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Voltammetric Determination of 2-Nitrobiphenyl and 4-Nitrobiphenyl Using a Mercury Meniscus Modified Silver Solid Amalgam Electrode

Eva Horakova, Daniela Smidova, Vlastimil Vyskocil[*]*, Jiri Barek*

Charles University in Prague, Faculty of Science, University Research Centre UNCE "Supramolecular Chemistry", Department of Analytical Chemistry, UNESCO Laboratory of Environmental Electrochemistry, Albertov 6, CZ-128 43 Prague 2, Czech Republic
[*]E-mail: vlastimil.vyskocil@natur.cuni.cz

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Direct current voltammetry (DCV), differential pulse voltammetry (DPV), and differential pulse adsorptive stripping voltammetry (DPAdSV) at a mercury meniscus modified silver solid amalgam electrode (m-AgSAE) were used for the determination of trace amounts of carcinogenic 2-nitrobiphenyl (2-NBP) and 4-nitrobiphenyl (4-NBP) in buffered aqueous-methanolic solutions (for 2-NBP: 0.01 mol L^{-1} LiOH (pH 12.0) – methanol (9:1), for 4-NBP: 0.25 mol L^{-1} acetate buffer (AB) of pH 4.8 – methanol (7:3)). Both nitrobiphenyls (NBPs) can be determined by DCV and DPV in the concentration range 0.2 – 100 μmol L^{-1} (limit of quantification (L_Q) of both NBPs for DCV is 0.2 μmol L^{-1}, L_Q of 2-NBP and 4-NBP for DPV is 0.1 and 0.2 μmol L^{-1}, respectively). An attempt to decrease the L_Q using DPAdSV was not successful, probably due to some competitive adsorption. The optimal medium for the simultaneous determination of 2-NBP and 4-NBP by DPV at the m-AgSAE was: 0.10 mol L^{-1} AB of pH 6.0 – methanol (7:3). The dependences of the peak current on the concentration of individual NBPs in the mixture were linear in the 10^{-6} and 10^{-7} mol L^{-1} concentration ranges. The practical applicability of the newly developed methods was verified using model samples of drinking and river water.

Keywords: Voltammetry, Silver solid amalgam electrode, Nitrobiphenyls, Simultaneous determination, Drinking and river water

1. INTRODUCTION

The studied compounds are nitrated aromatic hydrocarbons (NAHs), which are known as mutagenic agents [1,2]. NAHs are formed during incomplete combustion processes by reactions of aromatic hydrocarbons with atmospheric nitrogen oxides [3]. The toxic effects are attributed to

the formation of free anion-radicals formed by an enzyme activity [4]. Reduction of the nitro group leads to nitroso compounds, hydroxylamines, and amines [4,5] which can be detrimental to living organisms as well. The most important are their reactions with the cellular macromolecules. These facts show the importance of monitoring NAHs in the environment [6]. Moreover, the concentrations of NAHs in environmental samples are very low (pg m^{-3}) [7], so the monitoring of their levels is very difficult, and extremely sensitive methods of detection are needed [8].

Both 2-nitrobiphenyl (2-NBP) and 4-nitrobiphenyl (4-NBP) are biologically active genotoxic and ecotoxic compounds. 2-NBP is supposed to have mutagenic and other adverse effects [9]. Acute effects seen in animals are coma, shortness of breath, loss of weight [10]; it is not listed in any list of International Agency for Research on Cancer (IARC). Its occurrence in the environment is connected with its use as a dye intermediate and plasticizer [11]. 4-NBP is ranked in group 3 (not classifiable as to its carcinogenicity to humans) according to the IARC [12], and it has carcinogenic effects on animals [13]. Results of studies conducted with the rabbit liver preparations [14], rats [5], and samples of cytosol and microsomes of human lung [15] show that 4-NBP is metabolically converted by cytochrome P450 to 4-aminobiphenyl, which is by the IARC [16] included in group 1 (carcinogenic to humans) together with other suspected carcinogens (*N*-hydroxy-4-aminobiphenyl and 4-nitrosobiphenyl) [15], which are metabolites of investigated nitrobiphenyls. Results of studies in *Salmonella* Thyphimurium indicate that by the methylation of 4-NBP, its mutagenic effect can be eliminated [17]. Because of the location of a nitro substituent, 4-NBP is more toxic than 2-NBP [18].

For the determination of 4-NBP, sensitive methods based on chromatographic techniques, such as gas chromatography–mass spectrometric detection [19], gas chromatography–electron capture detection [20], and high performance liquid chromatography with UV spectrophotometric [21,22], electrochemical [22], fluorescent [22], or electrochemiluminescence detection [23] (electrochemical, fluorescent, and electrochemiluminescence detection were performed after reduction of 4-NBP to detectable 4-aminobiphenyl), have been developed. Electrochemical behavior and determination of nitrobiphenyls (NBPs) have been studied on mercury electrodes [24–28] and a glassy carbon rotating disk electrode [27]. For the determination of 2-NBP, the same methods as for 4-NBP are used, and also the limits of quantification are similar (*ca.* 0.1 μmol L^{-1}) [8,19]. The limits of quantification of NBPs achieved by electrochemical methods, namely linear scan adsorptive stripping voltammetry (LSAdSV) [26] and differential pulse adsorptive stripping voltammetry (DPAdSV) [28] on a hanging mercury drop electrode (HMDE), are of the order of nanomolar concentrations.

A mercury meniscus modified silver solid amalgam electrode (m-AgSAE) is a robust and reliable tool for analysis of environmental pollutants, such as chemical carcinogens, pesticides, and drugs present in water matrices [29–34]. Its main advantages are: fast and inexpensive determinations, a possibility of miniaturization, a simple electrochemical and/or mechanical cleaning, and, last but not least, a non-toxicity, which makes it a suitable substitute of mercury electrodes [35].

In this paper, direct current voltammetry (DCV) and differential pulse voltammetry (DPV) at the m-AgSAE were used for the development of sensitive analytical methods for the determination of trace amounts of carcinogenic 2-NBP and 4-NBP in simple environmental matrices – drinking and river waters (an attempt to increase the sensitivity of the newly developed methods using DPAdSV was carried out, too). As DPV is a useful technique for the mixture analysis [28] and 2-NBP and

4-NBP frequently occur simultaneously in the environment, the DPV method for the simultaneous determination of these NBPs using the m-AgSAE was also developed due to the fact that these pollutants have sufficiently different peak potentials [28]. The possibility to replace the HMDE [28] by the non-toxic m-AgSAE was thus verified, and the results obtained on both electrode materials were compared.

2. EXPERIMENTAL

2.1. Reagents

Methanolic stock solutions (1 mmol L^{-1}) of 2-NBP and 4-NBP (both 99%, Merck, Prague, Czech Republic) were prepared by dissolving 0.0199 g of pure substances in 100 mL of methanol (99.9%, p.a. purity, Merck, Prague, Czech Republic). Aqueous stock solutions (22 μmol L^{-1}) of 2-NBP and 4-NBP were prepared by dissolving 0.0088 g of pure substances in 2 L of deionized water. UV-Vis spectrophotometric study demonstrated that the methanolic stock solutions are stable for at least one month [36]. More dilute methanolic solutions were prepared by exact dilution of the methanolic stock solutions with methanol.

Boric acid, phosphoric acid, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dodecahydrate, sodium acetate trihydrate (all p.a. purity, Chemapol, Prague, Czech Republic), acetic acid, sodium hydroxide, and lithium hydroxide (all p.a. purity, Lach-Ner, Neratovice, Czech Republic) were used to prepare appropriate buffers. Britton-Robinson (BR) buffers were prepared by titration of a mixture of 0.04 mol L^{-1} boric acid, 0.04 mol L^{-1} phosphoric acid, and 0.04 mol L^{-1} acetic acid with 0.2 mol L^{-1} sodium hydroxide. Acetate buffers (AB, 0.25 mol L^{-1}, pH 4.8, and 0.10 mol L^{-1}, pH 6.0) were prepared from appropriate amounts of sodium acetate trihydrate and acetic acid dissolved in deionized water [37]. Phosphate buffers (PB) were prepared by dissolving 7.59 g of sodium dihydrogen phosphate monohydrate and 2.69 g of disodium hydrogen phosphate dodecahydrate in 250 mL of deionized water (for 0.25 mol L^{-1} PB of pH 6.0) and by dissolving 2.92 g of sodium dihydrogen phosphate monohydrate and 10.33 g of disodium hydrogen phosphate dodecahydrate in 500 mL of deionized water (for 0.10 mol L^{-1} PB of pH 7.0).

Deionized water produced by a Milli-Q Plus system (Millipore, Billerica, MA, USA) was used in all cases. All solutions were stored in glass vessels in the dark at the laboratory temperature.

2.2. Apparatus

Voltammetric measurements were performed with a computer controlled Eco-Tribo Polarograph with Polar Pro 5.1 software for Microsoft Windows operating systems (both Eco-Trend Plus, Prague, Czech Republic). Measurements were carried out in the three-electrode arrangement with the m-AgSAE (Eco-Trend Plus, Prague, Czech Republic) working electrode, a platinum wire auxiliary electrode, and a Ag|AgCl (1.0 mol L^{-1} KCl) reference electrode (both Monokrystaly, Turnov, Czech

Republic). All potentials in this paper are given with respect to the above mentioned reference Ag|AgCl electrode.

For both DCV and DPV, the polarization rate was 20 mV s^{-1}. For DPV, the modulation amplitude was –50 mV, the pulse duration was 100 ms (with current sampling for the last 20 ms), and the pulse period was 150 ms.

The pH measurements were carried out by the pH meter Jenway 4330 (Jenway, Chelmsford, UK) with a combined glass electrode by the same producer. The pH meter was calibrated with standard aqueous calibration buffers of pH 4.00, 7.00, and 10.00 (Sigma-Aldrich, Steinheim, Germany).

2.3. Procedures

2.3.1. Preparation and pretreatment of the working electrode

The m-AgSAE was prepared and pretreated using previously described methods [38]. Unless stated otherwise, an electrochemical regeneration was carried out in the analyzed solution while stirring and bubbling with nitrogen (purity 4.0, Linde, Prague, Czech Republic) before each analysis. It consisted of electrochemical potential jumps between regeneration potentials $E_{reg,1}$ and $E_{reg,2}$, the potential being kept for $E_{reg,1}$ and $E_{reg,2}$ for 0.1 s. The value of the $E_{reg,1}$ was selected 100 mV more negative than the potential of the anodic dissolution of the electrode material, while the $E_{reg,2}$ was selected about 100 mV more positive than the potential of the hydrogen evolution in the given supporting electrolyte (absolute value of the registered current $I \geq 1$ μA).

2.3.2. Measurement procedures

Because of the stability of analyzed solution, the applied volume ratio of the used buffer and methanol was 9:1 for 2-NBP, 7:3 for 4-NBP, and 7:3 for a mixture of studied compounds.

An appropriate volume of the stock solution of the investigated compound in methanol was measured into a 10 mL volumetric flask, an appropriate volume of methanol was added, and the solution was then filled up with the buffer of an appropriate pH and transferred into a voltammetric cell. Oxygen was removed by bubbling with nitrogen (purity class 4.0, Linde, Prague, Czech Republic) for 5 min before each measurement, and a nitrogen atmosphere was then maintained above the solution in the cell. All measurements were carried out at laboratory conditions.

All voltammetric curves were measured three times. The DCV peak height (all the voltammetric peak heights are represented by the same abbreviation I_p) was evaluated from the extrapolated linear portion of the voltammogram before the onset of the peak. The peak heights recorded using DPV and DPAdSV were evaluated from the straight lines connecting the minima before and after the peak. The DPV peak heights of individual components in a mixture of 2-NBP and 4-NBP were evaluated from the straight line connecting the minima before the first and after the second peak. The calibration curves were treated by linear regression. The parameters of the calibration curves (*i.e.*, slope, intercept, coefficient of determination (R^2), and confidence intervals) and other mathematical and statistical parameters (all for the significance level $\alpha = 0.05$) [39] were calculated

using Origin Pro 8.0 software (OriginLab Corporation, Northampton, MA, USA). The limit of quantification (L_Q) was calculated using the equation: $L_Q = 10s/b$, where s is the standard deviation of 10 repetitive determinations at the lowest measurable concentration of the analyte and b is the slope of the calibration curve [40].

2.4. Preparation of model samples of drinking and river water

Drinking water from a public water line in the building of the Faculty of Science of the Charles University in Prague, Czech Republic, or river water from the Vltava river in Prague, Czech Republic (both used without further pretreatment or purification), spiked with an appropriate amount of stock solutions of tested substances, were used for the preparation of model samples. The general procedure to obtain voltammograms was as follows: 9.0 mL of a drinking/river water sample spiked with an appropriate amount of 2-NBP or 4-NBP were filled up to 10.0 mL with the appropriate buffer. Oxygen was removed by bubbling with nitrogen for 5 min, and the corresponding voltammogram was recorded.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of the studied compounds on the m-AgSAE

Initially, the influence of a volume ratio of the BR buffer and methanol (1:1, 7:3, 8:2, and 9:1) was studied using DCV, DPV, and UV-Vis spectrophotometry in solutions containing 0.1 mmol L^{-1} NBP. The optimal ratio of the BR buffer and methanol was found to be 9:1 for 2-NBP and 7:3 for 4-NBP, because of the best repeatability of the peak current probably connected with a lower solubility of 4-NBP. Based on the above mentioned optimization results (4-NBP is less soluble in water than 2-NBP), the volume ratio 7:3 was chosen for a simultaneous determination of the studied compounds.

Table 1. Parameters of measured linear dependences of wave/peak potential of studied analytes on pH*.

Analyte	Method	Slope [mV/pH*]	Intercept [mV]	R^2
2-NBP	DCV	−40.70	−209.11	0.9582
2-NBP	DPV	−40.31	−262.75	0.9566
4-NBP	DCV	−49.79	−139.81	0.9942
4-NBP	DPV	−48.66	−104.65	0.9974

The influence of pH* (a pH value of a mixed buffer – methanol solution) on the voltammetric behavior of the studied compounds at the m-AgSAE was investigated using DCV and DPV in a solution of BR buffer – methanol (9:1 for 2-NBP, 7:3 for 4-NBP). For both voltammetric techniques,

it was found that both 2-NBP and 4-NBP give one well developed peak shifting towards more negative potentials with increasing pH* and 4-NBP gives, moreover, a second (much lower and poorly developed) peak at pH* > 10, appearing as a shoulder on the descending part of the first peak. Parameters of the measured linear dependences of the first peak potential of the studied compounds on the pH* are given in Table 1. The obtained slopes suggest a quasi-reversible nature of the studied redox process corresponding to the four-electron reduction of the present nitro group to corresponding hydroxylamino group [24].

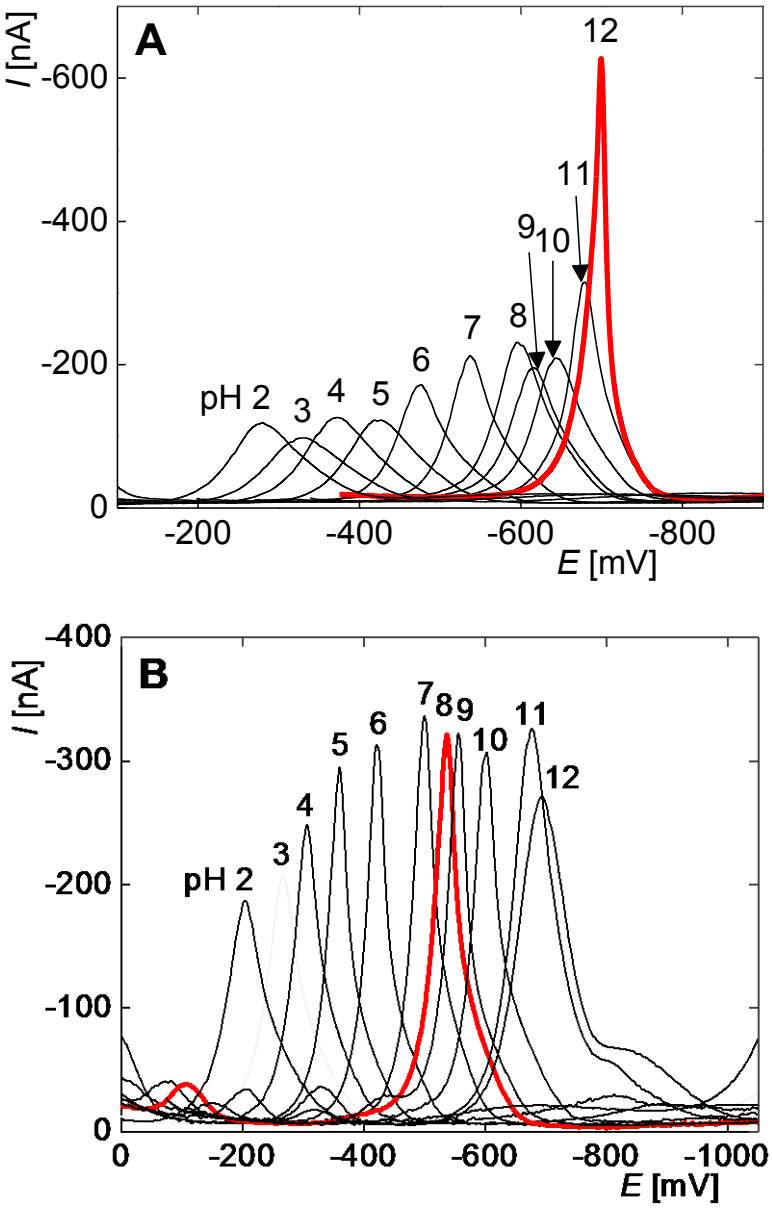

Figure 1. DP voltammograms of 2-NBP in BR buffer – methanol (9:1) (A) and of 4-NBP in BR buffer – methanol (7:3) (B), both c = 0.1 mmol L^{-1}, at a different pH of the BR buffer (pH is given above the voltammograms). Measured at the m-AgSAE, $E_{reg,1}$ and $E_{reg,2}$ were 100 mV more negative than the potential of amalgam dissolution ($E_{reg,1}$) and 100 mV more positive than the potential of hydrogen evolution ($E_{reg,2}$) in the used supporting electrolyte, E vs. Ag|AgCl. The bold line indicates the chosen optimal pH.

The best developed and the most easily evaluated peaks of 2-NBP for both DCV and DPV were obtained in BR buffer of pH 12.0 – methanol (9:1, pH* 12.0), as illustrated in Fig. 1A for DPV. For a simplification, the four-component BR buffer was replaced by the solution of 0.01 mol L^{-1} LiOH (pH 12.0), which gave practically identical results. The optimal medium for the voltammetric determination of 4-NBP was found to be BR buffer of pH 8.0 – methanol (7:3, pH* 8.3) (Fig. 1B). However, it was necessary to choose another buffer because of trace impurities in the BR buffer, which complicated the evaluation of voltammograms. The 0.25 mol L^{-1} AB of pH 4.8 was, therefore, used instead of the BR buffer of pH 8.0. As shown in Fig. 1B, the voltammograms of 4-NBP in the BR buffer of pH 8.0 and 5.0 are comparable.

The optimal conditions for the determination of 2-NBP were as follows: 0.01 mol L^{-1} LiOH (pH 12.0) – methanol (9:1, pH* 12.0), with the regeneration potentials $E_{reg,1}$ = −500 mV and $E_{reg,2}$ = −1700 mV (*RSD* = 1.5%), was used for DCV; the same supporting electrolyte, with the regeneration potentials $E_{reg,1}$ = 0 mV and $E_{reg,2}$ = −1800 mV, was used for DPV (*RSD* = 1.2%). For 4-NBP, the optimal conditions were: 0.25 mol L^{-1} AB of pH 4.8 – methanol (7:3, pH* 5.2), without the regeneration (*RSD* = 2.8%), was used for DCV; the same supporting electrolyte, with the regeneration potentials $E_{reg,1}$ = 0 mV and $E_{reg,2}$ = −1300 mV (*RSD* = 2.1%), was used for DPV. The repeatability of the determinations (expressed in terms of the relative standard deviation (*RSD*) of the peak current values) was evaluated from twenty subsequent voltammetric measurements at the concentration of both analytes of 0.1 mmol L^{-1}.

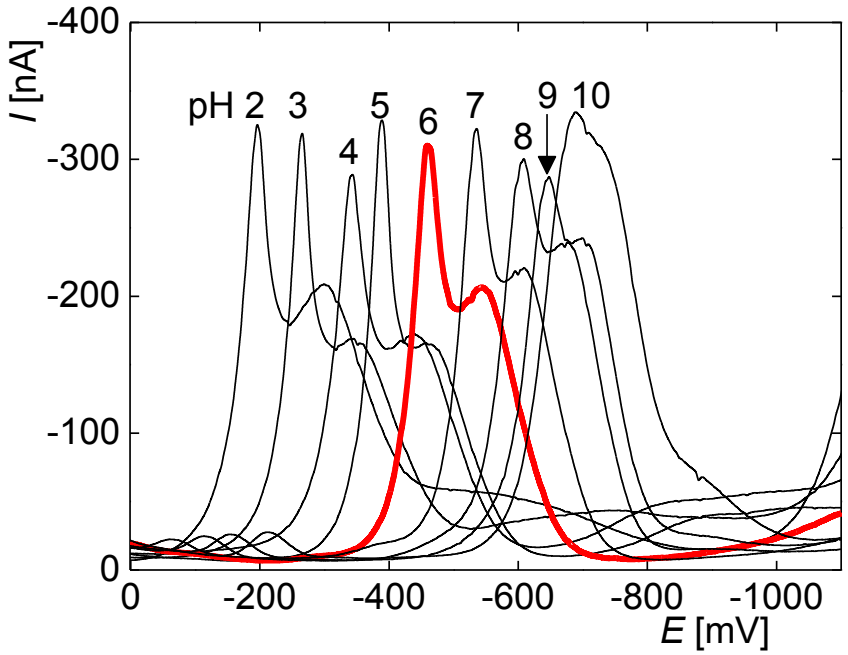

Figure 2. DP voltammograms of the mixture of 2-NBP (the second peak) and 4-NBP (the first peak), both c = 0.1 mmol L^{-1} in BR buffer – methanol (7:3), at a different pH of the BR buffer (pH is given above the voltammograms). Measured at the m-AgSAE, $E_{reg,1}$ and $E_{reg,2}$ were 100 mV more negative than the potential of amalgam dissolution ($E_{reg,1}$) and 100 mV more positive than the potential of hydrogen evolution ($E_{reg,2}$) in the used supporting electrolyte, E vs. Ag|AgCl. The bold line indicates the chosen optimal pH.

Dependences of the DPV peak heights of the studied compounds in their mixture on the pH* and of the potential difference between the DPV peaks of 2-NBP and 4-NBP (ΔE_p) on the pH* were investigated in BR buffer (pH 2.1 – 10.0) – methanol (7:3) media (see Fig. 2 and 3). For the pH higher than 8.0, the difference of the 4-NBP and 2-NBP peak potentials was not sufficient for the simultaneous determination, and for pH 9.0 and 10.0, it was not even possible to evaluate the peaks separately.

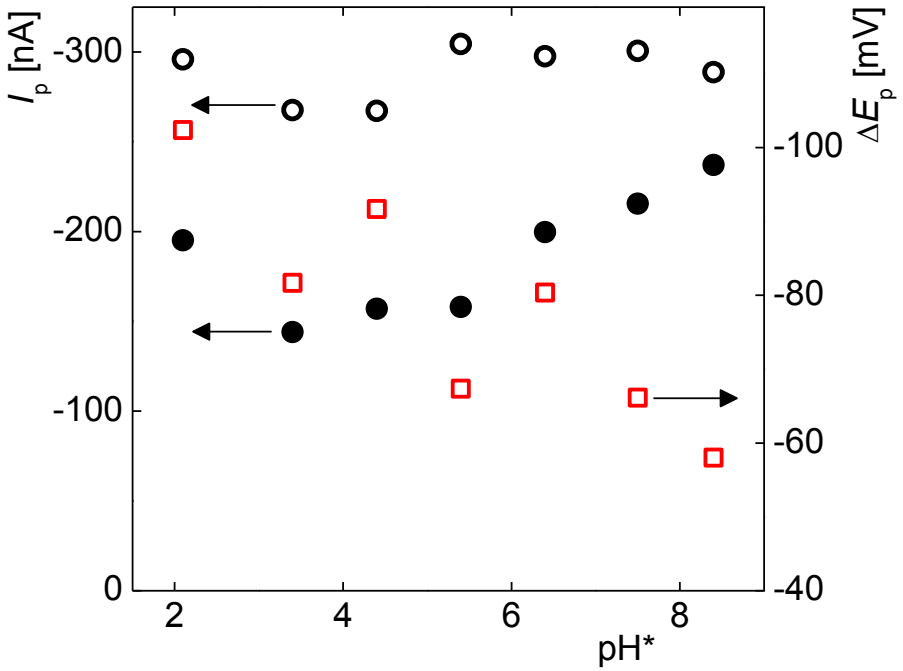

Figure 3. A dependence of the DPV peak current (I_p) of 2-NBP (●) and 4-NBP (○), both c = 0.1 mmol L^{-1} in BR buffer – methanol (7:3), and a dependence of the difference of their peak potentials (ΔE_p, □) on the pH* of the solution. Measured at the m-AgSAE, $E_{reg,1}$ and $E_{reg,2}$ were 100 mV more negative than the potential of amalgam dissolution ($E_{reg,1}$) and 100 mV more positive than the potential of hydrogen evolution ($E_{reg,2}$) in the used supporting electrolyte.

The influence of the composition of a supporting electrolyte on the simultaneous determination of 2-NBP and 4-NBP in their mixture was studied using DPV at the m-AgSAE in a selected buffer of pH 6.0 – methanol (7:3), concentration of both 2-NBP and 4-NBP was 0.1 mmol L^{-1}. Tested buffers (all of pH 6.0) were: 0.04 mol L^{-1} BR buffer, 0.25 mol L^{-1} PB and 0.10 mol L^{-1} AB. Series of measurements were made for each buffer, and the influence of an electrochemical regeneration on the repeatability of the determination was investigated for five measurements. Better results for all tested buffers were observed using DPV without the regeneration. When the BR buffer used, the repeatability of I_p (2-NBP) and I_p (4-NBP) was 0.7 and 3.9%, respectively, and ΔE_p = −73 mV. When the PB used, the repeatability of I_p (2-NBP) and I_p (4-NBP) was 2.2 and 11.6%, respectively, and ΔE_p = −79 mV. And finally, when the AB used, the repeatability of I_p (2-NBP) and I_p (4-NBP) was 1.6 and 2.2%, respectively, and ΔE_p = −178 mV. Comparing the repeatability and the separation of the peaks, the 0.10 mol L^{-1} AB of pH 6.0 was chosen as optimal.

For twenty DPV measurements without the regeneration, the *RSD* of the peak height was 0.8% for 2-NBP and 1.9% for 4-NBP. For analogous twenty measurements with the regeneration ($E_{reg,1}$ = 0 mV and $E_{reg,2}$ = −1400 mV), the *RSD* of the peak height was 2.3 and 3.5% for 2-NBP and 4-NBP, respectively. Therefore, it can be concluded that the regeneration of the m-AgSAE is not necessary under these conditions. However, when measured without the regeneration step, some impurities, probably adsorbed on the non-regenerated electrode surface, caused interfering signals observable at voltammograms of the supporting electrolyte at the potentials at which 4-NBP is reduced, which made the evaluation of the peaks of 4-NBP recorded at its lower concentrations significantly difficult. Hence, the electrochemical regeneration of the electrode surface was further used.

Based on the results of optimization, all following measurements of the mixture of 2-NBP and 4-NBP using DPV were carried out in the medium of 0.10 mol L^{-1} AB of pH 6.0 – methanol (7:3), with the regeneration potentials $E_{reg,1}$ = 0 mV and $E_{reg,2}$ = −1400 mV.

3.2. Voltammetric determination of the studied compounds at the m-AgSAE

Table 2. Analytical parameters of the 2-NBP and 4-NBP determination using DCV and DPV at the m-AgSAE, calculated from 3 repeated determinations.

Analyte	Technique	Matrix	Concentration [mol L^{-1}]	Slope [nA mol^{-1} L]	Intercept [nA]	R^2	L_Q [mol L^{-1}]
2-NBP	DCV	0.01 mol L^{-1} LiOH – methanol (9:1)	$(2 – 10) \times 10^{-5}$	$–1.2 \times 10^6$	–16.5	0.9937	-
		0.01 mol L^{-1} LiOH – methanol (9:1)	$(2 – 10) \times 10^{-6}$	$–1.2 \times 10^6$	–0.95	0.9969	-
		0.01 mol L^{-1} LiOH – methanol (9:1)	$(2 – 10) \times 10^{-7}$	$–1.3 \times 10^6$	–0.87	0.8870	2×10^{-7}
	DPV	0.01 mol L^{-1} LiOH – methanol (9:1)	$(2 – 10) \times 10^{-5}$	$–1.5 \times 10^6$	–2.85	0.9995	-
		0.01 mol L^{-1} LiOH – methanol (9:1)	$(2 – 10) \times 10^{-6}$	$–1.5 \times 10^6$	–0.23	0.9998	-
		0.01 mol L^{-1} LiOH – methanol (9:1)	$(2 – 10) \times 10^{-7}$	$–1.7 \times 10^6$	–0.07	0.9994	1×10^{-7}
	DCV	Spiked DW – 0.01 mol L^{-1} LiOH (9:1)	$(2 – 10) \times 10^{-7}$	$–1.7 \times 10^6$	–0.50	0.9916	5×10^{-7}
	DPV	Spiked DW – 0.01 mol L^{-1} LiOH (9:1)	$(2 – 10) \times 10^{-7}$	$–4.7 \times 10^6$	–0.25	0.9915	1×10^{-7}
	DCV	Spiked RW – 0.01 mol L^{-1} LiOH (9:1)	$(2 – 10) \times 10^{-7}$	$–2.2 \times 10^6$	–0.39	0.9970	4×10^{-7}
	DPV	Spiked RW – 0.01 mol L^{-1} LiOH (9:1)	$(2 – 10) \times 10^{-7}$	$–2.4 \times 10^6$	–0.06	0.9977	1×10^{-7}
4-NBP	DCV	Acetate buffer pH 4.8 – methanol (7:3)	$(2 – 10) \times 10^{-5}$	$–4.5 \times 10^6$	37.4	0.9945	-
		Acetate buffer pH 4.8 – methanol (7:3)	$(2 – 10) \times 10^{-6}$	$–2.7 \times 10^6$	–0.22	0.9736	-
		Acetate buffer pH 4.8 – methanol (7:3)	$(2 – 10) \times 10^{-7}$	$–5.0 \times 10^6$	0.33	0.9893	2×10^{-7}
	DPV	Acetate buffer pH 4.8 – methanol (7:3)	$(2 – 10) \times 10^{-5}$	$–6.1 \times 10^6$	64.5	0.9794	-
		Acetate buffer pH 4.8 – methanol (7:3)	$(2 – 10) \times 10^{-6}$	$–3.6 \times 10^6$	0.02	0.9970	-
		Acetate buffer pH 4.8 – methanol (7:3)	$(2 – 10) \times 10^{-7}$	$–5.1 \times 10^6$	0.99	0.9606	2×10^{-7}
	DCV	Spiked DW – acetate buffer pH 4.8 (9:1)	$(2 – 10) \times 10^{-7}$	$–6.0 \times 10^6$	0.13	0.9983	2×10^{-7}
	DPV	Spiked DW – acetate buffer pH 4.8 (9:1)	$(2 – 10) \times 10^{-7}$	$–6.2 \times 10^6$	–0.48	0.9990	2×10^{-7}
	DCV	Spiked RW – acetate buffer pH 4.8 (9:1)	$(2 – 10) \times 10^{-7}$	$–3.2 \times 10^6$	0.25	0.9943	1×10^{-7}
	DPV	Spiked RW – acetate buffer pH 4.8 (9:1)	$(2 – 10) \times 10^{-7}$	$–2.9 \times 10^6$	–0.31	0.9866	2×10^{-7}

DW – drinking water, RW – river water.

Under the optimal conditions found (see Section 3.1.), the calibration curves were measured using DCV and DPV, and for both studied compounds, the linear dynamic range was 0.2 – 100 μmol L^{-1}. Parameters of the calibration straight lines are summarized in Table 2. Representative DP voltammograms of 2-NBP and DC voltammograms of 4-NBP, corresponding to the lowest attainable concentration range, are depicted in Fig. 4.

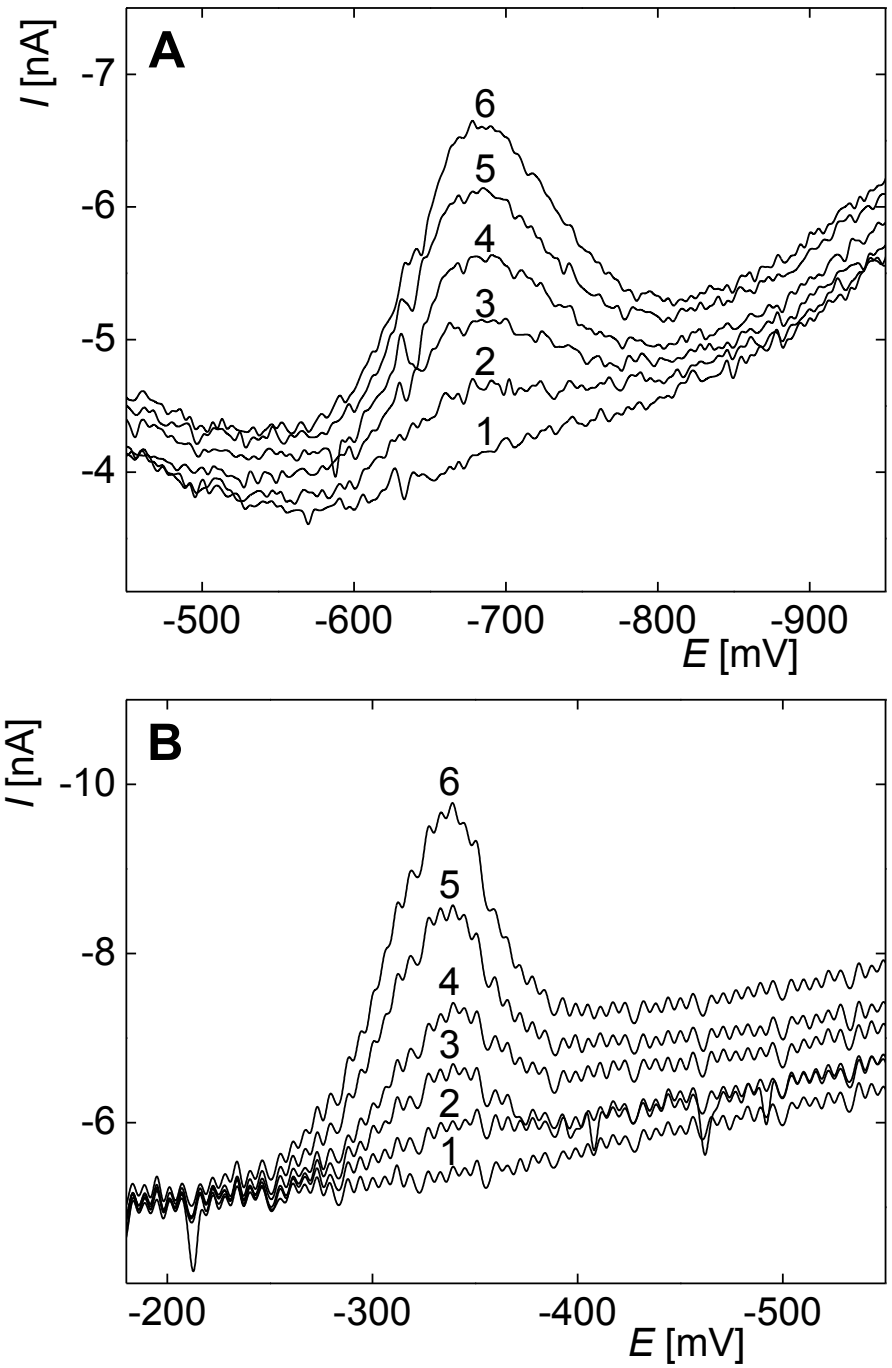

Figure 4. DP voltammograms of 2-NBP in 0.01 mol L^{-1} LiOH (pH 12.0) – methanol (9:1) (A), $E_{reg,1}$ = 0 mV and $E_{reg,2}$ = −1800 mV, and DC voltammograms of 4-NBP in 0.25 mol L^{-1} AB of pH 4.8 – methanol (7:3) (B), as a function of the concentration of the analyte c = 0 (1), 0.2 (2), 0.4 (3), 0.6 (4), 0.8 (5), and 1.0 (6) μmol L^{-1}. Measured at the m-AgSAE, E vs. Ag|AgCl.

In an attempt to increase the sensitivity of the determination of the studied compounds, their adsorptive accumulation at the m-AgSAE was tested. The above mentioned optimal conditions for each substance for DCV and DPV were used. Moreover, the media representing other pH values (0.10 mol L^{-1} PB of pH 7.0, BR buffers of pH 2.1, 4.0, 7.0, 9.0, 11.0, and 12.0) were also tested to cover the whole pH range. Different accumulation potentials (E_p + 0, E_p + 100, E_p + 200, and E_p + 300 mV) were tested with the accumulation time of 0 – 5 min. Because methanol can adsorb on the electrode surface, it was not contained in the supporting electrolyte and in the stock solution of studied compounds. However, under the examined conditions, the effect of the adsorption on the m-AgSAE surface was not sufficient to significantly increase the voltammetric response of NBPs.

3.3. DCV and DPV determination of the studied compounds in drinking and river water

The newly developed methods were applied on the model samples of drinking and river water using the procedure outlined in Section 2.4. Measurements were carried out using the volume ratio of water sample − appropriate buffer of 9:1. Supporting electrolytes 0.01 mol L^{-1} LiOH (pH 12.0) and 0.25 mol L^{-1} AB of pH 4.8 were used for the determination of 2-NBP and 4-NBP, respectively.

In the concentration orders of 10^{-6} and 10^{-7} mol L^{-1}, the calibration curves are linear. The L_Q of 2-NBP and 4-NBP achieved in drinking and river water are summarized in Table 2. DPV gave lower L_Q for both drinking and river water (around 0.1 μmol L^{-1}).

3.4. Simultaneous determination of 2-NBP and 4-NBP

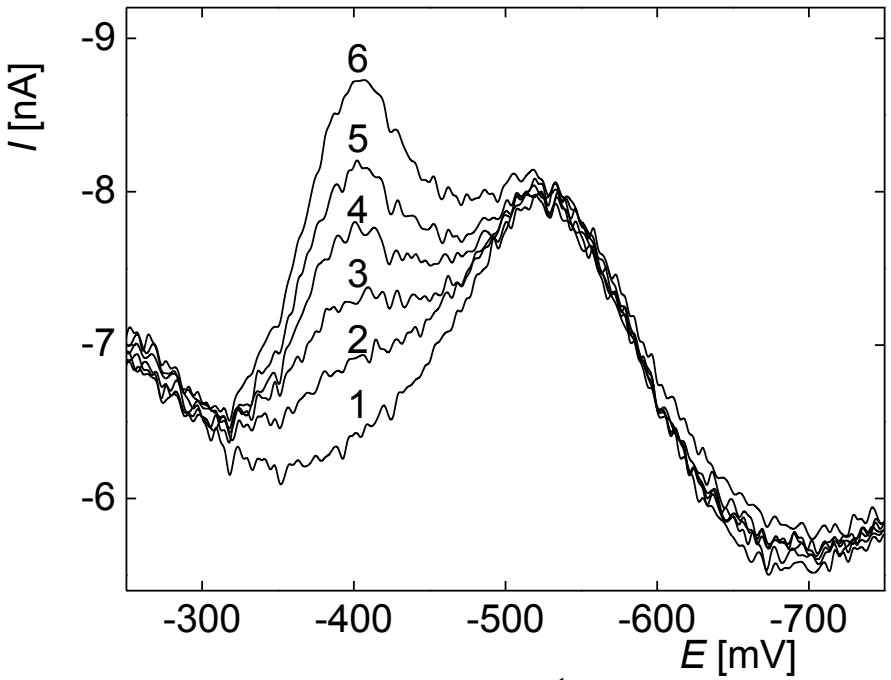

Figure 5. DP voltammograms of 2-NBP (c = 1.0 μmol L^{-1}) and 4-NBP (c = 0 (1), 0.2 (2), 0.4 (3), 0.6 (4), 0.8 (5), and 1.0 (6) μmol L^{-1}) in 0.10 mol L^{-1} AB of pH 6.0 – methanol (7:3). Measured at the m-AgSAE, $E_{reg,1}$ = 0 mV, $E_{reg,2}$ = −1400 mV, E vs. Ag|AgCl.

DPV at the m-AgSAE was applied for the simultaneous determination of 2-NBP and 4-NBP in their mixture under optimized conditions: the medium of 0.10 mol L^{-1} AB of pH 6.0 – methanol (7:3), with the regeneration potentials $E_{reg,1}$ = 0 mV and $E_{reg,2}$ = −1400 mV. Voltammograms of the mixture of 2-NBP and 4-NBP in the concentration range 0.2 – 1.0 μmol L^{-1} were measured when concentration of 4-NBP was changed, whereas the concentration of 2-NBP was constant, and vice versa. DP voltammograms of 2-NBP (c = 1.0 μmol L^{-1}) and 4-NBP (c = 0 – 1.0 μmol L^{-1}) obtained under the above mentioned optimal conditions are given for the sake of illustration in Fig. 5. A representative graph of dependences of the peak current of 4-NBP on its concentration, when the concentration of 2-NBP is changing in the micromolar concentration range, is depicted in Fig. 6.

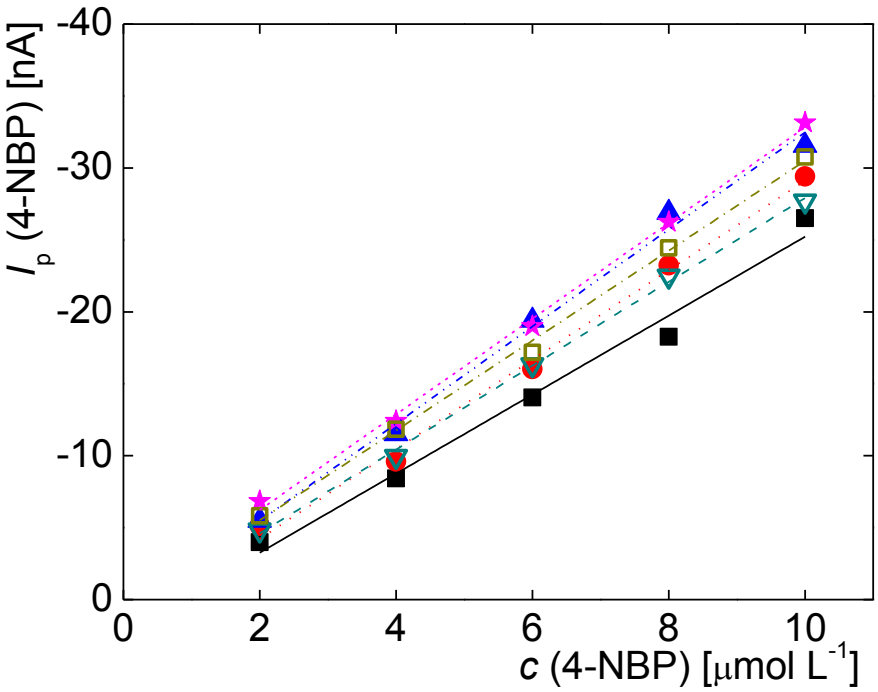

Figure 6. A dependence of the peak current of 4-NBP (I_p (4-NBP)) on its concentration (c (4-NBP)) when the changing concentration of 2-NBP was: 0 (■), 2.0 (●), 4.0 (▲), 6.0 (▽), 8.0 (★), and 10.0 (□) μmol L^{-1}. Measured at the m-AgSAE using DPV in 0.10 mol L^{-1} AB of pH 6.0 – methanol (7:3), $E_{reg,1}$ = 0 mV, $E_{reg,2}$ = −1400 mV.

The evaluation of the peak currents in the concentration order of 10^{-7} mol L^{-1} is limited by the L_Q of both studied substances. Both the dependence of the peak current of 4-NBP on its concentration at a constant concentration of 2-NBP and the dependence of the peak current of 2-NBP on its concentrations at a constant concentration of 4-NBP were linear. It can be seen that the influence of the second NBP on the slope of the calibration curve of the first NBP is not too large. Therefore, the analysis of the mixture of 2-NBP and 4-NBP by the standard addition method is possible.

4. CONCLUSIONS

New voltammetric methods for the determination of two hazardous environmental pollutants – 2-NBP and 4-NBP – in simple environmental matrices were developed and described in this paper. In

comparison with other polarographic and voltammetric methods for the determination of these compounds at mercury electrodes, the voltammetric determination at the m-AgSAE using DCV and DPV provides similar or higher L_Qs (see Table 3).

Table 3. A comparison of the limits of quantification (L_Q) of 2-NBP and 4-NBP of the newly developed methods and of other polarographic and voltammetric methods.

Analyte	Method*	L_Q [mol L^{-1}]	Reference
2-NBP	DCV at m-AgSAE	2.0×10^{-7} [a]	this work
	DPV at m-AgSAE	1.0×10^{-7} [a]	this work
	DCTP at SMDE	2.9×10^{-7} [b]	[25]
	DPP at SMDE	2.4×10^{-7} [b]	[25]
	DPV at HMDE	3.1×10^{-8} [b]	[28]
	DPAdSV at HMDE	2.9×10^{-9} [b]	[28]
4-NBP	DCV at m-AgSAE	2.0×10^{-7} [a]	this work
	DPV at m-AgSAE	2.0×10^{-7} [a]	this work
	DCTP at SMDE	2.8×10^{-7} [b]	[25]
	DPP at SMDE	3.3×10^{-8} [b]	[25]
	LSAdSV at HMDE	2.1×10^{-9} [c]	[26]
	FSDPAdSV at HMDE	2.5×10^{-9} [c]	[26]
	DPV at HMDE	2.5×10^{-8} [b]	[28]
	DPAdSV at HMDE	3.3×10^{-9} [b]	[28]
	SWAdSV at HMDE	6.0×10^{-10} [d]	[41]
	DPAdSV at HMDE	4.0×10^{-10} [d]	[41]
	DCV at RGCDE	3.3×10^{-5} [e]	[27]
	DPV at RGCDE	3.0×10^{-6} [e]	[27]

* DCTP – direct current tast polarography, DCV – direct current voltammetry, DPAdSV – differential pulse adsorptive stripping voltammetry, DPP – differential pulse polarography, DPV – differential pulse voltammetry, FSDPAdSV – fast scan differential pulse adsorptive stripping voltammetry, HMDE – hanging mercury drop electrode, LSAdSV – linear scan adsorptive stripping voltammetry, m-AgSAE – mercury meniscus modified silver solid amalgam electrode, RGCDE – rotating glassy carbon disc electrode, SMDE – static mercury drop electrode, SWAdSV – square wave adsorptive stripping voltammetry, [a] $L_Q = 10s/b$, where s is the standard deviation of 10 repetitive determinations at the lowest measurable concentration of the analyte and b is the slope of the calibration curve, [b] L_Q was calculated using Adstat 2.0 software according to Ebel [42], [c] L_Q was calculated as 10 times the standard deviation of 10 determinations of the analyte at a concentration of 2×10^{-x} mol L^{-1}, [d] L_Q corresponding to 10σ (σ being the relative standard deviation of the background noise), [e] L_Q was calculated as 3.3 times limit of detection (calculated according to Skogerboe and Grant [43]).

Nevertheless, the m-AgSAE is more user-friendly, compatible with the concept of "green analytical chemistry", and very robust and thus better compatible with field measurements. In comparison with a rotating glassy carbon disc electrode (RGCDE), the m-AgSAE provides substantially lower L_Q and thus should be preferred. For 4-NBP, the L_Qs attained at the m-AgSAE are also comparable with those attained using high performance liquid chromatography with UV

spectrophotometric (4.0×10^{-7} mol L^{-1}), electrochemical (4.0×10^{-6} mol L^{-1}), and fluorescent detection (2.0×10^{-7} mol L^{-1}) [22]. Moreover, DPV at the m-AgSAE is a suitable tool for the simultaneous determination of 2-NBP and 4-NBP in the concentration orders of 10^{-6} and 10^{-7} mol L^{-1}, as it was shown in this paper, too.

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