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Voltammetric determination of antibiotics in pharmaceuticals

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***Abstract*—This work outlines the scientific data concentrating on the determination of various antibiotics in medical drugs and substances by different physicochemical methods. The electrochemical behavior of widely used antibiotics such as streptomycin, tetracycline, and azithromicyn dihydrate from the macrolide group was studied for the purpose of selecting the determination conditions in pharmaceutical preparations by voltammetric methods. The voltammetric techniques for quantitative determination of streptomycin, tetracycline, and azithromicyn in different pharmaceutical preparations were developed and validated.**

Keywords— antibiotics, determination, medicinal facilities, voltammetry

I. INTRODUCTION

Medical diagnostics and quality control of medicines are based on analysis by various methods. One of the first methods of determining antibiotics began to use microbiological methods, based on their ability to delay sensitive strains of microorganisms. They are relatively simple, but extremely long (the analysis time is more than 36 hours). To determine antibiotics, a number of chemical methods based on their ability to enter into various reactions due to the presence of certain groupings in the molecule are proposed.

There are several variants of optical methods for the determination of different groups of antibiotics in drugs based on the property of many substances to give a characteristic absorption spectrum in visible or ultraviolet light (spectrophotometry) [1,2] or on the conversion of the preparation or its individual groupings to colored compounds (colorimetry).

Recently, the method of high-performance liquid chromatography (HPLC) has been widely used. To increase the sensitivity of the HPLC method, tandem mass spectrometry with an ion trap [4], chemiluminescent [5], ultraviolet [6] and electrochemical [7] detection are used.

One of the most promising methods for the determination of antibiotics, in our opinion, is the voltammetric method due to its high sensitivity, the ability to work with small samples of mass and volume with high expressiveness, selectivity and resolving power. The authors summarized the literature data in the publication [8] on the possible definition of antimicrobial, antiviral and antiparasitic agents; Drugs that regulate metabolic processes; Funds acting on the cardiovascular system; Drugs used to treat cancer; Funds, as well as preparations of different pharmacological groups of medicines by methods of voltammetry.

Although the range of drugs to be determined using voltammetric methods of analysis is quite wide, in many cases it is a question of developing conditions for the determination of certain pharmaceutical products, rather than the development of specific certified or validated methods for analyzing samples of real objects.

The purpose of this work is to study the electrochemical behavior of a number of widely used antibiotics: streptomycin, tetracycline and from the macrolide group - azithromycin dihydrate and the choice of conditions for their determination in pharmaceutical preparations.

II. EXPERIMENTAL PART

The voltammetric complex for analytical measurements was used in the work. - The analytical voltammetric complex STA (Russia, Tomsk, ITM LLC), which is a compact device consisting of an electronic unit, a measuring unit with three electrochemical Cells. Complex STA is completely computerized. Two types of electrochemical cell were used in the work. To determine azithromycin dihydrate and tetracycline on a glassy carbon electrode, this was a variant of a three-electrode cell, and when a mercury-film electrode was used to determine levomycitin and streptomycin, a two-electrode electrode was used.

The glass-carbon electrode is a glass-carbon rod with a diameter of 1.5-2.0 mm pressed into a fluoroplastic holder with a diameter of 5-6 mm so that the length of the protruding part of the glass-carbon rod (working surface) is 8-12 mm. The contact of the electrode with the device was carried out using a metal current lead and a standard connector.

To prepare the electrode for work, polished it with diamond paste to a mirror gloss, degreased with ethyl alcohol and washed with bidistilled water. As necessary, with a decrease in the sensitivity and reproducibility of the measurements, the glassy carbon electrode was subjected to mechanical treatment. For this purpose, the electrode was washed in 96% ethyl alcohol, followed by cleaning the electrode surface with filter paper.

Between the analyzes the electrode was stored in air or in alcohol.

The mercury-film electrode is a fluoroplastic rod with a pressed silver wire 2.0 mm in diameter and 9 - 10 mm in length, the surface area of which is about 15.0 mm2.

Preparation of the indicator electrode for work was carried out by amalgamating the working part of the electrode for this purpose, it was lowered into metallic mercury for several seconds, then triturated with filter paper until the mercury was uniformly distributed over the surface of the silver wire. The amalgamation procedure was repeated when non-amalgamated regions appeared on the electrode surface. A saturated silver chloride electrode was used as the reference electrode. The electrode was stored by immersing it in a saturated solution of potassium chloride.

In the variant of the three-electrode cell, a glass-carbon electrode served as an auxiliary electrode.

Deaeration and mixing of the solutions under analysis in the cell in the electrolytic accumulation stage was carried out by nitrogen gas with an oxygen content of less than 0.001%.

The pH of the solution was determined using a portable pH

meter-millivoltammeter, pH-673 in the usual manner. The error in determining the pH of the solution did not exceed ± 0.1%.To select the background electrolyte for streptomycin and azithromycin, various solutions were used: NaOH, KOH, Na_2HPO_4, Na_3PO_4, $NaClO_4$ (concentrations from 0.01 to 0.1 mol / l), Na_3Citr, Buffer solutions: Britton-Robinson pH (8.00 ÷ 11.00), boron - alkaline $Na_2B_4O_7$ - NaOH (pH 9.23 ÷11,02). All these electrolytes can be used to quantify streptomycin and azithromycin. However, the best background electrolyte for streptomycin is 0.01 mol / dm3 NaOH and for azithromycin 0.1M Na_3Citr.

The linearity of the calibration curves for the studied antibiotics on the selected background electrolytes is preserved in the following concentration ranges: ($8.0 \cdot 10^{-9}$ ... $1.0 \cdot 10^{-7}$) mol / l for streptomycin, ($1.0 \cdot 10^{-8}$ ... 1, $0 \cdot 10^{-5}$) mol / l for tetracycline and ($3,4 \cdot 10^{-10}$ ... $1,0 \cdot 10^{-5}$) mol / l for azithromycin. At concentrations of antibiotics exceeding the above, deviation from the linearity of the calibration curves is observed, which, apparently, is associated with saturation of the surface of the indicator electrode. The relative standard deviation of the results of measurements of analytical signals for the specified concentration ranges does not exceed 0.05.

The dependence of the magnitude of the analytical signals of antibiotics on the potential of preliminary electrolysis and on the time of preliminary electrolysis was studied. In Fig. 1 and 2, these dependences are shown using azithromycin as an example.

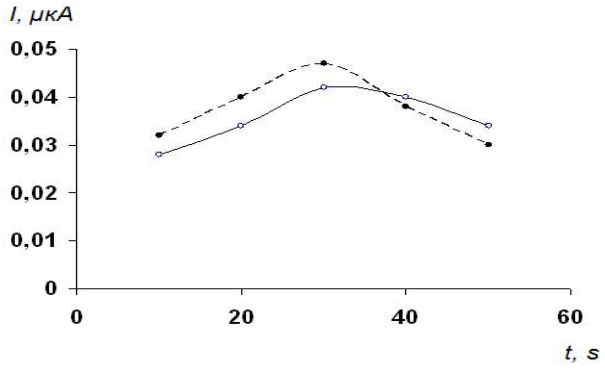


Fig. 1. Dependence of azithromycin reduction current on electrolysis time in Na2HPO4 solution, pH 8.2; W = 30 mV / s, CAz = 1.01 · 10-8 mol / L, 1 - Ee = 0.2 V, 2 - Ee = 0.15 V.

An investigation of the influence of the electrolysis time showed that the working time of the preliminary electrolysis (τe) is 30 ÷ 50 s. At less than 30 s, the sensitivity of its determination decreases (Fig. 1). Also, for sufficiently long times, there was a deviation from the linearity of the calibration dependences of the magnitude of the current on the concentration. Apparently, this was due to an increase in the influence of the adsorption processes proceeding at the electrode.

Estimation of the electrolysis potential at which the maximum concentration of azithromycin dihydrate occurs during the electroreduction has shown that the working range for the electrolysis potential is -0.95 ÷ -1.25 V (Fig.2)

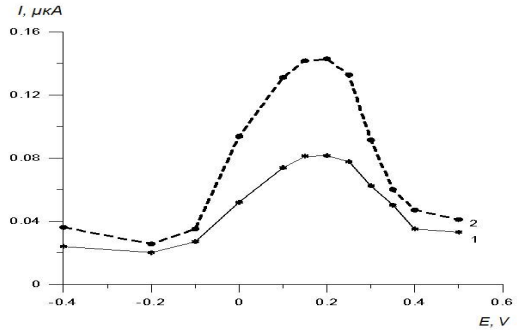


Fig. 2. Dependence of the oxidation current of azithromycin on the electrolysis potential. (Background: 0.2 M Na2HPO4; τe = 20 s; W = 30 mV / s). 1. C = 2.98 · 10-8 mol / L; 2. C = 4.97 · 10-8 mol / L

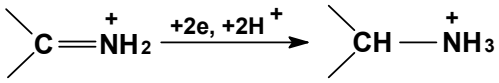
It can be seen from Fig. 2 that the peak current of azithromycin peak on the glassy carbon electrode reached a maximum value in the potential range of 0.15-0.25 V and increased approximately 2.4 times. When choosing the conditions for the determination of azithromycin in various dosage forms, the use of this operating range The electrolysis potential does not allow the concentration of trace amounts of interfering matrix components that are not removed during sample preparation.

An important factor in determining practically all organic substances is the pH of the solution, which affects not only the speed of the electrode process, but also its mechanism. It was found that the change in the pH of the solution affects the value of the peak potential of antibiotic peaks, and these dependences are complex. This is shown in more detail below, for example, the oxidation of azithromycin and the recovery of streptomycin.

Studies have shown that with an increase in the pH of the Britton-Robinson buffer solution above 8.0 the potential of the azithromycin oxidation peak shifts to a more positive range of potentials from 0.774 to 0.850 V. At pH greater than 9.0, an additional peak with En = 0.90 V, Which hampers its analytical definition. At pH less than 8.0, the azithromycin molecule is in the ionized state and therefore oxidizes at a less positive potential, at pH less than 6.0 the oxidation peak is not recorded. PH values from 8.0 to 9.0 are optimal for the quantitative determination of azithromycin. According to the results obtained and described by us earlier [9], we concluded that the electrooxidation reaction of azithromycin is electrophilic. The role of the electrophilic reagent with electrophilic deficiency is performed by the anode, and the role of the substrate is the molecule of azithromycin. From the obtained cyclic voltammograms azithromycin it follows that the process of its electrooxidation under the studied conditions is irreversible.

When the cathode peak of streptomycin was detected, an increase in the pH of the solution led to a displacement of the potential toward more negative values, that is, to a difficulty in the process of its reduction. With strong alkaline solutions, whose pH is more than 9.8, registration of voltammograms is difficult. The optimal pH for the quantitative determination of streptomycin in aqueous solutions is recommended in the range of 9.0 to 9.5. According to semilogarithmic graphs E - ln (I / Ipre - I), effective transfer coefficients are calculated, which have different values and change as the electrode

process proceeds from 0.8 to 0.7. This indicates a complex character of the electroreduction of streptomycin, apparently proceeding through the formation of intermediate reaction products. In general, the electrode process is not strictly diffusive and can be complicated by both adsorption phenomena and previous and subsequent reactions involving organic matter. Based on the conducted studies, a probable scheme of the process of electroreduction of protonated guanidine groups of streptomycin under optimal conditions in aqueous media is proposed:



The established values of the parameters influencing the analytic signals of antibiotics (background electrolyte and its acidity, values of accumulation potential and time of preliminary electrolysis, potential sweep rate) are shown in Table 1.

TABLE I. CONDITIONS FOR THE VOLTAMMETRIC DETERMINATION OF STREPTOMYCIN, TETRACYCLINE AND AZITHROMYCIN

Measurement parameters	**Streptomycin**	**Tetracyclin**	**Azithromycin**
Electrodes: -working - comparisons - auxiliary	mercury-film silver chloride platinum	glassy carbon silver chloride	glassy carbon silver chloride
Background electrolyte	0,01 M NaOH (pH 9,0÷9,5)	0,1 M Na_2HPO_4 +Na_3Citr (pH 7,0)	0,1 M Na_2HPO_4 pH 8,6
Potential of accumulation, *V*	minus 1,2	minus 0,4	plus 0,2
Range of potential sweep, V	minus 1,0 minus 1,8	from 0,2 to 0,9	from 0,2 to 1,1
Electrolysis time, s	30	20	20
Velocity of potential sweep, mV	50	20	30
Peak potential, V	minus (1,45±0,05)	plus 0,70 ± 0,05	plus 0,60 ± 0,05

When determining the content of antibiotics as the main component in medicinal preparations (tablets, capsules, vaccines, etc.), high measurement accuracy is required at significant concentrations of antibiotics in prepared solutions. As a rule, in this case, there is no need for preliminary isolation of antibiotics and separation of concomitant substances.

The essence of the method consists in diluting the samples of the drug with the subsequent voltammetric determination of the antibiotic.

Preparation of samples of tablets and capsules containing antibiotic was carried out as follows. The tablets, freed from the shell, were ground to a powdery state. Capsules containing a powdery substance were opened. 96% ethanol was added to the sample weighed on the analytical balance, intensive stirring was carried out for 20-30 minutes, after which it was filtered through a paper filter. For voltammetric measurements, an aliquot of the obtained filtrate was taken. The algorithm for sample preparation of drugs for the determination of antibiotics for levomycetin, streptomycin, and tetracycline using the voltammetric method is shown in Fig. 3

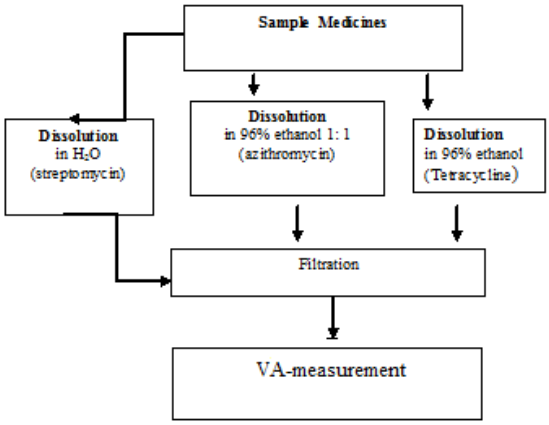


Fig. 3. The algorithm for sample preparation of drugs for the determination of antibiotics for levomycetin, streptomycin, and tetracycline

Methods for the quantitative determination of streptomycin, tetracycline and azithromycin in medicinal preparations (eye drops, tablets, capsules) have been developed and validated. Substances-fillers (starch, sugars, stearates) do not interfere with the determination. The method of determination of tetracycline hydrochloride in tablets and capsules, as well as tetracycline in tablets with nystatin was certified. The possibility of tetracycline determination in mixtures with rifampicin, thiamine bromide and riboflavin used in antibiotic therapy is also shown. The method of quantitative determination of medicinal preparations is developed: tablets and capsules "Sumamed".

TABLE II. THE RANGE OF DETECTABLE CONCENTRATIONS OF ANTIBIOTICS IN DRUGS AND BIOLOGICAL OBJECTS

The analysis object	**The component to be determined**	**The range of detectable concentrations**
Medications		
Tablets, capsules	Tetracycline azithromycin	from 40 to 300 mg / table from 8 to 800 mg / table
Eye drops, injectable solutions	Tetracycline	from 0,40 to 3,0 %
Biological objects		
Urine, blood, tissues	Azithromycin	from 3 to 150 mg / dm^3 (mg / kg)

The correctness indicators (the characteristic of the systematic error) of the developed methods were evaluated using standard samples, high-purity substances, by the method "introduced-found" and by comparison with the results obtained by standard methods. The values of the error

characteristics (accuracy indicators) of the measurement results are determined by the calculation method according to the established values of the characteristics of the random and systematic error components.

III. CONCLUSION

The performed investigations expand the possibilities of using the voltammetric method for quantitative determination of antibiotics in medicinal preparations. The developed techniques allow for rapid and rapid quality control of the analyzed objects with high sensitivity. At the same time, the analysis is significantly cheaper due to a reduction in the number of reagents used (5 to 6 instead of 30 according to previously known methods), and scarce and expensive substances are not required. The analysis time of samples taking into account sample preparation does not exceed 2 hours against 2 - 3 days according to the recommended methods.

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