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Research Article

A New Approach for the Determination of Benzocaine and Procaine in Pharmaceuticals by Single-Sweep Polarography

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A new polarographic method for the determination of benzocaine and procaine based on the polarographic reduction of their chemically obtained oxidation products with potassium peroxymonosulfate is developed. Experimental conditions affecting quantitative yield of benzocaine and procaine oxidation products such as pH, oxidation time, reagents' concentration, and temperature are explored. It is shown that the reduction current changes in a linear fashion (R=0.999) with increasing concentration of anesthetics over a concentration range of $1{\cdot}10^{-6}$ - $5{\cdot}10^{-5}$ mol L^{-1}. The calculated limits of detection (LOD) for benzocaine and procaine are found to be $5.6{\cdot}10^{-6}$ and $6{\cdot}10^{-6}$ mol L^{-1}, respectively. In the present study, quantitative polarographic determination of benzocaine in Farisil tablets and "Septolete Plus" lozenges and procaine in solution for injections is performed. The results of the analysis are in good agreement with the product specifications described in the quality certificates. The possibility of quantitative determination of benzocaine and procaine in pharmaceuticals is confirmed.

1. Introduction

Local anesthetics (LA) are the group of natural and synthesized substances that have the ability to cause a reversible, temporary blocking/interruption of the excitability, and conductivity of nerve receptors and conductors in direct contact with them. Therefore, they induce a local loss of sensitivity and eliminate the sensation of pain or so-called pain sensitivity. The structural basis of modern anesthetics is paraaminobenzoic acid. It exhibits a high biological activity. Esters of *p*-aminobenzoic acid have anesthetic effect and are synthetic substitutes for cocaine, which historically was the first anesthetic.

Benzocaine (BC) and procaine (PC) (Figure 1) are widely used local anesthetics. They are active constituents of many drugs and medications. PC is used for local, infiltration, spinal anesthesia, and in therapeutic blockade [1]. The main area of BC application is as a component of some free-sale formulations for topical use, for example, in skin creams, as a dry powder for skin ulcers, as throat lozenges, and as teething formulations for young children [1].

However, these drugs have many side effects, in particular, cardiovascular, allergic reactions, even capable of causing anaphylactic shock [1, 2]. Therefore, the quantitative determination of local anesthetics in pharmaceuticals, blood, and other biological materials is crucially important.

The chemical structure of LA makes it possible to use different methods for qualitative and quantitative determination of these analytes. British and European Pharmacopoeia [3, 4] suggests the use of the nitritometric titration method to determine the content of the substance in the BC and PC substrates.

The most selective are chromatographic methods. They allow simultaneous quantification of LA and their metabolites in the same mixture and can be used for the analysis of biological objects and foodstuffs. However, these methods are not always available and require expensive equipment and reagents [5–7]. There are also simple and cheap

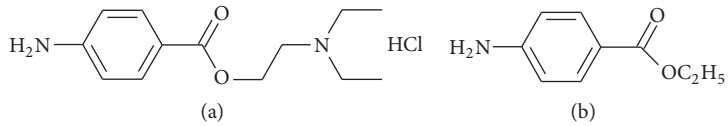

Figure 1: Structure formulas of procaine (a) and benzocaine (b).

spectrophotometric methods [8–12] that are significantly less selective and sensitive than chromatographic ones.

A good alternative is electrochemical methods that are increasingly used in the pharmaceutical industry. In principle, they are easier, quicker, and cheaper in performance than chromatographic methods. In addition, the sensitivity of electrochemical methods is often higher than of the spectrophotometric ones [13–15]. However, they are not very commonly employed techniques for the LA determination.

Plasticized ion-selective electrodes exhibit selectivity to cations of procaine and lidocaine. These electrodes work based on the formation of ion associates, i.e., *anesthetic—tetraphenylborate ion* [16]. Simultaneous determination of procaine and lidocaine in mixtures with ceftriaxone and cefazolin was performed in the range of 10^{-2} - 10^{-5} mol·L^{-1} [17]. However, these electrodes have some limitations, such as a short lifetime and, in addition, their response time depends on the analyte's concentration in the probe.

The authors of [18] developed a sensor for the determination of the procaine and lidocaine content in aqueous solutions and dosage forms, whose analytical signal is the Donnan potential (DP sensor). Concentration range for procaine was $1.0 \cdot 10^{-4}$-$7.3 \cdot 10^{-2}$ mol·L^{-1}.

Voltammetric methods for determination of LA are significantly more sensitive and more selective than potentiometric ones. The authors of works [19–25] used various carbonaceous modified electrodes. For instance, in short communication [21] Komorsky-Lovrić et al. showed the possibility of local anesthetics (namely, benzocaine, cinchocaine, lidocaine, and procaine) detection and their semiquantitative determination by immobilization of solid microparticles of anesthetics on paraffin impregnated graphite electrode. Also, the results of high-performance liquid chromatography (HPLC), flow injection analysis (FIA) [24], and batch injection analysis (BIA) [25] with amperometric determination were shown. Earlier we applied the miniaturized thick-film boron-doped diamond electrode as advanced and facile electrochemical sensor for simple, sensitive, and reliable quantification of BC [26]. However, stationary electrodes have some disadvantages, particularly, their utilization require labor-intensive renewing of electrode surface. The brief description of some methods of BC and PC determination is presented in Table 1.

Polarography is also used today in many control laboratories as a simple, commercially available, highly sensitive, and selective method for the determination of therapeutically active substances in medications and biological fluids; see for instance [27–33]. The main advantage of dropping mercury electrode (DME) against stationary electrodes is the high repeatability of measurements since each drop has a smooth and uncontaminated surface free from any adsorbed analyte or impurity. Thus, polarographic analysis gives very highly reproducible results. In the case when the substance cannot be reduced at DME, the molecule of this compound is modified by introducing electrochemically active functional groups, which in fact is used in further analysis. In particular, for the quantitative determination of compounds containing the amino group, it is proposed that they are previously oxidized by strong oxidants to form corresponding azo-, azoxi-, nitroso, and nitroderivatives and N-oxides, which are easily reduced on DME. Thus, a universal and simple technique for determination of LA belonging to the amide group was successfully developed [34–36].

We investigated the products obtained after BC and PC oxidation using $KHSO_5$ and their reduction on a mercury drop. The aim of this study is to develop a simple electrochemical method for the quantitative determination of BC and PC in pharmaceuticals.

2. Material and Methods

2.1. Reagents. Benzocaine (ethyl ester of 4-aminobenzoic acid) was purchased from Changzhou Sunlight Pharmaceutical Co., Ltd., China. The BC stock solution was prepared by dissolving its appropriate amount in double-distilled water with addition of 1 ml 0.25 mol·L^{-1} hydrochloric acid (p.a., Sfera sim, Ukraine).

Procaine (β-diethylaminoethyl ester of 4-aminobenzoic acid hydrochloride) was purchased from Guangxi Shentai Chemical Co., Ltd., China. The PC stock solution was prepared by dissolving its appropriate amount in double-distilled water.

The concentration of both anesthetics stock solutions was $1 \cdot 10^{-3}$ mol L^{-1}.

Aqueous solutions of BC and PC possess acidic reaction and are stable during storage. The working solutions of both anesthetics were obtained by diluting stock solution with double-distilled water.

Borate, carbonate, phosphate, and Britton-Robinson buffer solution were used for preliminary studies. In further investigations phosphate buffer solution was used. It was prepared in the following way: 15.00 g of KH_2PO_4 (p.a., Sfera sim, Ukraine) was dissolved in a 250 mL volumetric flask; then 2.5 mol·L^{-1} of sodium hydroxide (p.a., Sfera sim, Ukraine) was added to achieve the necessary pH and, finally, the flask was filled with double-distilled water up to the mark. Phosphate buffer is available to maintain pH value in wide ranges: at pH 4.8–8.0 $H_2PO_4^-/HPO_4^{2-}$ buffer system works and at pH>8.5 – HPO_4^{2-}/PO_4^{3-} system. In addition, the nature of ions and ionic strength does not change substantially. This is an important feature in voltammetric analysis. The buffer capacity at pH 8.0–9.0, which is relatively

Table 1: Brief description of some methods for determination of BC and PC.

Anesthetic	Method	Linear range	LOQ	LOD	Objects analyzed	References
PC	HPLC-UV	0.05 – 5.0 μg /mL	0.05 μg/mL	–	human plasma	[6]
PC	HPLC – MS – ESI	10 – 100 ng / mL	10.0 ng/mL	0.100 ng/mL	serum of human blood	[7]
BC	SP	10 – 25 μg / L	–	–		[8]
PC	DPV	3 – 50 μM	–	0.91 μM	commercial pharmaceutical samples	[22]
PC	DSWV	1 – 250 μM	1.35 μM	0.4 μM	commercial pharmaceutical samples	[22]
BC		1.0 – 100 μM	0.83 μM	0.25 μM	pharmaceutical products	[23]
BC	FIA–AD HPLC–AD	0.2 – 100 0.2 – 100 μM	– –	0.19 0.20 μM		[24]
BC	BIA–AD	0.1 – 8 μM	–	0.0302 μM		[25]
BC	DPV SWV	0.1 – 400 μM 0.4 – 200 μM	0.27 μM 0.32 μM	0.08 μM 0.1 μM	pharmaceutical samples, spiked urine samples	[26]

BIA–AD: batch injection analysis with amperometric detection; DPV: differential pulse voltammetry; DSWV: differential square wave voltammetry; FIA–AD – flow injection analysis with amperometric detection; HPLC: AD with amperometric detection; HPLC–MS–ESI: high-performance liquid chromatography–tandem mass spectrometry with electrospray ionization; HPLC-UV: high-performance liquid chromatography with ultraviolet detection; SP: spectrophotometry.

low, can be increased by increasing the buffer concentration [38].

"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, $KHSO_5$ (PMS) (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 [39]), and sufficient durability during exploitation and storage [DuPont™ Oxone[5] Technical Attributes] [37, 40, 41]. 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.

Purified argon was used to remove dissolved oxygen.

2.2. Apparatus. Voltammetric measurements were carried out on digital device equipped with personal computer [41] and temperature-controlled three-electrode cell, volume 10 mL. An indicator dropping mercury electrode (DME), a saturated calomel reference electrode, and platinum wire auxiliary electrode were used. The employed DME had the following characteristics: m=5.94·10^{-4} g·s^{-1}; τ_k=10 s in 0.2 mol L^{-1} NH_4Cl with open circuit.

The pH of the solutions was measured potentiometrically using MV 870 DIGITAL-pH-MESSERÄT pH-meter.

2.3. Voltammetric Procedure and Sample Preparation. After optimization of the experimental parameters for the proposed method, the analytical curve was obtained in the following way: 2 mL of 1.25 mol L^{-1} phosphate buffer with pH 9.0 was introduced into 25 mL volummetric flask, and then 2.5 mL of 10^{-3} mol L^{-1} PMS and aliquot of anesthetic were added to the flask. The concentration of anesthetic must be in the range from 1·10^{-5} to 5·10^{-5} mol L^{-1}. The obtained solution should stay during 5-6 min. Then 1.25 mL of 2.5 mol L^{-1} H_3PO_4 was added to obtain pH 4.0 (should be checked with pH-meter). Finally, the flask was filled with double-distilled water up to the mark. The obtained working solutions were introduced into the cell, and deoxygenated with argon for 10 min. The voltammogram was recorded by applying a linear potential scan from 0.0 to -1.5 V.

2.4. Preparation of Pharmaceutical Samples and Procedure for Their Analysis. The working investigated sample (WIS) was prepared as follows: four tablets were dissolved in 4 mL of 0.25 mol·L^{-1} hydrochloric acid, and then the double-distilled water was added to the mark followed by continuous stirring the solution with an electromagnetic stir bar. The solution was then filtered through the filter paper (the pore size of 1-2.5 nm) in order to remove insoluble excipients. The precipitate was washed on the filter in several portions of 10^{-2} mol·L^{-1} hydrochloric acid and then with double-distilled water. The contents were quantitatively transferred to a 200.0 mL flask and the double-distilled water was added to the mark. The concentration of anesthetic in such WIS, according to quality certificate, is 6.053·10^{-4} mol L^{-1}.

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

Anesthetics	Peak	pH range	Equation	Correlation coefficient, R
PC	P1	2.1-4.3	E=(-0,047±0.007)+(0.082±0.002)·pH	0.9984
		5.0-9.1	E=(0.021±0.002)+(0.065±0.003)·pH	0.9961
	P3	5.0-8.1	E=(-0.16±0.02)+(0.069± 0.003)·pH	0.9971
	P4	5.0-8.1	E=(0.011±0.020)+(0.078±0.003)·pH	0.9977
BC	P1	2.0-4.5	E=(-0,018±0.009)+(0.071±0.002)·pH	0.9957
		5.0-9.0	E=(0.03±0.03)+(0.062±0.004)·pH	0.9951

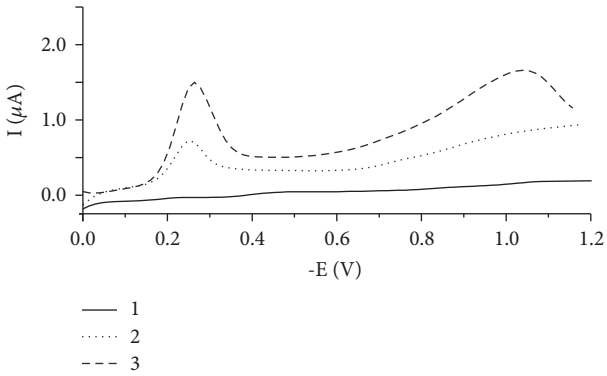

Figure 2: Polarogramms in a PMS solution without BC or PC (1) and the solutions of BC (2) and PC (3) after oxidation at pH 9 and heating to 60°C. $C_{PMS} = 10^{-3}$ mol·L^{-1}, $C_{BC} = C_{PC} = 5{\cdot}10^{-5}$ mol·L^{-1}. Phosphate buffer was used as supporting electrolyte, C_{bufer}=0.2 mol·L^{-1} and pH=4.

An aliquot of 1.00 mL of the dosage solution was taken into a 25.0 mL volumetric flask, and then 2 mL of a 1.25 mol·L^{-1} phosphate buffer solution with pH 9 and 2.5 mL of 10^{-2} mol L^{-1} of PMS were added and stirred. The obtained mixture was heated for 10 min at 40-60°C and cooled. Then the pH was adjusted to the value 4.0 by adding a 2.5 mol·L^{-1} solution of H_3PO_4. Finally, double-distilled water was added to the mark. The obtained solution was introduced into the cell and deoxygenated with argon for 10 min. The polarogramm was recorded by applying a linear potential scan from 0.0 to -1.5 V.

3. Results and Discussion

The polarogramms of $5{\cdot}10^{-5}$ mol L^{-1} BC and PC oxidation products in a phosphate buffer solution at DME are depicted in Figure 2. The reduction process of both BC and PC oxidation products is irreversible.

3.1. Effect of pH and Supporting Electrolyte. Oxidation products of amines are formed in alkaline medium. Acidification of reaction mixture leads to stop the oxidation process. An optimum pH for oxidation (pH_{ox}) of BC and PC is in the range from 8.7 to 9.3.

Phosphate, borate, carbonate, and Britton-Robinson buffer solutions were investigated as electrolytes for the oxidation reaction. Higher reduction currents of oxidation products were obtained using phosphate buffer as supporting electrolytes. For further experiments phosphate buffer was selected as the supporting electrolyte since it is more appropriate to change pH and to adjust weakly acidic medium required for polarographic measurement.

The shape of polarogramms of BC and PC derivatives reduction significantly depends on pH of polarographic scanning (pH_{pol}) (Figures 3 and 4).

At pH < 4.5 oxidation products of PC are reduced yielding two peaks: -0.13 – -0.3 V (first peak P1) and approximately -1.15 V (second peak, P2) (Figure 3). Peak P2 is broad and the maximum is blurred. At > 4.5 the peak P1 current dropped down and the two new peaks P3 and P4 appeared near P1.

On the polarogramm of BC on a phosphate buffer, one distinct peak P1 was observed within the studied pH region (Figure 4). At pH> 8, this peak splits similarly to PC. The peak, which corresponds to P2 for the PC, was not observed on the polarogramms of BC derivative.

The maximum value of the reduction current for P1 derivatives of BC and PC is observed at pH about 4.0; therefore, this value was selected for all further experiments.

Since the process is rather complicated, the potentials of the reduction peaks of the corresponding derivatives are shifted to a negative direction with increasing the pH. This behavior demonstrates that the electrochemical reduction of BC and PC involves proton transfer stage. The dependence of -E vs. pH of the buffer was found to be linear in the whole pH range. The obtained dependences can be expressed by the equation presented in Table 2.

3.2. Effect of Temperature, Oxidation Time, and Reagents' Concentration. The colorless products of oxidation of the BC and PC were always obtained, regardless of the reaction conditions used.

The oxidation reaction is slow at room temperature. The maximum current can be reached after 60 minutes of oxidation, which is too long. Therefore, all studies were performed with the heating of solutions during oxidation. It affects the rate of the oxidation reaction. All the reagents were mixed (phosphate buffer with pH 9, BC or PC, and PMS) in the appropriate ratio in a glass, and then the glass was immersed in a water bath until an appropriate temperature was established. The temperature of the solution was additionally controlled by a thermometer. After reaching the appropriate temperature, phosphate acid was added to pH 4 and cooled to room temperature. For the BC oxidation product, the optimal heating temperature is 40-60°C (Figure 5(a)) and for the oxidation product of PC (Figure 5(b)),

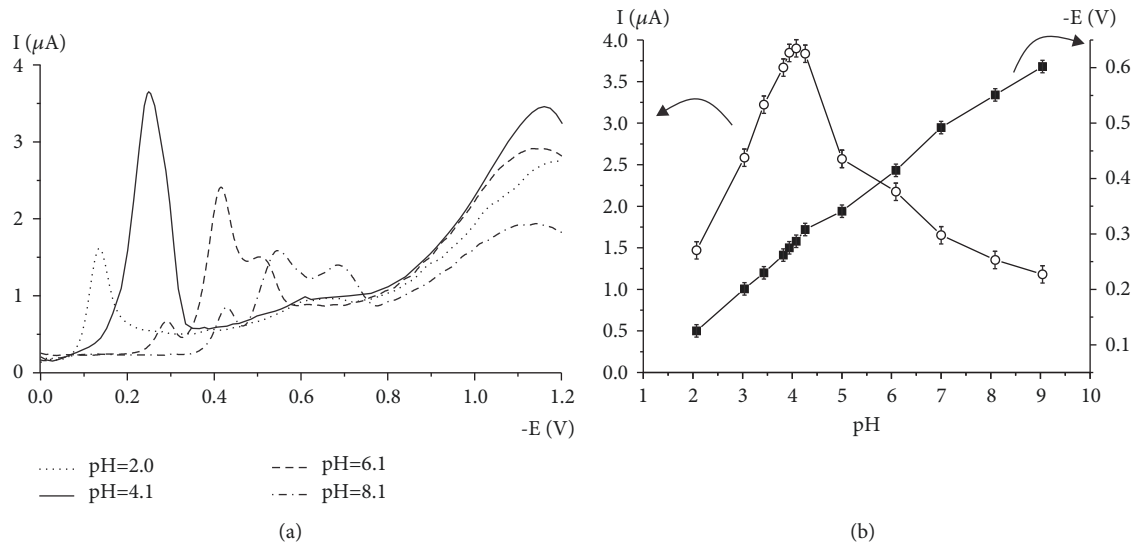

Figure 3: Polarogramms PC (a) and dependence of polarographic characteristics of derivatives at different pH (b) using phosphate buffer, $C_{PC}= 5{\cdot}10^{-5}$ mol·L^{-1}, $C_{PMS}= 10^{-4}$ mol·L^{-1}, C_{buffer}=0.2 mol·L^{-1}, duration of oxidation 10 min., and T= 70°C.

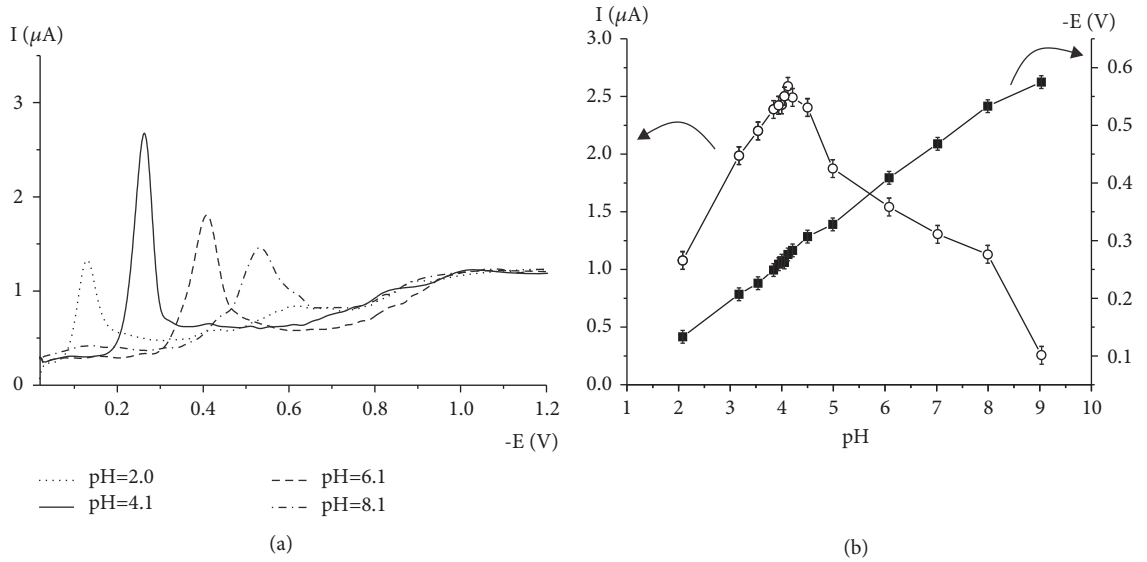

Figure 4: Polarogramms BC (a) and polarographic characteristics of derivatives at different pH (b) using phosphate buffer, $C_{BC}= 5{\cdot}10^{-5}$ mol·L^{-1}, $C_{PMS}= 10^{-4}$ mol·L^{-1}, C_{buffer}=0.2 mol·L^{-1}, duration of oxidation 10 min., and T= 60°C.

within 70-100°C. All further studies were performed in these temperature ranges.

An important factor affecting the quantitative yield of the corresponding oxidation products is the oxidation time. Its effect on the yield of the derivative occurs within first 10 minutes and after the amount of the oxidation product does not change. Therefore, further anesthetics were oxidized within 10 minutes (Figure 6(a)).

For the maximal yield of BC derivatives a 2-fold excess of PMS is sufficient. For the oxidation of PC a larger excess of oxidant should be used (Figure 6(b)). Figure 6(b) shows a fragment of these dependencies. The reduction current of anesthetic derivatives does not change up to a 200-fold excess of PMS. However, the concentration of PMS in the solution should not exceed 10^{-3} mol·L^{-1} because of PMS reduction leading to the residual current increase and polarogram background line distortions.

3.3. Effect of Scan Rate. The scan rate (ν) was changed from 0.1 to 1.0 V·s^{-1}. With increasing ν the peak height also increases and the potential shifts to the cathodic region (Figure 7).

The slope of $\log I$ versus $\log \nu$ for P1 of BC (Figure 7) in various conditions is in the range from 0.33 to 0.54 and indicates the diffusion-controlled current with minor kinetic issues that increase with increasing pH of the solution. The slope of $\log I$ versus $\log \nu$ for P1 of PC (the data are not shown here) under different conditions at pH less than 5 is 0.50 and also suggests the diffusion-controlled current. At pH 7.5

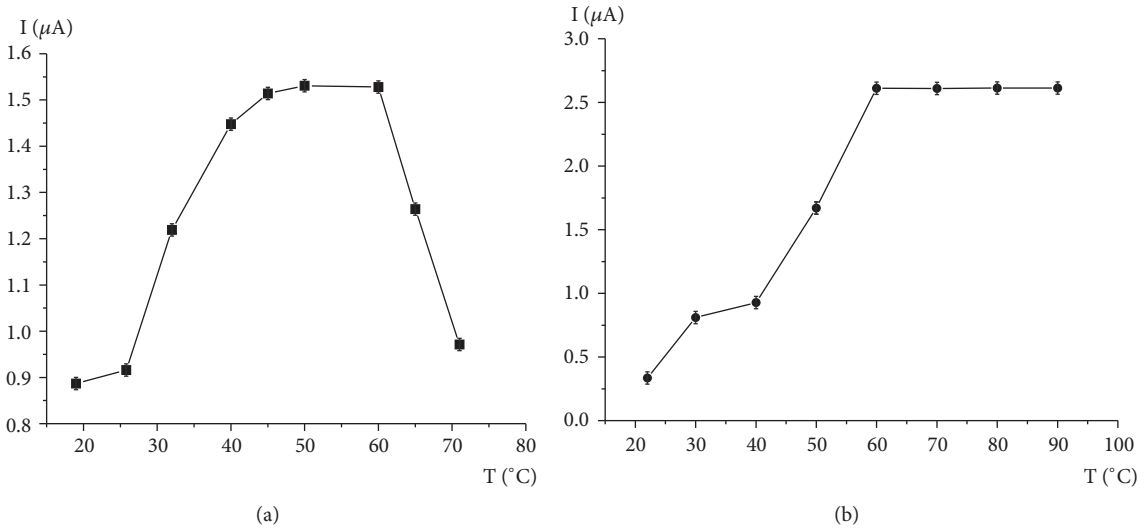

Figure 5: Effect of temperature on the oxidation product yield (for peak P1) of BC (a) and PC (b) $C_{PMS} = 10^{-3}$ mol·L^{-1}, $C_{BC} = C_{PC} = 5 \cdot 10^{-5}$ mol·L^{-1}; C_{buffer} ~0.2 mol·L^{-1}, and pH=4.0.

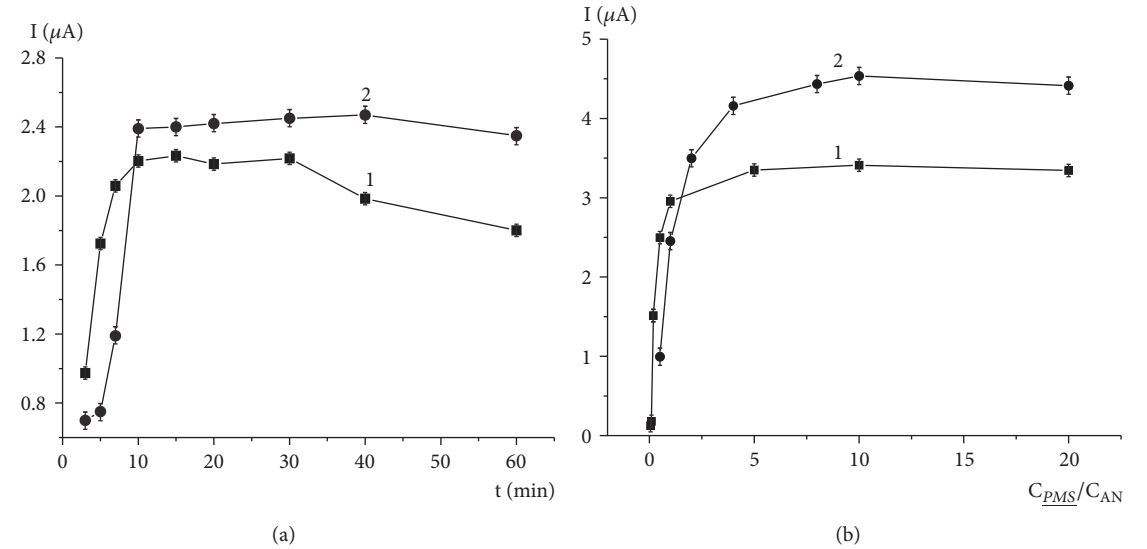

Figure 6: Dependence of the peak P1 current of BC (1) and PC (2) derivatives on the oxidation time (a), $C_{PMS} = 10^{-3}$ mol·L^{-1}, and on the molar excess of PMS (b), $C_{BC} = C_{PC} = 5 \cdot 10^{-5}$ mol·L^{-1}, pH 4.0.

for three peaks P1, P3, and P4 for PC the slope is close to 1 indicating the adsorption effect.

The linear relationship between I_p and the square root of the scan rate ($v^{1/2}$) clearly reflects a diffusion-driven mechanism of the electrode reaction at this pH.

3.4. The Possible Mechanism of Electrochemical Reaction. The polarogramms for derivatives of BC and PC have an analogous shape, and various factors affect their characteristics in a similar way. This indicates the participation of the same functional groups in electrochemical reactions and the same mechanism of transformation. Such a joint group for the BC and the PC is the primary amino group. In the case of excessive use of the oxidizing agent the peak of P1 appears on the polarogramms within some period of time. The current reaches a maximum with an excess of oxidizing agent, so no further oxidation of this group occurs. The soft oxidation of primary amines with peroxide compounds gives hydroxylamine as the initial product followed by its further oxidation to nitroso compounds. Under more stringent conditions, in particular when heated, primary amines can be oxidized to nitro compounds. It is also known that, in the concentrated sulfuric acid medium, the BC can be oxidized to the formation of colored products.

From the quantitative parameters of the polarogramm one can calculate the number of electrons n that participate in the electrode process [42]:

$$\alpha n = -\frac{47.7}{\left(E_p - E_{p/2}\right)}\ (\text{mV}), \tag{1}$$

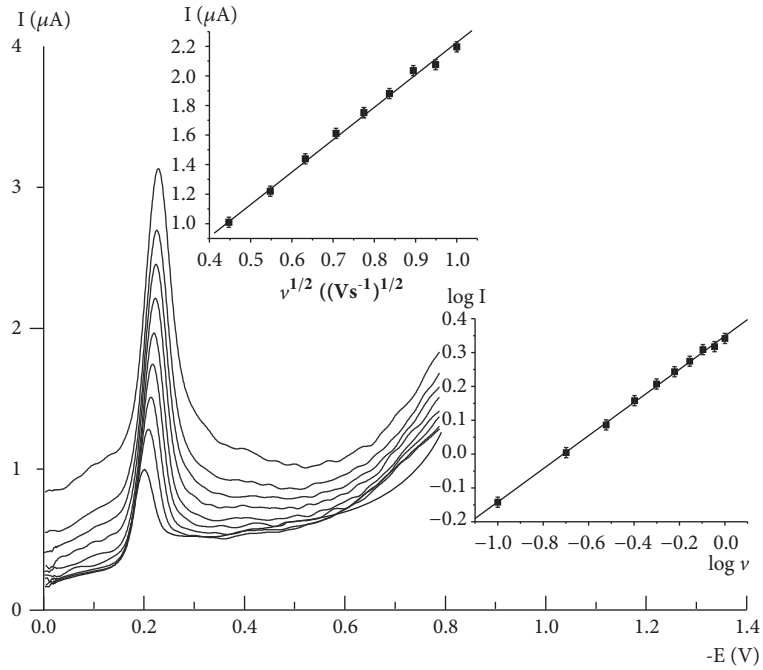

FIGURE 7: Polarogramms of $5{\cdot}10^{-5}$ mol·L^{-1} BC in the potential range from 0.0 to -1.4 V for the scan rate values from 0.1 to 1.0 V s^{-1}, C_{PMS}= $1{\cdot}10^{-3}$ mol·L^{-1}, pH 4.0, and C_{buffer}=0.2 mol·L^{-1}. Insets: the dependences of I_p vs. $v^{1/2}$ and log I_p vs. log v.

where α the charge transfer coefficient and n the number of electron transferred in a stage of electrode process. The coefficient α for irreversible systems equals 0.5.

The number of electrons can also be determined from the dependence of $E_p = f(\log v)$ (not shown here): the slope of this dependence equals ($2.3RT/\alpha nF$). The results of both calculations are consistent with each other (Table 3).

The number of H^+ ions (zH^+) participating in electrochemical process can be estimated from the slope of the peak potential vs. pH (Table 2) according to the following equation $dE/\text{pH} = (2.3RT{\cdot}zH^+)/\alpha nF$. The results of these calculations are presented in Table 3.

We assume that in the presence of the excess of oxidant and heating the complete oxidation of the primary amino group of anesthetics to the nitro group occurs. Then, the nitro group gaining four electrons is reduced to the hydroxylamine on the electrode. This is in good agreement with the data reported in the literature [43–45]. On the other hand, we do not exclude that the amino group is first oxidized to the nitroso compound and then reduced back to the amino group on the electrode. In the alkaline medium, the reduction is stepwise and also complicated by adsorption for PC.

Cathodic peak P2 of PC derivative can be caused by the reduction of N-oxide of tertiary amine, but this peak does not have a well-defined profile shape, which makes its precise measurements rather difficult. Thus, it was not used in further experiments.

For a detailed elucidation of the oxidation mechanism of the BC and PC and the reduction of their oxidation products, the spectral analysis (NMR or mass spectrometry) and coulometry are recommended for the future investigations.

4. Determination of Analytical Parameters

The previously optimized experimental parameters were employed to record the corresponding analytical curves for BC and PC on the phosphate buffer. Thus, the analytical parameters obtained by proposed methods are summarized in Table 4. The limits of detection (LOD) and quantification (LOQ) were estimated taking three and ten times the standard deviation of the blank ($3.3S_a/b$, $10S_a/b$), respectively (S_a: residual standard deviation or standard deviation of the y-intercept, n = 9) [46].

4.1. Analysis of Pharmaceutical Dosages. Based on the obtained results, we have developed a polarographic technique for the determination of BC in "Farisil" tablets (manufactured by Alcala Pharma, SL, Spain), "Septolete Plus" (KRKA dd, Novo mesto, Slovenia), and PC in solution for injections ("Darnytsya", Ukraine).

Presence of other compounds in solution for injection does not affect the determination of PC. The matrix of tablets is complex; in particular, it contains a lot of sugar. Matrix substances slightly influence the double electric layer leading to a minor change in shape of the polarogramm and the reduction current of the PC and BC derivatives by less than 10% in comparison to the pure solution. However, the dependence of the current on the concentration of BC on the background of the matrix of the tablets remains linear (Figure 8). Thus, the content of BC in "*Farisil*[5]" tablets and "*Septolete plus*[5]" lozenges was determined using the method of standard addition. Preparation of the samples is described in detail in the Section 2.4. In the same way the procedure with addition from 0.10 mL to 0.80 mL of standard BC

Table 3: The calculated number of electrons (n) and ions of H^+ (zH^+) involved in electrochemical reduction of BC and PC derivatives at various pH C_{PMS}= 1·10^{-3} mol·L^{-1}, $C_{BC} = C_{PC}$ = 5 · 10^{-5} mol·L^{-1}, phosphate buffer C_{buffer}=0.2 mol·L^{-1}, and ν=0.5 V·s^{-1}.

Anesthetic	pH	Peak	n		zH^+
			According to formula [37]	by $E_p = f(\log \nu)$	
PC	4.0	P1	4	4	3
	6.1	P1	2	–	1
		P3	3	–	3
		P4	2	–	1
BC	4.0	P1	4	4	3
	7.5	P1	2	2	1

Table 4: Characteristics of quantitative polarographic determination of BC and PC, pH 4.0. C_{PMS}= 1·10^{-3} mol·L^{-1} ν=0.5 Vs^{-1}.

Analytical parameter	BC	PC
Peak potential, V	-0.24	-0.51
Linear concentration range, mol·L^{-1}	1·10^{-6} - 5·10^{-5}	1·10^{-6} - 5·10^{-5}
Slope (μA·L/ mol)	4.1·10^4	9.1·10^4
Intercept (μA)	1.7·10^{-2}	4.2·10^{-2}
Correlation coefficient, R	0.9996	0.9996
RSD (%)	2.12	1.13
Limit of quantitation (LOQ), mol·L^{-1}	1.8·10^{-6}	1.9·10^{-6}
Limit of detection (LOD), mol·L^{-1}	5.6·10^{-6}	6·10^{-6}

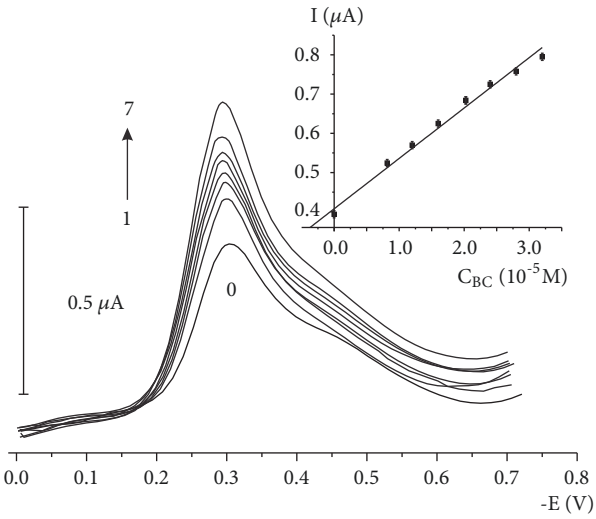

Figure 8: Polarogramms for the pharmaceutical dosage *Septolete plus* analysis with declared content of 5 mg BC using standard addition method. The corresponding standard additions: from 0.10 mL to 0.80 mL (C_{BC} = 1·10^{-3} mol L^{-1}). The quantification of BC by standard addition method is depicted in the inset.

solution was performed. The results of quantitative determination of BC in "*Farisil*" tablets and "*Septolete plus*" lozenges and PC in solution for injections are presented in Table 5.

We compared our results with the data obtained by the control laboratories of the State Administration of Ukraine on Medicinal Products, which provided us the quality certificates. In certification procedure analysis was carried out according to Quality Control Methods No. UA/L 48565 and No. UA 16/6052-L8 (Ukraine). The characteristics we determined are in the range from 97.0 to 102.6%. These results prove that our technique does not suffer from any significant matrix effects.

The developed method is simple and cheap and has a wide linear range. Sensitivity of our method is comparable to that of chromatographic method. Thus, this work extends the possibility to select the appropriate method among the available ones for determination of the PC and the BC in specific real objects such as drugs.

5. Conclusions

Modern voltammetric methods provide reliable and reproducible quantitative determination of substances in a complex matrix. In earlier reported works stationary bare electrodes and chemically-modified electrodes were used for local anesthetics quantitations. The procedure of modification and surface renewing of such electrodes is time consuming and labor-intensive. In this regard the mercury electrodes have advantages. Thus our methods are instrumentally simple and portable and have moderate cost, high reproducibility and are comparable in sensitivity to other methods. Therefore, we have developed a new polarographic method for the determination of BC and PC based on the electrochemical reduction of their chemically oxidized products obtained by the reaction with PMS. Moreover, the achieved results show that this method can be successfully applied for quantitative polarographic detetmination of anesthetics in pharmaceuticals, in particular, BC in tablets "*Farisil*" and "*Septolete plus,*" as well as PC in a freshly prepared solution for injections. Consequently, the presented method paves the way for accurate and reliable quantitative analysis of local anesthetics in medicinal products.

The developed technique is universal. Previously, we reported the determination of three other amide anesthetics, namely lidocaine, mepivacaine, and trimecaine, using the same approach. Althought these anesthetics were oxidized by PMS, the reduction process was different. Thus, our technique is also capable of identifying other drugs containing the amine functional groups. Obviously, some selectivity issues can occur in the real samples, for example, in patients' urine after taking medication. Then, it is necessary to use extraction procedures or HPLC. Nevertheless, the

Table 5: The analysis of the pharmaceutical dosages using the proposed method ($n = 3$).

Anesthetic	Pharmaceutical dosage	Requirement of ND, mg (%)	Declared amount, mg	Declared in quality certificate, mg	Determined amount, mg	Relative determination error, %
BC	"*Farisil®*"	4.75-5.25 (95-105)	5.00	5.10	4.9±0.3	3.03
	"*Septolete plus®*"	4.50-5.50 (90-110)	5.00	4.98	5.11±0.23	2.54
PC	Solution for injections	4.75-5.25 (95-105)	5.00	4.97	4.9± 0,4	1.62

proposed electrochemical detection will improve analytical performance.

Data Availability

The [polarogramms, dependences of analytical signal on variety factors (pH, temperature, concentration of reagents, oxidation time, and scan rate)] data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declares that they have no conflicts of interest.

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