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Amperometric Biosensors for the Determination of Tetracycline

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Abstract—Amperometric biosensors based on an immobilized tyrosinase enzyme and planar graphite electrodes modified with multiwalled carbon nanotubes (MWCNTs) in chitosan, reduced graphene oxide (RGO), and gold nanoparticles (Au NPs) in chitosan and nanocomposites based on them are developed for rapid and accurate determination of tetracycline. It is found that tetracycline is a tyrosinase inhibitor, which makes it possible to determine it using a tyrosinase biosensor in a range of concentrations of 1 nmol/L to 1 μmol/L. It is found on the basis of the results of kinetic studies of the reaction of enzymatic conversion of phenol that uncompetitive inhibition is observed on the tyrosinase biosensor in the presence of tetracycline. A combination of carbon nanomaterials with metal nanoparticles can form a nanocomposite with a synergistic effect. The use of carbon nanomaterials and metal nanoparticles as modifiers of the electrode surface makes it possible to improve the analytical characteristics of the developed sensors: to expand the range of determined concentrations and to decrease the limit of quantification (50 pmol/L for a biosensor with MWCNTs/Au NPs, 0.7 nmol/L for a biosensor with RGO/Au NPs). The relative standard deviation of the obtained using biosensors results does not exceed 0.08. Procedures for the determination of tetracycline using the proposed biosensors in milk and a cosmetic product are tested. The compounds present in these samples which are structurally unrelated to tetracycline do not interfere with its determination.

Keywords: determination of antibiotics, tyrosinase biosensor, tetracycline, carbon nanotubes, graphene oxide, gold nanoparticles, milk

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INTRODUCTION

Antibiotics are medicinal products capable of selectively suppressing the viability of microorganisms. They are subdivided into groups depending on the chemical structure. The representatives of one group differ only with respect to substituents.

Some of the widely used antibiotics are tetracyclines. This group is characterized by the similarity of the chemical structures (a four-membered naphthacene nucleus) and similar mechanism and broad spectrum of antibacterial action. Tetracycline ([4*S*-(4α,4α,5α,6β,12α)]-4-(dimethylamino)-1,4,4α,5,5α,6,11,12α-octahydro-3,6,10,12,12α-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacene carboxamide) (Fig. 1) is an antibiotic applied in the form of tablets, eye and skin ointments, and injections; it is the most widely used in medical practice.

Tetracycline is a bacteriostatic preparation. It inhibits protein synthesis in bacterial cells and violates the permeability of a microbial cell. The preparation is highly active towards gram-positive and gram-negative bacteria [1].

This compound belongs to tetracyclines of natural (biosynthetic) origin. The preparation has a broad spectrum of side effects; in particular, it induces hepatotoxicity, disbacteriosis, nausea, photosensitization, disturbance of the formation of bone and dental tissues, change in the color of the dental enamel in children, increase in the intracranial pressure, etc. The appearance of resistance of an organism to one representative of this group induces resistance to the entire group of tetracycline antibiotics [2]. Because of this, its presence in water and food products can negatively affect the human body. Currently, this preparation is applied in veterinary medicine for the treatment of cattle as well as in the production of meat, vegetables, and milk to increase their shelf life.

Currently, optical [3, 4], chromatographic [5, 6], electrochemical [7–9], and biosensor [10] devices are used for the determination of tetracycline, which are distinguished by high sensitivity, selectivity, and rapidity.

The use of amperometric biosensors modified with nanomaterials of different nature is one of the modern approaches to the determination of drug compounds. Biosensors based on vegetable and animal tissues can be more preferable than expensive methods owing to the availability and cheapness, providing high sensitivity and, in certain cases, selectivity of determination. The variety of the methods of modification of the surface of primary transducers for imparting certain properties to them provides the possibility of improv-

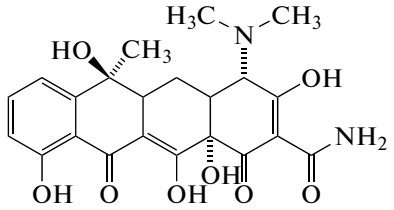


Fig. 1. The structural formula of tetracycline.

ing the characteristics of novel amperometric biosensors. Nanomaterials, e.g., multiwalled carbon nanotubes (MWCNTs) and reduced graphene oxide (RGO), are very promising materials in the development of biosensor devices owing to their exceptional electronic properties [11]. A relevant approach to improving the properties of the electrode surface is the use of nanocomposites based on carbon nanomaterials and metal nanoparticles, in particular, gold nanoparticles (Au NPs). This is determined by their high electrical conductivity, chemical resistance, and simplicity of preparation of stable gold sols [12].

The aim of this work is the development of amperometric biosensors based on graphite electrodes modified with MWCNTs, RGO, Au NPs, and an immobilized tyrosinase enzyme for the determination of tetracycline and assessment of their analytical capabilities for the control of the concentration of tetracycline in food (in particular, dairy) and cosmetic products.

EXPERIMENTAL

Chemicals and Equipment

The developed biosensors were based on a system consisting of working and auxiliary graphite electrodes and a silver/silver chloride reference electrode obtained on a polymer substrate by the method of screen-printing technologies (Department of Analytical Chemistry, Kazan (Volga Region) Federal University).

Graphite ink (Gwent Electronic Materials, United States) was the material of the working electrode surface, on which the modifier and enzyme were immobilized. The auxiliary electrode was also fabricated from graphite ink.

The volume of the working cell was 2000 μL. A 204 N potentiostat/galvanostat (Autolab, Netherlands) was used for electrochemical detection.

Electrochemical stabilization and cleaning of the electrode surface were carried out by cyclic variation of the potential in a range of 0 to 1000 mV; for this, five to seven cyclic voltammograms were recorded in a 0.15 M solution of KCl at a potential sweep rate of 100 mV/s.

Reagent grade phenol was used as the substrate. Its solutions were prepared using a precisely weighed amount. A phosphate buffer with pH of 7.1 ± 0.05 was used as the solvent. The shelf life of the specified solution did not exceed 3 h.

To prepare a dispersion of multiwalled carbon nanotubes (MWCNTs) (an external diameter of 10–15 nm, an internal diameter of 2–6 nm, a length of 0.1–10 μm; Sigma-Aldrich, United States), dispersion was executed in an S30H ultrasonic (US) bath (Elmasonic, Germany) at a frequency of 37 kHz.

A 1% solution of glutaraldehyde (GA) (ICN, United States) and bovine serum albumin (BSA) (Reanal, Hungary) were used. Reagent grade sodium citrate, reagent grade chitosan, reagent grade HCl, reagent grade $HAuCl_4 \cdot 4H_2O$, chemically pure grade $SnCl_2$, and polyethylene glycol (PEG-3000) (Sigma) were used for the preparation of gold nanoparticles (Au NPs).

A chromatographically pure tetracycline preparation (Sigma-Aldrich, United States) was used to obtain working solutions by dissolution in 0.1 M sulfuric acid. The values of pH of the aqueous solutions were determined using a pH-150 pH meter equipped with a glass electrode calibrated by standard buffer solutions.

The Preparation of Carbon Nanotubes for the Modification of Electrodes

Prior to the modification of the electrode surface, the MWCNTs were mixed with a 0.5 wt % solution of chitosan in 0.05 M acetic acid. To achieve homogeneity, the mixture was dispersed in a US bath at room temperature. The concentration of the dispersion of MWCNTs was 1 mg/mL. To maintain the homogeneity of the solutions of MWCNTs used for the modification, they were periodically sonicated.

The Preparation of Graphene Oxide for the Modification of the Electrode Surface

The initial reduced graphene oxide (RGO) was an aqueous solution with a concentration of 2 mg/mL (Sigma-Aldrich). For better fixation of RGO on the electrode surface, a 0.75% solution of chitosan in 2% acetic acid was added. The obtained solution was sonicated at 35°C to obtain a dispersion with a concentration of 1.5 mg/mL. In the case of an increase in the temperature above the set temperature, the water in the US bath was cooled down to the required temperature. The homogeneity of the surface solution of RGO used for the modification of the electrode was maintained by periodic sonication.

The Preparation of Tyrosinase from a Fungal Homogenate

Ten grams of finely cut and preliminarily frozen vegetable material (champignon mushrooms *Agaricus bisporus*) was brought to a paste-like texture in a frozen-out mortar. After this, 10 mL of a phosphate buffer solution with pH of 7.00 ± 0.05 was added to the homogenate, and it was stirred using a magnetic stir-

rer. The liquid fraction was separated by filtration through a double gauze layer. The filtrate was a direct source of tyrosinase.

To determine the catalytic activity of tyrosinase, a mixture containing a phosphate buffer solution (pH 7.00 ± 0.05), a 1 mM solution of phenol, and an aliquot of the homogenate was used [13, 14]. The absorbance was measured at a wavelength of 280 nm for about 20 min. The activity of the enzyme was determined as the increase in the absorbance (λ = 280 nm) over a certain time under these conditions.

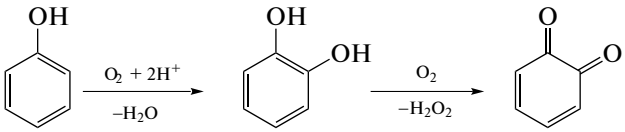
According to the spectrophotometric data, the catalytic activity of tyrosinase from the mushrooms was 2751 ± 133 ncat/mL.

The Preparation of the Biosensitive Part of the Amperometric Tyrosinase Biosensor Based on a Screen-Printed Graphite Electrode

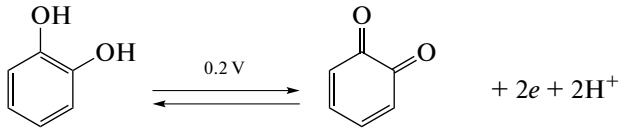
The biosensitive part of the biosensors was immobilized on the working electrode surface with tyrosinase prepared from mushrooms. A mixture consisting of a solution of the enzyme, distilled water, a phosphate buffer solution, a solution of BSA, and a 1% solution of GA which was added at the very end upon vigorously stirring the mixture was prepared for application on the electrode surface. This mixture was applied to the electrodes in the amount of 1 μL each. The obtained biosensors were kept in a closed Petri dish overnight at $t = +4°C$. After 12 h, the biosensors were rinsed with water and dried. They were further stored in a refrigerator at $t = 4°C$.

RESULTS AND DISCUSSION

It is known from the published data that phenol undergoes biocatalytic oxidation under the action of a tyrosinase enzyme, during which quinone is formed:



(an enzymatic reaction [15]),



(an electrochemical reaction).

An additional peak is observed on the tyrosinase biosensor in a region of potentials of 0.65–0.70 V, which belongs to oxidation of hydrogen peroxide. In accordance with the published data [16], electrochemical oxidation of hydrogen peroxide occurs according to the following scheme:

$$H_2O_2 \xrightarrow{0.7\ V} O_2 + H^+ + 2\bar{e}.$$

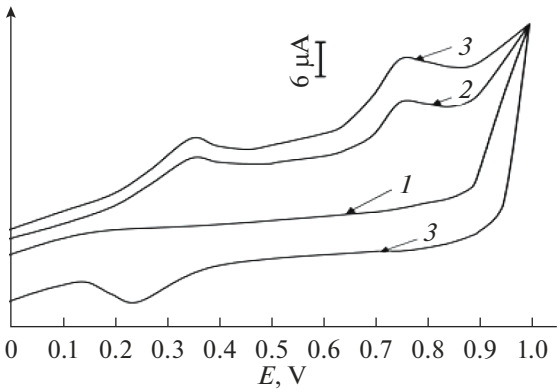


Fig. 2. Cyclic voltammograms of the conversion of the tyrosinase substrate of phenol (1×10^{-3} mol/L) on a screen-printed graphite electrode in the (*3*) absence and (*2*) presence of tetracycline (1×10^{-8} mol/L); (*1*) the supporting electrolyte is a phosphate buffer solution with pH of 7.00 ± 0.05.

The peak at a potential of 0.20 V probably corresponds to the electrochemical oxidation of phenol to quinone (Fig. 2).

The catalytic activity of the enzyme was maximal in the case of the use of a phosphate buffer with pH of 7.00 ± 0.05 as the medium [17]. The used concentration of phenol—the tyrosinase substrate—was 1 mmol/L.

The Action of Tetracycline on the Catalytic Activity of Immobilized Tyrosinase

The study of the effect of tetracycline on tyrosinase in the composition of the amperometric biosensor has shown that a linear decrease in the analytical signal of the developed biosensor in a range of concentrations of 1 nmol/L–10 μmol/L is observed in the presence of tetracycline. Therefore, a conclusion can be drawn that tetracycline is an inhibitor of the enzymatic conversion of phenol, which detected for the first time (see Fig. 2, curve *2*).

The maximum degree of inhibition for tetracycline in the studied range of concentrations upon acting on the tyrosinase–phenol enzyme–substrate system is 85 ± 1%.

Nanostructured Materials in the Composition of the Developed Biosensors

To apply modifiers to the surface of the used screen-printed electrodes, a method of dropwise evaporation was applied using dispersions of RGO and MWCNTs in chitosan or Au NPs in chitosan.

Owing to their unique electronic, electrophysical, thermal, optical, and mechanical properties, carbon nanomaterials (MWCNTs, RGO) turned out to be very promising for use as a base for miniature biosen-

Table 1. Analytical capabilities of determination of tetracycline by tyrosinase biosensors modified with MWCNTs and RGO ($n = 5$, $P = 0.95$)

Range of working concentrations, mol/L	Calibration curve equation $I^* = (A \pm \delta) + (B \pm \delta)(-\log C)$			c_n, mol/L	Maximum degree of inhibition, %
	$(A \pm \delta)$	$(B \pm \delta)$	r		
Tyrosinase biosensor					
1×10^{-6}–1×10^{-9}	120 ± 8	−4.2 ± 0.5	0.9823	5×10^{-10}	85 ± 1
Tyrosinase biosensor, modified with RGO					
1×10^{-6}–1×10^{-9}	60 ± 7	−15.6 ± 0.7	0.9901	5×10^{-10}	86.0 ± 0.8
Tyrosinase biosensor, modified with MWCNTs					
1×10^{-6}–1×10^{-10}	101 ± 7	−15.9 ± 0.7	0.9882	7×10^{-11}	88.0 ± 0.9

* $I = I_p/I_0 \times 100$, $(I_p = I_0 - I_s)$, where I_s is the current in the presence of the inhibitor and I_0 is the current in its absence.

sor devices [18, 19]. The presence of a large number of oxygen-containing functional groups provides the possibility of crosslinking and/or fixation of the enzyme on the surface of RGO, which, in combination with a large surface area, makes RGO an ideal platform for covalent immobilization of proteins. Composites based on RGO can be used as an electrode material for improving the electrochemical properties of sensors and biosensors [20]. RGO can deposit onto any substrate, transforming its properties.

Then a solution of tyrosinase was immobilized on the already modified surface of the working electrode. The use of modifiers makes it possible to increase the area and roughness of the working electrode surface, which reflects on the amount of the immobilized enzyme and leads to the improvement of the analytical characteristics of the sensor in the first place.

The Tyrosinase Biosensor Modified with MWCNTs and RGO for the Determination of Tetracycline

The study of the action of tetracycline on tyrosinase immobilized on electrodes modified with MWCNTs and RGO in chitosan has shown that tetracycline still has a reversible inhibiting action on tyrosinase. Here, just an insignificant change in the values of the oxidation potential is observed, in particular, a shift of the potential from 0.70 to 0.73 V.

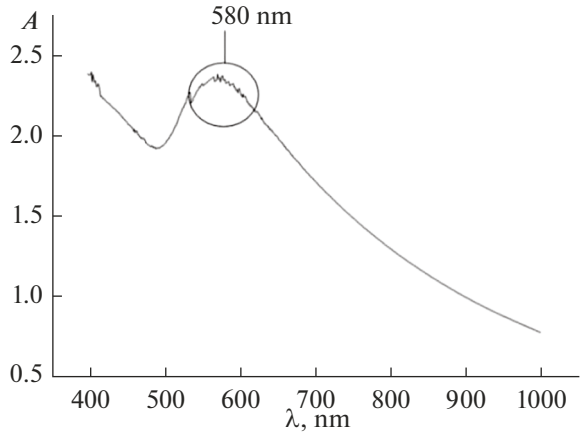


Fig. 3. Absorption spectrum of Au NPs in solution of chitosan.

Biosensors modified with MWCNTs in chitosan make it possible to expand the range of determined concentrations of tetracycline and to improve the correlation coefficient (Table 1). The maximum degree of inhibition of tetracycline in the case of acting on the tyrosinase–phenol enzyme–substrate system under these conditions was about 90% in the studied range of concentrations.

Gold Nanoparticles as the Modifiers of the Screen-Printed Electrode Surface

One of the methods of modification of the electrode surface is the use of nanocomposites based on various carbon nanomaterials and metal nanoparticles. To improve the analytical properties of biosensors, nanocomposites based on MWCNTs, RGO, and Au NPs (MWCNTs/Au NPs, RGO/Au NPs) were used.

Reduction of chloroauric acid with tin(II) chloride was used for the synthesis of Au NPs [21]. To obtain dispersions of nanoparticles, solutions of $SnCl_2$, polyethylene glycol, and $HAuCl_4$ were used at a certain ratio. Chitosan acted as the stabilizer [22].

The corresponding absorption spectra confirm the presence of Au NPs with a certain size in the solutions (Fig. 3) [23].

The Effect of the Modification of the Electrode Surface with Nanocomposites of MWCNTs/Au NPs and RGO/Au NPs on the Analytical Capabilities of the Tyrosinase Biosensor

The study of the effect of tetracycline on the tyrosinase biosensor modified with MWCNTs, RGO, and Au NPs has shown that tetracycline has an inhibiting action on tyrosinase in a range of concentrations of

Table 2. Analytical capabilities of determination of tetracycline by tyrosinase biosensors modified with MWCNT/Au NPs and RGO/Au NPs ($n = 5$, $P = 0.95$)

Range of working concentrations, mol/L	Calibration curve equation $I = (A \pm \delta) + (B \pm \delta)(-\log C)$			c_n, mol/L	Maximum degree of inhibition, %
	$(A \pm \delta)$	$(B \pm \delta)$	r		
Tyrosinase biosensor, modified with RGO					
1×10^{-6}–1×10^{-9}	63 ± 6	-20.6 ± 0.8	0.9925	7×10^{-10}	90 ± 1
Tyrosinase biosensor, modified with MWCNTs					
1×10^{-6}–1×10^{-10}	131 ± 8	-21.6 ± 0.7	0.99926	5×10^{-11}	92 ± 1

Table 3. Results of determination of tetracycline using tyrosinase biosensors ($n = 5$; $P = 0.95$)

Introduced, mol/L	Found, mol/L	S_r	Degree of recovery, %
Tyrosinase biosensor			
5×10^{-8}	$(5.1 \pm 0.4) \times 10^{-8}$	0.078	94–110
3×10^{-9}	$(2.8 \pm 0.2) \times 10^{-9}$	0.071	93–107
Tyrosinase biosensor—MWCNTs in chitosan			
5×10^{-9}	$(4.8 \pm 0.3) \times 10^{-9}$	0.062	94–106
7×10^{-10}	$(6.7 \pm 0.5) \times 10^{-10}$	0.060	93–107
Tyrosinase biosensor—MWCNTs and Au NPs in chitosan			
8×10^{-8}	$(7.6 \pm 0.4) \times 10^{-8}$	0.053	95–105
3×10^{-10}	$(2.9 \pm 0.2) \times 10^{-10}$	0.069	93–107
Tyrosinase biosensor—RGO in chitosan			
7×10^{-8}	$(6.7 \pm 0.5) \times 10^{-8}$	0.075	93–107
3×10^{-9}	$(2.6 \pm 0.2) \times 10^{-9}$	0.077	92–108
Tyrosinase biosensor—RGO in chitosan and Au NPs in chitosan			
7×10^{-7}	$(6.6 \pm 0.5) \times 10^{-7}$	0.076	92–109
5×10^{-8}	$(4.8 \pm 0.2) \times 10^{-8}$	0.042	96–104

0.1 nmol/L–1 μmol/L in the case of MWCNTs/Au NPs and 1 nmol/L–1 μmol/L, in the case of RGO/Au NPs (Table 2). It follows from the obtained results that the use of the nanocomposite modifiers makes it possible to decrease LOD by almost an order of magnitude and to improve the correlation coefficient.

The accuracy of determination of tetracycline in the specified ranges of concentrations using tyrosinase biosensors has been estimated by the spike/recovery method (Table 3).

It should be noted that the modification of the electrode surface with MWCNTs/Au NPs and RGO/Au NPs composites made it possible to improve the analytical characteristics of the developed biosensors, in particular, to expand the range of determined concentrations and to decrease LOD in comparison with the unmodified analog.

Analyzing the results of determination of tetracycline by biosensors modified with carbon nanomaterials and composites based on them, we can conclude that the main contribution to the improvement of the analytical characteristics of the developed biosensors is made by MWCNTs and RGO. However, in individual cases, e.g., for more sensitive determination, biosensors modified with the corresponding nanocomposites can also be used.

Kinetic Parameters of the Reaction of Enzymatic Conversion of Phenol in the Presence of Tetracycline

The values of the characteristic kinetic parameters (the Michaelis constant, maximum rate of enzymatic reaction, type of inhibition) of an enzymatic reaction in the presence of the effector under determination can be used for choosing the conditions providing the acquisition of the maximum analytical signal upon determining microscopic amounts of an analyte (Table 4).

Table 4. Kinetic parameters of the reaction of tyrosinase conversion of phenol in the presence of tetracycline ($C_{phenol} = 1 \times 10^{-3}$ mol/L, pH 7.00, $n = 3$)

Concentration of tetracycline, $C_{tetracycline}$, mol/L	Michaelis constant $K_m \times 10^5$, mol/L	Maximum rate $V_{max} \times 10^{-7}$, mol L^{-1} s^{-1}	Ratio of the parameters K_m and V_{max}	Type of inhibition	Inhibition constant K_I, mol^{-1}
Tyrosinase biosensor—MWCNTs/Au NPs					
0	3.2 ± 0.5	1.7 ± 0.2	$K_m' < K_m$	Two-parametrically discordant inhibition (uncompetitive inhibition)	$(6.8 \pm 0.6) \times 10^{-9}$
1×10^{-8}	1.3 ± 0.3	1.1 ± 0.2	$V_{max}' < V_{max}$		

K_m is the apparent Michaelis constant in the absence and K_m is the constant in the presence of a tyrosinase inhibitor; V_{max} is the maximum reaction rate in the absence and V_{max} is the maximum reaction rate in the presence of a tyrosinase inhibitor.

Table 5. Results of determination of tetracycline in milk samples by various methods and with the use of a tyrosinase biosensor modified with MWCNTs/Au NPs ($n = 5$, $P = 0.95$)

Method	Result of determination of tetracycline in milk	Reference
Magnetic solid-phase extraction in combination with HPLC	Detected in one milk sample at a concentration of 75.8 ± 3.8 ng/mL	[25]
Magnetic solid-phase extraction in combination with HPLC	Not detected*	[26]
Chemiluminescence enzyme-linked immunosorbent assay	Detected in three milk samples at concentrations of 15, 18, and 46 ng/mL	[27]
Tyrosinase biosensor (the modifier is MWCNTs/Au NPs)	Not detected*	Results of this study

* Concentration below c_n.

To determine the kinetic parameters of a reaction such as the apparent Michaelis constant K_m, maximum rate V_{max}, and inhibition constant K_I, integral analysis of the full kinetic curve of action of immobilized tyrosinase on phenol in the absence and presence of tetracycline was used [24]. To determine the reaction rates, the initial section of this curve was used. As is shown by the results of kinetic studies, two-parametrically discordant inhibition (uncompetitive inhibition) is observed in the case of the use of the modified with MWCNTs and Au NPs biosensors in the presence of tetracycline at a concentration of the substrate of 1 mmol/L. Under the same action leading to a decrease in the affinity of the substrate and enzyme, the effector in the presence of 1 mmol/L phenol decreases the rate of its enzymatic conversion.

The Determination of Tetracycline in Milk

Tetracycline, being a broad-spectrum antibiotic, is used in veterinary medicine for the treatment of cattle and, hence, can be present in milk and dairy products. The concentration of this preparation is regulated according to the standards of the European Union. For milk, the maximum allowable concentration (MAC) of tetracycline is 100 μg/L (4.4×10^{-7} mol/L).

The sample preparation of a milk sample (1.5% fat) for the analysis consisted in 50-fold dilution with a phosphate buffer solution. This dilution was sufficient to eliminate the effect of the matrix components. At the same time, the analytical characteristics of the proposed biosensors make it possible to successfully work in the required ranges of concentrations.

It has been found that the linear dependence between the concentration of tetracycline and value of the analytical signal against the background of milk in the case of an amperometric tyrosinase biosensor modified with MWCNTs/Au NPs is observed in the same range of concentrations as against the background of the buffer solution. Hence, any of the earlier obtained calibration curves can be used (see Table 4) for the determination of the residual amounts of medicinal products in milk samples.

The results of determination of tetracycline in the samples of dairy products are presented in Table 5.

During the analysis of milk samples, other medicinal products that are also used in veterinary medicine but have a structurally unrelated structure, in particular, colchicine and levomycetin, do not have an interfering action during the determination of tetracycline by the developed biosensors.

The Determination of Tetracycline in a Salicylic Lotion

According to the results of the analysis of widely used antiacne lotions, tetracycline is often detected in their composition which is added to these products (without specifying it on the label) because this antibiotic can help cure skin diseases but can aggravate them as well. In our country, the use of antibiotics in decorative cosmetics is illegal. In this connection, it was of interest to assess the possibility of using the developed biosensor for the estimation of the presence or absence of tetracycline by way of example of a Propeller salicylic antiacne lotion—an inexpensive cosmetic product that is present in many stores.

The Procedure for the Determination of Tetracycline in a Salicylic Lotion

Into a 2000-μL cell, 100 μL of a solution of a sample of the lotion under study obtained by preliminary 25-fold dilution, a 0.1 M solution of phenol, a phosphate buffer (pH of 7.00), and a modified with RGO/Au NPs tyrosinase biosensor were introduced. The solutions were incubated for 10 min, and the value of the current was measured.

No tetracycline was found in the lotion under study ($c_{\text{tetracycline}} < c_{\text{n}}$).

CONCLUSIONS

Therefore, biosensors unmodified and modified with various nanomaterials on the basis of an immobilized tyrosinase enzyme have been proposed; their main analytical characteristics have been determined; and the optimum conditions of preparation of the sensors for conducting measurements in aqueous and biological media have been found. It should be noted that there are no examples of biosensors for the determination of tetracycline in the published sources. Multiwalled carbon nanotubes in chitosan, reduced graphene oxide, and also gold nanoparticles in chitosan have been used as the modifiers. It has been shown that the modification of the electrode surface with these nanocomposites makes it possible to expand the range of determined concentrations and to decrease the limit of quantification of tetracycline.

An advantage of planar sensors is the possibility of their use for conducting measurements in microvolumes of samples, which is important in the analysis of biological media. A possibility of application of tyrosinase biosensors for the determination of tetracycline in milk and a salicylic lotion has been shown.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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