

Study of Voltammetric Determination of Carcinogenic 1-Nitropyrene and 1-Aminopyrene Using a Glassy Carbon Paste Electrode

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Abstract: Linear scan voltammetry (LSV), differential pulse voltammetry (DPV) and voltammetry with adsorptive accumulation (AAV) at a glassy carbon paste electrode (GCPE) based on mixing glassy carbon spherical microparticles with an organic pasting liquid were used for the determination of trace amounts of carcinogenic 1-nitropyrene (1-NP) (the limit of determination around $2 \cdot 10^{-5}$ mol L⁻¹ for LSV and $2 \cdot 10^{-6}$ mol L⁻¹ for DPV) and 1-aminopyrene (1-AP) (the limit of determination around $3 \cdot 10^{-6}$ mol L⁻¹ for LSV, $1 \cdot 10^{-6}$ mol L⁻¹ for DPV and $1 \cdot 10^{-7}$ mol L⁻¹ for AAV) using a Britton-Robinson buffer – methanol mixture as a base electrolyte. The main advantage of this new type of electrode is its full compatibility with media containing a high amount of organic solvent (methanol, acetonitrile)

Keywords: Glassy carbon paste electrode, linear scan voltammetry, differential pulse voltammetry, voltammetry with adsorptive accumulation, 1-nitropyrene, 1-aminopyrene.

Introduction

1-nitropyrene is one of the most abundant representatives of nitroaromatic compounds in the environment which has also been detected in automobile exhaust, urban air, incinerators exhalations and in certain food items such as grilled meats or teas (see reviews [1,2,3]). It is listed in the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans [4]. 1-Aminopyrene was found to be 1-nitropyrene's most frequent cell metabolite [5]. Therefore, there is an ever-increasing demand for the determination of trace amounts of these substances. So far, mostly chromatographic methods such as GC-MS or HPLC with fluorimetric detection are used for these purposes [3]. However, these methods are characterized by high investment and running costs. Modern electroanalytical methods, especially differential pulse voltammetry (DPV) and adsorptive stripping voltammetry (AdSV) at mercury [6] or carbon paste [7] electrodes satisfy even the highest demands on sensitivity required for the determination of extremely dangerous chemical carcinogens. Their main advantage being much lower investment and running costs.

Carbon paste electrodes earned wide use as working electrodes for voltammetric techniques [7,8]. A new carbon paste electrode material combining the electrochemical properties of glassy carbon with the attractive advantages of composite paste electrode materials based on mixing glassy carbon microparticles, is originally employed for FIA (flow-injection analysis) as a disposable and renewable electrode system [9] with an oil binder and was introduced recently [10,11]. The resulting glassy carbon paste electrode (GCPE) combines the electrochemical properties of glassy carbon with the various advantages of composite electrodes. Glassy carbon pastes offer high electrochemical reactivity, a wide accessible potential window, a low background current, and are inexpensive, easy to prepare, modify, and renew. The new material has a lower double-layer capacitance compared to conventional carbon pastes [11]. Scanning electron microscopy (SEM) images indicated significant differences in the structure of the spherical glassy carbon paste and classical graphite carbon paste electrodes [10,11]. Glassy carbon electrodes offer attractive electrochemical reactivity, negligible porosity, and good mechanical rigidity, while carbon paste electrodes are popular due to their low background current, simplicity of modification, renewal, and miniaturization. Girault [12] proposed a composite based on glassy carbon microparticles and a polystyrene polymer which exhibits electrochemical properties dependent on the carbon/polymer ratio and ranging from insulating films through isolated and closely spaced random array microelectrodes to microporous macro-electrode. Rivas [13] used a glassy carbon paste electrode modified with polyphenol oxidase for the determination of dopamine, acetaminophen and polyphenols. Quite recently, two types of glassy carbon powder (i.e., Sigradur K and Sigradur G) have been used to obtain a glassy carbon paste electrode which was then modified with Prussian Blue [14].

The main drawback of classical carbon paste electrodes is the fact that they can not be used in a medium with high content of organic solvents (such as methanol or acetonitrile), which dissolve the organic pasting liquid thus, making the paste friable. However, we have found [15] that this new type

of GCPE is much more resistant towards organic solvents than classical carbon pastes. The applicability of GCPE for the determination of trace amounts of 1-NP and 1-AP in mixed water-methanol medium is demonstrated in this paper. The possibility to use GCPE for both oxidation and reduction of organic substances is another advantage of this type of electrode. It enables to use a single electrode for the determination of both 1-NP and its reduction product 1-AP.

Experimental

Reagents

1-nitropyrene (97%, C.A.S. Name: Pyrene,1-nitro-, C.A.S. Reg.number: [5522-43-0], abbreviation: 1-NP) and 1-aminopyrene (99%, C.A.S. Name: 1-Pyrenamine, C.A.S. Reg.number: [1606-67-3], abbreviation: 1-AP) were obtained from Sigma-Aldrich. $1 \cdot 10^{-3}$ mol.L⁻¹ methanolic stock solutions of the analytes were kept refrigerated in dark. More diluted solutions were prepared by exact dilution of the stock solution with methanol. Mineral oil (Fluka) and spherical glassy carbon microparticles powder 0.4-12 μ m type 2 (Alfa Aesar, Ward Hill, MA, USA) were mixed in 20:80 mass ratio for the preparation of the glassy carbon paste electrode. Sodium hydroxide, glacial acetic acid, phosphoric acid, and methanol (all p.a. purity) were obtained from Lachema (Brno, Czech Republic). Solutions of Britton-Robinson buffers were prepared in 0.2 mol. L⁻¹ concentration. Deionized water (Millipore Q-plus System, Millipore, USA) was used for all experiments.

Apparatus

A polarographic analyzer EcoTriboPolarograph controlled by PolarPro v. 2.0 software (both PolaroSensors, Prague, Czech Republic) was used. A three-electrode arrangement was used with a saturated calomel reference and a platinum foil auxiliary electrode. A scan rate of 10 mV/s was used for all experiments. The pulse height was 50 mV for DPV experiments. A lab-made working glassy carbon paste electrode was used with a 2 mm inner diameter Teflon tube holder of the carbon paste. The contact was established via a steel screw dipped by its end into the carbon paste. The GCPE was stored after preparation and experiments for several days in a 50 mL beaker under a thin layer of water. The electrode was polished prior to use by a wetted filtration paper. A prebubbler containing a methanolic solution of the same water: methanol ratio as the analyzed solution was placed prior the voltammetric cell. pH of the solutions was measured by a Conductivity and pH meter 4330 (Jenway, Great Britain) with a combined glass electrode. All experiments were carried out under laboratory temperature.

Procedures

The general procedure to obtain corresponding voltammograms was as follows: A required amount of the stock solution of the test substance in methanol was placed in a 10 ml volumetric flask, an appropriate volume of methanol was added and the system was diluted to volume with a Britton-Robinson buffer of the required pH. (A different order of mixing the solutions resulted in a precipitation of the test substance.) The solution was transferred into a voltammetric vessel, oxygen was removed from the measured solution by bubbling with nitrogen for five minutes and the voltammogram was recorded. Calibration curves were measured in triplicate and their statistical parameters (e.g., slope, intercept, limit of decision, limit of detection, limit of determination L_D) were calculated from the confidence bands ($\alpha=0.05$) according to Oppenheimer [16], Schwartz [17], and Ebel [18] using statistic software Adstat, version. 2.0 (TriloByte, Czech Republic).

Results and Discussion

Linear scan voltammetry of 1-aminopyrene

The influence of pH on the LS voltammetric behavior of 1.10^{-4} mol.L⁻¹ 1-AP at GCPE in mixtures of Britton-Robinson buffer : methanol (9:1 v/v) was investigated for a pH range of the buffer 2-13. It can be seen from Fig. 1 that 1-AP provides one well-resolved irreversible anodic peak. Its position shifts towards negative potentials with increasing pH and its height is not significantly influenced by the change of pH. In the region of higher potentials, another smaller peak can be observed, which can not be evaluated. For pH 2-6, a third anodic peak can be found.

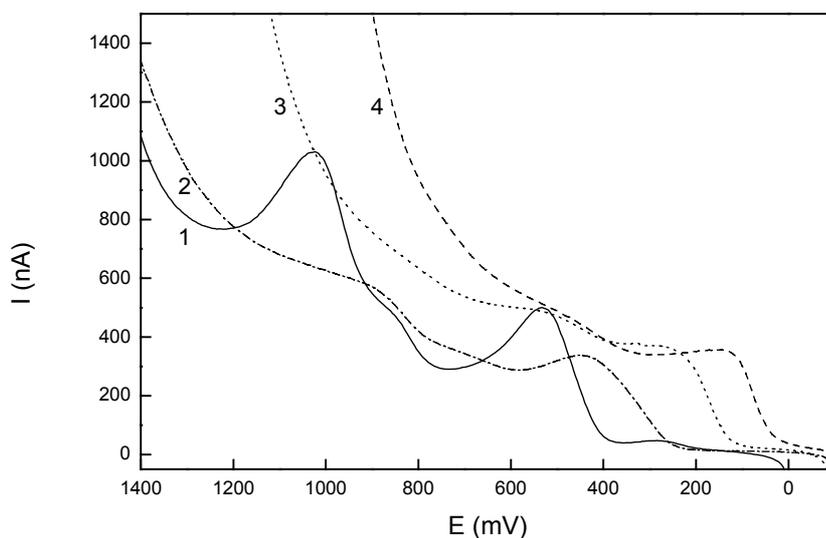


Figure 1. Selected LS voltammograms of 1-AP ($c=1.10^{-4}$ mol.L⁻¹) at GCPE in mixture of BR buffer : MeOH (9:1) at buffer pH 2 (1), 6 (2), 10 (3), 12 (4).

Best-developed and most easily evaluated LS voltammograms were obtained for BR buffer pH 2 : methanol (9:1 v/v), where a peak-shaped signal was obtained. Calibration curves were measured under these conditions in the concentration ranges of $(2-10) \cdot 10^{-5} \text{ mol.L}^{-1}$ and $(2-10) \cdot 10^{-6} \text{ mol.L}^{-1}$. The first peak was used for a quantitative evaluation (its height was measured from the straight line parallel with the potential axis constructed as a prolongation of the linear part of the LS voltammogram in the region 200-400 mV, i.e. before the onset of the peak.) Parameters of the calibration curves are summarized in Table 1.

Table 1. Calibration data for voltammetric determination of 1-AP at GCPE in a BR buffer : MeOH mixture.

c (mol.L ⁻¹)	Slope (mA.mol ⁻¹ .L)	Intercept (nA)	R	L ₀ (mol.L ⁻¹)
LSV, BR buffer pH 2: MeOH (9:1 v/v)				
$(2-10) \cdot 10^{-5}$	6.86	80.0	0.9942	--
$(2-10) \cdot 10^{-6}$	7.91	6.2	0.9994	$3 \cdot 10^{-6}$
DPV, BR buffer pH 2: MeOH (9:1 v/v)				
$(2-10) \cdot 10^{-5}$	6.33	43.01	0.9963	--
$(2-10) \cdot 10^{-6}$	9.98	-0.85	0.9958	$1 \cdot 10^{-6}$
AAV, BR buffer pH 2: MeOH (9:1 v/v), t _{acc} = 180 s				
$(2-10) \cdot 10^{-7}$	20.5	0.3	0.9967	$1 \cdot 10^{-7}$

Differential pulse voltammetry of 1-aminopyrene

The influence of pH on the DP voltammetric behavior of $1 \cdot 10^{-4} \text{ mol.L}^{-1}$ 1-AP at GCPE in mixtures of Britton-Robinson buffer : methanol (9:1 v/v) was again investigated for a buffer pH range 2-13 (Fig 2).

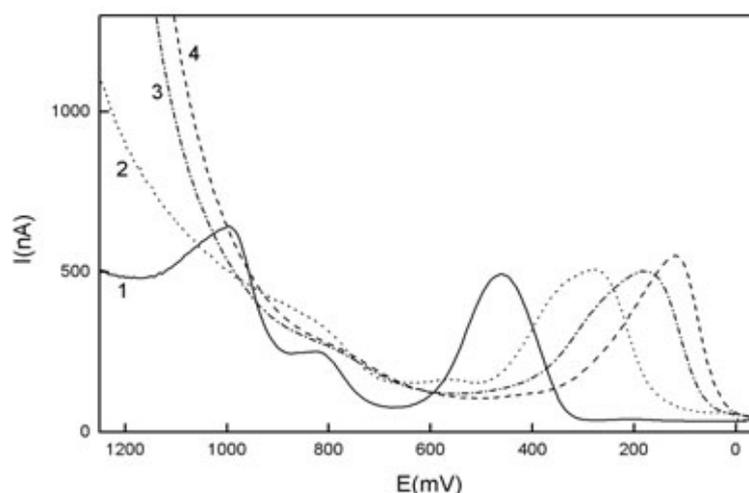


Figure 2. Selected DP voltammograms of 1-AP ($c=1 \cdot 10^{-4} \text{ mol.L}^{-1}$) at GCPE in a mixture of BR buffer : MeOH (9:1) at buffer pH 2(1), 6 (2), (3) 10, 12 (4).

It can be seen from Fig. 2 that 1-AP gives one well-developed irreversible anodic peak, which shifts towards positive potentials with decreasing pH, its height being practically independent of pH. For pH 2-6, there is a second peak, followed by a third peak that is observable only in the region of pH 2-4 (see Fig. 2.).

Best-developed and most easily evaluated DP voltammograms were obtained for a BR buffer, pH 2 : methanol (9:1 v/v). Calibration curves were measured under these conditions in the concentration ranges of $(2-10) \cdot 10^{-5} \text{ mol.L}^{-1}$ and $(2-10) \cdot 10^{-6} \text{ mol.L}^{-1}$. The first peak was used for the quantitative evaluation. Parameters of the calibration curves are also summarized in Table 1.

Voltammetry of 1-aminopyrene with adsorptive accumulation

Based on the above described DPV experiments, a Britton-Robinson buffer pH 2: methanol (9:1 v/v) mixture was selected for first measurements of 1-AP by voltammetry with adsorptive accumulation at GCPE. Under these conditions, 1-AP gave a well-developed anodic peak observed at $E_p = 430 \text{ mV}$. First, the influence of the accumulation potential E_{acc} on the peak current I_p of $2 \cdot 10^{-7} \text{ mol.L}^{-1}$ 1-AP was studied for accumulation potentials from -200 to +200 mV in a BR buffer pH 2:methanol (9:1 v/v) mixture using accumulation time $t_{acc} = 60 \text{ s}$ in a stirred solution. $E_{acc} = 200 \text{ mV}$ was found optimal. Further, the influence of t_{acc} for the voltammetric determination of $2 \cdot 10^{-7} \text{ mol.L}^{-1}$ 1-AP with adsorptive accumulation was studied for $E_{acc} = 200 \text{ mV}$ and $t_{acc} = 0, 30, 60, 120, 180$ and 300 s in Britton-Robinson buffer pH 2 : methanol (9:1 v/v) (see Fig. 3).

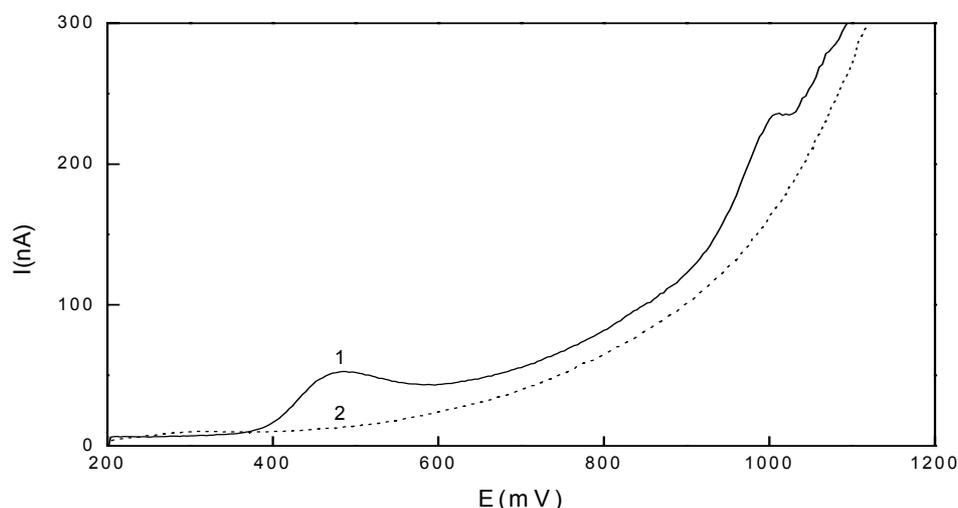


Figure 3. Influence of the t_{acc} for AAV of $2 \cdot 10^{-7} \text{ mol.L}^{-1}$ 1-AP in stirred solution of a BR buffer pH 2 : MeOH (9:1 v/v), $E_{acc} = 200 \text{ mV}$.

For the sake of illustration, voltammogram of $2 \cdot 10^{-7} \text{ mol.L}^{-1}$ 1-AP with adsorptive accumulation is depicted in Fig. 4. To achieve higher sensitivity, 180 s accumulation time was selected for the measuring calibration curves. The peaks were well developed and reproducible under these conditions.

A further increase in the accumulation time did not enhance the signal. Calibration curves were measured in the range of $(2-10) \cdot 10^{-7} \text{ mol.L}^{-1}$ (see also Table 1).

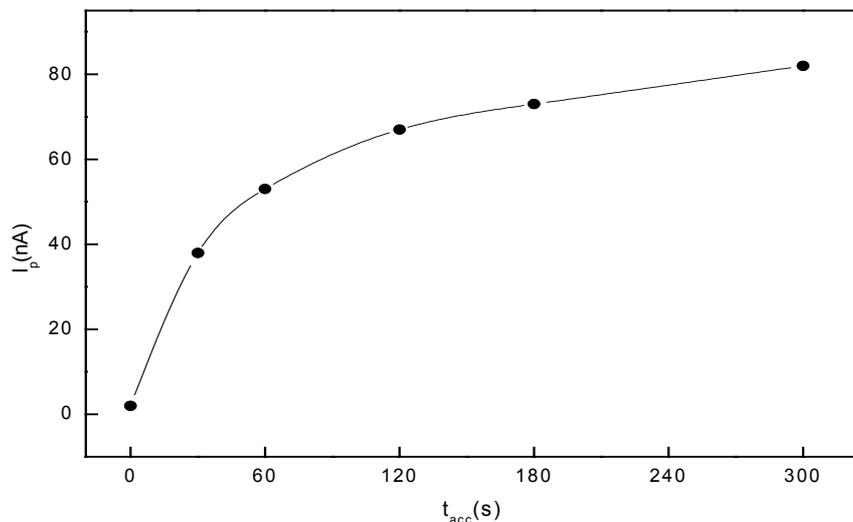


Figure 4. Voltammogram of $2 \cdot 10^{-7} \text{ mol.L}^{-1}$ 1-AP with adsorptive accumulation in stirred solution of a BR buffer pH 2 : MeOH (9:1 v/v) (1) and of the supporting electrolyte (2), $E_{acc} = 200 \text{ mV}$, $t_{acc} = 60 \text{ s}$.

Linear sweep voltammetry of 1-nitropyrene

The influence of pH on the LS voltammetric behavior of $1 \cdot 10^{-4} \text{ mol.L}^{-1}$ 1-NP at GCPE in mixtures of Britton-Robinson buffer pH 2-13 : MeOH (1:9 v/v) was investigated (see Fig. 5).

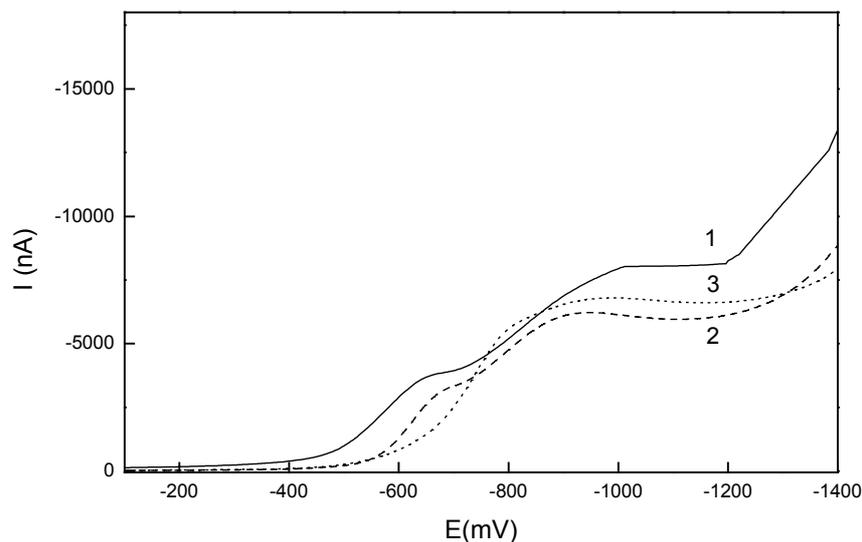


Figure 5. Selected LS voltammograms of 1-NP ($c = 1 \cdot 10^{-4} \text{ mol.L}^{-1}$) at GCPE in mixture of a BR buffer : MeOH (1:9 v/v) with buffer pH 3 (1), 5 (2) and 8 (3).

Precipitation of the analyte from the solution was observed for solutions with lower content of methanol. It was found that 1-NP gives two well-developed waves. Their half-wave potential $E_{1/2}$ shifts towards negative potentials with increasing pH and the dependence of their limiting current on pH is not too pronounced. At higher pH, the two waves merge. Best-developed voltammograms were obtained for a BR buffer pH 3 : methanol (1:9 v/v) mixture. Calibration curves were measured under these conditions in the concentration range of $(2-10) \cdot 10^{-5} \text{ mol.L}^{-1}$ and their parameters are summarized in Table 2.

Table 2. Calibration data for voltammetric determination of 1-NP at GCPE.

c (mol.L ⁻¹)	Slope (mA.mol ⁻¹ .L)	Intercept (nA)	R	L _Q (mol.L ⁻¹)
LSV, BR buffer pH 3: MeOH (1:9 v/v)				
$(2-10) \cdot 10^{-5}$	26.2	267.8	0.9924	$2 \cdot 10^{-5}$
DPV, BR buffer pH 3: MeOH (1:9 v/v)				
$(2-10) \cdot 10^{-5}$	9.13	17.9	0.9935	--
$(2-10) \cdot 10^{-6}$	11.40	5.3	0.9901	$2 \cdot 10^{-6}$

Differential pulse voltammetry of 1-nitropyrene

It was found that 1-NP gives one well-resolved peak. Its E_p shifts towards negative potentials with increasing pH and its current I_p does not change with pH too much (see Fig. 6).

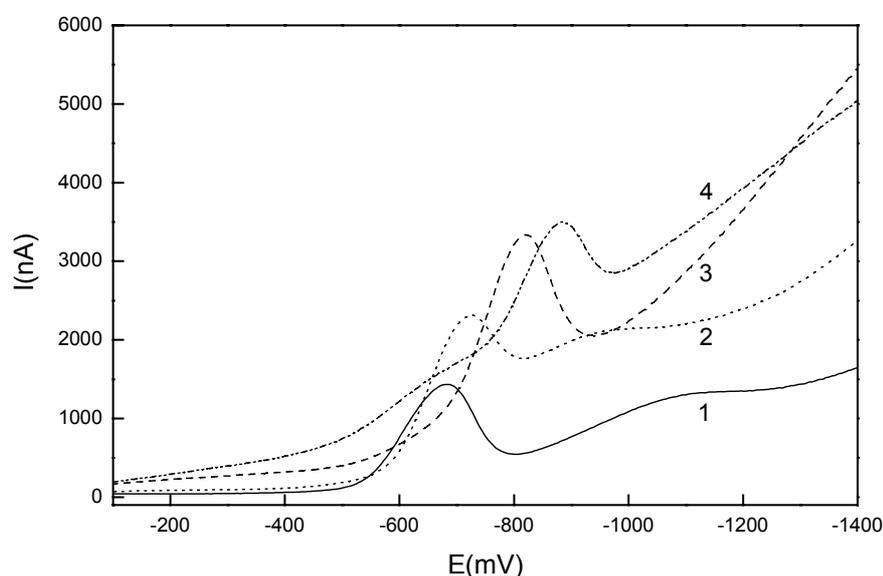


Figure 6. Selected DP voltammograms of 1-NP ($c=1.10^{-4} \text{ mol.L}^{-1}$) at GCPE in mixture of a BR buffer : MeOH (1:9 v/v) mixture with buffer pH 3 (1), 5 (2), 8 (3) and 12 (4).

Best-developed voltammograms were obtained for a BR buffer pH 3 : methanol (1:9 v/v). Calibration curves were measured under these conditions in the concentration ranges of $(2-10) \cdot 10^{-5} \text{ mol.L}^{-1}$ and $(2-10) \cdot 10^{-6} \text{ mol.L}^{-1}$. Parameters of the calibration curves are summarized in Table 2, too. The attempt to increase the sensitivity of the determination by adsorptive stripping voltammetry was not successful.

Conclusions

Glassy carbon paste represents a versatile electrode material offering a variety of applications for electrochemical detection of environmentally significant organic compounds. The main advantage of this type of electrode material is its compatibility with organic solvents. It has been shown that this electrode can be used for the determination of 1-AP based on its anodic oxidation using either LSV, DPV or AAV with a limit of determination around $3 \cdot 10^{-6} \text{ mol.L}^{-1}$, $1 \cdot 10^{-6} \text{ mol.L}^{-1}$, and $1 \cdot 10^{-7} \text{ mol.L}^{-1}$, respectively. Determination of 1-NP based on its cathodic reduction signal can be successfully carried out thanks to the high stability of this type of electrode in methanolic solutions. Under optimal conditions, $1 \cdot 10^{-6} \text{ mol.L}^{-1}$ detection limit was achieved for 1-NP using DPV. The higher detection limit in the cathodic region is obviously connected with a relatively high background current connected with traces of oxygen entrapped in the paste during its preparation. In the cathodic region, the carbon paste electrodes are outperformed by mercury electrodes. Nevertheless, in some cases (such as monitoring of the destruction of nitro compounds based on their reduction to amino compounds followed by their oxidation with permanganate, etc.) they provide an advantageous tool to determine both oxidizable and reducible substances with a single electrode. Similarly, the application of this type of electrode for monitoring the efficiency of the photolytic destruction of 1-NP and 1-AP is under investigation.

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