Full Paper

Wiley Online Library

ELECTROANALYSIS

DOI: 10.1002/elan.201900595

A New Approach for Voltammetric Determination of Nefopam and its Metabolite

Liliya Dubenska,*[a] Olha Dushna,[a] Solomiya Pysarevska,[b] and Mykola Blazheyevskiy[c]

Abstract: In present paper we described a new simple voltammetric method of determination of nefopam alkaloid and its metabolite – N-oxide. N-oxide of nefopam is reduced at the dropping mercury electrode (DME) and silver solid amalgam electrodes (AgSAE), which can effectively replace mercury and chemically modified electrodes. The reduction consists of two one-electron stages each accompanied with one proton transfer. N-oxide of nefopam can be obtained from nefopam substance by oxidation with potassium peroxymonosulfate. It was studied the effect of various factors on N-oxide quantitative yield (pH, oxidation duration, reagents concentration) as well as on the reduction of N-oxide at DME and p-AgSAE (pH, the nature of background electrolyte, potential and time of accumulation). It was showed that the reduction current linearly increased with increasing of concentration of analgesic. Limit of quantiation is 10^{-6} mol L^{-1} at DME and 10^{-7} mol L^{-1} at p-AgSAE. The developed method was applied for the analysis of commercial drug solution for injection "Nefopam" with recovery of 96.7 %, as well as for the spiked human urine samples. Excellent repeatability with a relative standard deviation below 5 % was achieved.

Keywords: nefopam · analgesic · cyclic voltammetry · N-oxide · oxidation

1 Introduction

Nowadays the actual and important issue of modern medicine is searching for medicals that can be used for pain relief during surgery and postoperative period without drug dependence. Thus, narcotic and non-narcotic analgesics and non-steroidal anti-inflammatory drugs with strong analgesic effect are used in the medical treatment. These drugs can be used both in a mixture and as individual medicines. Morphine, tramadol and nefopam are the drugs the most frequently used for pain relief.

Nefopam hydrochloride (Figure 1) was designed in the early 1970s as antidepressant, which also was used as muscle relaxant for the treatment of muscle spasms. It belongs to non-narcotic analgesics of central activity, which inhibits the back capture serotonin, noradrenaline and dopamine. Nefopam is widely used in many countries around the world for the treatment of acute and chronic pain syndromes of malignant and non-malignant etiology, the prevention of muscle tremor and its therapy, treatment of hiccups of neurogenic origin, postoperative analgesia [1].

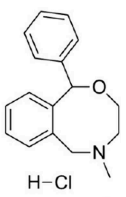


Fig. 1. Structure formula of nefopam hydrochloride.

According to chemical structure nefopam is alkaloid from the group of benzoxazine derivatives (Figure 1). Methods of nefopam hydrochloride determination in pharmaceutical preparations and biological materials are scarce. Spectrophotometry (SP) and thin-layer chromatographic (TLC) were used for identification of nefopam in substances and simple medicines [2–4]. The most selective methods for the determination of nefopam and its metabolite are chromatographic one, in particular high performance liquid chromatography (HPLC) [5–6]. The brief description of some methods of nefopam determination is presented in Table 1.

A good alternative to known methods of nefopam determination is voltammetric methods, which are becoming more popular in pharmaceutical industry [7]. Electrochemical methods combine several useful features such as

[a] L. Dubenska, O. Dushna
Ivan Franko National University of L'viv, Department of Analytical Chemistry, 79005, Kyryla i Mephodia Str. 8, L'viv, Ukraine,
E-mail: dubenskyy@gmail.com
[b] S. Pysarevska
Ivan Franko National University of L'viv, Department of Life Safety, 79000, Doroshenka Str. 41, L'viv, Ukraine,
E-mail: pysarevska_s@yahoo.com
[c] M. Blazheyevskiy
National Pharmaceutical University, Department of Physical and Colloid Chemistry, 61168, Bljuhera Str. 4, Kharkiv, Ukraine,
E-mail: blazejowski@ukr.net

Supporting information for this article is available on the WWW under https://doi.org/10.1002/elan.201900595

Table 1. The methods of nefopam determination.

Method	Linear range	LOQ	LOD	Objects analyzed	References
SP	0.5–30 μg mL^{-1}	1.96 μg/mL	0.49 μg/mL	Tablets, injection	[2]
TLC	0.05–1.00 mg/mL	3.16 μg	0.95 μg	Tablets	[3]
UV-SP	50–400 μg/mL	–	–	Polymethacrylate nanospheres	[4]
LC-ED	1–100 ng/mL	1 ng/mL	–	Plasma	[5]
HPLC	1–60 ng/mL	1 ng/mL	–	Plasma	[6]
HPLC	2–25 ng/mL	1 ng/mL	–	Globule	[6]
HPLC	25–250 ng/mL	5 ng/mL	–	Urine	[6]

HPLC – high performance liquid chromatography, LC-ED – liquid chromatography with electrochemical detection, SP – spectrophotometry, TLC – thin layer chromatography, UV-SP – UV spectrophotometry

high selectivity and sensitivity, relatively not expensive instruments, they are fast to use, can be automated, meet the requirements of green chemistry because they do not require large amounts of organic solvents. There are also reported works where voltammetric detectors were used in chromatography [8–10]. In addition, it is assumed that there is a similarity between electron transfer process that occurs on the electrode and in the cells of living organisms. Knowledge of the redox properties of pharmaceuticals makes it possible to evaluate and predict the ways of their metabolism during redox reactions *in vivo*, as well as their pharmacological activity [11]. Therefore, it is advisable to use voltammetry in investigations of properties of biologically active molecules.

Earlier we have reported the work on polarographic determination of amide group anesthetics based on the obtaining of polarographically active derivatives – N-oxides [12]. Amide group of nefopam is easily oxidized forming its N-oxide. Authors [5] used the same way of derivatization for LC-ED determination of nefopam in human plasma. It is also known that one of the nefopam metabolites is its N-oxide. Thus, all these issues determined our purpose and subjects of the studies. We elaborated the procedure, which was based on the oxidation of nefopam hydrochloride to its metabolite using potassium peroxymonosulfate, and then the obtained N-oxide was electrochemically reduced.

2 Experimental

2.1 Reagents

The substance of nefopam hydrochloride (Genkevilliers, France, c. 1701040039) with content of the main substance not less than 99 % was used in the work. The standard sample solution (SSS) was prepared as follows: the exact amount of substance (the calculated amount 0.0284 g) was introduced into the 100 mL volumetric flask and dissolved in 70 mL of distilled water. The volume of SSS was brought to 100 mL at 20 °C and mixed thoroughly. In this case, the concentration of SSS was $1.0 \cdot 10^{-3}$ mol L^{-1}. SSS was stable during two weeks when stored in the fridge.

"Extra pure" commercial triple potassium salt of Caro's acid – Oxone was purchased from Acros Organics and used as oxidizing agent. The active ingredient of Oxone is potassium peroxymonosulfate, $KHSO_5$ (KPMS) (CAS 10058-23-8), commonly known as potassium monopersulfate, which is present as a component of a triple salt with the formula $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ potassium hydrogen peroxymonosulfate sulfate, (CAS 70693-62-8). This reagent was chosen because of its availability, sufficient solubility in water, high oxidative ability ($E_{HSO_5^-/HSO_4^-}$ changes from 1.82 ± 0.03 V at pH 0 to 1.44 V at pH 11 [13]) and sufficient durability during exploitation and storage [DuPont™ Oxone® Technical Attributes] [14–16]. Stock solution of Oxone was prepared by dissolving its appropriate amount in 70 mL of double-distilled water in a 100 mL volumetric flask; then it was filled with double-distilled water to the mark and shaken.

The Britton-Robinson (BR) buffer was used to provide the required pH. 20.2 g $Na_2B_4O_7 \cdot 10H_2O$, 28.7 mL of glacial acetic acid CH_3COOH and 17.6 mL of H_3PO_4 orthophosphoric acid were dissolved in volumetric flask. The pH 8.0 was obtained by adding 2.5 M sodium hydroxide (controlled by pH meter) and distilled water up to 1 L of solution. All these reagents were "pure for analysis" qualifications acquired in Ukraine.

The dissolved oxygen was removed from electrochemical cell by purified argon during 10–15 min.

2.2 Apparatus

All voltammetric measurements were performed by computer controlled digital device MTech OVA-410 with three-electrode cell (working dropping mercury electrode, a saturated calomel reference electrode and platinum wire auxiliary electrode) and MTech UVA-410 [17] with three-electrode cell, where silver solid liquid mercury free polished (p-AgSAE) amalgam electrode was used as the working electrode, saturated silver/silver chloride electrode as the reference and platinum wire as the auxiliary electrode. Silver solid amalgam electrodes (AgSAEs) represent non-toxic alternative to the traditional mercury electrodes due to outstanding properties such as wide negative potential window, low noise, easy construction and regeneration of electrode surface [18–21]. Moreover, AgSAEs are mechanical stable and can be successfully used for continuous control in flow.

The DME had the following characteristics: $m = 5.9 \cdot 10^{-4}$ g/s, $\tau = 10$ s in 0.2 M NH_4Cl with open circuit.

The accuracy of potential measurements is 1 mV. The uncertainty of current measurement is 0.1 %.

Cyclic voltammetry (CV) was employed for the initial experiments, but for the development of analytical protocol a rapid screening method namely linear sweep voltammetry was applied, which can be characterized by a low definition limit and fast response.

The pH measurements were performed using pH-meter MV 870 DIGITAL-pH-MESSERAT with silver chloride reference electrode.

2.3 Voltammetric Procedure and Sample Preparation

Working solution of N-oxide preparation: an aliquot of SSS was added into a 25 mL volumetric flask to obtain a solution with needed concentration, then 4 mL of BR buffer with pH 8.0 and 0.2 mL 10^{-2} mol L^{-1} KPMS were added to the volume. The obtained solution was stirred and kept for 10 min, then 0.37 mL of 2.5 M HCl solution was added to decrease the pH of the solution to 5.5 (controlled by pH meter), then bring the volume with distilled water to the mark. The obtained working solutions were introduced into the cell, and deoxygenated with argon for 10 min. Polarogramms were recorded in the range of potentials from 0.0 to −1.5 V.

The solution for injection "Nefopam" manufactured by "COOPER.S.A." (Athens, Greece) was used to verify the accuracy of our method. This solution contains 19–21 mg/mL of nefopam according to regulatory documents. One ampoule contains 2.0 mL of solution. SSS of "Nefopam" was prepared as follows: 1.00 mL of solution was taken from ampoule with a measuring pipette and transferred to 25 mL volumetric flask, then it was diluted with water to the mark and stirred. Concentration of this SSS according to regulatory documents of this drug is $1.38 \cdot 10^{-3}$ mol L^{-1}. Aliquot 0.15–0.30 mL was taken from SSS and transferred to 25 mL volumetric flask. Then 4 mL of BR buffer with pH 8.0 and 0.2 mL 10^{-2} mol L^{-1} KPMS were added to the volume. The mixture was stirred and kept for 10 min, then 0.37 mL of 2.5 mol L^{-1} HCl solution was added to decrease the pH of the solution to 5.5 (controlled by pH meter), then bring the volume with distilled water to the mark. The obtained solutions were introduced into the cell, and polarogramms were recorded in the same way as for standard sample solution.

2.4 Preparation of Urine Samples and Procedure for their Analysis

Drug-free human urine samples were taken from one non-smoking and healthy volunteer (female, 20 years old) on an empty stomach on the day of the experiment. In this sense, these experiments were undertaken in compliance with respective law with the informed consent obtained from the volunteer prior to the experiments. Besides, at the time of experiments and shortly before, volunteer did not undergo any treatment with multivitamin formulations and other drug dosages.

Samples of urine were prepared as follows: 1 mL of fresh urine, 4 mL of BR buffer solution with pH 5.5, 0.2 mL 10^{-2} mol L^{-1} of KPMS and 1 mL of saturated KCl solution were transferred into 25 mL volumetric flask. Then, the obtained solution was enriched with aliquots of nefopam N-oxide obtained as stated in 1.3 in the concentration range $2 \cdot 10^{-5}$–$4 \cdot 10^{-6}$ mol L^{-1} and filled with water to the mark. Prepared solution was transferred into the electrochemical cell, purified with argon to remove dissolved oxygen during 10 min. and polarogramms were recorded in the range from −0.5 to −1.5 V.

3 Results and Discussion

Figure 2 A displays polarogramms in nefopam solution before oxidation and in the absence of KPMS (1), in solution of KPMS and in the absence of nefopam (2), and also in solution after oxidation of nefopam (3). Figure 2 B

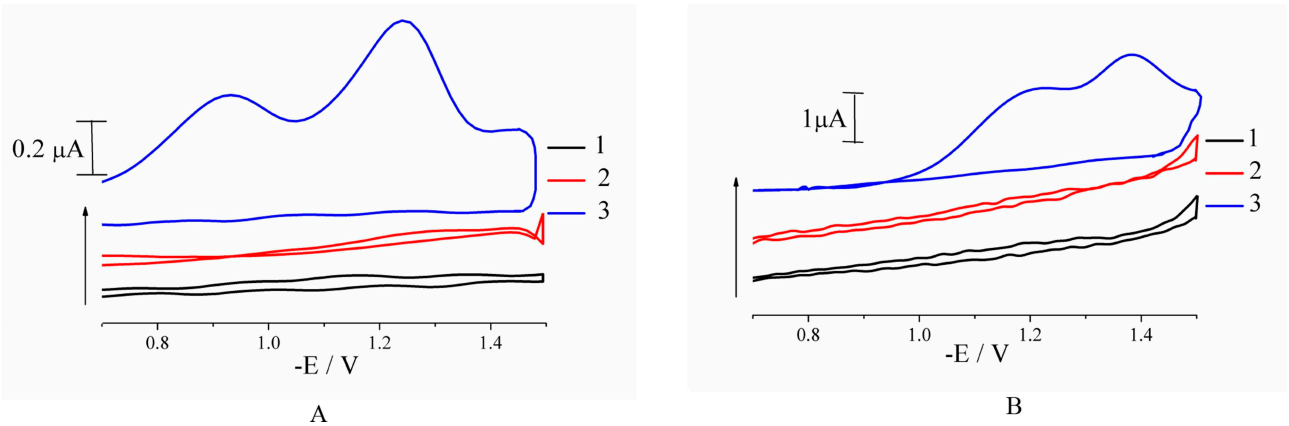


Fig. 2. Polarogramms in solution of nefopam before (1) and after (3) oxidation, as well as in solution of KPMS without nefopam (2) at the BR buffer solution, $C_{buffer} = 0.4$ mol L^{-1}, $C_{KPMS} = 1.0 \cdot 10^{-4}$ mol L^{-1}, $C_{nefopam\ at\ DME} = 4.0 \cdot 10^{-5}$ mol L^{-1} (A), $C_{nefopam\ at\ p\text{-}AgSAE} = 1.0 \cdot 10^{-6}$ mol L^{-1} (B), pH 5.5; $v = 0.5$ V/s at DME, $v = 3.0$ V/s at p-AgSAE.

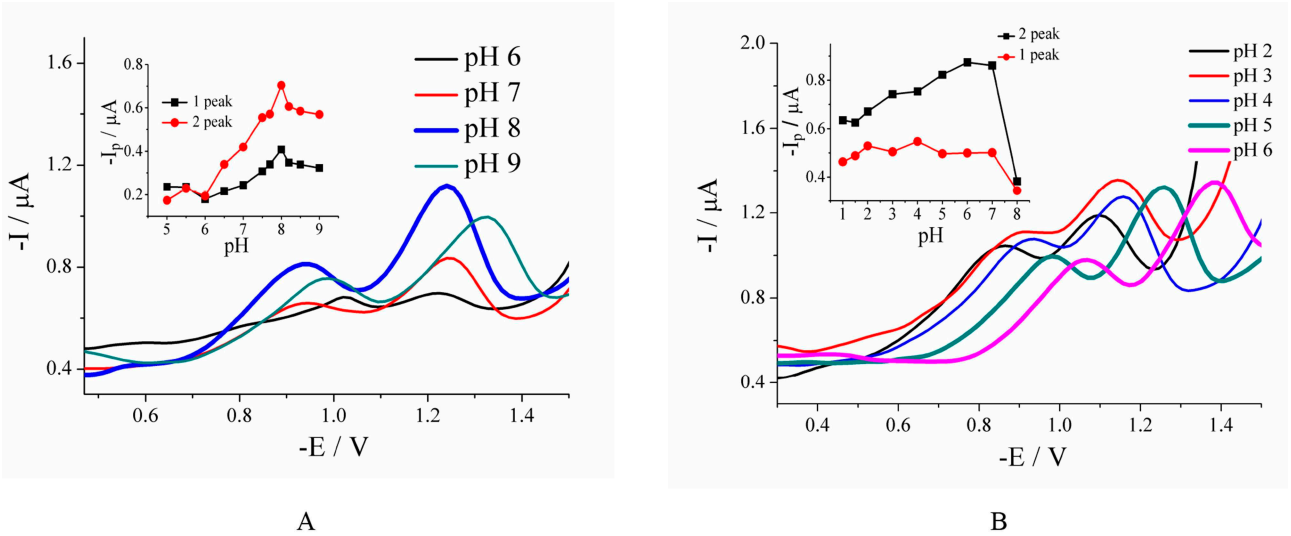


Fig. 3. Polarogramms and dependences of current (input) of nefopam hydrochloride reduction on $pH_{oxid.}$ (A) and pH_{pol} (B), $C_{nefopam} = 4.0 \cdot 10^{-5}$ mol L^{-1}, $C_{KPMS} = 1.0 \cdot 10^{-4}$ mol L^{-1}, $t_{oxid.} = 10$ min; $pH_{pol} = 5.5$ (for A) and $pH_{oxid.} = 8.0$ (for B).

shows curves obtained in the same conditions, but using p-AgSAE as working electrode. Polarogramms 1 and 2 are practically identical with those obtained in the solution of background electrolyte. The arrow on Figure 2 indicates that polarogramms 2 and 3 were shifted up towards axis Y for better view. The reduction of nepofam derivative at DME is characterized by two peaks at potentials −0.92 V and −1.24 V (polarogramm 3 Figure 2A). The shape of voltammograms at p-AgSAE is similar (Figure 2B), but at high scan rates (see item 3.4) only one peak is clearly registered. Anodic peaks are absent that means irreversibility of electrochemical process. When changing the conditions no changes at anodic brunch of voltammogram were observed. Therefore, analytical parameters and optimal conditions were determined using cathodic linear sweep voltammetry.

3.1 Effect of pH

N-oxides of tertiary amines are formed in alkaline medium. Acidation of reaction mixture leads to stop the oxidation process. An optimum pH for oxidation ($pH_{oxid.}$) of nefopam is pH 8.0 (Figure 3A).

The shape of polarogramms of nefopam derivatives reduction significantly depends on pH of polarographic scanning (pH_{pol}) (Figure 3B).

The shape of polarogramms, current and potential of nefopam derivative reduction are dependent on solution pH. The reduction peak increased till pH reached 7.0, but at pH 8.0 and higher it decreased sharply, and disappeared at pH 9.0. Peak potentials were shifted to a negative direction while pH increased. The least changes of polarogramms were observed within pH 5–6 (Figure 3B), thus for further investigations we have chosen pH 5.5.

Therefore, the well-shaped and high peaks can be obtained using the following conditions: nefopam was oxidized at pH 8.0 and then for recording polarogramms the obtained solution was acidified to pH 5.5.

Since the process is rather complicated, the potentials of the reduction peaks of the corresponding derivatives are shifted to a negative direction with increasing pH. This behaviour demonstrates that the electrochemical reduction of nefopam derivatives involves proton transfer stage. The dependence of −E vs. pH of the buffer consists of three linear segments. The figure is not displayed in the work, however, we used the same experimental polarogramms as for plotting the dependence of *I* vs. pH (inset Figure 3B). The obtained dependences can be expressed by the equation presented in Table 2. The most strictly linear dependence −E vs. pH observed for pH range from 5 (for peak 1) or 4 (for peak 2) to pH 7.

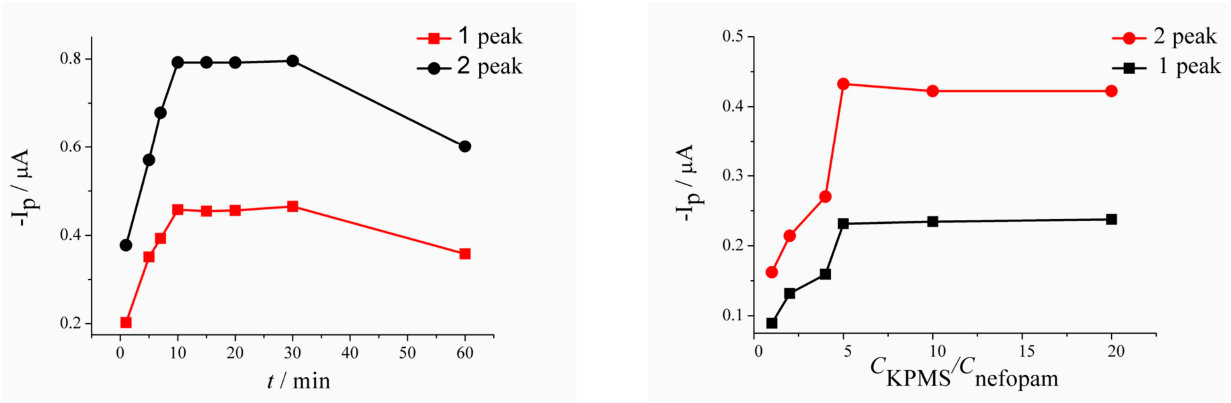
3.2 Effect of Oxidation Time and Reagents Concentration

The quantitative yield of nefopam derivative strongly depended on oxidation time. The effect of oxidation time on quantitative yield of nefopam derivative was investigated in BR buffer at time range from 0 to 60 minutes. The obtained results (Figure 4A) revealed that in order to obtain the maximum peak current, oxidation should last not less than 10 minutes. After 30 min. of oxidation the peak current slightly decreased probably because of forming the other oxidation products. Thus, the best results can be achieved when oxidation lasts from 10 to 30 min.

Another studied factor was the concentration of KPMS. As can be seen from Figure 4B, the highest peak current of the product can be achieved at 5-fold excess of KPMS. However, it is desirable that the concentration of KPMS does not exceed 10^{-4} mol L^{-1}, since higher concen-

Table 2. The equations of linear dependence of E, V on pH on a BR buffer.

Peak	pH range	Equation	Correlation coefficient, R
1	1–2	$E = (1.11 \pm 0.05) + (-0.12 \pm 0.03)$ pH	0.8464
	2–5	$E = (0.789 \pm 0.008) + (0.038 \pm 0.002)$ pH	0.9912
	5–7	$E = (0.553 \pm 0.008) + (0.0854 \pm 0.0013)$ pH	0.9995
2	1–2	$E = (1.38 \pm 0.06) + (-0.13 \pm 0.04)$ pH	0.8313
	2–4	$E = (1.04 \pm 0.03) + (0.029 \pm 0.008)$ pH	0.8451
	4–7	$E = (0.79 \pm 0.07) + (0.094 \pm 0.012)$ pH	0.9536



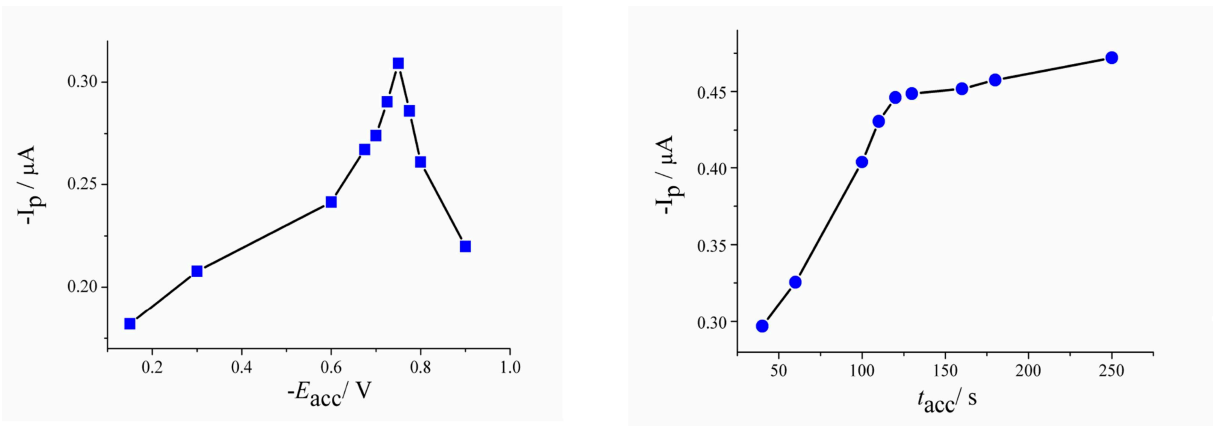
A B

Fig. 4. The effect of oxidation time (A) and molar ratio of reaction components (B), $pH_{oxid.}$ 8.0, $pH_{pol.}$ 5.5, $C_{nefopam} = 4.0 \cdot 10^{-5}$ mol L^{-1}.

trations of KPMS lead to enhancement and fluctuating of background current.

3.3 Effect of Accumulation Parameters

To improve the sensitivity of determination, the utilization of adsorptive accumulative step of nefopam at the surface of p-AgSAE was tested. Hence, the parameters such as accumulation potential and accumulation time were examined. The influence of accumulation potential (E_{acc}) on the nefopam signal was examined by varying it over a range from −150 mV to −900 mV, while the accumulation time of 20 s was kept constant. As shown on the Figure 5A the current intensity increased with the enhancement of E_{acc} up to −750 mV, then gradually decreased. As a consequence, the accumulation potential of −750 mV was chosen for all subsequent measurements. The dependence of voltammetric response of nefopam on the accumulation time (t_{aac}) is displayed on Figure 5B. It was found that peak current increased linearly as the



A B

Fig. 5. The effect of accumulation potential (A) and accumulation time (B) on peak current of nefopam N-oxide at p-AgSAE, pH_{oxid} 8.0, pH_{pol} 5.5, $C_{nefopam} = 8.0 \cdot 10^{-6}$ mol L^{-1}, $C_{KPMS} = 1.0 \cdot 10^{-4}$ mol L^{-1}, $t_{oxid} = 10$ min, $v = 1.0$ V/s.

value of t_{aac} increased from 40 s to 120 s confirming the adsorption of nefopam N-oxide onto the working electrode surface. Moreover, no significant variations of current for larger accumulation time were registered, indicating that the electrode surface was saturated.

3.4 Effect of Scan Rate

In order to ascertain the effect of scan rate (v) on voltammetric behaviour of nefopam, scan rates studies were performed in the range from 0.3 to 4.0 V/s at DME and from 0.1 to 5.0 V/s at p-AgSAE. The heights of the reduction signals recorded on DME and p-AgSAE increased with scan rate and the peak potentials shifted toward more negative values. Moreover, no oxidation signals were registered, demonstrating the irreversibility of the electrochemical process. At concentration of nefopam $C_{nefopam} \geq 1.0 \cdot 10^{-5}$ mol L^{-1} and $v \geq 1$ V/s for p-AgSAE two reduction peaks merge into one and potential can be measured at maximum near −1.3 V.

The relationship between logI_p and log$v$ for both peaks at DME consisted of two linear segments, indicating that a change in the scan rate leads to alteration the nature of reduction current. The slope values of this dependence for the range from 0.3 to 1 V/s are 0.55 and 0.39 for the first and the second peak, respectively. It is possible to conclude that the reduction of nefopam N-oxide at DME is controlled by the diffusion with minor kinetic difficulties (for second peak). In addition, according to Randles-Sevcik equation for irreversible system, linear dependence between the peak current (I_p) and the square root of the scan rate ($v^{1/2}$) confirms the diffusion-controlled electrode reaction. Furthermore, the small values of parameter a of the linear regression equation $I_p = f(v^{1/2})$ (−0.036 and −0.13 for first and second peak, respectively) are clearly evident of the weak influence of adsorption in the electrochemical process.

However, the contribution of adsorption increases with the enhancement of the scan rate in the range 1–4 V/s. The slopes for dependences log$v$–logI_p were 0.96 for the first peak and 0.91 for the second peak respectively.

logI_p is linearly dependent on log$v$ in the range from 0.1 to 5.0 V/s at p-AgSAE. The slope of this dependence is 0.84. The adsorption nature of the current at high scan rates promotes the use of adsorption voltammetry to accumulate the analyte at the electrode surface and to improve the analytical parameters of the determination method.

3.5 The Possible Mechanism of Electrochemical Reaction

Based on the quantitative parameters of the voltammograms, the electron number n involved in the electrode process was calculated by the following equation [22]:

$$\alpha n = -47.7/(E_p - E_{p/2})(mV) \quad (1)$$

where, α – the charge transfer coefficient, n – the number of electron transferred in a stage of electrode process.

The coefficient α for irreversible systems equals to 0.5.

The number of electrons was calculated using polarogramms obtained at different conditions (various values of pH for oxidation – from 7.0 to 9.0 and various values of pH when polarogramms were recorded – from 2.0 to 7.0 as well as different values of nefopam concentrations). The calculated n varied from 0.7 to 1.3 for each peak.

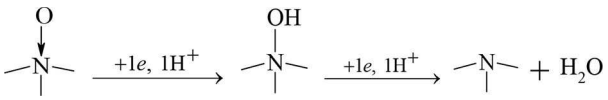
The number of electrons can also be determined from the dependence $E_p = f(\log v)$, where the slope of this dependence is equal to 2.3 RT/αnF. The value of 0.9 was determined in the range 0.3–1.0 V for the first and the second peak, and in the range 1.0–4.0 $n = 0.7$ – for the first peak and $n = 0.8$ – for the second peak. When using the same dependence for p-AgSAE working electrode the number of electrons was 0.7 for each peak. Thus we suggested that two peaks on the polarogramms corresponded to two one-electron stages of nefopam N-oxide reduction.

The number of H^+ ions (zH^+) participating in electrochemical process can be estimated from the slope of the peak potential vs. pH according to the following equation:

$$\frac{dE}{d\mathrm{pH}} = \frac{2.3\mathrm{R}T \cdot zH^+}{\alpha n\mathrm{F}} \quad (2)$$

The obtained values of zH^+ (calculated according to data from Table 2) were 1.1 and 1.2 for the first and second peaks respectively.

The possible mechanisms of the reduction of N-oxides of other substances, including 37 compounds of indolone-N-oxides and di-N-oxides derivatives of quinoxaline-2-carboxamide are described in literature [23–25]. Their results suggest a transfer of one electron during the reduction of the N-oxide forming a stable radical nitroxide anion [25]. Moreover, addition of small quantities of water facilitated the reduction process of indolone-N-oxide derivatives and it can be explained by the protonation of the nitroxide radical anion. The authors proposed the EC mechanism of these compounds reduction, when the one electron transfer stage precedes the protonation reaction with one proton. In aprotic solvents the anion radical can be relatively stable. On the contrary, in the presence of water it is unstable and undergoes rapid transformation attaching proton. However, in acid aqueous medium the protonation process of Oxygen of nefopam N-oxide precedes the electron transfer stage. The second stage is also accompanied by the transfer of one electron and one proton. Therefore, we suggest that the reduction of nefopam N-oxide could be described according to the scheme:



3.6 Determination of Analytical Parameters

In Figure 6A 0 means background line, and 1–8 – polarogramms, which correspond to concentrations of nefopam from $1.0\cdot10^{-6}$ to $2.0\cdot10^{-5}$ mol L^{-1}. In Figure 6C 0 means background line, and 1–6 – polarogramms, which correspond to concentrations of nefopam from $4.0\cdot10^{-6}$ to $2.0\cdot10^{-5}$ mol L^{-1}.

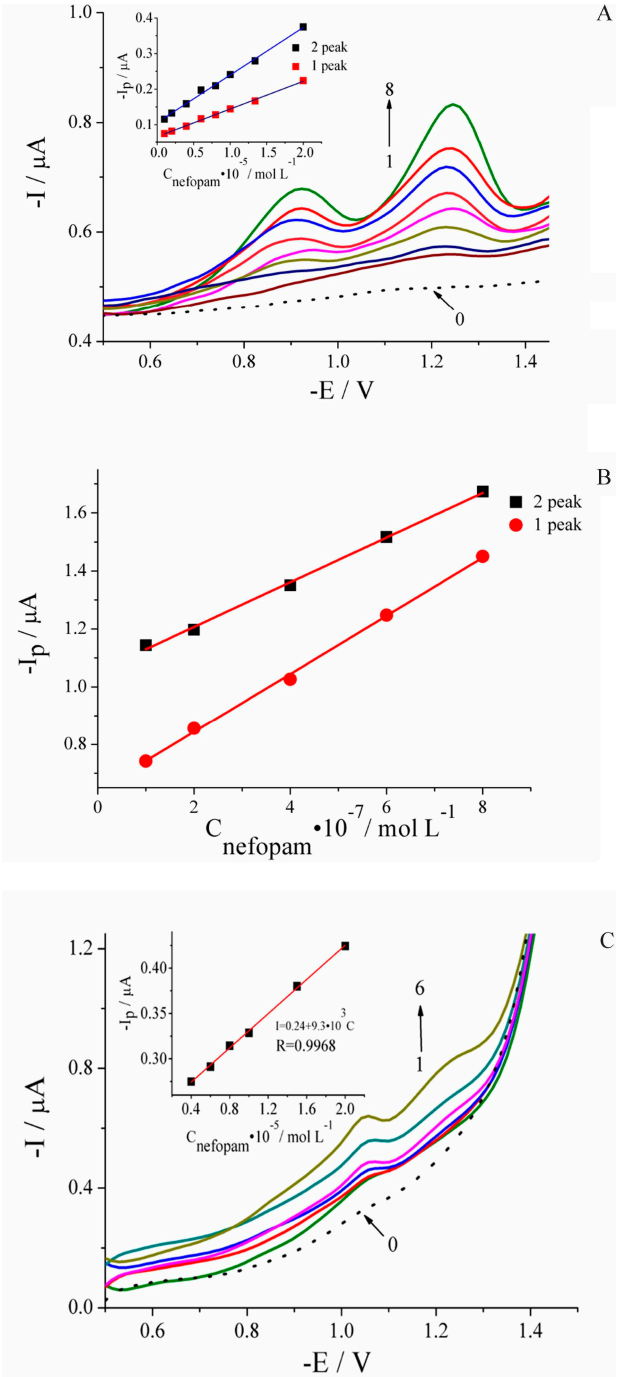


Fig. 6. The polarogramms of nefopam at the background of BR buffer and calibration graphs for nefopam determination at DME (inset) (A); calibration graphs for nefopam determination at p-AgSAE (B); polarogramms and calibration graphs for nefopam N-oxide in urine (inset) (C). pH_{oxid} 8.0, pH_{pol} 5.5, C_{KPMS} = $1.0\cdot10^{-4}$ mol L^{-1}; t_{oxid} = 10 min, v = 0.5 V/s. For p-AgSAE E_{acc} = −0.75 V and t_{acc} = 120 s.

Calibration graphs were constructed using solutions with appropriate concentrations of nefopam prepared according to 2.3. Current measured at the maximum of peak height was used for obtaining dependences of peak current (I, μA) vs. concentration of nefopam (C, mol L^{-1}) (Figure 6A). The analytical parameters are presented in Table 3. The LOD and LOQ for the determination based on three and ten times of the blank standard deviation ($3.3S_a/b$, $10S_a/b$) respectively (S_a – residual standard deviation or standard deviation of the y-intercept) [26]

3.7 Method of "Nefopam" Analysis

The preparation of SSS is described in 2.3. The concentration of nefopam was determined according to calibration graph obtained for each peak at DME (Figure 6A). Components of solution for injection did not interfere with nefopam determination. The obtained results of analysis were compared to those stated in quality certificate given by testing laboratory of Ukraine on medicines and drugs control. The content of nefopam stated in certificate was 20.48 mg/mL. We obtained in mg/mL: 19.4 ± 0.7 (S_r = 1.4 %) for the first peak and 19.8 ± 0.5 (S_r = 1.0 %) for the second peak. Measuring the signal of two peaks separately we obtained close results that fall within the acceptable range of nefopam content. However, for the result of the second peak, the reliable interval is narrower and the value closer to the nominal content 20 mg/mL. In addition, we used Student's criteria to compare these results as independent. The calculated value t_{exp} = 1.675 is significantly lower than critical value t (P = 0.95; f = 2). Therefore, these two samples are characteristic of one set.

In addition, we compared our results with those obtained from testing laboratory. The value indicated in certificate was taken as true value of nefopam content. In this case our result 19.4 mg/mL significantly differs from the true value (recovery 94.7 %). We suggested that this is regularly since the first peak is not sufficiently clear at low concentrations of nefopam. This makes it difficult to measure current and decreases the accuracy. Thus for analytical determination it is better to use only the second peak (recovery 96.7 %).

3.8 Analysis of Model Human Urine Samples

The developed method was also utilized for the determination of nefopam metabolite (N-oxide) in human urine samples. The detailed technique of samples preparation is presented in 2.4. N-oxide of nefopam is reduced at the background of urine at DME giving only one peak at potential −1.07 V. Obviously, this is because of matrix effect. The current of background line of urine sample (without nefopam N-oxide) strictly increased when potential of electrode became more negative, but no peaks

Table 3. Analytical parameters of calibration graph at background of BR buffere solution. pH_{oxid} 8.0, pH_{pol} 5.5, $C_{KPMS} = 1.0 \cdot 10^{-4}$ mol L^{-1}, $t_{oxid} = 10$ min. For p-AgSAE $E_{acc} = -0.75$ V and $t_{acc} = 120$ s.

Analytical parameter	DME		p-AgSAE	
	1 peak	2 peak	1 peak	2 peak
Scan rate v, V/s	0.5		3.0	
Peak potential E, V	−0.92	−1.24	−1.26	−1.38
Linear concentration range, mol L^{-1}	$1.0 \cdot 10^{-6}$–$2.0 \cdot 10^{-5}$		$1.0 \cdot 10^{-7}$–$8.0 \cdot 10^{-7}$	
Slope $b \pm \triangle b$, μA mol L^{-1}	$(7.78 \pm 0.15) \cdot 10^{3}$	$(1.33 \pm 0.03) \cdot 10^{4}$	$(1.16 \pm 0.05) \cdot 10^{6}$	$(8.9 \pm 0.3) \cdot 10^{5}$
Intercept $a \pm \triangle a$, μA	0.0778 ± 0.0015	0.106 ± 0.003	0.50 ± 0.03	0.962 ± 0.015
Corelation coefficient, R	0.9975	0.9957	0.9951	0.9971
RSD	0.01706	0.05024	0.0212	0.0125
Limit of quantiation (LOQ), mol L^{-1}	$1.9 \cdot 10^{-6}$	$2.5 \cdot 10^{-6}$	$2.2 \cdot 10^{-7}$	$1.7 \cdot 10^{-7}$
Limit of detection (LOD), mol L^{-1}	$6.2 \cdot 10^{-7}$	$8.2 \cdot 10^{-7}$	$7.3 \cdot 10^{-8}$	$5.6 \cdot 10^{-8}$

were registered (Figure 6C). This contributes to the selectivity of the determination, but has a negative effect on the sensitivity of the determination. The minimum quantity of nefopam hydrochloride, which can be detected using our method, is $1.03 \cdot 10^{-6}$ mol L^{-1} or 7.3 μg per 1 mL of urine. Limit of quantiation respectively is $3.1 \cdot 10^{-6}$ mol L^{-1} or 22.5 μg per 1 mL of urine. We introduced standard solution of nefopam N-oxide into urine and compared the data of quantitative determination obtained experimentally by method of standard additives with introduced amount. It was revealed that for N-oxide content within the range 25–40 μg per 1 mL urine, the recovery was 110 %. When the content of nefopam N-oxide was 55–100 μg per 1 mL urine, the recovery fluctuated from 95 % to 105 %. The entire analytical performance of the technique can be improved by entering the separation step (extraction procedures and/or utilization of HPLC with electrochemical detection).

4 Conclusions

The number of methods known in literature for nefopam determination is scarce. In this work, we used simple unified procedure for obtaining of nefopam N-oxide capable to electrochemical reduction. The oxidation of nefopam to its N-oxide using potassium peroxymonosulfate is very similar to the oxidation of other compounds containing tertiary amino group. The maximum yield of nefopam N-oxide was obtained in weakly alkaline medium, whereas it is advisable to reduce N-oxide in weakly acidic medium. New methods of voltammetric determination of nefopam characterized by wide linear range (1.5–2 orders of magnitude), low limit of determination (at the level of 10^{-6} mol L^{-1}), sufficient accuracy and selectivity, low cost, fast, portable and adequate concept of routine pharmaceutical analysis were developed. The main feature of the proposed method for the determination of nefopam using N-oxide as an analytical form is the ability to determine directly the metabolite of nefopam. A modern aspect of this work is the use of p-AgSAE, which effectively can replace mercury electrodes and chemically modified electrodes.

Acknowledgement

This work was financed by the Ministry of Education and Science of Ukraine (Grant number 0116U001541). We express our sincere gratitude to Bohdan Yosypchuk (J. Heyrovsky Institute of Physical Chemistry of the Czech Academy of Sciences, Dolejskova 3, 182 23 Prague8, Czech Republic) for the working electrode p-AgSAE used in this work.

References

[1] M. V. Bondar, M. M. Pylypenko, D. B. Areshnikov, *J. Emergency Med.* **2018**, *5*, 71.
[2] S. A. Shama, A. S. Amin, *Spectrochim. Acta Part A* **2004**, *60*, 1769.
[3] M. Starek, M. Dabrowska, M. Tarsa, *Acta Chim. Slov.* **2011**, *58*, 262.
[4] S. Singh, N. Sharma, Y. P. Singla, *Int. J. Pharm. Pharm. Sci.* **2016**, *8*, 414.
[5] L. C. Burton, N. J. Loftus, D. W. Vere, *J. Chromatogr.* **1990**, *526*, 159.
[6] G. Aymard, D. Warot, P. Démolis, *J. Pharm. Biomed. Anal.* **2002**, *30*, 1013.
[7] Q. Xu, A.-J. Yuan, R. Zhang *Curr. Pharm. Anal.* **2009**, *5*, 144.
[8] L. O. Dubenska, M. Y. Blazhejevskyj, S. I. Plotycya, *Methods Objects Chem. Anal.* **2017**, *12*, 61.
[9] S. A. Ozkan, B. Uslu, *J. Pharm. Biomed. Anal.* **2016**, *130*, 126.
[10] R. Flanagan, D. Perrett, R. Whelpton , *Electrochemical detection in HPLC analysis of drugs and poisons*, The Royal Society of Chemistry (RSC) **2005**, P. 239.
[11] J. Wang, *Electroanalytical techniques in clinical chemistry and laboratory medicine*, Wiley-VCH **1988**, P. 188.
[12] S. Plotycya, L. Dubenska, M. Blazhejevskyj, *Electroanalysis* **2016**, *28*, 2575.
[13] M. Blazheyevskiy, *Application of derivatization by means of peroxy acid oxidation and perhydrolysis reactions in pharmaceutical analysis*, Ivan Franko National University of L'viv, Lviv **2017**.
[14] J. Belej, J. Electroana, *Chem. Interfacial Electrochem.* **1986**, *214*, 481.
[15] W. V. Steele, E. H. Appelman, *J. Chem. Termodynamics* **1982**, *14*, 337.

[16] V. Zinchuk, O. Mytsuk, O. Stadnichuk, *Visnyk of the Lviv University. Series Chemistry* **2004**, *44*, 107 http://chem.lnu.edu.ua/visn44.htm.
[17] http://chem.lnu.edu.ua/mtech/devices.htm
[18] B. Yosypchuk, J. Barek, *Crit. Rev. Anal. Chem.* **2009**, *39*, 189
[19] B. Yosypchuk, L. Novotny, *Electroanalysis* **2002**, *14*, 1733
[20] B. Yosypchuk, L. Novotny, *Crit. Rev. Anal. Chem.* **2002**, *32,* 141
[21] B. Yosypchuk, J. Barek, O. Yosypchuk, *Electroanalysis* **2011**, *23*, 2226
[22] D. K. Gosser, *Cyclic voltammetry: Simulation and Analysis of Reaction Mechanisms.* New York: VCH Publishers, Inc. **1993**.
[23] A. M. O. Brett, C. M. A. Brett, *Electrochemistry Principles, Methods, and Applications, first ed. Oxford Science Publications*, Oxford, New York **1993**.
[24] K. Reybier, T. H. Y. Nguyen, H. Ibrahim, *Bioelectrochemistry* **2012**, *88,* 57.
[25] E. Moreno, S. Pérez-Silanesa, S. Gouravaram, *Electrochim. Acta.* **2011**, *56*, 3270.
[26] J. Ermer, J. H. M. Miller, *Validation in Pharmaceutical Analysis: A Guide to Best Practice*, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim **2006**.

Received: October 3, 2019
Accepted: November 21, 2019
Published online on December 9, 2019

