Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 10 (2014) 513 – 518

Procedia Chemistry

XV International Scientific Conference “Chemistry and Chemical Engineering in XXI century” dedicated to Professor L.P. Kulyov

Voltammetric sensor for total cholesterol determination

Ksenia V. Derina[a*], Elena I. Korotkova[a], Elena V. Dorozhko[a] , Olesya A.Voronova[a], Darya A. Vishenkova[a]

[a] *National Research Tomsk Polytechnic University, Lenin avenue 43a, Tomsk, 634050, Russia*

Abstract

We report on a voltammetric sensor for the detection of total cholesterol. The sensor was fabricated by co-immobilization of two enzymes: cholesterol oxidase (ChOx) and horseradish peroxidase (HRP) on porous graphite. The electrochemical behavior of the sensor was studied with the use of linear sweep voltammetry. It has been shown that the sensor has high stability and high sensitivity (16 μA mM^{-1} cm^{-2}). The biosensor exhibited a wide linear range up to 300 mol/dm^3 in a condition close to physiological (pH=6.86). Besides, the interferences of some key analytes containing in the blood were studied. As a matter of fact, making a fabricated sensor is rather promising for using in clinical practice.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
Peer-review under responsibility of Tomsk Polytechnic University

Keywords: Cholesterol; biosensor; Horseradish peroxidase; Cholesterol oxidase; Hydrogen peroxide

1. Introduction

Cholesterol plays a vital role in a human body. It is a precursor of vitamin D, bile acids and steroid hormones (such as, estrogens, aldosterone, progesterones and glucocorticoids)[1]. Moreover, cholesterol is one of the necessary elements of a cellular structure. It is provided by liver and somehow is a part of healthy dietary. Besides, cholesterol plays a key role in brain, immune and nervous systems. Nevertheless, high level of total cholesterol is a marker of diverse illnesses such as hypertension, myocardial infarction, atherosclerosis, cerebral thrombosis, nephrosis, jaundice, diabetes. It happens because excess cholesterol that is not taken up by body cells may deposit in the walls

* *Corresponding author. Tel.: +79061997237; fax 8(3822)56-38-32.*
E-mail address: derinaksenia@yandex.ru

1876-6196 © 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
Peer-review under responsibility of Tomsk Polytechnic University
doi:10.1016/j.proche.2014.10.087

of arteries. Therefore, cholesterol detection is widely used in clinical practice. Developing rapid, simple and sensitive methods for cholesterol detection has significance in practical applications. There are several methods of cholesterol detection. The most widely spread are chromatographic and enzymatic methods. Non-enzymatic techniques such as gas–liquid chromatography, [3] HPLC [4] and spectrophotometric[5] have been reported for the analysis of cholesterol in clinical samples and food. Some of the methods, for example, spectrophotometry based on the Liebermann–Burchard reaction[3], have several disadvantages such as lack of selectivity and specificity because of the interfering reactions and the use of unstable reagents. But, nevertheless, such methods have been widely used for a long time. For several years Centers for Disease Control and Prevention have used standardization of spectrophotometric cholesterol measurements by reference measurement procedure (RMP)[4]. This procedure is a modified version of the Abell–Levy–Brodie–Kendall (AK) colorimetric method and is accepted as an accuracy point. Clinical AK cholesterol measurements also refers to the NIST gas chromatography–isotope dilution mass spectrometry (GC-IDMS) primary RMP. It has been shown in published comparisons as well on NIST SRM certificates for SRM 1951a and 1951b [4]. The main disadvantage of the AK RMP is nonspecificity to cholesterol, because interfering compounds such as noncholesterol sterols, cholesterol precursors, and oxidation products produce chromophores[6] which are measured at the same wavelength as cholesterol.

Due to the simplicity, rapidity and cost, enzymatic procedures have gradually replaced chemical methods based on classical reactions concerning colored complexes formation. But, nevertheless, direct enzymatic methods have some disadvantages, for example, inaccuracy caused by storage conditions of reagents. At the same time electrochemical sensors propose several distinct advantages. These devices are uniquely qualified for meeting a small size, cost effectiveness, low volume and power requirements of decentralized testing and show great promise for a wide range of biomedical and environmental applications. Electrochemical methods based on the use of cholesterol oxidase (ChOx) seem to be very attractive for cholesterol determination because of low prices and, besides, absence of sample preparation procedures. Some biosensors are based on the detection of hydrogen

peroxide electrooxidation which is produced while a catalytic oxidation reaction of cholesterol is in the presence of ChOx [6-8]. Normally, such sensors require a high anodic potential[9]. That may start simultaneous oxidation of other electroactive analytes in the samples, for example, uric acid, ascorbic acid, and produce false positive signals as well. That is why, the second-generation enzyme biosensor, based on the use of an electron mediator, is widely used. Mostly, such sensors are based on the study of an amperometric signal. In recent years, some cholesterol electrochemical biosensor researches focused on the direct electron transfer between ChOx and electrode surfaces[12-14]. However, there are some difficulties which may restrict analytical efficiency of such sensors.

Voltammetry, as an analytical technique, provides more accuracy than amperometric methods. That is why, in this paper a voltammetric ChOx based sensor with the use of HRP as a supporting agent on a porous graphite has been studied.

2. Experimental

2.1. Reagents and materials

Cholesterol oxidase (from *Streptomyces species*, lyophilized powder, ≥20 units/mg protein), cholesterol and Triton® X-100 were purchased from *Sigma Aldrich* (St. Louis, the USA); horseradish peroxidase (Type VI, essentially salt-free, lyophilized powder, 250-330 units/mg solid), hydrogen peroxide, glucose, ascorbic acid, lactic acid, uric acid and potassium chloride were purchased from Carl Roth GMBH (Karlsrue, Germany); phosphate buffer (as solid standard substances) was obtained from «UralChemInvest» (Ufa, the Russian Federation). All reagents were of analytical grade.

Distillate water was used to prepare the most of stock solutions, except the stock solutions of enzymes and cholesterol. All other chemicals were of analytical grade and were used as received from commercial sources.

2.2. Instruments and cell

For running electrochemical experiments an analyzer of thiol substances in blood provided by LLC «SPE Polyant» (Tomsk, the Russian Federation) was used. The analyzer modified cells for the use of microelectrodes. The analyzer works as a polarography and could be used for stripping voltammetry such as linear (DC), cyclic, staircase,

differential pulse. It is possible to use the analyzer for detection of several substances (not only thiol). Besides, it could be used for element mass concentration measuring.

Graphite electrodes (GE) and silver chloride electrodes (SCHE), which are a silver wire in the tube filled with KCl solution, were obtained from “Technoanalyt” (Tomsk, the Russian Federation).

In that work a three-electrode cell was used. GE with immobilized enzymes was used as a working electrode. All potentials reported in this paper are referred to the Ag/AgCl (saturated KCl) reference electrode. Similar electrode was used as a supporting electrode for stabilizing a potential. Linear voltammetric measurements of cholesterol were carried out in a phosphate buffer solution (pH=6.86) at selected potential ranges. All measurements were conducted in 8 ml solution and at a room temperature (22±2◦C).

2.3 Procedure of sensor preparation and running experiments

For preparation ordinary adsorption in the pores of a graphite electrode was used. First, stock solutions of enzymes and cholesterol were prepared by dissolving of lyophilized powders in Triton® X-100. Then, bare GE was immersed to the enzymatic solution (equal mixture of ChOx and HRP) and was left there for 4 hours.

After immersing the sensor was dried in the air at a room temperature. After drying the sensor was stored at a room temperature immersed to a buffer solution to keep activity of enzymes more stable.

As a background solution and for the storage of prepared sensors a standard equal mixture of 0.025 M KH_2PO_4 and 0.025M Na_2HPO_4 dissolved in distilled water (pH = 6.86) was used.

After every series of experiments the sensor surface was washed with a phosphate buffer which was used as a background.

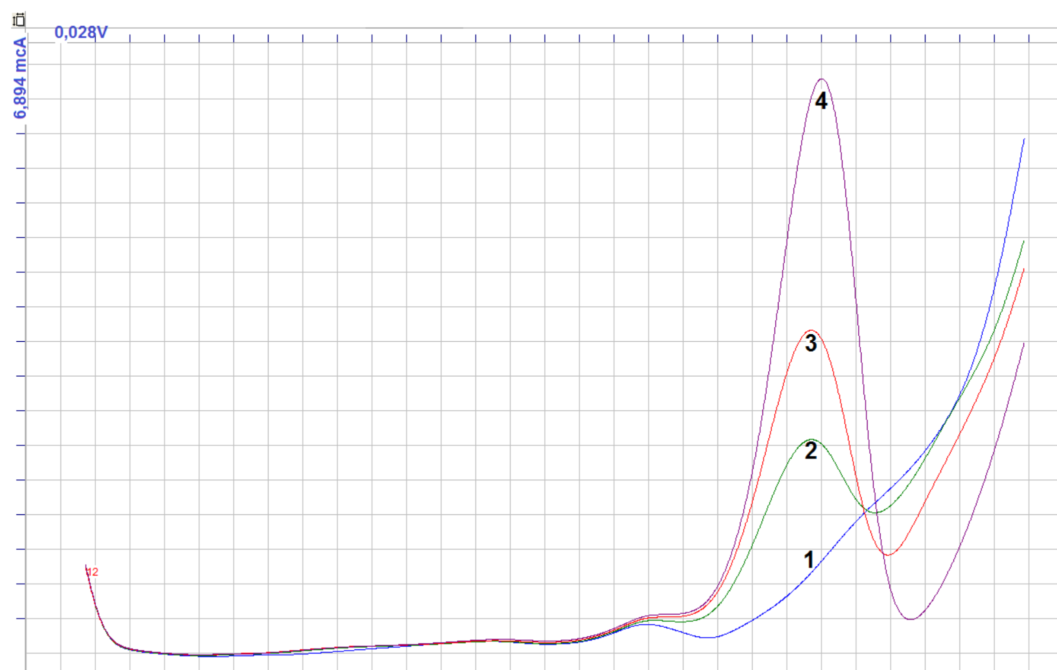

Fig. 1. Voltammogram without cholesterol (1), with $6\cdot10^{-3}$ mg/ml of cholesterol (2), with $12\cdot10^{-3}$ mg/ml of cholesterol (3), with $18\cdot10^{-3}$ mg/ml H_2O_2

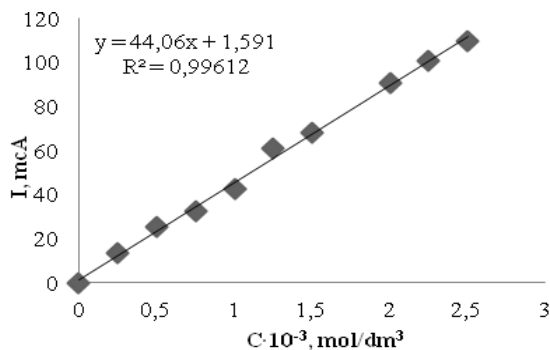

Fig. 2. Dependence of provided current on cholesterol containing

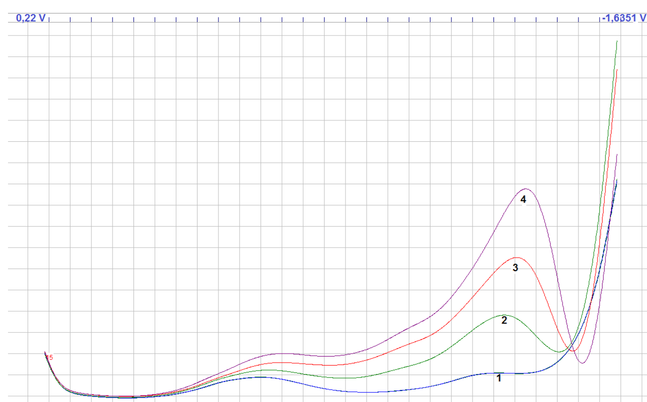

Fig. 3. Voltammogram without cholesterol (1), with $6{\cdot}10^{-3}$ mg/ml of cholesterol (2), with $12{\cdot}10^{-3}$ mg/ml of cholesterol (3), with $18{\cdot}10^{-3}$ mg/ml of H_2O_2

To prove that a received response correlated with hydrogen peroxide, which was obtained by oxidation of cholesterol, standard hydrogen peroxide was added to the cell. It was shown that the response correlated with hydrogen peroxide, and increase of cholesterol concentration in the cell correlated with the height of a hydrogen peroxide reduction peak. The correlation is shown in Fig. 2.

To prove the use of a silver chloride electrode as a supporting (auxiliary) the results received with the use of an original cell were compared with the results received with the use of a Pt-electrode as supporting one. Obtained results are shown in Fig. 3 (with Pt-electrode) and Fig. 1 (with original cell). A signal received with the use of an original cell was more stable.

Cholesterol oxidase is an alcohol of dehydrogenase/oxidase flavoprotein catalyzing the dehydrogenation of C(3)–OH of a cholesterol molecule to yield a corresponding carbonyl product. During the reduction half-reaction, an oxidized FAD (flavin adenine dinucleotide) cofactor accepts a hydride from the substrate and, in the ensuing oxidation half-reaction, the reduced flavin transfers redox equivalents to molecular oxygen yielding hydrogen peroxide. Than received molecular hydrogen peroxide decreases on the surface of GE.

2.4. Selectivity and stability of sensor

It is well known that blood contains different analytes which could provoke interference of the signal. That is why selectivity of the sensor was tested. For such an experiment four common interfering species were used. Fig. 4 presents a response of the ChO_X–HRP biosensor to 0.1 mM.

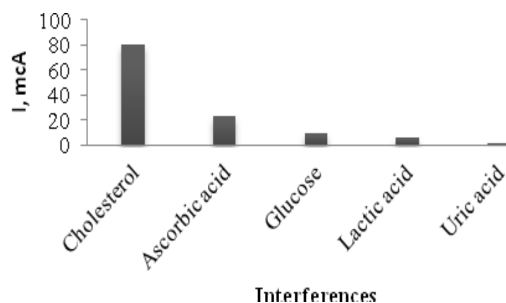

Fig. 4. Selectivity response of the ChO_X–HRP electrode after addition of ascorbic acid (0.1 mM), glucose (5 mM), lactic acid (5 mM), uric acid (0.1 mM) and cholesterol (5.2 mM) into a background solution independently (background current subtracted)

The sensor produces current signals for all analytes. The strongest response correlates with ascorbic acid, but, nevertheless, the obtaining response of cholesterol is significantly stronger.

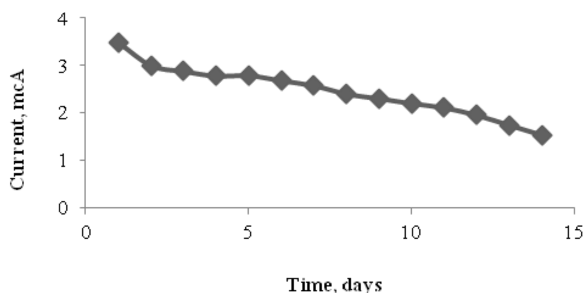

Fig. 5. Current changing in time

Stability of the sensor was tested by measurement of a 20 mg/ml standard cholesterol solution for 15 days. The tests were carried out once a day. In Fig. 5 dependence of a current provided by the sensor as a response on time is shown. During this period, the sensors were stored in a phosphate buffer solution at a room temperature. The biosensor demonstrated 80% of the initial current response after the 15 day period. After 15 days the active centers were blocked and there was no response from the sensor. To renew the sensor an enzymatic layer was deleted from the surface by dissolving with isopropanol. After that, the surface was properly washed with distilled water to remove isopropanol, then dried on air and polished.

3. Conclusions

A novel sensor for rapid detection of cholesterol was fabricated by co-immobilizing cholesterol oxidase and horseradish peroxidase on the porous graphite surface. The use of immobilized cholesterol oxidase effectively catalyzes the oxidation of cholesterol with providing of hydrogen peroxide. Meanwhile, the immobilized horseradish peroxidase facilitates the detection of the H_2O_2 generated while oxidative conversion of cholesterol to cholest-4-en-3-one. The fabricated sensor is highly selective and showed anti-interferent resistance as tested toward common interfering substances such as ascorbic acid, glucose, lactic acid and uric acid.

Under conditions close to physiological (pH=6.86), the developed sensor exhibited reliable linear voltammetric responses, high sensitivity, a wide linear range up to 300 mg dL^{-1} and high stability.

High-performance along with simple fabrication and low costs makes the fabricated sensor very promising for the detection of total cholesterol in clinical practice and food industry.

Acknowledgments

This work was financially supported by the Ministry of Education and Science of Russian Federation (project “Science” № 1.1310.2014).

References

1. Hirany S., Li D., Jialal I. *American Journal of Medicine*. 1997; **1:** 53-58.
2. Bongiovanni C., Ferri T., Poscia A., Varalli M., Santucci R., Desideri A. *Bioelectrochemistry.*2001; **54:** 17-22.
3. Chauhan N., Narang J., Pundir C.S. *American Journal of Analytical Chemistry*. 2010, **2**: 41-46.
4. Ahmadalinezhad, A., Kafi, A.K.M., Chen, A., *Electrochem. Commun.* 2009; **11**: 2048–2051.
5. Ferri T., Poscia A., Santucci R. *Bioelectrochem. Bioenergy*. 1998; **44**: 177-181.
6. Korotkova E.I., Avramchik O.A., Angelov T.M., Karbainov Y.A. *Electrochimica Acta*. 2005; **51** (2), p. 324–332
7. Mallesha M., Manjunatha R., Nethravathi C., Suresh G.S., Rajamathi M., Melo J.S., Venkatesha T.V., *Bioelectrochemistry*. 2011; **81:** 104–108.
8. Sungano M., Beynen A.C., *Dietary Proteins, Cholesterol Metabolism and Atherosclerosis,* S Karger AG: Basel; *1990.*
9. Ivanov A.A., Yudina N.V., Korotkova E.I., Lomovsky O.I. *Solid Fuel Chemistry*. 2008; **42:** 2, p.68-73.
10. M. Kooshki, H. Abdollahi, S. Bozorgzadeh, B. Haghighi, *Electrochim. Acta. 2011; **56**: 8618–8624.*
11. E. Laviron, *J. Electroanal. Chem.* 1979; **101:** 19–28.
12. J.J. Gooding, *Electrochim. Acta.* 2005; **50**: 3049.
13. A.Merkoci, M. Pumera, X. Llopis, B. Perez, M. del Valle, S. Alegret, *Trends Anal. Chem.* 2005; **24**: 826.
14. J. Wang, *Electroanalysis*. 2005;**17**: 7-13.
15. G. Rivas, M.D. Rubianes, M.C. Rodr_guez, N. Ferreyra, G.L. Luque, M.L. Pedano, C. Parrado, *Talanta*. 2007; **74**: 291.
16. C.E. Banks, A. Croosley, C. Salter, S. J. Wilkins, R.G. Compton, *Angew. Chem. Int. Ed.* 2006; **45:** 25=33.
17. J. Kruusma,V. Sammelselg,C.E. Banks., *Electrochem. Commun.* 2008; **10**: 1872.
18. Roy S., Vedala H., Choi W.B. *Nanotechnology*. 2006; **17**: 14-18.
19. Trojanowich M., Krawczyk T., Krawczynsky. *Microchim. Acta. 1995; **121**:.167-181.*
20. Yang, L., Bashir, R., *Biotechnol. Adv. 2008; **26**:135–150.*
21. Yang, L., Li, Y., Griffis, C.L., Johnson, M.G. *Biosens. Bioelectron. 2004;**19**:1139–1147.*
22. Yoon, J.-Y., Kim, B. *Sensors. 2012; **12**: 10713–10741.*
23. Zelada-Guillén, G.A., Bhosale, S.V., Riu, *J., Rius Anal. Chem. 2010; **82:** 9254–9260.*
24. Zhou, M., Zhai, Y.M., Dong, S.J. *Anal. Chem. 2009; **81:** 5603–5613*.