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DIAZOTATION AND AZO COUPLING AS DERIVATIZATION REACTIONS FOR POLAROGRAPHIC DETERMINATION OF SOME LOCAL ANESTHETICS

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We have determined that diazonium salts of anaesthesin and novocaine are suitable derivatives for the polarographic determination of these anesthetics. The dependence of the current of the reduction peak of diazonium salts and azo compounds on the concentration of anesthetics is linear with good metrological characteristics. The individual polarographic determination of anaesthesin and novocaine in their mixture in the form of diazo- or azo compounds is not possible because of overlapping the reduction peaks related to individual anesthetics. However, it is possible to determine the total amount of the investigated local anesthetics by summing the heights of the peaks or peak areas of the anaesthesin and novocaine derivatives using the calibration plot as well as the method of additives. The developed methods of polarographic determination of anaesthesin and novocaine in the form of their derivatives were checked by analyzing model solutions and pharmaceuticals (a solution for the injections of novocaine, a combined antiseptic drug «Farysil»). The metrological characteristics of lidocaine determination in the form of 2,6-dimethylaniline diazonium salt are poor, and the method of derivatization is complicated and long-continued.

Keywords: anesthetics, anaesthesin, novocaine, diazotation, azo coupling, polarography.

Introduction

Local anesthetics (LA) are medications that are widely used not only in anesthesiology but also in ophthalmology, ENT practice, treatment of chronic and oncological pain, etc. Mostly they are organic compounds in the form of well soluble salts. The mechanism of anesthetic action is associated with the alteration of electrochemical processes in the nerves or nerve fibers.

The use of LA is strictly dosed. Anesthetics in elevated concentrations negatively affect the human body, up to the fatal outcome. Even therapeutic doses of drugs can cause negative effects when there is individual intolerance. The maximum daily doze is 3, 10 and 21 mg/kg for lidocaine, novocaine and anaesthesin, respectively. Thus, there is a need to develop fast and reliable methods for the determination of anesthetics in drugs and biological objects with complex multi-component matrices [1]. Safe and effective use of drugs requires multi-level control of their quality at all stages of production, from the synthesis of the substance to the finished medications.

The State Pharmacopeia of Ukraine (SPU) and the British and European Pharmacopeia suggest determining the content of main compound in substances of anaesthesin and novocaine by nitritometric titration method, acidimetry in non-aqueous medium is suggested for lidocaine. The quantitative determination of anesthetics in a substance can be carried out by non-pharmacopoeial methods, such as inverse bromometric titration with iodometric endings and reverse iodochlorometric titration for anesthesin; alkalimetrical technique by quantitative determination of bound hydrochloric acid (titration is carried out in the presence of chloroform that extracts the released base) or argentometrical method for lidocaine and novocaine.

Classical methods for the determination of local anesthetics used in pharmacy are not sufficiently sensitive and selective; thus, different physicochemical methods are currently being used as follows: spectrophotometry, ionometry [2] and chromatography [3,4]. Since a lot of pharmacologically active compounds exhibit electrochemical activity, so they can participate in electron transfer reactions. For this reason, electroanalytical methods become more and more

widely used in pharmaceutical analysis. Thus, the first works dealing with polarography appeared in the fifties of the last century. The reviews [5–9] are devoted to the use of different types of voltammetry (VA) for the determination of medicinal substances (MS). Universal method seems to be cyclic voltammetry (CV). This method is used to study the mechanisms of reduction or oxidation, stability, adsorption capacity, kinetics of hydrolysis of MS, etc.; it is used as a method of analytical quantitative determination too. The efficiency of the method is determined by the rapid registration of oxidation-reducing processes in a wide range of electrode potentials. Along with the CV, linear VA (with fast scan) is very important to understand the electrode processes; it is characterized by a low limit of quantitation and a fast response. In spite of the variety of voltammetric methods and types of electrodes, many mercury-based electrodes continue to be used in many control laboratories: dropping mercury electrode (DME), stationary or hanging mercury drop, mercury film and amalgam electrodes. For the determination of substances that cannot be reduced on a mercury electrode or are reduced in too far cathodic region of potentials, unified methods of quantitative determination in the form of appropriate derivatives have been developed. These methods are based on the introduction of electrochemically active functional groups into the analyte molecule, which, in fact, is used for further determination [10–12].

Diazo- and azo compounds are ones of the most common classes of organic reagents used in analytical chemistry. These reagents are used in spectrophotometry and voltammetry. Colorless or pale yellow diazonium salts are formed during the interaction in an acidic medium with nitric acid; these undergo azo coupling with phenols in alkaline solutions to form azo dyes of various shades of red color. Diazo- and azo compounds are easily reduced on mercury electrodes. These derivatives are good analytical forms for the qualitative analysis and quantitative determination of medications of various pharmacological groups (for instance, derivatives of p-aminobenzoic acid (PABA) and p-aminosalicylic acid, sulfanilamides, etc.) by various physicochemical methods of analysis [13,14].

The aim of this work was to investigate the possibility of using diazotation and azo coupling for the polarographic determination of LA such as lidocaine, novocaine and anaesthesin, derivatives of PABA, and, further, develop a method for their quantitative determination in the form of their derivatives in finished dosage forms.

Experimental

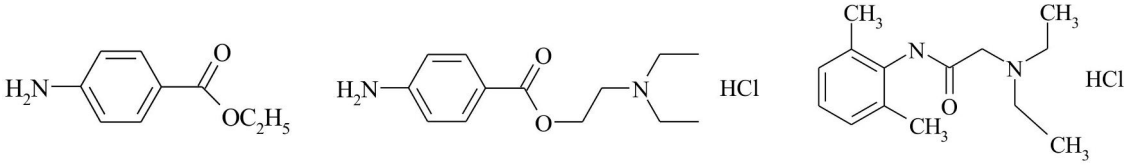
Materials

The structural basis of modern anesthetics is PABA. It exhibits high biological activity and is a structural fragment of folic acid. Also, PABA is a part of the enzymatic complex necessary for the life of living organisms, and in addition it is a factor in the growth of bacteria. Esters of PABA exhibit anesthetic effect and are synthetic substitutes for cocaine, which historically was the first anesthetic.

Novocaine (procaine, β-diethylaminoethyl ester of p-aminobenzoic acid hydrochloride) is one of the oldest local anesthetics used in medicine (Fig. 1). It was synthesized by Einhorn in 1898 as an alternative to cocaine. So, the name of novocaine originates from the «new cocaine». In the twentieth century, it was also used as a standard (equivalent) to determine the force of local anesthetics on the human body.

Novocaine exists in the form of colorless crystals or white odorless crystalline powder. It is very well soluble in water (1:1), easily soluble in ethanol (1:8), practically insoluble in ester and chloroform; its melting temperature is 154–156^{0}C. It is easily hydrolyzed in an alkaline medium. 0.1 M solution of hydrochloric acid was added to maintain pH 3.8–4.5 to stabilize novocaine solution [6]. The dosage forms of novocaine are powder, ointment, rectal suppository, and solutions for injection (0.25%, 0.5%, 1%, 2% in 1.25 mL and 10 mL).

Anaesthesin (ethyl ester of PABA) exists in the form of colorless crystals or white odorless crystalline powder, weakly bitter to taste. It is soluble in ethanol (1:5), chloroform (1:3), dichloroethane, worse soluble in toluene and benzene. It is difficult to dissolve in greasy oils and diluted hydrochloric acid, preferably in hydrochloric acid with a concentration of above 4.5–5%. Anaesthesin is very little soluble in cold water, a little better in hot water (89–91.5^{0}C). It is



a **b** **c**

Fig. 1. Graphic formulas of anaesthesin (a), novocaine hydrochloride (b), lidocaine hydrochloride (c)

used in the form of 5–10% ointment or powder. For anesthesia of mucous membranes, 5–20% oil solutions are used. It is orally prescribed in powders or tablets. Anaesthesin is an active component of many medicines, for example, it is a part of the «Relief Advance» candles and ointments, «Dentol» gel, «Menovazin» solution and ointment, «Belastezin» and «Farasil» tablets, «Anesthesol» suppositories and «Dentispray» spray, and «Amprovisol» aerosol.

Lidocaine hydrochloride (xycaine, diethylamino-2,6-dimethylacetanilide hydrochloride) is a white to slightly yellowish crystalline powder of bitter taste. It is soluble in water, alcohol and chloroform, insoluble in ester, its melting temperature is 74–79^{0}C. Unlike novocaine, it is not an ester; therefore, it is more slowly metabolized in the body and its action is more prolonged. In the body it does not form PABA; hence, it does not exhibit anti-sulfanilamide activity and can be used by patients taking sulfanilamide preparations. Dosage forms are as follows: ampoule solutions 1%, 2%, 10%, eye drops 2% and 4% in vials of 5 mL and dropper tubes of 1.5 mL. Abroad, the «Lidestin» aerosol for surface anesthesia is produced when changing bandages, disclosing abscesses, in dentistry and ENT-practice. Also, it is a constituent of the ointment «Aurodin», tablets «Trachisan» and «Strepsils plus».

The stock standard solutions of novocaine, anaesthesin and lidocaine were prepared from the substances by dissolving their precise amount. The following substances were used:

– novocaine hydrochloride, $C_{13}H_{21}ClN_2O_2$. Mol. wt. 272.8 g/mol; manufacturer – Guangxi Shentai Chemical Co.,Ltd. China;

– anaesthesin, $C_9H_{11}NO_2$. Mol. wt. 165.2 g/mol; manufacturer – Changzhou Sunlight Pharmaceutical Co., Ltd., China;

– lidocaine hydrochloride, $C_{14}H_{23}ClN_2O$. Mol. wt. 270.8 g/mol; manufacturer – Societa Italiana Medicinali, Italy.

Anaesthesin was dissolved in 0.25 M hydrochloric acid, lidocaine and novocaine were dissolved in double-distilled water, and finally double-distilled water was added to the mark. Aqueous solutions of lidocaine, novocaine and anaesthesin are acidic and resistant to storage.

Sodium nitrite in the presence of hydrochloric acid was used as a reagent for diazotation. The excess of sodium nitrite was destroyed by 1 M solution of urea. Alkaline solutions of β-naphthol, salicylic acid and resorcinol were used as the reagents for the azoic coupling component. $NaNO_2$, urea, β-naphthol, salicylic acid, and resorcinol were prepared by dissolving the precise amount in double-distilled water. The background electrolyte was a universal buffer mixture (UBM), Britton-Robinson's buffer, with an initial concentration of 0.42 M of each of the components. It was prepared by mixing 20 g sodium tetraborate, 12 mL of 17.5 M acetate and 18.5 mL of 15.4 M phosphate acid in 0.5 L volumetric flask and double-distilled water was added to the mark. pH of this buffer was 2.1, another required pH was adjusted with alkali according to pH meter. All reagents were qualified not less than «clear for analysis».

Equipment

Voltammetric measurements were carried out by means of a digital device equipped with personal computer and three-electrode cell (the reader is referred to http://chem.lnu.edu.ua/mtech/mtech.htm for more detailed information). An indicator dropping mercury electrode, a saturated calomel reference electrode and platinum wire auxiliary electrode were used. The dissolved oxygen from electrolytic cell was removed by purified argon for 10–15 min. All measurements were carried out at room temperature.

The solutions pH was controlled by MV 870 DIGITAL-pH-MESSERAT pH-meter with silver chloride reference electrode.

Class A of glassware was used in this work. To measure the volumes smaller than 0.5 mL, automatic single-channel pipettes «Thermo Scientific» were used.

Results and discussion

Investigation of the optimum conditions for the reaction of diazotation and reduction of novocaine, anaesthesin and lidocaine derivatives on DME

According to the SPU, novocaine and anaesthesin can be identified by the reaction of the primary aromatic amino group via diazotation followed azo coupling with в-naphthol in an alkaline solution. The reaction of diazotation is usually carried out for amine with sodium nitrite in an aqueous solution of mineral acid at cooling (~4^{0}C). The obtained diazonium salt of anaesthesin and novocaine is reduced on DME (Fig. 2).

Anaesthesin and novocaine undergo diazotation well both at 0^{0}C (in an ice bath) and at room temperature (18–20^{0}C), but at higher temperatures the process is complicated and the height of the derivatives reduction peaks decreases. Therefore, all further studies were performed at room temperature.

Hydrochloric, acetate, phosphate and sulfate acids were investigated as the medium for diazotation. When using hydrochloric acid, higher reduction currents of diazonium salts were obtained in

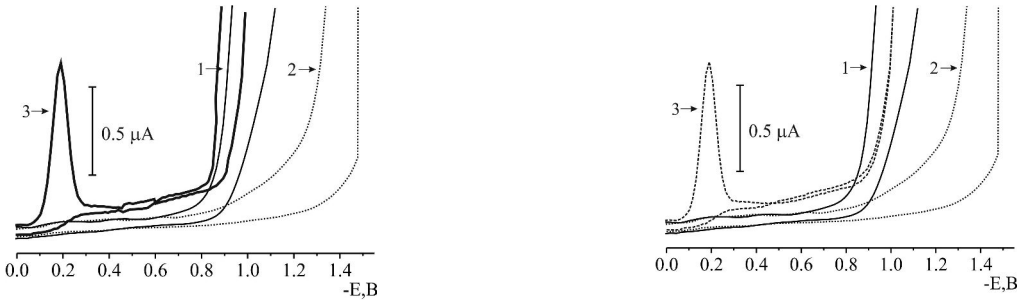


Fig. 2. Polarograms recorded in solution of $NaNO_2$ (1), (a) anaesthesin (2) and obtained diazonium salt of anaesthesin (3) and (b) novocaine (2) and diazonium salt of novocaine (3); the supporting electrolyte is 0.2 M UBM, $C(NaNO_2)=10^{-3}$ M, $C(HCl)=10^{-2}$ M, $C_{anaesthesin}=C_{novocaine}=5\cdot10^{-5}$ M, pH 3, $\upsilon=0.5$ V/s

comparison to other media. Anaesthesin and novocaine undergo diazotation also in acetate, phosphate and sulfate acids, but the value of the current is smaller, and the potential of reduction peak is also shifted. The sufficient concentration of hydrochloric acid for effective diazotation is ca. 10^{-2} M.

The change in the value of solution pH equally affects the polarographic characteristics of the anaesthesin and novocaine diazonium salts reduction using UBM as a supporting electrolyte (Fig. 3). The process of the reduction of anaesthesin and novocaine diazonium salts becomes more difficult: peaks are shifted to a negative region of potentials, which is characteristic of processes involving the H^+ ions. It is obvious that the «best» pH for the determination is pH in the range of 3 to 5, when the current and reduction potential do not substantially depend on the acidity of the medium. Further, all polarograms were recorded at these pH values.

The polarographic characteristics of the reduction of anaesthesin and novocaine diazonium salts do not substantially depend on the time of diazotation and the concentration of sodium nitrite. Sufficient time for diazotation is 3–5 min because the peak current did not increase after 5 min. The concentration of sodium nitrite should be 10^{-3} M.

Thus, the optimal conditions for anaesthesin and novocaine diazotation and for recording the polarograms of the corresponding derivatives are identical; therefore, we can propose a unified method for the determination of these anesthetics.

Method of polarographic determination of anaesthesin and novocaine in the form of their diazonium salts

This procedure is as follows:

– Add an aliquot of anesthetic solution, 1 mL of hydrochloric acid (C=0.25 M) and 0.25 mL of $NaNO_2$ solution (C=0.1 M) to a 50 mL beaker, and then leave the mixture for 3–5 min.

– Add 4 mL of UBM and use NaOH solution (C=2.5 M) to achieve pH 3 controlled by pH meter.

– Add double-distilled water to the mark to 25 mL volumetric flask. Then introduce the obtained solution into the cell, deoxygenate for 10 min, and record the polarogram by applying a linear potential scan from 0.0 to –1.0 V.

The dependence of the current (I, mA) of diazonium salts reduction on the concentration of anesthetics is linear with good metrological

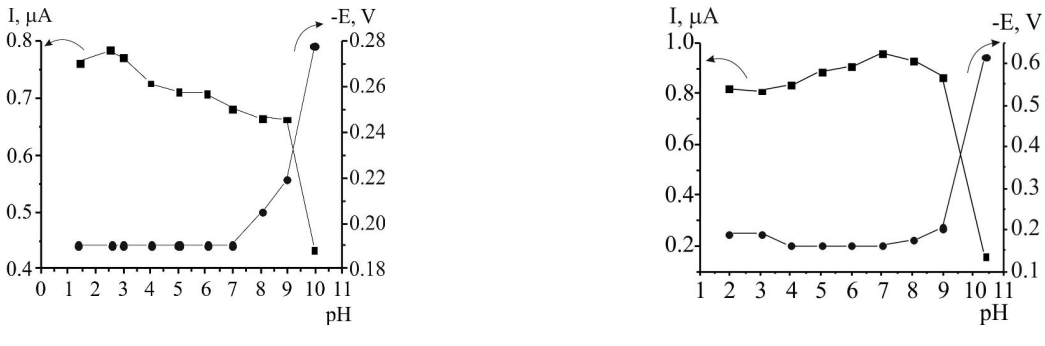


Fig. 3. Effect of pH value on the polarographic characteristics of anaesthesin (a) and novocaine (b) diazonium salts reduction. The conditions of diazotation are as follows: $C(NaNO_2)=10^{-3}$ M, $C(HCl)=10^{-2}$ M, $C_{anaesthesin}=C_{novocaine}=5\cdot10^{-5}$ M, t=5 min. The conditions of recording the polarograms are as follows: C(UBM)=0.2 M, $\upsilon=0.5$ V/s

Table 1

Parameters of calibration graph for anaesthesin and novocaine determination in the form of diazonium salts and azo compounds with resorcinol

Graph parameters	In the form of diazo compounds		In the form of azo compounds with resorcinol	
	Anaesthesin	Novocaine	Anaesthesin	Novocaine
Linearity range (I–C_{LA}, mol/L)	$4\cdot10^{-5}\div4\cdot10^{-6}$	$2\cdot10^{-5}\div8\cdot10^{-7}$	$1\cdot10^{-5}\div2\cdot10^{-6}$	Linearity range (I–C_{LA}, mol/L)
a	$-7.2\cdot10^{-2}$	$-9.1\cdot10^{-2}$	$1.9\cdot10^{-2}$	$1.07\cdot10^{-2}$
Δa	$3.0\cdot10^{-2}$	$1.4\cdot10^{-2}$	$1.6\cdot10^{-2}$	$1.2\cdot10^{-2}$
b	$2.5\cdot10^{4}$	$2.0\cdot10^{4}$	$5.8\cdot10^{4}$	$5.8\cdot10^{4}$
Δb	$1.3\cdot10^{3}$	$6.1\cdot10^{2}$	$2.6\cdot10^{3}$	$1.8\cdot10^{3}$
SD	$4.17\cdot10^{-2}$	$1.91\cdot10^{-2}$	$2.0\cdot10^{-2}$	$1.1\cdot10^{-2}$
LOQ, mol/L	$5.3\cdot10^{-6}$	$5.5\cdot10^{-6}$	$8.5\cdot10^{-7}$	$1.8\cdot10^{-6}$
r	0.9945	0.9983	0.9970	0.9986

characteristics (Table 1), and can be further used to determine anaesthesin and novocaine in medicinal products. However, we should note that when the concentration of LA decreases in one order of magnitude, the peak potential of diazonium salts shifted towards the negative region up to about 0.05 V.

Lidocaine is an anesthetic belonging to the group of amide compounds. Lidocaine is hydrolyzed during heating in a strongly acidic or strongly alkaline medium forming 2,6-dimethylaniline. 2,6-dimethylaniline can undergo diazotation with nitric acid and the formed diazonium salt can be used in azo coupling reaction. This principle is a basis of the method of the qualitative identification of lidocaine substance. In this method β-naphthol is used as an azo component, and, as a result, an orange or red azo dye is obtained.

Diazonium salt 2,6-dimethylaniline is reduced on the DME (Fig. 4).

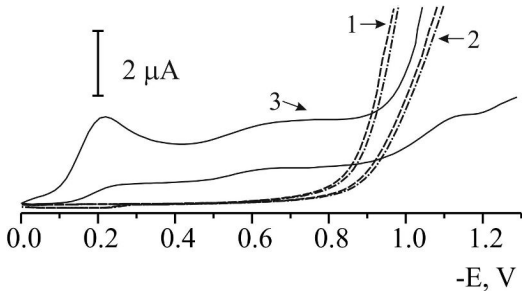


Fig. 4. Polarograms recorded in solutions $NaNO_2$ (1), lidocaine (2) and obtained diazonium salt 2,6-dimethylaniline (3) in UBM as a supporting electrolyte. The conditions are as follows: $C_{lidocaine}=5\cdot10^{-5}$ M; for hydrolysis $C(H_2SO_4)=0.5$ M; for diazotation $C(NaNO_2)=10^{-3}$ M, pH 2; $\upsilon=0.5$ V/s

However, the method of lidocaine derivatization is complicated and long-continued, since it involves heating in a medium of concentrated H_2SO_4 until the appearance of white vapor of acid. The metrological characteristics of lidocaine determination in this way are low (R=0.9928, LOQ=$1.5\cdot10^{-5}$ mol/L). Thus, the polarographic determination of lidocaine using such a way of a derivatization will not have any advantages over known methods for the determination of this anesthetic.

Selection of azo component for the polarographic determination of LA in the form of azo compounds

β-naphthol and salicylic acid were found to be unsuitable as azo components for the polarographic determination of anaesthesin and novocaine in the form of azo compounds. At pH>7, insoluble azo compounds were formed and several non-reproducible peaks appeared on the recorded polarograms. However, anaesthesin and novocaine diazonium salts easily undergo the reaction of azo coupling with resorcinol (1,3-dihydroxybenzene) forming a water-soluble azo compound of yellow-orange colour. The shape of polarograms depends on the pH of the azo coupling reaction. In an acidic or a neutral medium, solutions become turbid and two peaks appeared on polarograms (Fig. 5). The peak at the potentials of −0.18÷−0.20 V corresponds to the reduction of anesthetics diazonium salt. This is additional evidence that azo coupling is not full at pH<7. The potential of the peaks of the reduction of anaesthesin and novocaine azo compounds is shifted to a negative region with increasing pH.

Polarographic characteristics of the reduction of anaesthesin and novocaine azo compounds with resorcinol do not substantially depend on the duration of azo coupling. The reaction proceeds quickly, with sufficient time for the azo coupling equal to 5 min, the peak current does not further increase after 5 min.

At low concentrations of anesthetics, in addition to the peak of the LA azo compound reduction, we

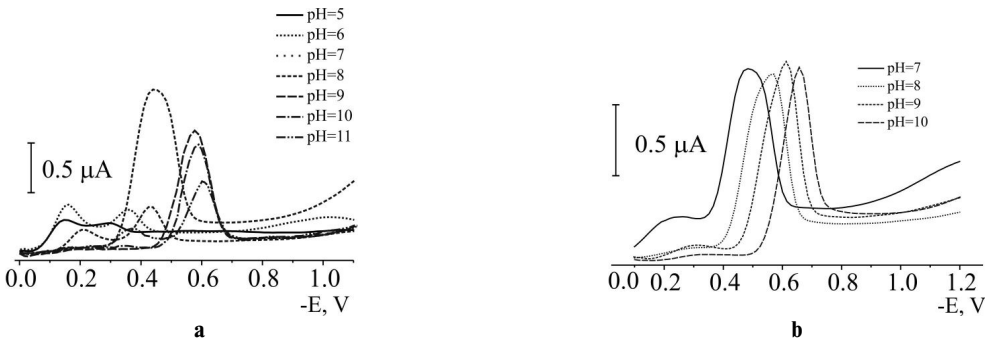


Fig. 5. Polarograms of reduction products in azo coupling reaction with resorcinol obtained at different pH for anaesthesin (a) and novocaine (b). The conditions of diazotation are as follows: $C_{anaesthesin}=C_{novocaine}=5\cdot10^{-5}$ M, $C(NaNO_2)=10^{-3}$ M, $C(HCl)=10^{-2}$ M, t=5 min. The conditions of azo coupling are as follows: $C(C_6H_4(OH)_2)=2\cdot10^{-4}$ M, $C(CO(NH_2)_2)=0.1$ M, t=10 min. The conditions of recording the polarograms are as follows: C(UBM)=0.2 M, υ=0.5 V/s

can also observe the reduction of the nitrite ion at −0.32÷−0.38 V (Fig. 6). Therefore, there is an excess of nitrite, which does not interact with LA; it is advisable to be removed from the reaction mixture with urea. For further studies, 0.1 M urea was used. This concentration is sufficient to destroy the excess of nitrite used for diazotation of LA.

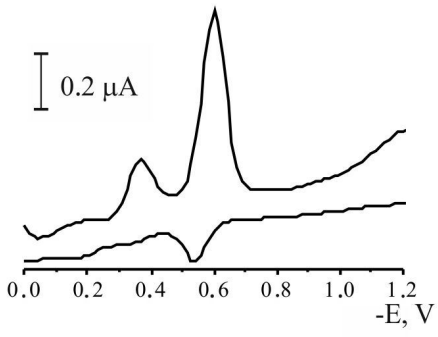


Fig. 6. Polarograms in solutions of the LA azo coupling product in the presence of a 50-fold excess of sodium nitrite

The polarographic characteristics of the reduction of anaesthesin and novocaine azo compounds are slightly influenced by the interaction time of the studied solution containing urea. The peak current of anaesthesin and novocaine azo compounds decreases smoothly (by 7−10% compared with the value of the current observed after three minutes of interaction in the presence of urea), and reaches the plateau after 10 minutes.

The peak current of novocaine and anaesthesin azo compounds increases slightly with increasing resorcinol concentration, and reaches certain stable value for a 10-fold excess of resorcinol.

Consequently, we can propose a new method for the determination of anaesthesin and novocaine.

The method of polarographic determination of anaesthesin and novocaine in the form of azo compounds with resorcinol

This procedure is as follows:

− Add an aliquot of anesthetic solution, 1 mL of hydrochloric acid (C=0.25 M) and 0.25 mL of $NaNO_2$ solution (C=0.1 M) to a 50 mL beaker, and then leave the mixture for 3−5 min.

− Add 2.5 mL urea, leave for 10 min, and then add 0.5 mL of resorcinol solution (C=0.01 M). Add 4 mL of the UBM solution to achieve the optimal pH for the azo coupling, after which the solution should be left for another 5 min.

− Add double-distilled water to the mark to 25 mL volumetric flask. Then introduce the obtained solution into the cell, deoxygenate for 10 min and record the polarogram by applying a linear potential scan from 0.0 to −1.0 V.

The dependence of the current of the reduction peak of the azo compound on the concentration of anesthetics is linear with satisfactory metrological characteristics (Table 1), and can be used to determine anaesthesin and novocaine in different medicines. However, it should be noted that with a decrease in the concentration of anesthetics, the potential of the reduction peak of azo compounds is shifted into a negative region from −0.59 V to −0.65 V.

Polarographic determination of anaesthesin and novocaine in finished dosage forms

The developed methods were tested on the model solutions of anaesthesin and novocaine by the «introduced-found» method (Table 2) as well as on the finished dosage forms. The content of novocaine was determined in a solution for injection of novocaine (procaine hydrochloride) containing 20 mg/mL of anesthetic, 2 mL per vial, ten ampoules in contour cell package (Arterium Corporation, manufacturer PAT Halychpharm). The solution of tested sample (STS) was prepared as follows: 1.00 mL of solution was collected by measuring pipette,

transferred to a volumetric flask of 50.0 mL, diluted with water to the mark and stirred. The concentration of this STS was $1.466 \cdot 10^{-3}$ M. The methods described above have been used to determine the content of novocaine by diazotation and azo coupling. The content was calculated using the parameters of the calibration plot of novocaine and the method of additives. The results of the determination are shown in Table 2.

Farysil tablets are a combined antiseptic drug for local use in the case of infectious and inflammatory diseases of the mouth cavity and throat. One tablet contains 5 mg of chlorhexidine dihydrochloride, 5 mg of benzocaine and auxiliary substances. STS was prepared as follows: a 0.6960 g of tablet was dissolved in double-distilled water with the addition of 0.25 M hydrochloric acid, the solution being stirred by an electromagnetic mixer all the time. The content was quantitatively transferred to a 50 mL volumetric flask and double-distilled water was added to the mark. Further, the solution was filtered (due to the presence of insoluble auxiliary substances). The anaesthesin concentration in this STS is $6.053 \cdot 10^{-4}$ M. The determination of anaesthesin was performed in the form of diazo- and azo compounds according to the methods described above. The content was calculated using the parameters of the calibration plot of anaesthesin as well as the method of additives. The results of the determination are shown in Table 3.

Consequently, the results of the determination of novocaine and anaesthesin in the finished dosage form confirmed the correctness of finished methods for quantification of LA.

Polarographic determination of anaesthesin and novocaine in the solution containing their mixture

Often dosage form contains simultaneously two or more medicinal substances. Therefore, during the quality assessment, the tests on the authenticity and quantitative determination of each medicinal substance that is part of the dosage form are performed. A characteristic feature of

Table 2

The results of testing the method of polarographic determination of anaesthesin and novocaine in solutions for injection, $C_{STS}=1.466 \cdot 10^{-3}$ M

Studied solution	Concentration of the introduced anesthetic + concentration of additive, M	Found, M	Content in a solution for injection, mg/ml	Error of determination, %
In the form of diazo compounds				
Model solution of anaesthesin	$2.63 \cdot 10^{-5}$	$2.71 \cdot 10^{-5}$	–	3.0
Model solution of novocaine	$2.40 \cdot 10^{-5}$	$2.43 \cdot 10^{-5}$	–	1.3
Aliquot of STS 0.5 mL	$2.93 \cdot 10^{-5}$	$2.97 \cdot 10^{-5}$	20.28	1.4
Aliquot of STS 0.5 mL+0.2 mL additive of model solution	$2.93 \cdot 10^{-5}+8.0 \cdot 10^{-5}$	$2.80 \cdot 10^{-5}$	19.09	4.6
In the form of azo compounds with resorcinol				
Model solution of anaesthesin	$4.8 \cdot 10^{-6}$	$5.03 \cdot 10^{-6}$	–	4.8
Model solution of novocaine	$4.8 \cdot 10^{-6}$	$5.04 \cdot 10^{-6}$	–	5.0
Aliquot of STS 0.15 mL	$8.79 \cdot 10^{-6}$	$8.64 \cdot 10^{-6}$	19.65	1.8
Aliquot of STS 0.15 mL+0.2 mL additive of model solution	$8.79 \cdot 10^{-6}+8.0 \cdot 10^{-6}$	$9.21 \cdot 10^{-5}$	20.96	4.8

Table 3

The results of testing the method of polarographic determination of anaesthesin in combined drug "Farysil", $C_{STS}=6.053 \cdot 10^{-4}$ M

	Concentration of the introduced anesthetic + concentration of additive, M	Found, M	Content, mg/tablet	Error of determination, %
In the form of diazo compounds				
Aliquot of STS 0.8 mL	$1.94 \cdot 10^{-5}$	$1.89 \cdot 10^{-5}$	4.88	2.4
Aliquot of STS 0.8 mL+0.3 mL of additive	$1.94 \cdot 10^{-5}+1.2 \cdot 10^{-5}$	$1.85 \cdot 10^{-5}$	4.77	4.6
In the form of azo compounds with resorcinol				
Aliquot of STS 0.5 mL	$1.21 \cdot 10^{-5}$	$1.27 \cdot 10^{-5}$	5.26	5.2
Aliquot of STS 0.5 mL+0.3 mL of additive	$1.21 \cdot 10^{-5}+1.2 \cdot 10^{-5}$	$1.09 \cdot 10^{-5}$	4.50	10

multicomponent analysis of dosage form is that methods for the determination of individual substances do not often yield positive results when used for the analysis of mixtures.

Separate polarographic determination of anaesthesin and novocaine in one mixture in the form of diazo- and azo compounds is not possible, since the potentials of derivatives reduction peaks are very close and the peaks are overlapped. It is possible to determine the total amount of LA by summing the heights of the peaks or peak areas of the anaesthesin and novocaine diazonium salts or azo compounds (Table 4, Fig. 7).

The «introduced-found» method was used to verify the possibility of determination the total amount of novocaine and anaesthesin, moreover the calibration graphs were used for pure (individual) anesthetics as well as the method of additives. The results are presented in Tables 5 and 6.

As can be seen from the obtained results, the total amount of anesthetics of novocaine and anaesthesin can be determined in two ways: by means of calibration plot and the method of additives. However, a lower error in the determination of LA is achieved when using the diazotation reaction. The transformation of LA to azo compounds requires more reagents consumption and is time-consuming. As a result, the error of determination of anesthetics increases.

Conclusions

The anaesthesin and novocaine diazonium salts and their azo compounds with resorcinol can be easily prepared; they are reduced on the DME in a wide pH range. The dependence of the current of the reduction peak of diazonium salts and azo compounds on the concentration of anesthetics is

Table 4

The optimal conditions for the determination of novocaine and anaesthesin in the form of diazo- and azo compounds

Conditions	Diazotation	Azo coupling
Concentration of reagents C, M	0.001 M $NaNO_2$, 0.01 M HCl pH≤3	0.001 M $NaNO_2$, 0.01 M HCl 0.1 M $CO(NH_2)_2$ 0.001 M $C_6H_4(OH)_2$) pH 8
Duration of the derivatization stage, min	5	20
$-E_p$, V	0.16–0.21	0.59–0.65

Fig. 7. Polarograms in solutions of anaesthesin (1) and novocaine (2) diazonium salts (a) and azo compounds (b) and in their mixture (3). $C_{anaesthesin}=C_{novocaine}=2\cdot10^{-5}$ M. The conditions see in Table 4.

Table 5

The determination of the total concentration of anaesthesin and novocaine in the mixture by the «introduced-found» method

Introduced anaesthesin+novocaine C, M	Found for novocaine / anaesthesin C, M	Error, %
In the form of diazonium salts		
$2.0\cdot10^{-5}+2.0\cdot10^{-5}$	$3.91\cdot10^{-5}$	2.25
	$4.07\cdot10^{-5}$	1.75
$2.4\cdot10^{-5}+1.6\cdot10^{-5}$	$3.96\cdot10^{-5}$	1.00
	$4.11\cdot10^{-5}$	2.75
In the form of azo compounds with resorcinol		
$4.0\cdot10^{-6}+4.0\cdot10^{-6}$	$7.22\cdot10^{-6}$	9.75
	$1.01\cdot10^{-5}$	6.13
$3.2\cdot10^{-6}+4.0\cdot10^{-6}$	$6.64\cdot10^{-6}$	7.76
	$6.93\cdot10^{-6}$	3.75

Table 6

The determination of the total concentration of anaesthesin and novocaine in the form of diazonium salts in the mixture by the method of additives

Concentration of mixture anaesthesin+novocaine C, M	Introduced of additive C, M	Found C, M	Error, %
In the form of diazonium salts			
$1.0\cdot10^{-5}+1.0\cdot10^{-5}$	$1.0\cdot10^{-5}$ novocaine	$1.94\cdot10^{-5}$	3.0
$1.2\cdot10^{-5}+0.8\cdot10^{-5}$	$1.2\cdot10^{-5}$ anaesthesin	$1.91\cdot10^{-5}$	4.5
$0.8\cdot10^{-5}+1.2\cdot10^{-5}$	$8\cdot10^{-6}+8\cdot10^{-6}$ anaesthesin+novocaine	$1.92\cdot10^{-5}$	4.0
In the form of azo compounds with resorcinol			
$4\cdot10^{-6}+4\cdot10^{-6}$	$2.4\cdot10^{-6}$ novocaine	$6.5\cdot10^{-6}$	18.8
$2\cdot10^{-6}+2\cdot10^{-6}$	$2{,}0\cdot10^{-6}$ anaesthesin	$4.8\cdot10^{-6}$	20.0
$2\cdot10^{-6}+2\cdot10^{-6}$	$4\cdot10^{-6}+2\cdot10^{-6}$ anaesthesin+novocaine	$4.5\cdot10^{-6}$	12.5
$4\cdot10^{-6}+4\cdot10^{-6}$	$4\cdot10^{-6}+4\cdot10^{-6}$ anaesthesin+novocaine	$8.6\cdot10^{-6}$	7.5

Note: The conditions see in Table 4.

linear with good metrological characteristics. The developed methods of the polarographic determination of anaesthesin and novocaine in the form of their derivatives were checked by analyzing model solutions and officinal (a solution for injections of novocaine, a combined antiseptic drug «Farysil»).

Separate polarographic determination of anaesthesin and novocaine in one mixture in the form of diazo- or azo compounds is not possible, since the potentials of derivatives reduction peaks are very close and the peaks are overlapped. It is possible to determine the total amount of LA by summing the heights of the peaks or peak areas of the anaesthesin and novocaine derivatives using the calibration plot as well as the method of additives. The lower error of the determination of LA can be achieved when using the diazotation reaction. The transformation of LA to azo compounds requires more reagents consumption and is time-consuming. As a result, the error of determination of anesthetics increases.

The metrological characteristics of lidocaine determination in the form of 2,6-dimethylaniline diazonium salt are not high enough, and the method of derivatization is complicated and time-consuming; therefore, the polarographic determination of lidocaine using such derivatization has no advantages over known methods for the determination of anesthetic.

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ДІАЗОТУВАННЯ ТА АЗОСПОЛУЧЕННЯ ЯК РЕАКЦІЇ ДЕРИВАТИЗАЦІЇ ДЛЯ ПОЛЯРОГРАФІЧНОГО ВИЗНАЧЕННЯ ДЕЯКИХ МІСЦЕВИХ АНЕСТЕТИКІВ

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У роботі досліджено використання діазонієвих солей анестезину і новокаїну як хороших похідних для полярографічного визначення цих анестетиків. Залежність струму піка відновлення діазонієвих солей та азосполук від концентрації анестетиків є лінійною з хорошими метрологічними характеристиками. Полярографічне визначення окремо без розділення анестезину і новокаїну за сумісної наявності у формі діазо- чи азосполук є неможливим через перекривання піків відновлення відповідних дериватів. Однак, можливим є визначення сумарної кількості досліджених місцевих анестетиків за сумуванням висоти чи площі піків похідних анестезину і новокаїну, використовуючи калібрувальний графік, а також метод добавок. Розроблені методики полярографічного визначення анестезину і новокаїну у формі їх похідних перевірені на модельних розчинах і лікарських препаратах (розчин новокаїну для ін'єкцій, комбінований антисептичний препарат «Фариспіл»). Метрологічні характеристики визначення лідокаїну у формі 2,6-диметиланілін діазонієвої солі не є високими, а метод деривативації є складним і тривалим.

Ключові слова: анестетики, анестезин, новокаїн, діазотування, азосполучення, полярографія.

DIAZOTATION AND AZO COUPLING AS DERIVATIZATION REACTIONS FOR POLAROGRAPHIC DETERMINATION OF SOME LOCAL ANESTHETICS

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We have determined that diazonium salts of anaesthesin and novocaine are suitable derivatives for the polarographic determination of these anesthetics. The dependence of the current of the reduction peak of diazonium salts and azo compounds on the concentration of anesthetics is linear with good metrological characteristics. The individual polarographic determination of anaesthesin and novocaine in their mixture in the form of diazo- or azo compounds is not possible because of overlapping the reduction peaks related to individual anesthetics. However, it is possible to determine the total amount of the investigated local anesthetics by summing the heights of the peaks or peak areas of the anaesthesin and novocaine derivatives using the calibration plot as well as the method of additives. The developed methods of polarographic determination of anaesthesin and novocaine in the form of their derivatives were checked by analyzing model solutions and pharmaceuticals (a solution for the injections of novocaine, a combined antiseptic drug «Farysil»). The metrological characteristics of lidocaine determination in the form of 2,6-dimethylaniline diazonium salt are poor, and the method of derivatization is complicated and long-continued.

Keywords: anesthetics; anaesthesin; novocaine; diazotation; azo coupling; polarography.

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