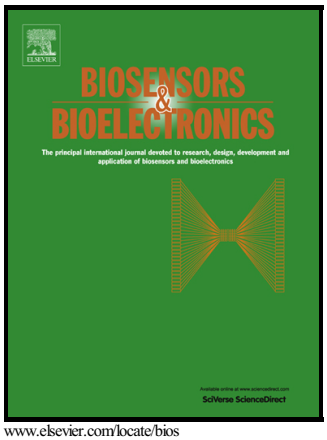

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ENZYME BIOSENSOR SYSTEMS BASED ON POROUS SILICON PHOTOLUMINESCENCE FOR DETECTION OF GLUCOSE, UREA AND HEAVY METALS

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ABSTRACT

A phenomenon of changes in photoluminescence of porous silicon at variations in medium pH is proposed to be used as a basis for the biosensor system development. The method of conversion of a biochemical signal into an optical one is applied for direct determination of glucose and urea as well as for inhibitory analysis of heavy metal ions. Changes in the quantum yield of porous silicon photoluminescence occur at varying pH of the tested solution due to the enzyme-substrate reaction. When creating the biosensor systems, the enzymes urease and glucose oxidase (GOD) were used as a bioselective material; their optimal concentrations were experimentally determined. It was shown that the photoluminescence intensity of porous silicon increased by 1.7 times when increasing glucose concentration in the GOD-containing reaction medium from 0 to 3.0 mM, and decreased by 1.45 times at the same increase in the urea concentration in the urease-containing reaction medium. The calibration curves of dependence of the biosensor system responses on the substrate concentrations are presented. It is shown that the presence of heavy metal ions (Cu^{2+}, Pb^{2+}, Cd^{2+}) in the tested solution causes an inhibition of the enzymatic reactions catalyzed by glucose oxidase and urease, which results in a restoration of the photoluminescence quantum yield of porous silicon. It is proposed to use this effect for the inhibitory analysis of heavy metal ions.

Keywords:
biosensor
luminescence
porous silicon
enzyme
heavy metal

1. Introduction

In recent years, semiconductor sensors and sensor arrays for the detection of chemical and biological substances have drawn much attention (Kohl, 2001; Sailor, 2012). The ultimate goal of the research in this field was to fabricate sensors that can determine the presence of a wide range of substances at the relevant concentration levels with sufficient selectivity and sensitivity. The research would produce the apparatus that could be applicable in many segments of human activity including food processing, environmental remediation, agriculture, medical diagnostics and defense. The main requirements to the devices developed, together with selectivity and sensitivity, are fast response, low fabrication costs, reliability and portability (Masrournia et. al., 2011).

The development of highly sensitive, low-cost, reliable glucose sensors having an excellent selectivity has been the subject of concern for decades, not only in medical science but also in the food industries (Newman, et al, 2005). Urea is widely distributed in nature and its analysis is of considerable interest in the clinical chemistry, agro-food chemistry, and environmental monitoring. Previously, series of enzyme-based biosensors were developed for glucose and urea detection, i.e. potentiometric, amperometric, impedimetric and conductometric (Pizzariello, et al, 2001; Eggenstein et al, 1999; Rahman et al, 2010).

Well-known, the most dangerous polluting substances are heavy metals (Golovanov, 2008; Zayets et. al., 2008). In humans and herbivorous animals, heavy metals come from plant food, and to plants - from the soil. The mechanism of the harmful effects of heavy metals is the formation of covalent bonds with the radicals of biologically active molecules, particularly due to binding with (-SH) groups of amino acid residues that are a part of active sites of enzymes (Soldatkin et. al., 2008; Kycherenko et. al., 2009). As a result, the three-dimensional structure of enzyme is broken, reducing its activity (irreversible inhibition).

Heavy metals affect the physiological functions of the body, violate the acid base balance of blood, alter the activity of enzymes, etc. (Amine et. al., 2006). At present, the toxicity of mixtures of heavy metals is of particular importance because of the anticipated increase in heavy metal concentrations in soil caused by sewage entering groundwater and organisms (Preston et. al., 2000; Yadav, 2010). Therefore, the development of biosensors, simple, easy-to-use and cheap devices for environmental monitoring, is an important task of modern biotechnology development (Bontidean et. al., 2003; Blake et. al., 2001; Corbisier et. al., 1999). Indirect determination of heavy metal ions can be ensured by inhibition of the reactions catalyzed with glucose oxidase (GOD) and urease (Chey et al, 2012; Upadhyay, 2012) that can be realized in enzyme biosensors.

An increasing number of researchers have explored the application of novel nano-scale metal oxides, noble metal-doped metal oxides for enzyme-based sensors. Novel analytical devices based on nanostructured metal oxides are cost-effective, highly sensitive due to the large surface-to-volume ratio of the nanostructure, and additionally show high selectivity when coupled to biorecognition molecules with simple design. Some metal oxides such as ZnO and CeO_2 show excellent biocompatibility that enables reliable immobilization of glucose oxidase (Liu, et al, 2008).

However, there are still some disadvantages of enzyme-based glucose determination. Biosensors are characterized by complicated enzyme immobilization, critical operating conditions such as optimum temperature and pH, the chemical instability, and high cost (Reitz, et al, 2008).