Catalase Biomimetic Sensor Based on Metal

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Abstract. The development of motivated biosensors associated with the study of a large number of different substances is one of the most important areas of development of analytical chemistry. This goal in the development of certain biosensors was necessary for a sufficiently clear, fast, qualitative and quantitative determination of the desired component. Chemical reactions in living organisms are carried out with the help of catalysts - enzymes. Enzymes are proteins with prosthetic groups, and proteins are polymers formed from amino acid links. The number of different enzymes in a cell is the same as the number of different chemical reactions that occur in it. This work is devoted to the study of biomimetic sensors, where metals (Pb, Ag, Al) were used as a converter. The active material was iron tetraphenylporphyrin adsorbed on Al_2O_3. It was found that biomimetic sensors made on the basis of intelligent material (TPhPFe3+/Al2O3) and metals have a number of technological advantages. The developed biomimetic sensors are characterized by high sensitivity, activity, stability and reproducibility. When detecting the catalase activity of biomimetic sensors, the maximum sensitivity to the concentration of H_2O_2 in an aqueous solution for $TPhPFe^{3+}/Al_2O_3$//Pb was 10^{-8}wt.%, for $TPhPFe^{3+}/Al_2O_3$//Ag – 10^{-8}wt.%, for $TPhPFe^{3+}/Al_2O_3$//Al – 10^{-6} wt.%.

Keywords: biomimetic; sensor; tetraphenylporphyrin of iron; catalase; smart material.

1 Introduction

In recent years, the development of biomimetic sensors has been developed, in which bioselectors are replaced by enzyme analogues that combine high sensitivity and stability.

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To improve efficiency, the biosensor design should allow analytes to freely interact with enzymes involved in catalytic reactions, which requires knowledge of both the properties of the medium being studied and the properties of the analytes themselves. To identify, recognize and assess the concentration of substances in the medium, it is also necessary to take into account factors that can suppress or distort the signal, which may be associated with the interaction of biosensor components with each other and with the molecules of the medium being studied. The requirements of modern analysis are sensitivity, selectivity, low cost, simplicity and rapidity. Biomimetic sensors ideally meet these requirements. They are simple, easy to use and allow continuous monitoring of key analytes, which is important for clinical diagnostics, industrial production and environmental monitoring. A promising direction in the field of modern biotechnology is the creation of highly sensitive biosensors and their mimetics, in which enzymes are used as working materials (bioselectors). Research in this direction was carried out in [1,2].

Biomimetics is a very important area, since by imitating living nature one can try to find chemical analogues of enzymes and create new industrial processes based on them. A feature of biomimetics is the copying of the amazing functions of living organisms and their use in the creation of technologies and things. However, in recent years, there has been growing attention to another feature of living organisms - energy-saving production processes used by living beings [3,4,5]. Biomimetic materials, which aim to mimic the desired properties of natural enzymes and biological systems, have become an attractive route for creating reliable and sensitive electrochemical biosensors.

Hydrogen peroxide sensors can be used to assess antioxidant activity, as well as to determine a wide range of toxic amines and phenols for environmental control and monitoring purposes. The ranges of determined concentrations of hydrogen peroxide vary widely, from submicromolar and micromolar to molar [6,7]. There are many methods for determining the concentration of hydrogen peroxide [8,9,10]. Methods vary in accuracy, complexity and range of detectable concentrations. Among the most common are permanganatometry, spectrophotometric methods, luminescent methods [11] and electrochemical methods [12]. The simplest method for the quantitative determination of hydrogen peroxide is titration with potassium permanganate [13,14]. This method can be used over a relatively wide range of H_2O_2 concentrations, but its main requirement is that the peroxide concentration does not change over time.

However, from the point of view of high sensitivity and selectivity, short response time, low cost and ease of analysis, electrochemical methods are the most convenient. Electrochemical methods hold great promise for the development of low-cost, miniaturized, easy-to-use portable devices for a wide range of applications, particularly in medical diagnostics and environmental monitoring [15-19].

Particular attention is paid to metal-based biosensors. This is due to their stability, technological ease of manufacture and modification [20,21]. This work is devoted to the study of a catalase biomimetic sensor using a smart material, where metals such as Pb, Ag, Al were used as transducers, and iron tetraphenylporphyrin (smart material) was used as an active material. The use of these materials made it possible to increase the sensitivity of the biomimetic sensor. Using the developed biomimetic sensor, the possibility of determining trace concentrations of hydrogen peroxide in an aqueous solution has been demonstrated.

2 Methods

Experimental studies of the electrode potential of the catalase reaction as a function of time were carried out using the potentiometric method. The experiments were carried out in an electrochemical cell consisting of a reference electrode ($Ag/AgCl/AgCl^-$) and biomimetic sensors. Double-distilled water served as a background solution. To create an equilibrium solution, the electrochemical installation was equipped with a magnetic stirrer. The studies were carried out in an electrochemical cell, which for clarity is presented in the form of infographics (Fig. 1). The experimental setup for these studies consisted of an electrode part, a cell and a B7-21A universal voltmeter. The electrode part of the installation consists of a reference electrode (Ag/AgCl/Cl) and a biomimetic sensor manufactured by us. In a cell filled with a certain amount of bidistilled water (background solution), we determine the e.m.f. (E) element and then adding different concentrations of H_2O_2 determined the changes in e.m.f. solution.

3 Results and Discussions

The experiments were carried out at various concentrations of H_2O_2. The presence of hydrogen peroxide in the system leads to a change in the value of ΔE, and an increase in the H_2O_2 concentration increases the jump in the electrochemical potential. At the beginning, we carried out our research with a Pb electrode. (Fig. 2).

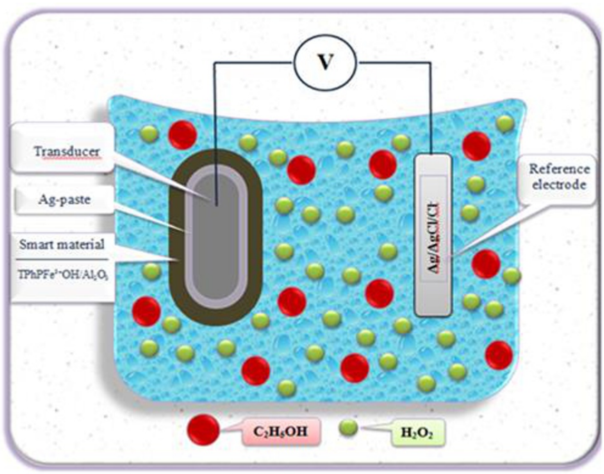


Fig. 1. Infographics.

Smart material $TPhPFe^{3+}/Al_2O_3$ is applied to this electrode by gluing (silver paste is used as an adhesive material). To study catalase activity, we used an extremely low concentration of H_2O_2. The results of a potentiometric study of the catalase activity of the biomimetic sensor $TPhPFe^{3+}/Al_2O_3/Pb$ are presented in Figure 2. The figure shows that when H_2O_2 is added, a sharp change in the electrode potential of the system is observed for both concentrations. The results showed that the $TPhPFe^{3+}/Al_2O_3/Pb$ biomimetic sensor exhibits high sensitivity and detects trace concentrations of hydrogen peroxide in an aqueous solution up to 10^{-8} wt%.

The next series of experimental studies was devoted to the immobilization of smart material on an Al - electrode and the determination of trace concentrations of hydrogen peroxide in an aqueous solution.

The results showed that the $TPhPFe^{3+}/Al_2O_3//Al$ biomimetic sensor exhibits high sensitivity and detects trace concentrations of hydrogen peroxide in an aqueous solution up to 10^{-6} wt% Figure 3.

Figure 4 shows experimental data obtained from studying low concentrations of H_2O_2 in an aqueous solution for $TPhPFe^{3+}/Al_2O_3//Ag$. As can be seen from Fig. 4, a biomimetic sensor prepared by gluing smart material – $TPhPFe^{3+}/Al_2O_3$ onto a transducer – Ag, showed very high sensitivity. The sensitivity threshold of the $TPhPFe^{3+}/Al_2O_3/Ag$ biomimetic sensor was 10^{-8}wt.%.

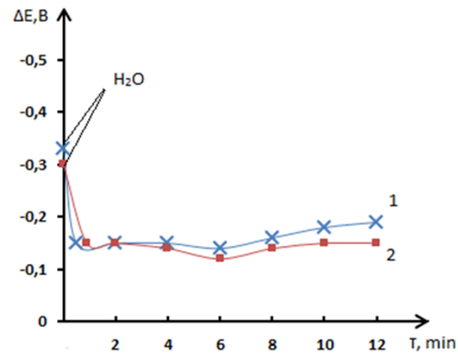


Fig. 2. Change in e.m.f. systems depending on time at low concentrations of H_2O_2 for $TPhPFe^{3+}/Al_2O_3//Pb$ electrode: 1.10^{-6} wt.%; 2. 10^{-8} wt.%.

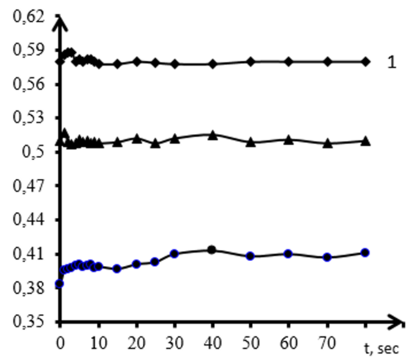


Fig. 3. Change e.m.f systems depending on time at low concentrations of H_2O_2 for $TPhPFe^{3+}/Al_2O_3//Al$ biomimetic sensor 1.10^{-6}wt.%; 2. 10^{-2}wt.%; 3. 10^{-4}wt.%

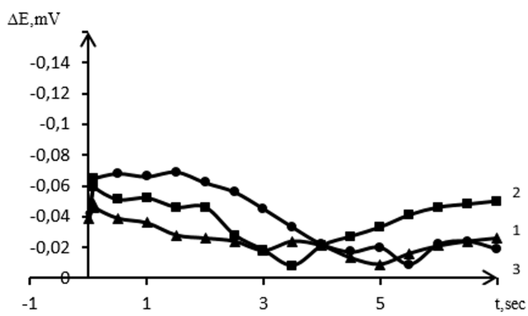
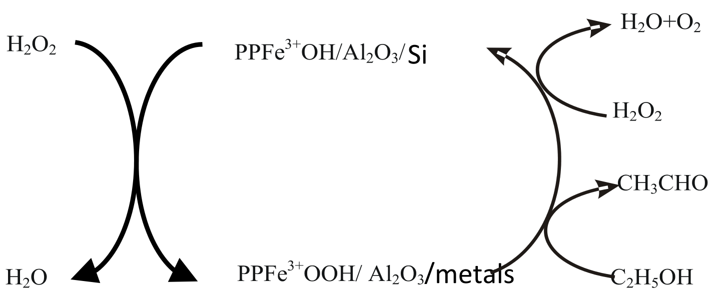


Fig. 4. Change in e.m.f. systems as a function of time at low H_2O_2 concentrations for a $TPhPFe^{3+}/Al_2O_3//Ag$ biomimetic sensor. 1.$C_{H_2O_2} = 10^{-4}$wt.%; 2. $C_{H_2O_2} = 10^{-6}$wt.%; 3. $C_{H_2O_2} = 10^{-8}$wt.%

Previously, we have carried out successful work in this area. As a result of the studies of the biomimetic sensor for catalase and peroxidase activity, it was found that the electrode with $TPhPFe^{3+}/Al_2O_3$ makes it possible to detect trace concentrations of hydrogen peroxide in an aqueous solution [22-24].

Scheme of the catalase reaction



(1)

electrochemical reaction

$$O_2 + 4e^- + 2H^+ \xrightarrow{electrode} 2OH^- \quad (2)$$

According to the literature, hydrogen peroxide is a weak dibasic acid. It can be assumed that, as a result of the catalase activity of the biomimetic sensor, the pH of the H_2O_2 solution should change due to reactions (1) and (2). Therefore, if the proposed reactions take place on a biomimetic sensor, then ultimately the pH of the solution should have a lower value than at the beginning of the experiment.

Figure 5 shows the proposed mechanism of the catalase reaction on the surface of the biomimetic electrode.

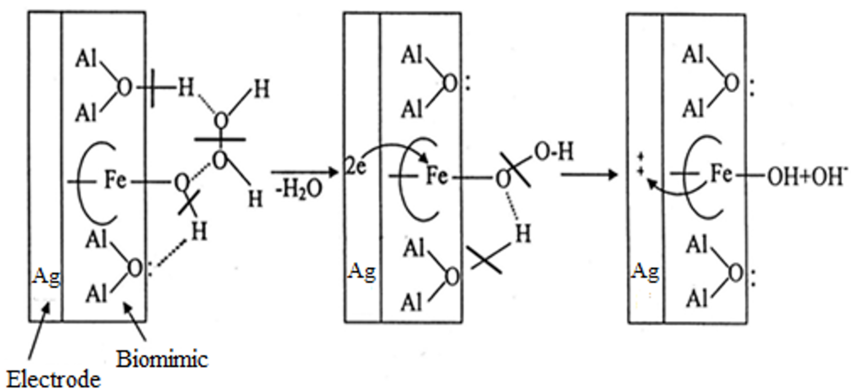


Fig. 5. The proposed mechanism of the catalase biomimetic sensor in electrocatalytic mode.

4 Conclusions

The developed biosensors $TPhPFe^{3+}/Al_2O_3//Pb$, $TPhPFe^{3+}/Al_2O_3//Ag$, $TPhPFe^{3+}/Al_2O_3//Al$ of the catalase type are active and make it possible to determine trace concentrations of H_2O_2 in an aqueous solution.

Synthesized catalase biosensors are characterized by long-term stability, high sensitivity and reproducibility. The maximum sensitivity to the concentration of H_2O_2 in an aqueous solution for $TPhPFe^{3+}/Al_2O_3//Pb$ was 10^{-8} wt.%, for $TPhPFe^{3+}/Al_2O_3//Ag$ – 10^{-8} wt. %, $TPhPFe^{3+}/Al_2O_3//Al$ –10^{-6} wt.%.

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