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POLAROGRAPHIC DETERMINATION OF ANABASINE IN A FORM OF ITS N-OXIDE IN URINE

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A new, simple method for the polarographic determination of the pyridine alkaloid – anabasine (AN) in the form of its N-oxide has been developed. Anabasine N-oxide (ANO) can be easily obtained in the laboratory, using potassium peroxymonosulfate (KPMS) as the oxidant reagent. In this work, the optimal conditions for obtaining the ANO are presented. Specifically, the pH of oxidation reaction is 9.1, the duration of the oxidation reaction is not less than 10 minutes, the temperature of the solution is 40 ºC, and there is a 5-fold excess of KPMS.

ANO is easily reduced in the Britton-Robinson buffer (BRB) medium at a dropping mercury electrode (DME), forming one peak at a potential of –0.83 V. The highest reduction current anabasine N-oxide, characterized by diffusion nature, can be achieved at pH 5.0.

A calibration curve for ANO in the concentration range of $2.0 \cdot 10^{-6}$–$4.0 \cdot 10^{-5}$ M was obtained. The limit of detection is $1.3 \cdot 10^{-6}$ M. The developed method was approbated on real human urine samples. An aliquot of ANO was injected into the urine samples.

Keywords: electrochemistry, polarography, alkaloids, anabasine N-oxide, medicinal substances.

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1. Introduction

Alkaloids are a large group of substances of plant origin that contain one or more nitrogen atoms, most often as part of a heterocyclic ring and reveal the main properties. Plants containing alkaloids have been used by people as medicinal and poisonous plants for a long time. Some of the most well-known alkaloids include morphine, caffeine, codeine, quinine, nicotine, anabasine, and atropine, among others. Towards the end of the 20th century, certain analogues of alkaloids with valuable pharmaceutical properties were successfully synthesized [1, 2].

Anabasine (AN) is an alkaloid of the pyridine group, serving as a structural isomer of nicotine. The plant that contains AN, anabasis, is poisonous to animals, as well as to humans (if it encounters the skin). In terms of pharmacological properties, the substance is like cytisine and lobeline. The AN is often prescribed for smoking cessation. In veterinary medicine, it is used for skin diseases in livestock, and in agriculture, as an insecticide for

disinfecting planting material, especially in citrus crops. The use of AN as a botanical insecticide may lead to its release into the environment, further causing the poisoning of humans and animals [3, 4].

AN and other tobacco alkaloids can be used as biomarkers for the use of tobacco, electronic cigarettes, or nicotine replacement therapy [5, 6]. There is a stable ratioof alkaloids AN /anatabine to the main metabolites of nicotine. So, it is possible to trace the pharmacokinetics and metabolism of nicotine by its content in human biological fluids in urine, as well as to study the tendency to use tobacco products by analysing domestic wastewater [7]. Such nicotine biomarkers are quite stable in wastewater because less than 30 % of AN is metabolized or transformed into other substances [8]. In Belgium and Australia, a study was conducted on the determination of AN and anatabine in wastewater [9–11], which showed that the use of tobacco products among the population can be traced by the content of AN and anatabine, regardless of therapeutically prescribed nicotine.

Different types of chromatography are used to control AN in-tobacco products, biological materials, vegetables, and wastewater [12–18]. However, most known chromatographic techniques are characterized by costly and/or time-consuming sample preparation. Additionally, the qualifications required for performers often limit the practical application of such methods in routine analytical practice. Therefore, the development of a fast, efficient, and cost-effective method that is user-friendly for everyday practice is a topical task. For these reasons, electrochemical methods provide a viable alternative to chromatography. Voltammetric methods of analysis offer high selectivity and sensitivity. They do not necessitate expensive equipment, are quick to use, can be automated, and align with the principles of green chemistry as they eliminate the need for organic solvents.

The objective of our work was to develop a new polarographic method for determining AN in the form of N-oxide. Anabasine N-oxide (ANO) can also be easily synthesised in the laboratory using an oxidation reaction. In our previous studies, we reported on the successful synthesis of alkaloids N-oxides using potassium peroxymonosulfate (KPMS) [19–22]. In this work, for the first time, we used the oxidation reaction of AN to its N-oxide using the oxidant reagent – KPMS.

2. Experimental

2.1. Apparatus

In this work, the digital device MTech OVA-410 with three-electrode cell (working dropping mercury electrode (DME), a saturated calomel reference electrode, and platinum wire auxiliary electrode) we used [23]. The applied DME had τ = 12 s in 0.1 M NH_4Cl with an open circuit. The current was measured at a fixed time (10 s) in the life of the drop.

The prepared solutions for measurement were transferred to an electrochemical cell, dissolved oxygen was removed with purified argon for 10 min, and polarograms were recorded in the potential range from 0 V to –1.5 V. The pH values of solutions were measured potentiometrically using pH-meter pH-150 MI with combined glass electrode.

2.2. Reagents

The substance AN (CAS No. 494-52-0) with the purity ≥ 99 % (Sigma-Aldrich) was used in the work. Stock standard solution (SSS) with AN concentration $1.0 \cdot 10^{-3}$ M was prepared as follows: the exact amount of AN substance was dissolved in double-distilled water in a 25.0 mL volumetric flask, the volume was brought to the mark, and the solution was mixed thoroughly. Stock standard solution was stored at the temperature +4 ºC for no longer than two weeks.

The Britton-Robinson buffer (BRB) was prepared using such reagents $Na_2B_4O_7 \cdot 10H_2O$, CH_3COOH, H_3PO_4 of analytical grade. The BRB was prepared according to [24]. Oxone ("extra pure" commercial triple potassium salt of Caro's acid) used as an oxidizing agent (ACROS ORGANICS, "extra pure", CAS 70693-62-8). The active ingredient of Oxone is potassium peroxymonosulfate $KHSO_5$, which is present as a component of a triple salt potassium hydrogen peroxymonosulfate sulfate (KPMS) The solution of $1.0 \cdot 10^{-2}$ M KPMS was prepared by diluting the exact amount of this substance in double-distilled water in a 100 mL volumetric flask, bringing the volume to the mark and mixing thoroughly.

Preparation of solution ANO as follows: 4 mL of BRB solution with pH 9.1, aliquot of SSS ANO (for example, 1 mL of $4.0 \cdot 10^{-5}$ M) was transferred into the beaker, and the beaker was immersed into a water bath at 40 °C until the necessary temperature of 40 °C was achieved (additionally, the temperature was controlled by thermometer). Then, 0.5 mL of $1.0 \cdot 10^{-2}$ M KPMS was added. The solution was stirred and kept for 10 min, and after that was added 2.0 M HCl to decrease the pH of the solution to 5.0 (controlled by pH-meter). The mixture was transferred into a 25 mL volumetric flask; and finally, the volume was brought with double-distilled water to the mark.

2.3. Sample preparation of urine samples for polarographic analysis

Urine samples of drug-free human were taken from one non-smoking and healthy volunteer (female, 24 years old), who did not use tobacco products and nicotine-containing therapy drugs, did not undergo any medical or multivitamin treatment, on an empty stomach on the day of the experiment. 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 BRB solution with pH 4.5, 0.2 mL of $1.0 \cdot 10^{-2}$ M KPMS, and 1 mL of saturated KCl solution were transferred into 25 mL volumetric flask. Then, the obtained solution was enriched with aliquots of ANO in the concentration range of $2.0 \cdot 10^{-6}$–$4.0 \cdot 10^{-5}$ M and filled with double-distilled water to the mark. Prepared solution was transferred into the electrochemical cell, purified with argon for 10 min to remove dissolved oxygen, and polarogramms were recorded in the range from 0 V to –1.5 V.

3. Results and discussion

Figure 1 shows polarogramms in AN solution before oxidation and in the absence of KPMS (*1*), in solution of KPMS and the absence of AN (*2*), and in solution after oxidation of AN (*3*). On the polarogramms 1 and 3, one unclear peak can be traced at a potential of –1.12 V, which probably corresponds to the electrochemical reduction of the pyridine fragment in the AN molecule [25]. The reduction of ANO at DME is characterized one peak at the potential –0.83 V.

No changes at anodic brunch of the polarogram were observed. Therefore, in further work, the study of the electrochemical behaviour of ANO and optimal conditions were determined using cathodic linear sweep voltammetry.

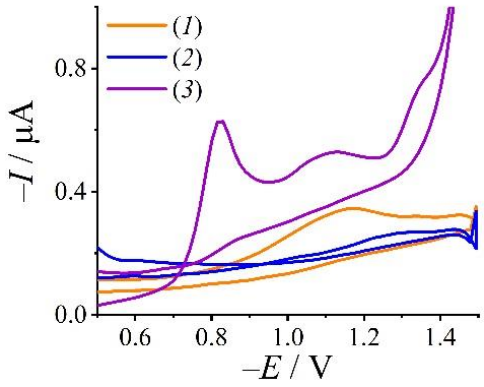
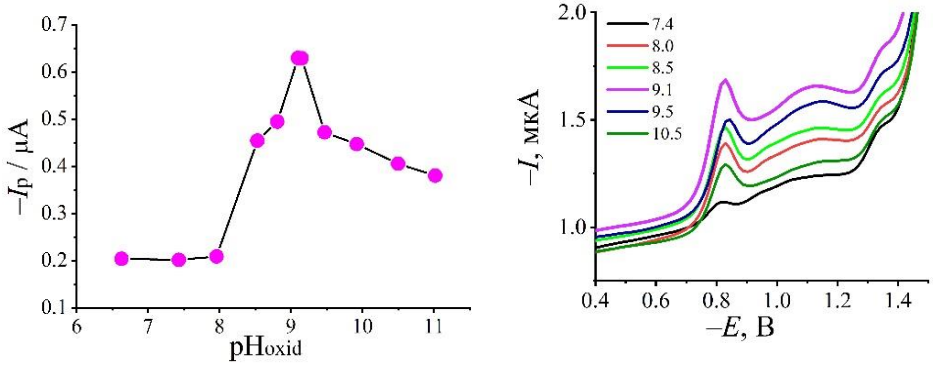


Fig. 1. Polarogramms in AN solution before oxidation and in the absence of KPMS (*1*), in solution of KPMS and the absence of AN (*2*), and in solution after oxidation of AN (*3*). C_{AN} = 4.0·10^{-5} M, C_{KPMS} = 1.0·10^{-4} M, pH = 5.0, V = 0.5 V/s

3.1. Optimization of optimal conditions for obtaining of ANO

N-oxides of alkaloids are formed in an alkaline medium. Acidation of reaction mixture leads to stop the oxidation process [19–22]. The appearance of the polarograms of the reduction of ANO depends on the pH at which AN was chemically oxidized. The dependence of the reduction current of ANO on the value of pH oxidation was studied in the pH range from 6.5 to 11.0 (Fig. 2, *a*). The optimal value of pH for AN oxidation is pH 9.1 (Fig. 2, *b*).

The shape of polarograms in solutions of ANO, particularly the value of the reduction current, is influenced by the time duration of chemical oxidation, the temperature of the solution during oxidation, and the molar excess of the KPMS.



a *b*

Fig. 2. The dependence of the reduction current of ANO on the values of pH oxidation (*a*) polarograms in solutions of ANO with different values of pH oxidation (*b*). C_{AN} = 4.0·10^{-5} M, C_{KPMS} = 1.0·10^{-4} M. Polarograms were registered at pH= 5.0 and V = 0.5 V/s.

AN oxidizes slowly at room temperature, and heating the reaction mixture favours the process. The effect of temperature solutions during the chemical oxidation of AN on the further reduction of N-oxide anabasine at the DME was studied as follows: all reagents (BRB with pH = 9.1, AN and KPMS) were mixed in the required ratio in a beaker, the beaker was immersed in a water bath with the appropriate temperature and the solution was kept for 10–15 minutes, after which hydrochloric acid was added to pH = 5.0 and cooled the solution to room temperature. To oxidize AN, it is sufficient to heat the solution to a temperature of 40 °C (Fig. 3, *a*).

Moreover, it is sufficient to heat the solution during oxidation to a temperature of 40 °C for 10–15 min (Fig. 3, *b*) – then the maximum value of the reduction current was observed, which did not change due to longer heating.

Another important studied factor was the concentration of KPMS. The maximum yield of the product can be achieved with no less than a 5-fold excess of KPMS (Fig. 3, *c*). However, it is desirable that the concentration of KPMS does not exceed 10^{-4} M since higher concentrations of KPMS lead to enhancement and fluctuating of background current.

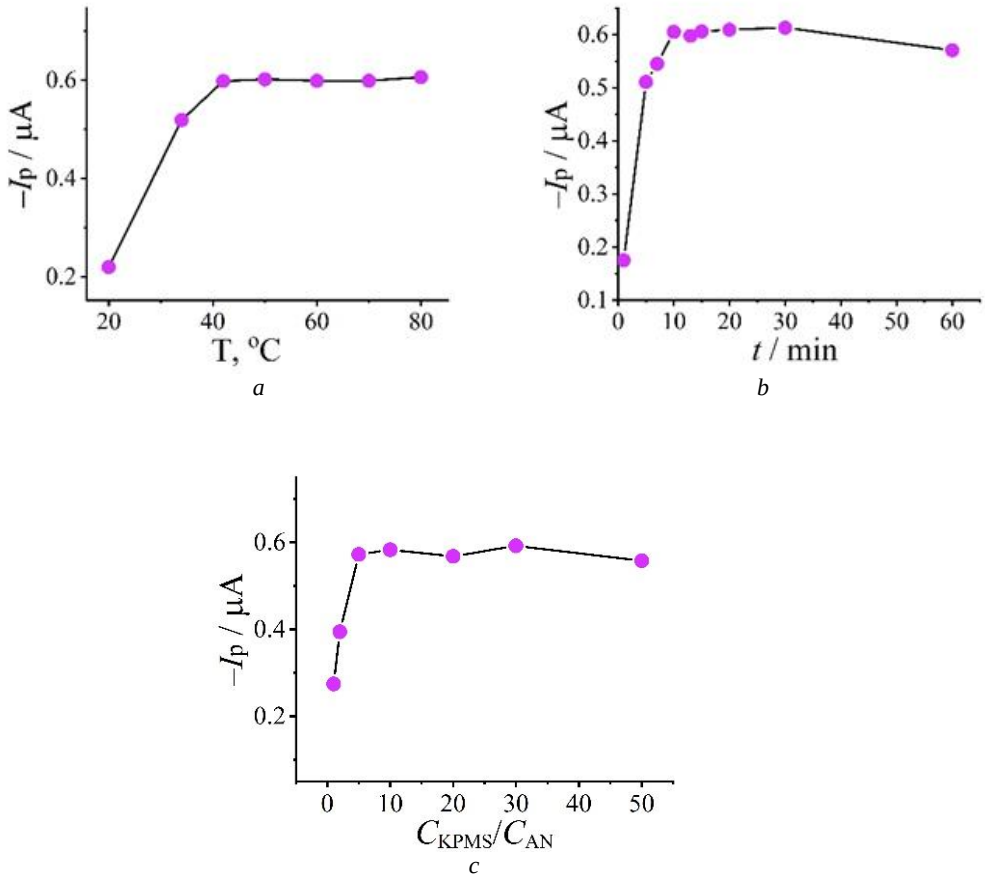
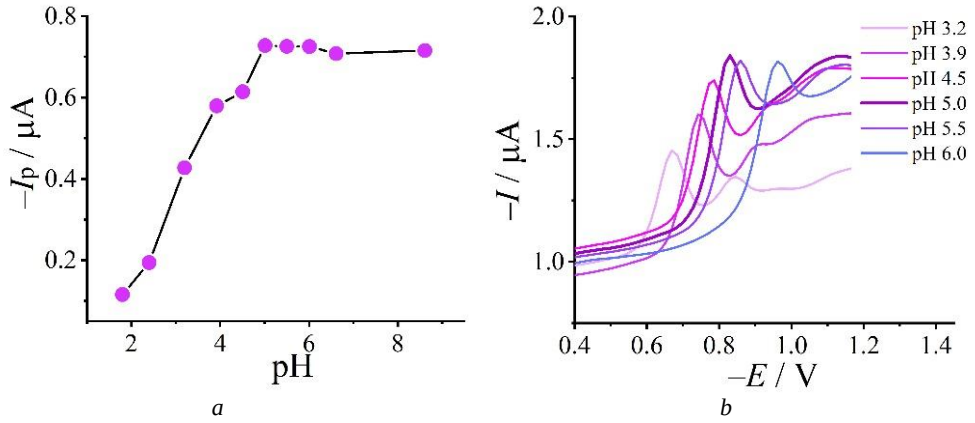


Fig. 3. The effect a temperature of solution during the chemical oxidation of AN (*a*) oxidation time at the temperature 40 °C (*b*) and molar ratio of reaction components (*c*) on reduction current of ANO pH_{oxid} = 9.1, pH = 5.0; $C_{AN} = 4.0 \cdot 10^{-5}$ M, V = 0.5 V/s

3.2. The electrochemical behaviour of ANO at the DME

3.2.1. The effect of pH on reduction ANO

The effect of pH on the reduction of ANO was studied in the pH range from 1.7 to 9.0 (Fig. 4, *a*). The shape of polarogramms, current and potential of ANO reduction are dependent on solution pH. (Fig. 4, *b*). The highest reduction current of ANO is traced from a weakly acidic to a weakly alkaline medium (pH = 5.0–8.0). For further investigations, we have chosen the value of pH 5.0 as the optimal value of pH reduction of ANO.



a *b*

Fig. 4. The dependence of the reduction current of ANO on the values of pH the solution (*a*) polarograms in solutions of ANO with different values of pH (*b*).
C_{KPMS} = 1.0 ·10^{-4} M, pH_{oxid} = 9.1, C_{AN} = 4.0 ·10^{-5} M, V = 0.5 V/s.

Since the process is rather complicated, the potentials of the reduction peaks of ANO are shifted to a negative direction with increasing pH. This behaviour demonstrates that the electrochemical reduction of ANO involves proton transfer stage. The dependence of $-E$ vs. pH is linearity in the range of pH from 2.4 to 5.5:

$$-E = (0.429 \pm 0.013) + (0.079 \pm 0.003)\ \text{pH} \qquad R = 0.9968$$

The ratio of the number of protons to the number of electrons (xH^+/n) involved in the electrochemical process was calculated based on a slope of the dependence: $dE/d\text{pH} = (2.3RT \cdot xH^+)/\alpha nF$ (where α is the charge transfer coefficient) [27]. The obtained value xH^+/n was about 0.7.

3.2.2. Effect of scan rate

An important factor affecting the electrochemical process is the scan rate. Scan rates studies were performed in the range from 0.2 to 4.0 V/s at DME. The heights of the reduction signals increased with scan rate and the peak potentials shifted toward more negative values (Fig. 5, *a*). This is characteristic of an irreversible electrode process.

According to the dependence log I_p – log V, the nature of the reduction current of the ANO was established (Fig. 5, *b*). The slope value of this dependence was 0.63. It is possible to conclude that the reduction of ANO at DME is controlled by the diffusion with minor adsorption [27].

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 (Fig. 5, *c*):

$$–I_p = (–0.061 \pm 0.011) + (0.377 \pm 0.008)\, V^{½} \qquad R = 0.9968$$

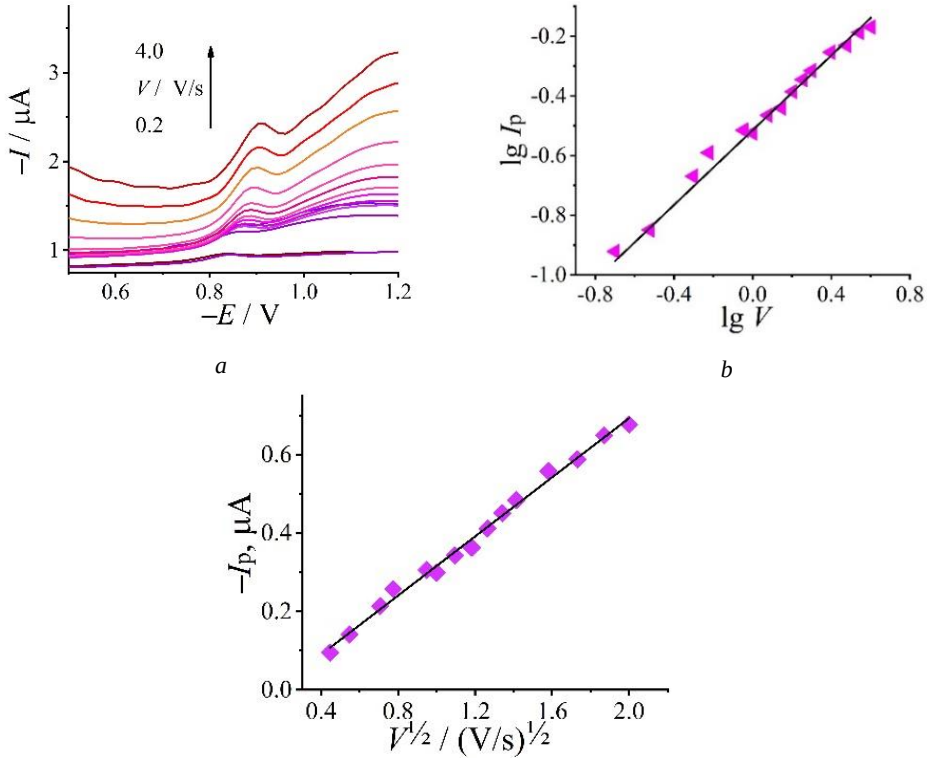


Fig. 5. Polarograms with different scan rates in solutions of ANO (*a*) з dependence lg I vs. lg V (*b*) and $–I_p$ vs. $V^{1/2}$ (*c*). C_{KPMS} = 1.0·10^{-4} M, pH_{oxid} = 9,1, C_{AN} = 1.0 ·10^{-5} M, pH = 5.0

The number of electrons (n) 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 [28]. The obtained value was 0.98. This result allows us to assume that one electron and one proton participate in the electrochemical reaction of the reduction of ANO.

3.2.3. Determination of analytical parameters

We obtained a calibration curve within the concentration range of $2.0 \cdot 10^{-6}$–$4.0 \cdot 10^{-5}$ M (Fig. 6). In Figure 6, *a* "0" means background line, and 1–9 – polarogramms, which correspond to concentrations of ANO from $2.0 \cdot 10^{-6}$ to $4.0 \cdot 10^{-5}$ M.

The analytical parameters are presented in Table 1. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using LOD = 3.3 S_a/b and LOQ = 10 S_a/b, where S_a is the standard error of the intercept value and b is the slope of the calibration curve [29].

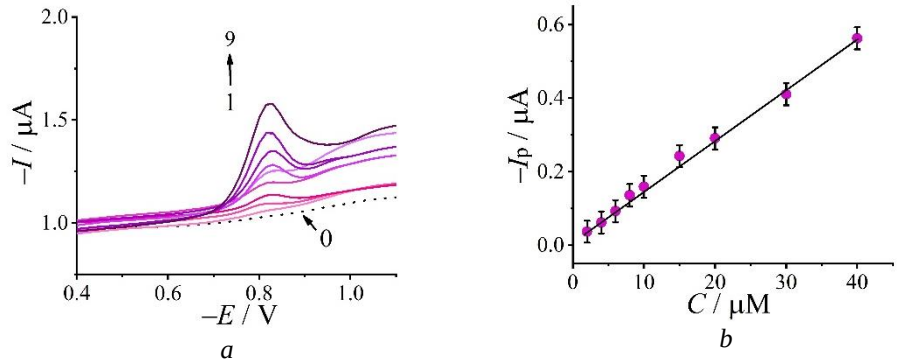


Fig. 6. Polarograms (*a*) and a calibration curve (*b*) obtained for the reduction of ANO in the medium BRB on DME. "0" means background line, and 1–9 – polarogramms, which correspond to concentrations of ANO from $2.0 \cdot 10^{-6}$ to $4.0 \cdot 10^{-5}$ M. $C_{KPMS} = 1.0 \cdot 10^{-4}$ M, $pH_{oxid} = 9.1$, pH = 5.0, V = 0.5 V/s

Table 1

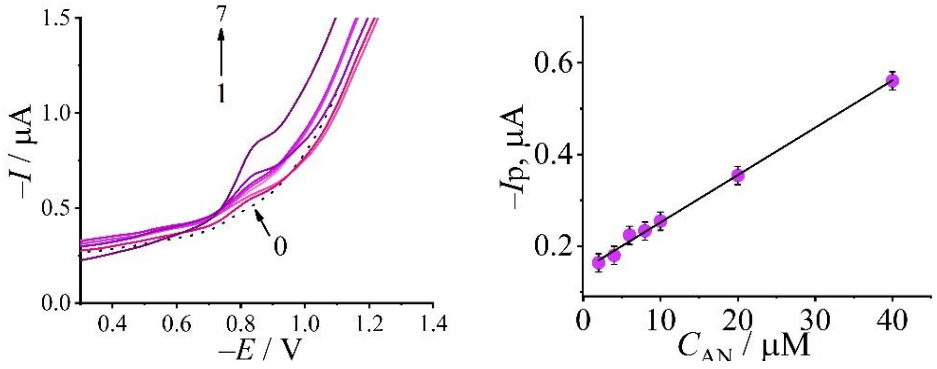
Analytical parameters of the methods of ANO determination at the background BRB and urine

Matrix / Analitycal parameters	BRB	Urine
Peak potential, E, V	–0.83	–0.87
Linear concentration range, M	$2.0 \cdot 10^{-6}$–$4.0 \cdot 10^{-5}$	$2.0 \cdot 10^{-6}$–$4.0 \cdot 10^{-5}$
Slope b, μA M	$1.36 \cdot 10^{4}$	$1.03 \cdot 10^{4}$
S_b	$0.029 \cdot 10^{4}$	$0.025\ 10^{4}$
Intercept a, μA	0.016	0.148
S_a	0.005	0.004
Correlation coefficient, R	0.99817	0.99858
RSD	0.011	0.008
LOQ, M	$3.90 \cdot 10^{-6}$	$4.25 \cdot 10^{-6}$
LOQ, μg/mL	15.91	17.21
LOD, M	$1.30 \cdot 10^{-6}$	$1.40 \cdot 10^{-6}$
LOD, μg/mL	5.30	5.67

3.2.4. Polarographic determination of ANO in urine

Human urine samples as the matrix for analysis were chosen for the approbation of the method. Because AN is a stable biomarker for controlling the use of tobacco products, which can be easily determined in urine.

ANO is reduced forming a single peak at the potentials of –0,87 at the background of human urine, however, the "quality" of this peak (signal) is much worse than for blank aqueous solutions. In Figure 7 *a* "0" shows the background line (without ANO additives) and polarogramms which were obtained for solutions with concentrations of ANO from $2.0 \cdot 10^{-6}$ M to $4.0 \cdot 10^{-5}$ M. The analytical parameters of calibration graphs are summarized in Table 1. Therefore, the minimum quantity (LOD) of ANO that can be detected in urine is LOD = $1.40 \cdot 10^{-6}$ M or 5.67 μg per 1 mL of urine. The LOQ, respectively, is equal to $4.25 \cdot 10^{-6}$ M or 17.21 μg per 1 mL of urine.



a *b*

Fig. 7. Polarograms (*a*) and a calibration curve (*b*) obtained for the reduction of ANO at the background of human urine on DME. "0" means background line, and 1–7 – polarogramms, which correspond to concentrations of ANO from $2.0 \cdot 10^{-6}$ to $4.0 \cdot 10^{-5}$ M. $C_{KPMS} = 1.0 \cdot 10^{-4}$ M, $pH_{oxid} = 9.1$, pH = 5.0, $V = 0.5$ V/s

To check the accurasy of the method, we added standard solutions of ANO into urine and compared the experimentally obtained data of ANO quantitative determination with the added amount, according to the method of standard additives (Table 2). The value of recovery is only 90.4 % for a low content of ANO (at the level of 8 μg/mL). This is obviously due to the influence of matrix components of urine. However, the value of recovery varies between 99.7 and 102.8 % for higher concentrations of ANO (from 24 to 160 μg/mL).

Table 2

Results of ANO determination in urine, according to the method of standard additives

Added ANO, μg/mL	Found ANO, μg/mL	Recovery, %
8.11	7.33 ± 0.04	90.4
16.22	15.29 ± 0.04	94.2
24.33	24.463 ± 0.021	100.5
32.45	33.25 ± 0.03	102.5
40.56	41.675 ± 0.017	102.8
81.12	80.723 ± 0.027	99.5
162.23	161.76 ± 0.03	99.7

4. Conclusions

A new simple method of polarographic determination of the pyridine alkaloid – AN in the form of its N-oxide has been developed. ANO can easily be obtained in the laboratory using KPMS. This work focused on optimizing the conditions for obtaining ANO, specifically the pH during oxidation, the duration of the oxidation process, the solution temperature, and the concentration of reagents.

It was determined that the highest yield of ANO is achieved under the following conditions: the pH during oxidation of 9.1, employing no less than a 5-fold excess of KPMS, an oxidation duration of no less than 10 minutes at a temperature of 40 °C.

Under the conditions of polarography, ANO is easily reduced at the background of the BRB (pH = 5.0), in the form of a single peak at a potential of – 0.83 V. The reduction current for ANO exhibits a diffusion-controlled nature. A calibration curve was constructed within a concentration range of $2.0 \cdot 10^{-6}$–$4.0 \cdot 10^{-5}$ M, with a limit of detection was $1.3 \cdot 10^{-6}$ M.

This newly developed technique was tested for its applicability in the analysis of human urine. We introduced standard solution of ANO into urine and compared the data of quantitative determination obtained experimentally by method of standard additives with introduced amount. It was determined that for an introduced ANO content, the recovery was 90.4 % for ANO at a concentration of 7–10 μg per 1 mL of urine and 102.7 % for ANO at a concentration of ≥150 μg per 1 mL of urine. Therefore, the developed method is recommended for monitoring AN as a biomarker of tobacco use in human urine.

5. Acknowledgment

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ПОЛЯРОГРАФІЧНЕ ВИЗНАЧЕННЯ АНАБАЗИНУ У ФОРМІ ЙОГО N-ОКСИДУ У СЕЧІ

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Розроблено нову просту методику полярографічного визначення алкалоїда піридинового ряду анабазину, яка ґрунтується на перетворенні анабазину в його N-оксид. Анабазин є структурним ізомером нікотину, який так само як інші алкалоїди тютюну можна використовувати як біомаркери використання тютюну, електронних сигарет або нікотинзамісної терапії. N-оксид анабазину можна легко отримати в лабораторії, використовуючи як окисник калій пероксимоносульфат (КПМС). У цій праці подано оптимальні умови отримання N-оксид анабазину, зокрема для окиснення анабазину треба створити pH 9,1, реакція окиснення повинна тривати не менше 10 хв, температура розчину 40 ºС та не менше, ніж 5-кратний надлишок окисника.

На ртутному краплинному електроді N-оксид анабазину легко відновлюється в середовищі універсальної буферної суміші. На полярограмі простежується один за потенціалу –0,83 В. Найвищого струму відновлення можна досягти за pH 5,0. Згідно з логарифмічною залежністю струму від швидкості розгортки напруги поляризації з'ясовано, що струм відновлення N-оксиду анабазину має дифузійну природу.

Отримано градуювальний графік у діапазоні концентрацій N-оксиду анабазину від $2{,}0\cdot10^{-6}$ М до $4{,}0\cdot10^{-5}$ М. Межа виявлення N-оксиду анабазину становить $1{,}3\cdot10^{-6}$ М. Розроблену методику апробували на реальних зразках сечі людини. У сечу вносили стандартизований розчин N-оксиду анабазину та порівнювали дані кількісного визначення, отримані експериментально методом стандартних добавок, із уведеною кількістю. Визначено, що для уведеної кількості N-оксиду анабазину від 7 мкг до 10 мкг на 1 мл сечі ступінь визначення становив 90,4 %, а для умісту понад 150 мкг на 1 мл сечі – 102,7 %.

Розроблений метод можна рекомендувати для моніторингу анабазину як біомаркера вживання тютюну в сечі людини. Ця методика може стати важливим інструментом для ефективного контролю вживання тютюнових виробів у сучасному суспільстві.

Ключові слова: електрохімія, полярографія, алкалоїди, N-оксид анабазину, лікарські речовини.

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