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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 the 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 the practical application of the new LSBiF-AgSAE, the voltammetric behavior of genotoxic 5-nitrobenzimidazole (5-NBIA) was investigated using direct current voltammetry (DCV) and differential pulse voltammetry (DPV), and optimum conditions were found for its determination in the concentration range from 0.2 to 1000 µmol L^{-1} in the 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. The 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 ≈ 0.1µmol L^{-1} mol L^{-1}.

Keywords: Large-surface bismuth film electrode; Silver 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 a 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 the voltammetric determination of an organic nitro-compound. However, the low surface area of this electrode with small diameter of the electroactive part (0.5 mm) resulted in relatively low current signals. Therefore, in this paper, a large-surface bismuth film electrode was fabricated on a silver solid amalgam substrate resulting in the 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].

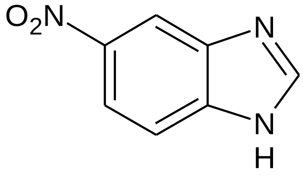


Figure 1. Structural formula of 5-nitrobenzimidazole.

The environment is increasingly polluted with various genotoxic nitro-compounds [30]. Therefore, we have selected 5-nitrobenzimidazole (NBIA, see Fig. 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 fuel combustion [32]; it was polarographically determined as a part of photographic processing solutions [33] and its properties were extensively studied in connection with its application in the area of metal corrosion protection [34]. Furthermore, the practical applicability of the 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 water contamination by this genotoxic compound after its application as anti-corrosive agent [34].

2. EXPERIMENTAL

2.1 Reagents

The stock solution of 5-NBIA ($c = 1.0\times10^{-3}$ mol L^{-1}; CAS Registry 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, Darmstadt, Germany), 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 auxiliary electrode (type PPE, Monokrystaly, Turnov, Czech Republic) and silver|silver chloride reference electrode (type RAE 113, 3 mol L^{-1} KCl, Monokrystaly, Turnov, Czech Republic). The LSBiF-AgSAE (disc diameter of 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 of –50 mV and pulse width of 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 a 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), Merck, Darmstadt, 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 on a 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

The surface topography of the electrochemically deposited bismuth film of the LSBiF-AgSAE was characterized by AFM (Multimode Nanoscope IIIa, Veeco, Plainview, NY, USA) in tapping mode. The silicon cantilevers OTESPA (Veeco) oscilating at ca. 300 kHz were used to minimize the tip interaction with the examined surface. The surface imaging was performed with the rate of 0.5 and 1.0 Hz, respectively. The 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 the optimization procedure, an appropriate amount of 5-NBIA stock solution was filled up to 10.0 mL with the BR buffer of an 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 interfering cations.

3. RESULTS AND DISCUSSION

3.1 Bismuth film deposition

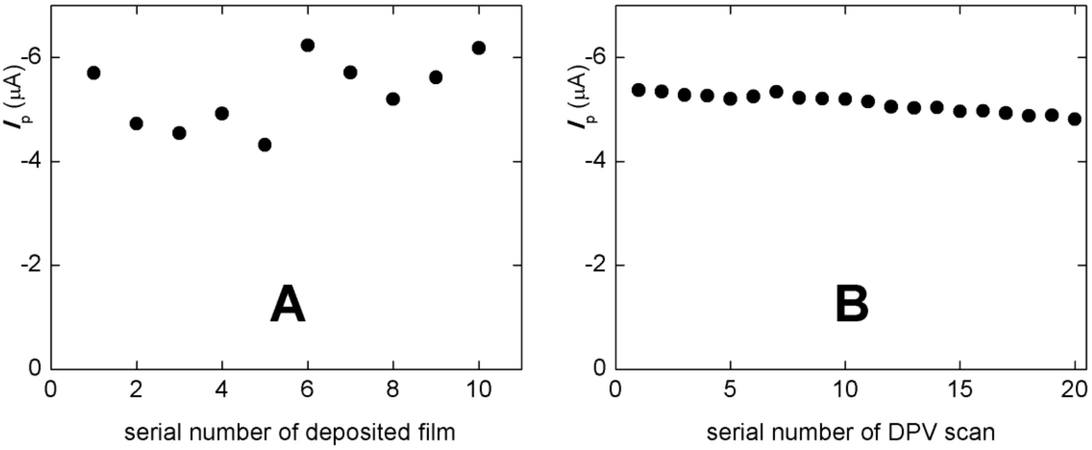


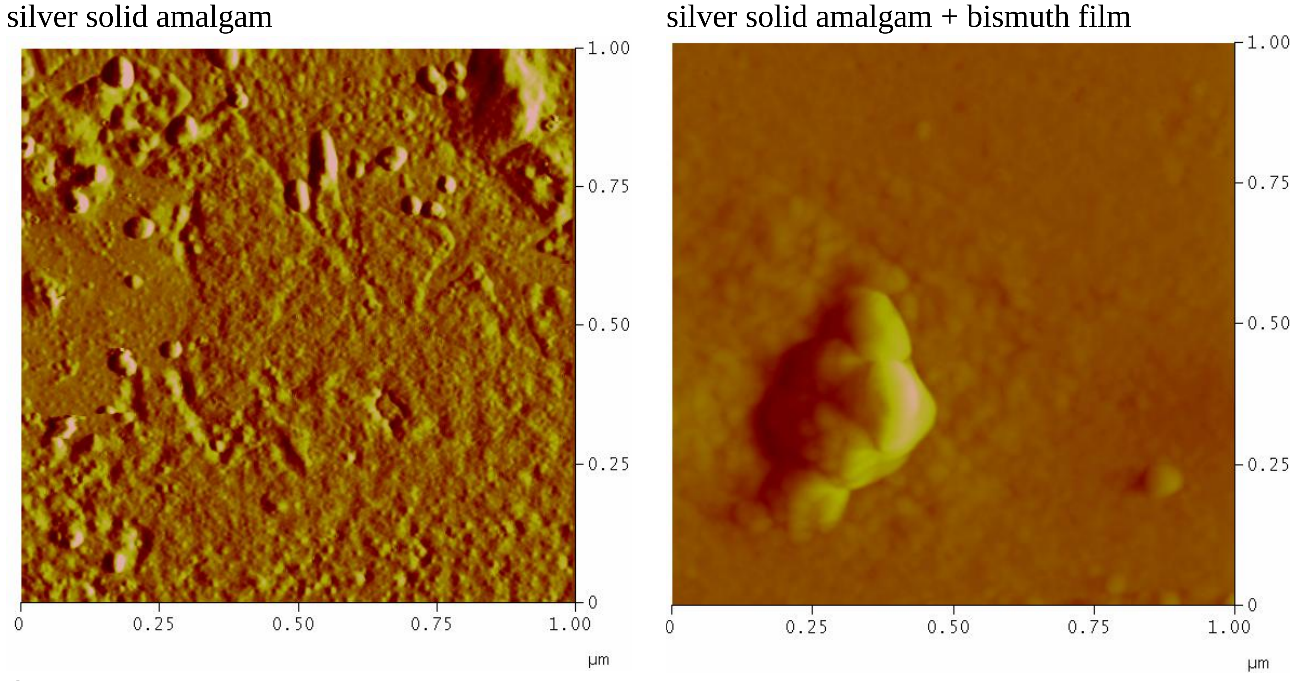
Figure 2. (A) Repeatability of DPV determination of 5-NBIA ($c = 1.0\times10^{-4}$ mol L^{-1}) at consecutively prepared LSBiF-AgSAEs in BR buffer pH 7.0 with deposition time 1800 s. (B) Repeatability ($n = 20$) of DPV peaks of 5-NBIA ($c = 1.0\times10^{-4}$ mol L^{-1}) at one LSBiF-AgSAE in BR buffer pH 7.0 with deposition time 1800 s.

Initially, the deposition time of the bismuth film was studied. Deposition times (t_{dep}) of 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, a 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 the small-surface AgSAE substrate [28], where the 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 the BR buffer pH 7.0 (this medium was selected on the basis of the 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 satisfactory 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.

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 the silver solid amalgam substrate with alumina and depositing the 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 Fig. 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.


silver solid amalgam

silver solid amalgam + bismuth film

A

B

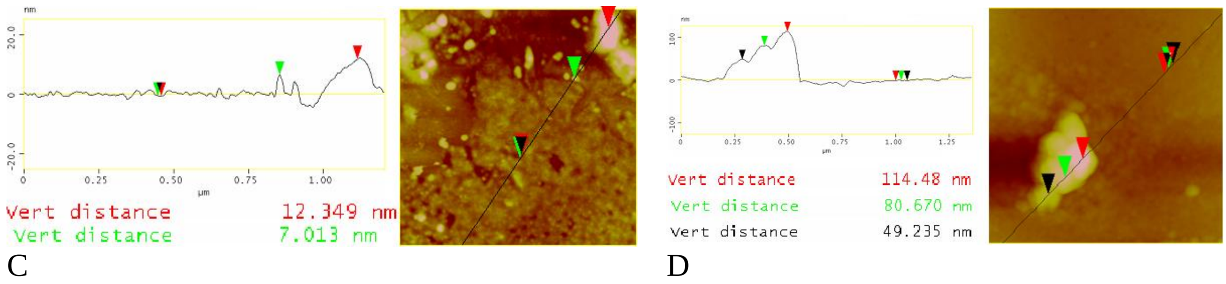


Figure 3. AFM images of tested electrode surface: **(A)** Topographic image of silver solid amalgam support (1×1 µm) without modification, **(B)** topographic image of the support (1×1 µm) modified by bismuth film, **(C)** line analysis of image A, **(D)** line analysis of image B.

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.

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 the LSBiF-AgSAE is depicted in Fig. 4. The differences in peak shapes, positions, and 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.

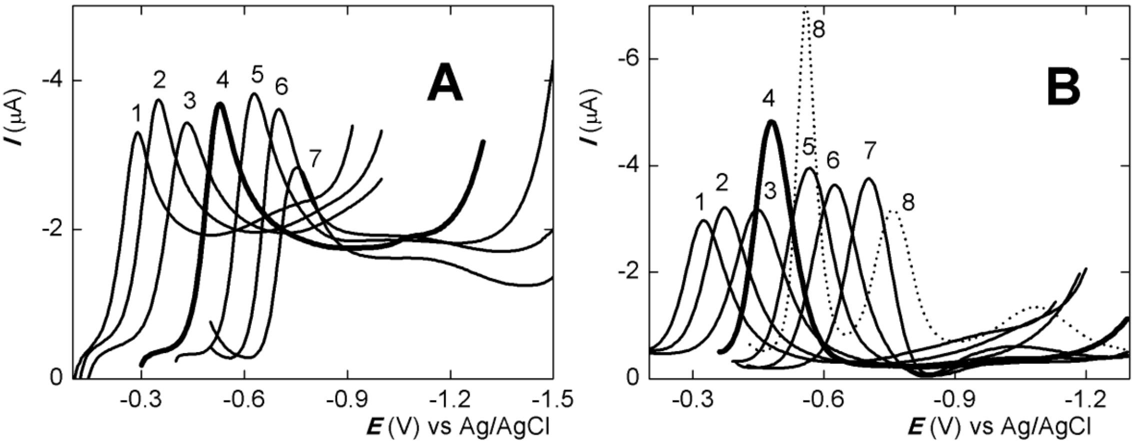


Figure 4. DC (**A**) and DP (**B**) voltammograms of 5-NBIA (c = 1.0×10^{-4} mol L^{-1}) at LSBiF-AgSAE (t_{dep} = 1800 s) in BR buffer. **(A)** pH 2.0 (1), 3.0 (2), 5.0 (3), 7.0 (4), 8.0 (5), 10.0 (6), 11.0 (7); **(B)** pH 2.0 (1), 4.0 (2), 6.0 (3), 7.0 (4), 8.0 (5), 9.0 (6), 10.0 (7), 12.0 (8). The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

The second peak probably corresponding to the 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 in 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].

3.3 Voltammetric determination of 5-NBIA at the 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 the 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 the LSBiF-AgSAE in the concentration range of 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 the LSBiF-AgSAE are more favorable than those values obtained on the bismuth film electrode with smaller surface [28]. Therefore, the LSBiF-AgSAE should be preferred for trace environmental analysis.

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 in more acidic (BR buffer pH 3.0) or more alkaline (BR buffer pH 10.0) solutions and at different accumulation potentials.

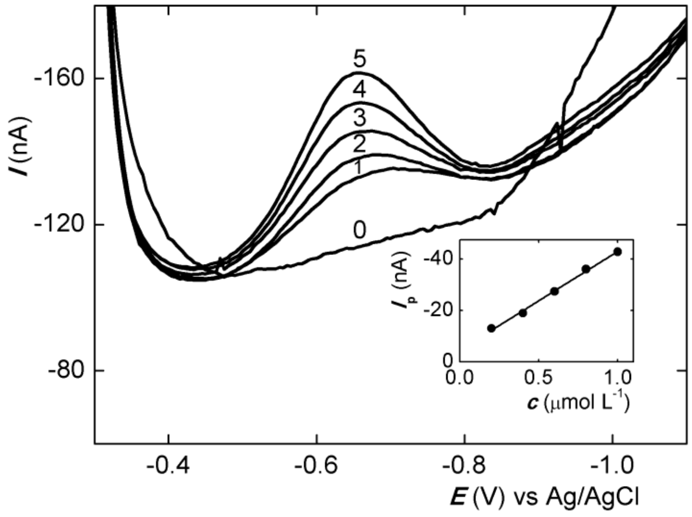


Figure 5. DP voltammograms of 5-NBIA ($c_{5\text{-NBIA}}$ = 0 (0), 0.2 (1), 0.4 (2), 0.6 (3), 0.8 (4), and 1 (5) μmol L^{-1}) at LSBiF-AgSAE in BR buffer pH 7.0; the corresponding calibration straight line is in the inset.

Table 1. Parameters of the calibration straight lines (± standard errors) for the 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	$(2–10)\times10^{-5}$	$–(3.70 \pm 0.89)\times10^{7}$	102 ± 59^{a}	–0.9991	–
	$(2–10)\times10^{-6}$	$–(3.13 \pm 1.05)\times10^{7}$	$–35 \pm 7$	–0.9983	–
	$(2–10)\times10^{-7}$	$–(5.00 \pm 1.23)\times10^{7}$	$–9.4 \pm 0.8$	–0.9991	3.7×10^{-7}
DPV	$(2–10)\times10^{-5}$	$–(3.48 \pm 0.59)\times10^{7}$	$–83 \pm 39^{a}$	–0.9996	–
	$(2–10)\times10^{-6}$	$–(2.55 \pm 0.49)\times10^{7}$	6.2 ± 3.2^{a}	–0.9995	–
	$(2–10)\times10^{-7}$	$–(3.84 \pm 1.34)\times10^{7}$	$–4.6 \pm 0.9$	–0.9982	–
	$(2–10)\times10^{-8}$	$–(1.20 \pm 6.72)\times10^{8}$	$–2.0 \pm 0.5$	–0.9953	6.8×10^{-8}

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

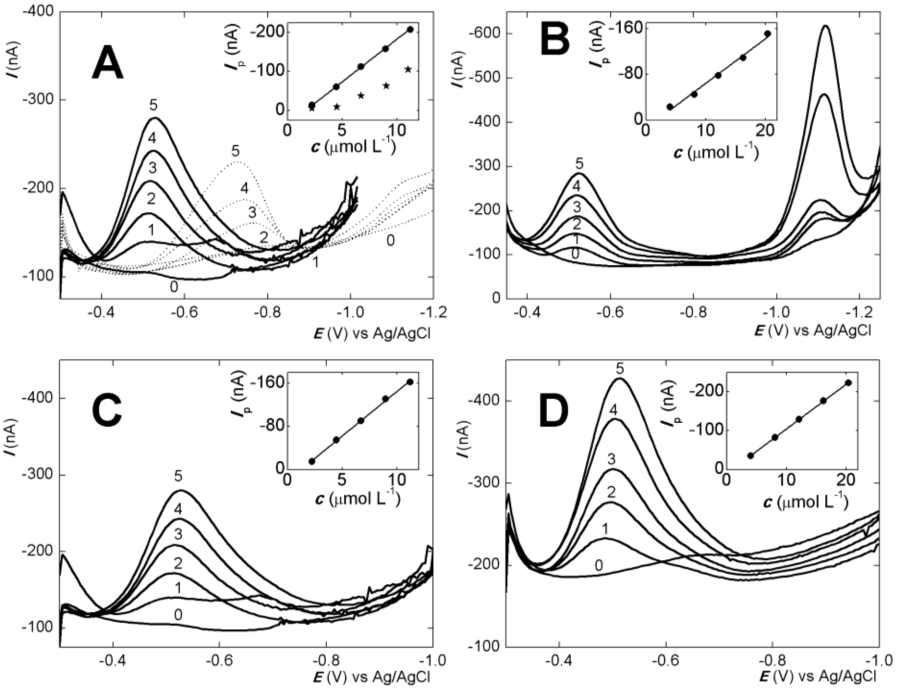


Figure 6. DP voltammograms of 5-NBIA at LSBiF-AgSAE. **(A)** Spiked tap water with addition of 1 g of EDTA to 50 mL of water (full line) and without EDTA (dotted line); BR buffer pH 7.0 – tap water (1:9). **(B)** Spiked tap water without addition of EDTA; BR buffer pH 7.0 – tap water (5:5). **(C)** Spiked mineral water with addition of EDTA (1 g to 50 mL of water); BR buffer pH 7.0 – mineral water (1:9). **(D)** Spiked mineral water with addition of EDTA (1 g to 50 mL of water); BR buffer pH 7.0 – mineral water (5:5). **(A-D)** $c_{5\text{-NBIA}}$ in measured solutions was: 0 (0), 2.0 (1), 4.0 (2), 6.0 (3), 8.0 (4), and 10.0 (5) μmol L^{-1}; i.e. the concentration in model samples was 1.1 times (in the case of ratio of buffer and model sample 1:9) or 2 times (in the case of ratio of buffer and model sample 5:5) higher; the corresponding calibration straight lines based on 5-NBIA concentration in model samples are always given in the inset.

A practical application of the proposed new LSBiF-AgSAE was the determination of 5-NBIA in model samples of tap and mineral water in the 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 were 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. The 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 the EDTA addition was impossible. The effect of the addition of EDTA on the voltammetric determination of 5-NBIA is demonstrated in Table 2.

Table 2. Parameters of the calibration straight lines (± standard errors) for the DCV and DPV determination of 5-NBIA at the BiF-AgSAE in model samples of tap and mineral water (measurement in the 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	$(2–10)\times10^{-5}$	$–(3.40 \pm 0.87)\times10^{7}$	6.7 ± 5.8[a]	–0.9990	–
		$(2–10)\times10^{-6}$	$–(1.58 \pm 0.84)\times10^{7}$	–37.0 ± 5.6	–0.9957	–
		$(2–10)\times10^{-7}$	$–(6.41 \pm 1.90)\times10^{7}$	4.5 ± 1.3	–0.9987	6.8×10^{-7}
	DPV	$(2–10)\times10^{-5}$	$–(3.10 \pm 2.24)\times10^{7}$	–193 ± 149[a]	–0.9922	–
		$(2–10)\times10^{-6}$	$–(1.60 \pm 0.98)\times10^{7}$	14.9 ± 6.5[a]	–0.9944	–
		$(2–10)\times10^{-7}$	$–(5.13 \pm 2.04)\times10^{7}$	6.3 ± 1.4	–0.9976	9.8×10^{-7}
Tap water[c]	DCV	$(2–10)\times10^{-5}$	$–(2.37 \pm 0.31)\times10^{7}$	61.5 ± 20.4[a]	–0.9997	–
		$(2–10)\times10^{-6}$	$–(2.73 \pm 0.81)\times10^{7}$	27.5 ± 5.4	–0.9987	–
		$(2–10)\times10^{-7}$	$–(2.81 \pm 1.25)\times10^{7}$	–5.1 ± 0.8	–0.9970	2.3×10^{-7}
	DPV	$(2–10)\times10^{-5}$	$–(2.12 \pm 1.01)\times10^{7}$	133 ± 67[a]	–0.9966	–
		$(2–10)\times10^{-6}$	$–(2.43 \pm 0.21)\times10^{7}$	35.4 ± 1.4	–0.9999	–
		$(2–10)\times10^{-7}$	$–(1.72 \pm 0.84)\times10^{7}$	3.62 ± 0.55	–0.9965	6.6×10^{-7}
Mineral water[d]	DCV	$(2–10)\times10^{-5}$	$–(2.92 \pm 0.56)\times10^{7}$	6 ± 37[a]	–0.9995	–
		$(2–10)\times10^{-6}$	$–(3.21 \pm 0.64)\times10^{7}$	–27 ± 4	–0.9994	–
		$(2–10)\times10^{-7}$	$–(8.43 \pm 3.92)\times10^{7}$	–3.0 ± 2.6[a]	–0.9968	7.8×10^{-7}
	DPV	$(2–10)\times10^{-5}$	$–(2.62 \pm 0.26)\times10^{7}$	6 ± 17[a]	–0.9999	–
		$(2–10)\times10^{-6}$	$–(2.35 \pm 0.07)\times10^{7}$	11.6 ± 0.5	–1.0000	–
		$(2–10)\times10^{-7}$	$–(4.80 \pm 0.80)\times10^{7}$	2.8 ± 0.5	–0.9996	4.2×10^{-7}
Mineral water[c]	DCV	$(2–10)\times10^{-5}$	$–(2.72 \pm 0.80)\times10^{7}$	123 ± 53[a]	–0.9987	–
		$(2–10)\times10^{-6}$	$–(2.94 \pm 0.32)\times10^{7}$	–16.4 ± 2.1	–0.9998	–
		$(2–10)\times10^{-7}$	$–(7.92 \pm 4.79)\times10^{7}$	–0.8 ± 3.2[a]	–0.9945	5.5×10^{-7}
	DPV	$(2–10)\times10^{-5}$	$–(2.76 \pm 1.01)\times10^{7}$	14 ± 66[a]	–0.9980	–
		$(2–10)\times10^{-6}$	$–(1.85 \pm 0.43)\times10^{7}$	20.5 ± 2.9	–0.9992	–
		$(2–10)\times10^{-7}$	$–(2.70 \pm 0.49)\times10^{7}$	3.8 ± 0.3	–0.9993	3.6×10^{-7}

R, correlation coefficient; L_Q, limit of quantification (10σ; $\alpha = 0.05$). *Concetration refers to values in measured solution (not in water samples) for easier comparison of slopes;

[a] Intercepts are not statistically significantly different from zero at the significance level $\alpha = 0.05$;

[b] 5:5 model water sample : buffer ratio in the measured solution;

[c] 9:1 model water sample : buffer ratio in the measured solution with the addition of EDTA;

[d] 5:5 model water sample : buffer ratio in the measured solution with the addition of EDTA.

Without the addition of EDTA, both DCV and DPV determination are possible only when using a lower volume ratio of water sample to BR buffer, namely 1:1. DP voltammograms of 5-NBIA at the 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 the 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. For the sake of easy comparison of the slopes, concentration refers to the concentration in measured solution, not in the model water sample. These results confirm the applicability of the LSBiF-AgSAE for the determination of submicromolar concentrations of 5-NBIA (and possibly of many other electrochemically reducible organic pollutants) in both tap and mineral waters.

4. CONCLUSIONS

It has been shown that the newly developed LSBiF-AgSAE (disc diameter of 2.64 mm, deposition time of 1800 s) is a suitable sensor for the 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 the 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 the 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 the 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 of 10^{-7} mol L^{-1}. Therefore, it can be concluded that the 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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