4 ± 2.0	−0.9990	–
					$(2–10) \times 10^{-7}$	$-(3.06 \pm 0.38) \times 10^{7}$	2.36 ± 0.26	−0.9998	1.9×10^{-7}
				DPV	$(2–10) \times 10^{-5}$	$-(1.45 \pm 0.26) \times 10^{7}$	−253 ± 18	−0.9995	–
					$(2–10) \times 10^{-6}$	$-(1.09 \pm 0.15) \times 10^{7}$	−15.42 ± 0.98	−0.9997	–
					$(2–10) \times 10^{-7}$	$-(1.56 \pm 0.42) \times 10^{7}$	−0.38 ± 0.28	−0.9992	1.3×10^{-7}

R, correlation coefficient; L_Q, limit of quantification (10σ; α = 0.05).

Calibration curves were measured using a mixture of 9.0 mL of a spiked model waters samples and 1.0 mL of the BR buffer of appropriate pH. DP voltammograms at CFE representing direct determination in spiked drinking (Fig. 4A and B for ANBT and C for 5-NBIA) water, in the concentration range 2–10 μmol L^{-1}, are depicted in Fig. 4.

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The obtained parameters of the calibration curves are summarized in Table 2. These results confirm the possible application of tested electrode for the determination of proposed method for measurement of ANBT and 5-NBIA in both drinking and river waters.

4. Conclusions

It has been shown that DC voltammetry (DCV) and differential pulse voltammetry (DPV) at carbon film electrode (CFE) on silver solid amalgam electrode (disc diameter 0.50 mm) as a substrate can be used for the determination of submicromolar concentrations of genotoxic 2-amino-6-nitrobenzothiazole (ANBT) and 5-nitrobenzimidazole (5-NBIA). In the optimum medium found (BR buffer pH 4.0-methanol (9:1) for ANBT and BR buffer pH 7.0 for 5-NBIA), the measurable concentration range was 0.2–100 μmol L^{-1} of ANBT for DCV reduction (the limit of quantification, $L_Q \approx 0.89$ μmol L^{-1}) and DPV reduction (the limit of quantification, $L_Q \approx 0.77$ μmol L^{-1}), concentration range 2–100 μmol L^{-1} of ANBT for DCV oxidation (the limit of quantification, $L_Q \approx 5.12$ μmol L^{-1}) and DPV oxidation (the limit of quantification, $L_Q \approx 3.96$ μmol L^{-1}). For determination of 5-NBIA at the CFE in concentration range 0.2–1000 μmol L^{-1} using DCV the limit of quantification was $L_Q \approx 0.62$ μmol L^{-1}) and using DPV the limit of quantification was $L_Q \approx 0.31$ μmol L^{-1}.

The applicability of the tested electrode for DCV and DPV determination of ANBT (in the concentration range from 0.2 to 100 μmol L^{-1} of ANBT for reduction and 2.0–100 μmol L^{-1} of ANBT for oxidation) and of 5-NBIA (in the concentration range from 0.2 to 1000 μmol L^{-1}) in model samples of drinking and river water has also been verified, with the L_Qs in the concentration range 10^{-7} mol L^{-1} for reduction and 10^{-6} mol L^{-1} for oxidation. Therefore, it can be concluded that CFE can be successfully used for the determination of trace amounts of ANBT and 5-NBIA as suitable non-toxic and environmentally friendly alternative to mercury electrodes. CFE prepared on p-AgSAE is very stable and it can be used for several days without any problem. If its performance deteriorates because of passivation, preparation of a new film take just a few minutes.

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12. APPENDIX VII – CONFIRMATION OF PARTICIPATION

1. **Deylova, D.**, Barek, J., Vyskocil, V. Voltammetric determination of 6-nitrobenzimidazole in the presence of surfactants. *Collection of Czechoslovak Chemical Communications*, **2011**, 76, 1317.

   Impact Factor (WOS, 2011): **1.000**; percentage of participation of Mgr. Dana Deýlová ~ **75**. **%**

2. **Deylova, D.**, Yosypchuk, B., Vyskocil, V., Barek, J. Voltammetric determination of 4-nitrophenol and 5-nitrobenzimidazole using different types of silver solid amalgam electrodes - a comparative study. *Electroanalysis*, **2011**, 23, 1548.

   Impact Factor (WOS, 2011): **2.817**; percentage of participation of Mgr. Dana Deýlová ~ **75**. **%**

3. **Deylova, D.**, Vyskocil, V., Barek, J. Voltammetric determination of 2-amino-6-nitrobenzothiazole at two different silver amalgam electrodes. *Electrochim. Acta*, **2012**, 62, 335.

   Impact Factor (WOS, 2012): **3.777**; percentage of participation of Mgr. Dana Deýlová ~ **75**. **%**

4. **Deylova, D.**, Vyskocil, V., Barek, J., Economou, A. Bismuth film electrode at a silver solid amalgam substrate as a new tool for voltammetric determination of electrochemically reducible organic compounds. *Talanta*, **2012**, 102, 68.

   Impact Factor (WOS, 2012): **3.498**; percentage of participation of Mgr. Dana Deýlová ~ **75**. **%**

5. **Deylova, D.**, Vyskocil, V., Economou, A., Mansfeldova, V., Barek, J. New tipe of large surface Bismuth film electrode at a silver solid amalgam substrate and its application for voltammetric determination of 5-Nitrobenzimidazole. *Int. J. Electrochem. Sci.*, **2014**, in press.

   Impact Factor (WOS, 2013): **2.672**; percentage of participation of Mgr. Dana Deýlová ~ **75**. **%**

6. **Deylova, D.**, Vyskocil, V., Barek, J. Voltametric determination of 2-amino-6-nitrobenzothiazole and 5-nitrobenzimidazole using a silver solid amalgam electrodes modified by microcrystalline natural graphite–polystyrene composite film. *J. Electroanal. Chem.*, **2014,** http://dx.doi.org/10.1016/j.jelechem.2014.01.017.

Percentage of participation of Mgr. Dana Deýlová ~ **75**. **%**

I declare that the percentage of participation of Mgr. Dana Deýlová at the above given papers corresponds to above given numbers.

In Prague, 28.2.2014

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Prof. RNDr. Jiří Barek, CSc.

13. APPENDIX VIII – LIST OF PUBLICATIONS, ORAL AND POSTER PRESENTATIONS

DIPLOMA THESIS

1. **Deýlová, D.** Voltammetric determination of 5-nitrobenzimidazole. Diploma Thesis, Charles University in Prague, Prague, 2008.

CHAPTER IN BOOK

1. Vyskocil, V.; Danhel, A.; Fischer, J.; Novotny, V.; **Deylova, D.**; Horackova, E.; Barek, J.; Yosypchuk, B.; Wang, J., Silver amalgam electrodes – A look back at the last five years of their development and applications. In *Sensing in Electroanalysis*, Vytřas, K.; Kalcher, K.; Švancara, I., Eds. University of Pardubice: Pardubice, 2010; Vol. 5, pp 13-31.

ARTICLES IN IMPACTED JOURNALS

1. **Deylova, D.**, Barek, J., Vyskocil, V. Polarographic and voltammetric determination of 6-nitrobenzimidazole and mechanism of its electrochemical reduction. *Collection of Czechoslovak Chemical Communications*, **2009**, 74, 1443.
2. Vyskocil, V., Danhel, A., Fischer, J., Novotny, V., **Deylova, D.**, Musilova-Karaova, J., Peckova, K., Barek, J. The beauty and usefulness of novel electrode materials (Krása a užitečnost nových elektrodových materiálů). *Chemicke Listy*, **2010**, 104, 1181.
3. **Deylova, D.**, Barek, J., Vyskocil, V. Voltammetric determination of 6-nitrobenzimidazole in the presence of surfactants. *Collection of Czechoslovak Chemical Communications*, **2011**, 76, 1317.
4. **Deylova, D.**, Yosypchuk, B., Vyskocil, V., Barek, J. Voltammetric determination of 4-nitrophenol and 5-nitrobenzimidazole using different types of silver solid amalgam electrodes - a comparative study. *Electroanalysis*, **2011**, 23, 1548.
5. **Deylova, D.**, Vyskocil, V., Barek, J. Voltammetric determination of 2-amino-6-nitrobenzothiazole at two different silver amalgam electrodes. *Electrochim. Acta*, **2012**, 62, 335.
6. **Deylova, D.**, Vyskocil, V., Barek, J., Economou, A. Bismuth film electrode at a silver solid amalgam substrate as a new tool for voltammetric determination of electrochemically reducible organic compounds. *Talanta*, **2012**, 102, 68.
7. **Deylova, D.**, Vyskocil, V., Economou, A., Mansfeldova, V., Barek, J. New tipe of large surface Bismuth film electrode at a silver solid amalgam substrate and its application for voltammetric determination of 5-Nitrobenzimidazole *Int. J. Electrochem. Sci.*, **2014,** in press.
8. **Deylova, D.**, Vyskocil, V., Barek, J. Voltametric determination of 2-amino-6-nitrobenzothiazole and 5-nitrobenzimidazole using a silver solid amalgam electrodes modified by microcrystalline natural graphite–polystyrene composite film. *J. Electroanal. Chem.*, **2014,** http://dx.doi.org/10.1016/j.jelechem.2014.01.017.

ORAL PREZENTATIONS

1. **Deylova, D.**, Barek, J., Yosypchuk, B. In *Voltammetric determination of 4-nitrophenol and 5-nitrobenzimidazole on different amalgam electrodes* Moderní elektrochemické metody - Sborník přednášek z XXIX. mezinárodní odborné konference, Jetřichovice, 25. - 29.5. **2009**, p 19.
2. **Deylova, D.**, Barek, J., Yosypchuk, B. In *Voltammetric determination of 4-nitrophenol and 5-nitrobenzimidazole on different amalgam electrodes*, 5th Proceedings of the International Students Conference "Modern Analytical Chemistry", Prague, 21. -22.9. **2009**, p 15.
3. **Deylova, D.**, Barek, J. In *Determination of 5-nitrobenzimidazole using HMDE and AgSE in presence of the surfactants*, Moderní elektrochemické metody - Sborník přednášek z XXX. mezinárodní odborné konference, Jetřichovice, 24. - 28.5. **2010**, p 30.
4. **Deylova, D.**, Barek, J. In *Voltammetric determination of 2-amino-6-nitrobenzothiazole at different amalgam electrodes*, Proceedings of the 6th International Students Conference "Modern Analytical Chemistry", Prague, 23. - 24.9. **2010**, p 101.
5. **Deylova, D.**, Barek, J. In *Voltammetric Determination of 5-Nitrobenzimidazole at a Carbon Film Electrode Deposited on a Silver Solid Amalgam Electrode (Voltametrické stanovení 5-nitrobenzimidazolu na uhlíkové filmové elektrodě deponované na stříbrné pevné amalgámové elektrodě)*, XXXII. Modern Electrochemical Methods, Jetrichovice, 21.-25.5. **2012**, p 27.

POSTER PREZENTATIONS

1. Vyskocil, V., **Deylova, D.**, Musilova, J., Peckova, K., Barek, J. In *Polarografické a voltametrické stanovení submikromolárních množství genotoxického 5-nitrobenzimidazolu*, 59. Zjazd chemikov, Tatranské Matliare, Slovensko, 2.-6.9. **2007**, p 160.
2. **Deylova, D.**, Barek, J., Peckova, K., Vyskocil, V. In *Voltammetric determination of 5-nitrobenzimidazole (Voltametrické stanovení 5-nitrobenzimidazolu)*, 60. sjezd chemických společností, Olomouc, 1.-4.9. **2008**, p 708.
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4. **Deylova, D.**, Barek, J., Yosypchuk, B. In *Voltametrické stanovení 4-nitrofenolu a 5-nitrobenzimidazolu na různých amalgamových elektrodách* 61. Zjazd chemikov, ChemZi 5/9, Tatranské Matliare, Slovensko, 7.-11.9. **2009**, p 135.
5. **Deylova, D.**, Barek, J. In *Vliv povrchově aktivních látek na voltametrické stanovení 5-nitrobenzimidazolu*, ACP 2010 - Súčasný stav a perspektívy analytickej chémie v praxi, Bratislava, 9.5.-12.5. **2010**, p s569.
6. **Deylova, D.**, Barek, J., Chladkova, B. In *Voltametrické stanovení 5-nitrobenzimidazolu na špičkové amalgamové pastové elektrodě*, 62. sjezd chemických společností, Pardubice, 28.-30.6. **2010**, p 451.
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