ISSN 1070-3632, Russian Journal of General Chemistry, 2019, Vol. 89, No. 3, pp. 466–469. © Pleiades Publishing, Ltd., 2019.
Russian Text © L.K. Sal'keeva, E.I. Korotkova, K.V. Dyorina, E.K. Taishibekova, E.V. Minaeva, A.A. Muratbekova, A.K. Sal'keeva, 2019, published in Zhurnal Obshchei Khimii, 2019, Vol. 89, No. 3, pp. 441–446.

Electrochemical Study of the Complex-Forming Properties of Phosphorylated Glucoluril

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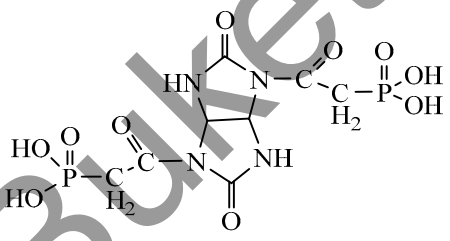
Received August 30, 2018; revised September 20, 2018; accepted September 26, 2018

Abstract—Complex-forming ability of glucolurildiphosphonic acid synthesized via the classical Arbuzov reaction has been studied using natural fatty lipophilic alcohol cholesterol. As a result of voltammetric examinations, an approach for the production of new sensitive electrochemical sensor for cholesterol detection has been proposed; this sensor exhibits much higher efficiency in comparison with other existing samples.

Keywords: glucoluril (bisurea), diphosphonic acid, cholesterol, electrochemical sensor

DOI: 10.1134/S1070363219030162

Glucoluril derivatives attract great attention of researchers due to the possibility of their functionalization and diverse practical applications [1–11]. Previously we have demonstrated the synthesis of {(2,5-dioxohexahydroimidazo[4,5-*d*]imidazole-1,4-diyl)bis(2-oxoethane-2,1-diyl)}bis(phosphonic acid) **1** via classical Arbuzov reaction of 1,4-bis(bromoacetyl)-tetrahydroimidazo[4,5-*d*]imidazole-2,5(1*H*,3*H*)-dione and triethyl phosphite [12].



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The structure of the obtained diphosphonic acid **1** suggests its strong ability to the complex formation and apparently capability to create the supramolecular *host-guest* systems, where several acidic molecules **1** might be coordinated, resulting in the formation of a new inclusion complex. Therefore, herein we investigated the complex formation of diphosphonic acid **1** with different compounds. In particular, we considered the possibility to use compound **1** as a surface modifier of carbon-containing electrodes for the cholesterol detection.

Cholesterol is a natural fatty alcohol. This compound plays significant role in human organism: it is involved in the synthesis of vitamin D, different steroid hormones (including, female sex hormones estrogen and progesterone, and also male sex hormone testosterone), in the functioning of nervous and immune systems, and in lipid metabolism [13]. However, despite the positive role of this sterol for human organism, it might be one of the markers of cardiovascular diseases (atherosclerosis, hypertension, cerebral thrombosis, ischemia, stroke and etc.), distortions of lipid metabolism and diabetes mellitus [14]. Thus, the monitoring of cholesterol concentration in a patient blood is essential for clinical diagnostics and therapy of cardiovascular diseases.

The topicality of the issue of the determination of cholesterol promotes active development of the related analytical methods, including electrochemical methods and the corresponding sensors. The application of electrochemical sensors is promising for the preparation of test systems and diagnostics of clinical

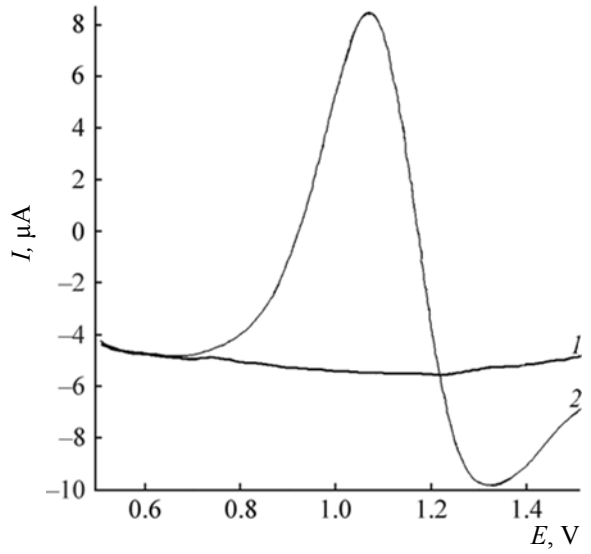

Fig. 1. Voltammogram of cholesterol (10 μmol/L): (*1*) pristine glassy carbon electrode and (*2*) modified electrode. Background electrolyte: phosphate buffer with pH = 6.86, v = 0.04 V/s, E_p = +1.06 V.

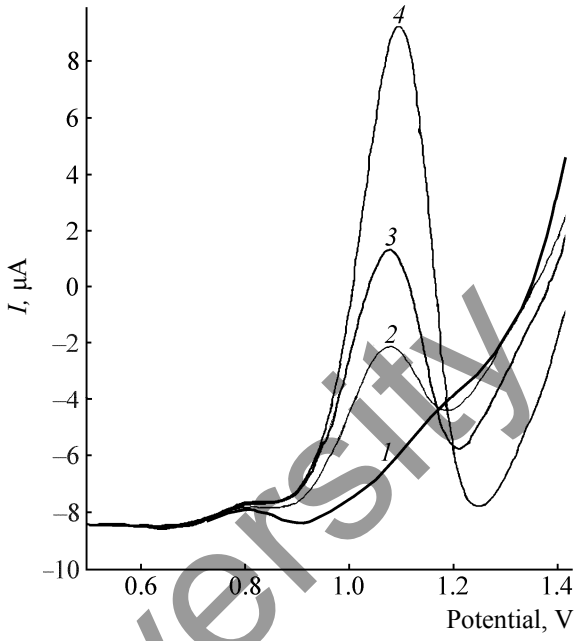

Fig. 2. Volammograms registered in the absence (*1*) and in the presence (*2–4*) of cholesterol in the cell with modified electrode (E_p = +1.06 V). Cholesterol concentration, μmol/L: 6 (*2*), 12 (*3*), 18 (*4*).

analysis at the place of treatment due to its simplicity and speed of implementation, low cost and possibility of miniaturization along with high sensitivity. Most of the existing sensory methods of cholesterol detection are based on the use of enzymatic systems or metal nanoparticles (mainly, silver and gold ones). However, the currently available methods suffer from a number of drawbacks. Enzymatic systems, possessing high selectivity and relative production simplicity, are extremely sensitive to the ambient conditions (the change in external medium temperature, medium pH, etc.), owing to enzymes propensity to denaturation [15]. This feature adversely affects the reproducibility and accuracy of the enzymatic sensors. In turn, sensors based on nanoparticles possess lower selectivity in comparison with the enzymatic ones. Besides this, the synthesis of nanoparticles can hardly be standardized, which complicates the industrial-scale production of such devices [16]. Hence, selection and development of new electrode surface modifiers are scientific-technical challenges.

Previously it has been shown that voltammetric determination depends on the type of electrode material and on the potential at which the reaction with the analyzed substance is conducted on the electrode [17]. With the aim of exploring new ways of cholesterol detection and development of its determination methods, we probed glassy carbon electrodes modified with polyfunctional azaheterocyclic compound of the bicyclic bisureas class, namely, diphosphonic acid **1**.

The electrochemical reactions were conducted using a three-electrode cell consisting of working (modified glassy carbon), counter, and reference (silver chloride electrode) electrodes. The measurements were carried in phosphate buffer at pH = 6.86. Thus, the measurements were conducted under conditions close to physiological.

It was shown that the use of the modifier allowed to obtain the signal of cholesterol oxidation at the anodic potential region (Fig. 1). Deposition of the modifier was conducted via two methods: physical adsorption from a solution and electrochemical deposition. It was stated that the electrochemical deposition gave more stable response using glassy carbon electrode. The potential of the detected electrooxidation peak equaled +1.06 V. The modifier was electrochemically inactive in that potential range. Besides that, the application of phosphate buffer as a background electrolyte requires the use of additional emulsifying agent. *tert*-Octylphenoxypolyethoxyethanol (Triton X-100) (Fig. 2) was used in this study.

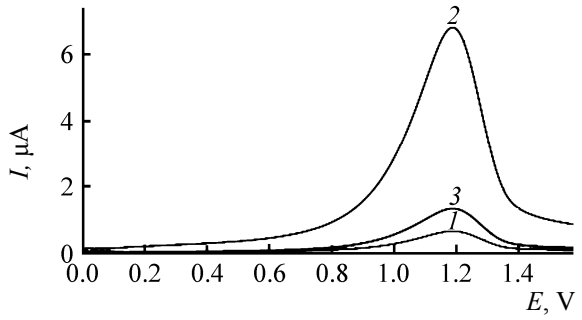

Fig. 3. The current of cholesterol oxidation as a function of the applied potential, registered using the modified electrode at different pH values (composition of the system: cholesterol, Triton X-100, isopropyl alcohol). pH = 1.65 (*1*), 6.86 (*2*), 12.43 (*3*).

It was shown that the peak of cholesterol electrooxidation was observed when emulsifier (Triton X-100) (Fig. 3) was present in the system. If the emulsifying agent was absent, the signal was not observed. That fact was explained by the presence of the modifier in the aqueous phase due to its hydrophilicity. Thus, no interaction of cholesterol with the modifier was observed in the absence of the amphiphilic emulsifier. Moreover, isopropanol, despite being an alcohol, did not interact with diphosphonic acid **1**.

It was also confirmed that the emulsifying agent did not exhibit any electrochemical activity in the operating potential range. The peak of cholesterol electrooxidation was absent if any of system components was absent (cholesterol, Triton X-100, or isopropyl alcohol), which could be explained by the insufficient probe dilution in the absence of isopropyl alcohol and impossibility of cholesterol interaction with the modifier in the absence of the emulsifying agent.

The current dependence on the cholesterol concentration in the cell was linear in the 1×10^{-6}–1×10^{-5} mol/L range. That linear plot allowed the determination of cholesterol in foodstuffs as well as in biological fluids (blood serum). The obtained signal was characterized by high stability in the model media corresponding to investigating objects. The composition of the model media is given in the table. Also, the measurements were conducted using the electrodes prepared by screen printing method.

Hence, the suggested method of cholesterol detection using {(2,5-dioxohexahydroimidazo[4,5-d] imidazole-1,4-diyl)bis(2-oxoethane-2,1-diyl)}bis-(phosphonic acid) as a modifier allowed to decrease the potential of cholesterol oxidation and obtain stable signal in the region available for the measurements. It opens up extensive prospects for the modifier application with the aim to determine cholesterol in foodstuffs and human biological fluids to provide early diagnostics of cardiovascular diseases and therapy.

Composition of the model media for the cholesterol content determination

Blood serum		Foodstuffs	
component	content, mM	component	content, mM
Glucose	6.40	Glucose	2.50
Ascorbic acid	0.06	Galactose	2.50
Pyruvic acid	0.50	Lactose	0.03
Lactic acid	0.20	Ascorbic acid	0.01
Aspartic acid	0.02	Tocopherol acetate	0.01
Uric acid	0.50	Casein	0.50
Glutamic acid	0.09	Albumin	0.50
Alanine	0.31	Globulin	0.50
Creatinine	1.00	Thiamine	1.00
Leucine	0.19	Pyridoxin	1.50
Glycine	0.32	Cyanocobalamin	2.00
Urea	5.00	Retinol acetate	0.01
Cholecalciferol	0.10	Choline	3.50
Cholesterol	5.00	Cholesterol	0.05

Further, the approbation of obtained system on real subjects such as foodstuffs will be performed.

EXPERIMENTAL

Diphosphonic acid **1** was synthesized as described elsewhere [12].

Electrochemical measurements were conducted using an AT universal electrochemical analyzer (ITM, Russia). The analyzer is a universal electrochemical station for the detection of the current changes upon introduction of an electroactive compound in the system. The changes in the current value were registered by plotting cathodic and anodic potential sweep. DC, square wave, and differential pulse methods are available. This analyzer is intended for qualitive and quantitative determination of different types of compounds. Phosphate buffer (a mixture of 1 M monopotassium phosphate and 1 M disodium phosphate, pH = 6.86, Uralkhiminvest, Russia) was used as background electrolyte. Glassy carbon (Tom'analit, Russia) was used as the working electrode. Counter and reference electrodes were saturated silver chloride electrodes (Tom'analit, Russia). To prepare a standard cholesterol solution, cholesterol powder obtained from sheep wool (at least 99.5% of the main substance, Sigma Aldrich, USA), was used. Isopropyl alcohol (>99.7%, Sigma Aldrich, USA) was used as solvent. Emulsifying agent Triton X-100 with the content of the main substance >99% and aggregation number 120 (Sigma Aldrich, USA) was introduced to the system to study its behavior in aqueous medium. Triton X-100 and isopropanol were used in the 1 : 1 mass ratio. 10 μL portion was sampled from the mixture and introduced into a 15 mL quartz glass containing 10 mL of the working electrolyte. Then the mixture was stirred during 20 s by stationary vibration of the working electrode, and the system was left for 30 s. Electrooxidation current was registered by DC method with the differentiation, the scan rate being v = 0.05 V/s. The working electrode potential range was +0.5 to +1.0 V. The accumulation in the near-electrode volume was not performed. Further calculation of cholesterol content in the cell was accomplished using the calibration curve.

CONFLICT OF INTEREST

No conflict of interest was declared by authors.

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