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High Sensitive Voltammetric Determination of Paroxetine on Glassy Carbon Electrode Modified with Nafion/MWCNTs

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A glassy carbon electrode modified with a Nafion-MWCNT composite is shown to enable the high sensitive determination of paroxetine using differential pulse voltammetry in phosphate buffer of pH 6.5. At a preconcentration time of 15 s, the calibration graph is linear in the 0.1 μM (0.033 $mg \cdot L^{-1}$) to 2.5 μM (0.82 $mg \cdot L^{-1}$) concentration range with a correlation coefficient of 0.998. The detection limit at a preconcentration time of 60 s is as low as 2.6 $\mu g \cdot L^{-1}$. The repeatability of the method at a 0. 16 $mg \cdot L^{-1}$ concentration level, expressed as the RSD, is 4.1% (for n = 5). The method was successfully applied and validated by analyzing paroxetine in drug and urine samples.

Keywords: Paroxetine; MWCNTs; Nafion; Voltammetry.

1. INTRODUCTION

Paroxetine, (3s-trans)-3-[(1,3-benzodioxol-5-yl-oxy)methyl]-4-(4-fluorophenyl) piperidine, is a new generation drug which acts as a potent selective serotonin re-uptake inhibitor (SSRI) in the central nervous system [1]. Its action appears to account for the antidepressant activity observed with this class of drugs [2] that is safe and effective for treatment of related disorders, such as obsessive-compulsive disorder, panic fits, social phobia, and posttraumatic stress [3]. Paroxetine is devoid of sedative effect and remarkably safe in overdose. Paroxetine takes 5.2 hours to reach the peak, with extended half-life (21 hours) that allowed the introduction of formulations for once-daily dosing [4]. Thus a sensitive, specific, fast and cheap method of determining paroxetine is necessary for studying the presence of paroxetine in drugs and human body fluids.

Several analytical procedures have been developed to quantify paroxetine in various samples i.e. high performance liquid chromatography with ultraviolet, fluorescence and electrochemical

detection [5-13] gas chromatography [14,15], spectrophotometry [14-19], capillary electrophoresis [20] and spectrofluorimetry [21,22], but electrochemical methods as voltammetry [23,24] are rarely used.

The aim of this work was to study the high sensitive determination of paroxetine by means of linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV) with the use of glassy carbon (GC) electrode modified with Nafion/multi-walled carbon nanotubes (MWCNTs). The new procedure was examined and successfully used for the determination of a low paroxetine concentration in urine and tablets. Potential interferences from selected metal ions, ascorbic acid, citric acid, glucose and surface-active substances were checked.

2. EXPERIMENTAL

2.1. Measuring apparatus and software

A multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-ANKO, Poland) were used for all voltammetric measurements. The classical three-electrode quartz cell, volume 20 mL, consisting of a GC electrode (diameter 3 mm, Mineral, Poland) modified with Nafion/MWCNTs as the working electrode, a double junction reference electrode Ag/AgCl/KCl (3M) with replaceable outer junction (3 M KCl) and a platinum wire as an auxiliary electrode. pH measurements were performed with laboratory pH-meter (N-512 elpo, Polymetron, Poland). Stirring was performed using a magnetic bar rotating at approximately 500 rpm. All experiments were carried out at room temperature. The MTM-ANKO *EAGRAPH* software enabled electrochemical measurements, data acquisition and advanced processing of the results.

2.2. Chemicals and glassware

All reagents used were of analytical grade. KH_2PO_4, K_2HPO_4 were obtained from Merck and H_3PO_4 was obtained from CHEMAN (Poland). In measurements a 0.1 M phosphate buffer solution was used (prepared using double distilled water). Standard stock solutions of paroxetine (0.01 M) were prepared by dissolving paroxetine hydrochloride (Aldrich) in double distilled water. Solutions with lower paroxetine concentrations were made by appropriate dilution of the stock solution. The multi-walled carbon nanotubes (purity >95%, diameter 40-60 nm, length 5-15 μm) were obtained from Nanostructured & Amorphous Materials Inc., (USA). Nafion 5 wt. % solution in a mixture of lower aliphatic alcohols and water was purchased from Aldrich.

Prior to use, glassware were cleaned by immersion in a 1:10 aqueous solution of HNO_3, followed by copious rinsing in distilled water.

2.3. Preparation of the electrode

Prior to modifying the GC electrode was mechanically polished with Al_2O_3 (0.05 μm), and then rinsed and sonicated 5 min in distilled water. Next 10 mg of MWNTs was added to 10 mL ethanol and Nafion (final Nafion concentration 0.1 %), and then sonicated for 2 hours to obtain a homogenous suspension. The prepared GC electrode was coated with 10 μl homogenous Nafion/MWCNTs and allowed to evaporate the solvent at room temperature in the air. Prior to use Nafion/MWCNTs GC electrode was stabilized in phosphate buffer (pH 6.5) with addition of 1 μM paroxetine by scanned within potential range from 300 mV to 1100 mV of cyclic voltammetry until stability and reproducibility of the signal. Next electrode was rinsed in distilled water and appropriate measurements were made.

2.4. Standard procedure of measurements

The electrochemical behavior of the Nafion/MWCNTs glassy carbon modified electrode was investigated using cyclic voltammetry. The voltammograms were recorded in the potential range from 300 to 1100 mV. Before each registration scan the potential of 1100 mV (2 s) was applied to clean the surface of the electrode. The electrode conditioned in this way was used to determine paroxetine in the supporting electrolyte: 0.1 M phosphate buffer (pH 6.5) (total volume 10 mL) contained in a quartz voltammetric cell. In the case of DP measurements the potential of the electrode was changed in the following sequence: cleaning potential 1100 mV for 2 s and preconcentration potential E_{acc} = 300 mV for t_{acc} = 20 s. During the preconcentration step paroxetine was collected while the solution was being stirred (ca. 500 rpm) using a magnetic stirring bar. Next a differential pulse voltammogram was recorded in the anodic direction from 300 to 1100 mV. The other experimental parameters were as follows: step potential, 5 mV; pulse potential, 50 mV; time step potential, 40 ms (20 ms waiting + 20 ms sampling time). The measurements were carried out from undeaerated solutions. Quantitative measurements were performed using the standard addition procedure.

2.5. Sample preparation

2.5.1. Urine

For *DPV* determination of paroxetine in urine, 200 μL of the fresh urine sample spiked with paroxetine was added directly into voltammetric cell with supporting electrolyte (total volume 10 mL).

2.5.2. Tablets

For the determination of paroxetine in tablet, 3 tablets (20 and 40 mg paroxetine per tablet) were dissolved in 50 mL volumetric flask and additionally sonicated for 15 min. Next appropriate volume of the sample was added to the voltammetric cell.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry studies

The influence of the scan rate (v) on the peak current and peak potential at the GC electrode modified with *Nafion/MWCNTs* was investigated in the range of 5 mVs^{-1} to 500 mVs^{-1} (Fig. 1). During the scan at pH of 6.5 in phosphate buffer the single anodic peak has appeared. The absence of reduction peak in the reverse step indicates the irreversibility of the electrode reaction. The peak current vs. square root of scan rate gave a straight line up to 500 mVs^{-1} (Fig.1 inside). The obtained linear regression equation is:

$I_p = 0.32v^{1/2} + 0.17$ [μA], r = 0.994

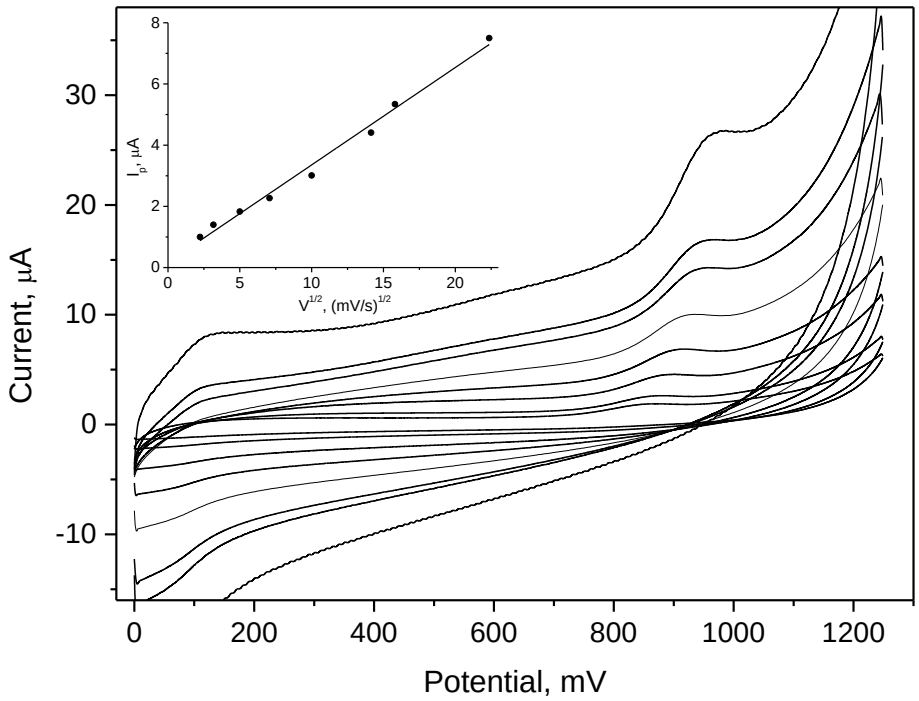

Figure 1. The Cyclic voltammograms obtained for 50 μM paroxetine at the GC electrode modified with 10 μL Nafion/MWCNTs in 0.1 M phosphate buffer (pH 6.5). Scan rate in the range from 5 to 500 mVs^{-1} (Fig. inner – dependence of the paroxetine peak current on square root of scan rate).

This suggest that the process of electrode reaction is controlled by diffusion of paroxetine. The anodic peak potential was shifted in the positive direction with the increasing scan rate. The peak potential vs. ln scan rate gave a straight line (Fig. 2). The obtained linear regression equation is:

$E_p = 0.022ln(v) + 0.774$ [V], r = 0.998

Based on the theory for an irreversible electrode reaction [25]:

$$E_p = E^0 + \frac{RT}{\alpha nF}\left[0.780 + \ln\left(\frac{D_p^{\frac{1}{2}}}{k_s}\right) + \ln\left(\frac{\alpha nFv}{RT}\right)^{\frac{1}{2}}\right] \quad (1)$$

from the slope of E_p vs. $ln(v)$, αn = 0.58 could be obtain and the number of the electron transfer for α assuming 0.5 could be calculate to 1.

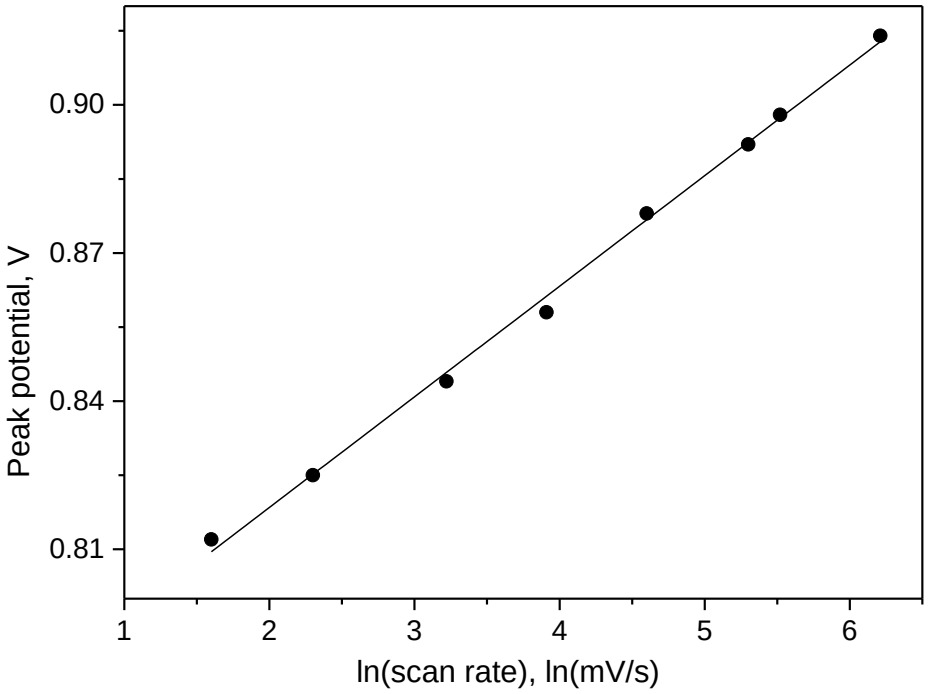

Figure 2. Dependence of the paroxetine peak potential on ln of the scan rate in the range from 5 to 500 mVs^{-1} for 50 μM paroxetine in 0.1 M phosphate buffer (pH 6.5).

3.2. Influence of DPV parameters on technique on paroxetine peak

The important parameters of the *DPV* technique are pulse amplitude (ΔE), potential step amplitude (E_s), waiting time (t_w) and sampling time (t_s). Consequently, these parameters were investigated. To optimize the conditions for paroxetine measurements, the following instrumental parameters were systematically varied: ΔE in the range 5 – 100 mV (both positive and negative mode), E_s in the range 1 – 7 mV, t_w and t_p from 10 to 50 ms.

The best results were obtained for the amplitude of 50 mV (the peak current was ~2.4 μA for 5 μM paroxetine). Higher pulse amplitude (>50 mV) caused the major growth of the background current. For further work, the pulse amplitude of 50 mV was applied.

Changes of the step potential cause influence on peak current. For a step potential equal to 1 mV the peak current was 1.5 μA, and for a step potential of 7 mV the peak current was 2.9 μA. The step potential of 5 mV was applied in further work.

The waiting time and sampling time were changed in the range from 10 to 50 ms. The best result was obtained for waiting time and sampling time of 20 ms, and this was the value chosen for further work.

3.3. Influence of the volume of Nafion/MWCNTs on paroxetine peak

The mixture of Nafion/MWCNTs coated on the GC electrode is necessary to obtain a high sensitive determination of paroxetine. The paroxetine peak current depends on the volume of Nafion/MWCNTs (Fig. 3). For bare GC electrode the paroxetine peak current was 0.5 μA. Presence and increase the amount of Nafion/MWCNTs on the GC electrode is accompanied by an increase of the paroxetine peak. The optimal volume of Nafion/MWCNTs was for 10 μL (with the peak current reaching values approx. 2.4 μA). Higher volumes of Nafion/MWCNTs cause worse repeatability of the paroxetine signal however this effect is accompanied by an increase of the analytical signal. The presence of Nafion/MWCNTs also had an influence on the peak potential. For bare GC electrode the DPV paroxetine peak potential was 845 mV and for modified electrode with 10 μL Nafion/MWCNTs the paroxetine peak potential was 830 mV. The negative shift of the peak potential suggests catalytic effect caused by Nafion/MWCNTs. For further work, the volume of 10 μL was used.

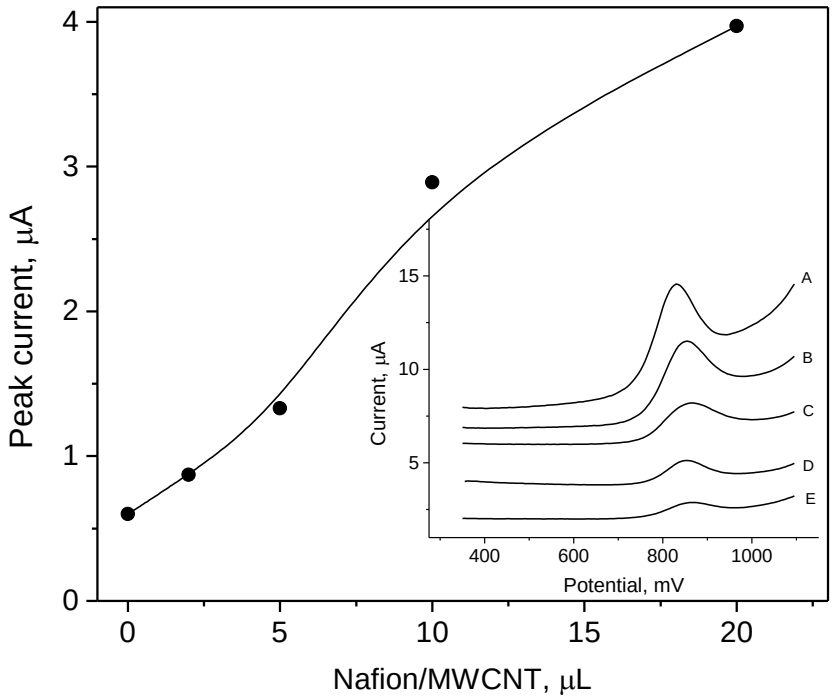

Figure 3. Dependence of the peak current on volume of Nafion/MWCNTs on GC electrode in the range of 0 to 20 μL for 1 μM paroxetine in 0.1 M phosphate buffer (pH 6.5) and obtained voltammograms for: (A) – 20; (B) – 10; (c) – 5; (d) – 2; (e) – 0 μL Nafion/MWCNTs. Instrumental parameters: ΔE = 50 mV, E_s = 5 mV, t_w, t_s = 20 ms. Preconcentration potential 300 mV, preconcentration time 30 s, stirring rate, 500 rpm.

3.4. Influence of preconcentration potential and time on paroxetine peak

The influence of preconcentration potential and time are usually important factors on the sensitivity and detection limit of the stripping methods. In the case of paroxetine determination the

preconcentration potential has practically no influence on the peak current. For further work, the 300 mV preconcentration potential was applied.

The changes in magnitude of the paroxetine current vs. preconcentration time are presented in Fig. 4. The peak current increased with the increase of the preconcentration time from 0.57 μA (t_{acc} = 0 s) to 11.2 μA (t_{acc} = 240 s).

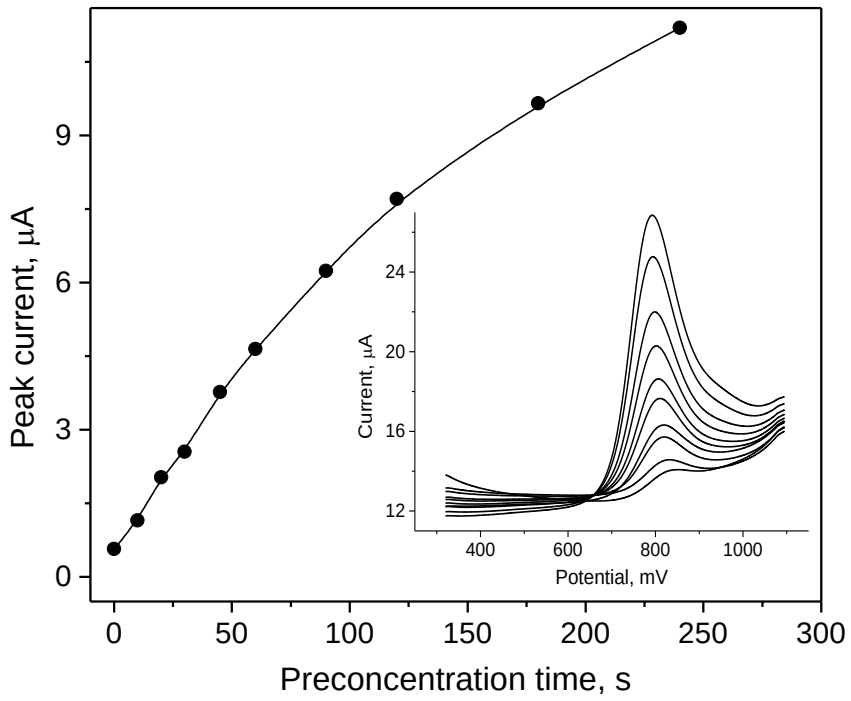

Figure 4. Dependence of the peak current on preconcentration time in the range from 0 to 240 s for 1 μM paroxetine in 0.1 M phosphate buffer (pH 6.5), volume of Nafion/MWCNTs 10 μL and obtained voltammograms. All other conditions are as in Fig. 3.

3.5. Influence of electrolyte composition and pH on paroxetine peak

The electrochemical oxidation of paroxetine has been studied in 0.1 M KCl, KNO_3, $KClO_3$, borate buffer and phosphate buffer (Fig.5). The best results were obtained in phosphate buffer (pH 6.5). Determination of paroxetine on GC electrode modified with Nafion/MWCNTs in phosphates requires a neutral condition in order to obtain a high peak. The optimal pH for the quantity determination of small amounts of paroxetine was in the range from 5.2 to 7 (peak current reaching value about 2.1 μA for 1 μM paroxetine). More acidic and more alkaline conditions caused a decrease in the peak current, e.g. for the pH of 2.2 the peak current was 0.9 μA and for the pH of 8 the peak current was 1.7 μA (Fig. 6). For further study, the pH of 6.5 was applied.

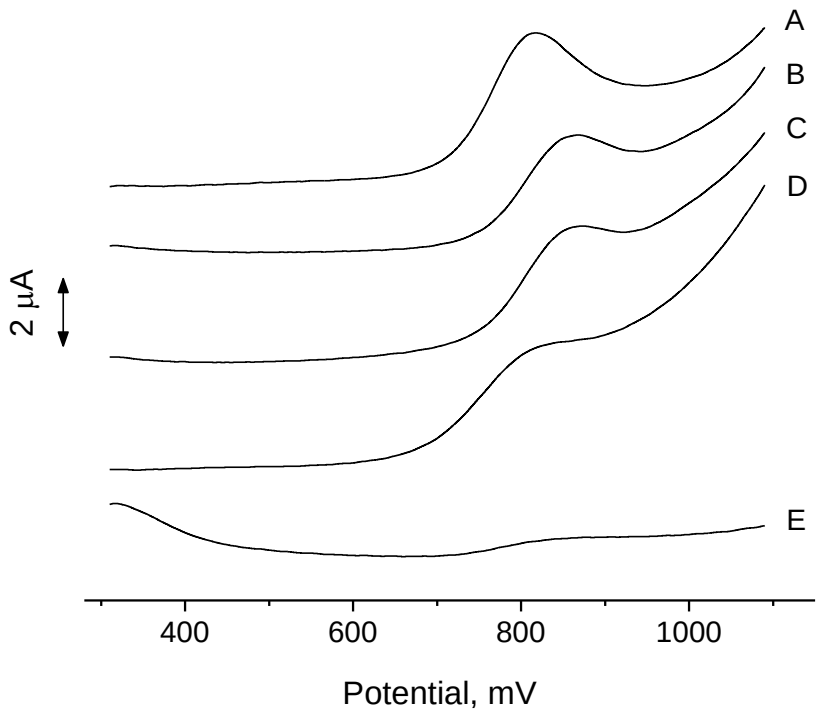

Figure 5. Comparative voltammograms obtained for 1 μM paroxetine in 0.1 M: (A) – phosphate buffer (pH 6.5), (B) – KNO_3, (C) – KCl, (D) – $KClO_3$, (E) – borate buffer (pH 9.1); volume of Nafion/MWCNTs 10 μL. All other conditions are as in Fig. 3.

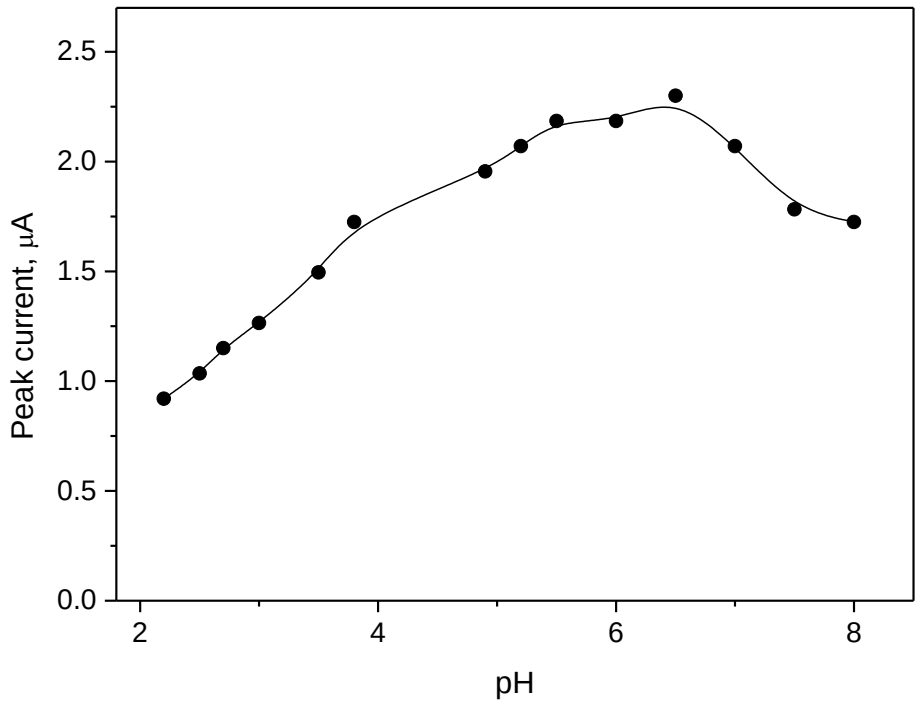

Figure 6. Dependence of the peak current on pH in the range from 2.2 to 8 for 1 μM paroxetine in 0.1 M phosphate buffer, volume of Nafion/MWCNTs 10 μL. All other conditions are as in Fig. 3.

3.6. Interferences

The examined ions, such as: Ca(II), Mg(II) in a 100-fold excess, and Zn(II), Mn(II) in a 10-fold excess and Pb(II), Cd(II), Cu(II) in a 5-fold excess did not interfere. Organic compounds such as: citric acid, caffeine in a 20-fold excess and glucose 25 $mg \cdot L^{-1}$ did not interfere. For 5-fold excess of ascorbic acid, no suppress of the signal was observed. However, it was observed that for 20-fold excess of ascorbic acid the paroxetine signal decreased by 35%.

3.7. Analytical performance

The *DP SV* voltammograms of paroxetine for the 25-200 nM concentration range and preconcentration time of 60 s are presented in Fig. 7.

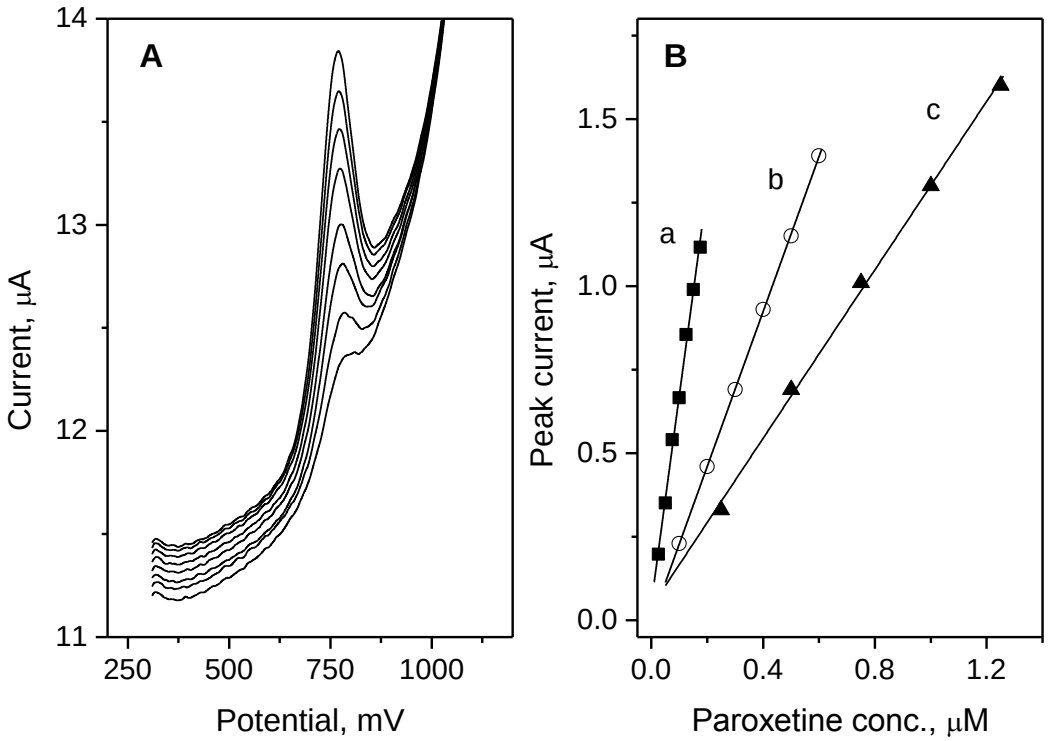

Figure 7. (A) – The *DP SV* paroxetine calibration voltammograms for: 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175 and 0.2 μM paroxetine obtained for preconcentration time 60 s in 0.1 M phosphate buffer (pH of base electrolyte 6.5), volume of Nafion/MWCNTs 10 μL, **(B)** – Paroxetine calibration curves obtained for preconcentration time: (a) – 60; (b) – 30 and (c) – 15 s. All other conditions are as in Fig. 3.

The detection limit obtained for short preconcentration time (15 s) was 65 nM with the linearity up to 2.5 μM (slope of the regression line was 1.13 ± 0.11 [$\mu A \cdot \mu M^{-1}$], intercept 0.13 ± 0.12 μA, correlation coefficient 0.998). A longer preconcentration time results in a lower detection limit (for example when the preconcentration time of 30 s was used during the measurement the detection limit was 21 nM, and for the preconcentration time of 60 s the detection limit was 8 nM). In comparison to

the described so far results for voltammetric determination of paroxetine [24] (the detection limit 2 μM) and [23] (the detection limit 62 nM) the obtained detection limit is lower and is similar for HPLC methods.

The slopes for regression lines were [$\mu A \cdot \mu M^{-1}$]: 2.35 ± 0.01 and 6.23 ± 0.16, intercepts [μA]: -0.02 ± 0.04 and 0.05 ± 0.02, the correlation coefficients 0.999 and 0.998 for preconcentration times of 30 and 60 s, respectively. The linearity was up to 1 μM (t_{acc} = 30 s) and 0.4 μM (t_{acc} = 60 s).

To validate the method the urine and drugs were investigated.

The samples, spiked with paroxetine were analyzed according to the described procedure using the GC electrode modified with Nafion/MWCNTs. Determinations of paroxetine were performed using the standard addition method (three additions of the standard solution). Results from paroxetine determination are presented in Table 1. The recovery of paroxetine ranged from 87–106%. The analytical usefulness, of the presented method for the determination of paroxetine in the samples was confirmed.

Table 1. Results of paroxetine determination in various samples.

Paroxetine added	Paroxetine found $\bar{x}$ ± s (recovery %)		
	Urine (μM)	Paxtin[1] (mg/tablet)	Paxtin[2] (mg/tablet)
0	0	42.4 ± 3.7	20.8 ± 1.2
0.05 μM	0.043 ± 0.004 (87)	-	-
0.1 μM	0.092 ± 0.06 (92)	-	-
0.15 μM	0.147 ± 0.06 (98)		
10 mg	-	53.0 ± 3.3 (106)	31.2 ± 2.8 (104)
20 mg	-	63.6 ± 4.1 (102)	42.8 ± 2.7 (105)

[1] – product declared 40 mg/tablet

[2] – product declared 20 mg/tablet

4. CONCLUSIONS

The presented *DPV* method for the electrochemical determination of paroxetine using a GC electrode modified with Nafion/MWCNTs allows to determine paroxetine at trace level, in concentrations as low as 8 nM (2.6 $\mu g \cdot L^{-1}$), calculated according to [26] for a preconcentration time of 60 s. The obtained detection limit is much lower for voltammetric results described so far in the

literature and is comparable for HPLC methods. The reproducibility of the method is good, i.e. when measured as RSD is 4.1%. Acceptable recovery (87–106%) shows that the method can be used for the determination of paroxetine in drugs and urine.

The preparation of GC electrode modified with Nafion/MWCNTs is simple, short and economically suitable. The obtained results confirm that method may be used into out-of-laboratory systems.

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