Sensors and Actuators B 251 (2017) 746–752

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Inkjet printing of the chromogen free oxidase based optical biosensors

Sofia M. Safaryan, Aleksandr V. Yakovlev*, Alexandr V. Vinogradov, Vladimir V. Vinogradov

ITMO University, Saint Petersburg, 191002, Russian Federation

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form 17 May 2017
Accepted 18 May 2017
Available online 22 May 2017

Keywords:
Biosensor
Sol–gel
Inkjet printing
Redox nanoparticles
Titanium dioxide

ABSTRACT

This paper describes a new method for preparation of chromogen free oxidase based optical biosensors. Oxidases enzymes were deposited on the titania film consisting of uniformly distributed nanoparticles reacting with hydrogen peroxide thus providing formation of yellow color due to complex Ti (IV)-H_2O_2. Using ink jet printing and specially developed titania ink we have created a universal platform for the oxidase based biosensors and have successfully tested it on glucose and cholesterol detection. The paper fully discusses new biomaterials as well as biosensor creation technology and describes the mechanism of action, study on stability and working concentration limits and adjusting composition of the inks for good printability and scale-up production.

1. Introduction

Recent years, low-cost, simple and automated biomaterials production methods are widely recognized in enzymatic biosensor development [1,2]. In most cases, this requires the application of sensitive liquid components containing enzymes on the substrate surface [3]. For example, such methods include additive technology that can accurately control the amount of components applied to a substrate [4]. At the moment, there are different additive methods, ranging from microdispensing to printing [5]. Inkjet printing is a perspective direction for material science development as it allows to apply different reagents from active substances solutions up to complex sol-gel systems with unique properties in the ink form [6]. A distinctive feature of this method is the possibility of a high-precision deposition of variety materials, the creation of ordered layers and complex three-dimensional structures of an active substance [7].

A relatively new direction in the field of inkjet printing is the producing of biomaterials and biosensors [8,9]. The possibilities of accurate dosing and positioning along with the autonomous manufacturing largely determine the advantages of a printing method for the production of high-precision diagnostic biosensors [10]. There is a great variety of types of biomolecules [11], bio-inks [12] and based on them biosensors [13]. The simplest and most widely recognized biosensing method is still a qualitative enzymatic reaction based on oxidases due to their high selectivity and sensitivity [14]. Oxidases catalyze the oxidation-reduction reactions [15]. They specifically react with the substrate and transfer the hydrogen from the substrate to atmospheric oxygen to form water or hydrogen peroxide. There are many oxidases meaningful for biochemical analysis and biosensors, such as Glucose oxidase, Lactate oxidase, Urate oxidase, Cholesterol oxidase, Glycerol-3-phosphate oxidase, Choline oxidase, Alcohol oxidase and others [14].

In the widely used method, the additional reaction is used to visualize the substrate oxidation products. In this reaction a chromogenic substrate (dye) and peroxidase are used for detection the formed peroxide [16]. Peroxidase transfers oxygen from the peroxide to the dye molecule. As a result, the oxidized form of the chromogen acquires or changes the color. However, many chromogenic dyes are not stable in the light [17], they are readily oxidized in the air and require additional components to protect against undesirable oxidation at storage conditions [18]. This multicomponent system overloads and complicates the sensor, requires careful calculation of optimal concentrations and ratios of each component for the reproducibility of the results and increases the costs of creating a biosensor.

In addition to the use of chromogenic dyes, colorimetric biosensors based on nanoparticles were reported [19]. These assays usually use noble metals nanoparticles [20] such as gold or silver. The detection is based on the principle of particles aggregation or dispersion [21]. However, most of these nanoparticles are used to DNA analysis [22], only a few of them being adapted to the enzy-

* Corresponding author.
E-mail address: mailto.yakovlev@gmail.com (A.V. Yakovlev).

http://dx.doi.org/10.1016/j.snb.2017.05.112
0925-4005/

matic reaction. For example, the use of redox ceria nanoparticles as colorimetric probes in bioassay was reported [23]. The method is based on changes in the physicochemical properties of cerium oxide nanoparticles operating as a chromogenic indicator.

Along with ceria nanoparticles, the color qualitative reaction to titanium with a solution of hydrogen peroxide is stated to exist [24]. This method is highly sensitive which allows the detection of titanium trace amounts, due to the yellow complex Ti (IV) $-H_2O_2$ formation with the tetravalent titanium and hydrogen peroxide [25]. In this work we have used titanium dioxide (titania) for the detection of hydrogen peroxide. Since hydrogen peroxide is formed in substrate oxidation reactions, oxidase enzymes are used for glucose and cholesterol determination.

In this work we for the first time propose an approach to obtain biosensors based on redox nanostructured material applied by desktop inkjet printing.

Using ink jet printing and special titania ink we have created a universal substrate for the oxidase biosensors and successfully tested it on glucose and cholesterol. The application of inkjet printing titania substrate as a colorimetric component achieves high repeatability with improved surface properties for subsequent layer deposition. The minimum concentration of peroxide that is possible to detect using this method is 0.4 mM.

2. Experimental section

2.1. Reagents

Nitric acid, titanium isopropoxide, 2-propanol, glucose oxidase (GOD) (15000 U/L) TRIS buffer pH 7.4, cholesterol oxidase (CHOD) (300 U/L), potassium phosphate buffer pH 6.8, H_2O_2 30% solution, glucose water solution 100 mg/dL, cholesterol solution of 200 mg/dL, ethylene glycol 99.5% were obtained from Sigma Aldrich. Slightly cationic fluorosurfactant DX4010N was obtained from DYNAX company.

2.2. Production of titania sol

To a flask containing 100 ml of deionized water the 0.7 ml of concentrated nitric acid was added with constant stirring. Then the flask was covered with foil and heated to 70 °C. At the same time the solution of titanium isopropoxide in 2-propanol mixture was prepared, the components being in the amount of 16 ml and 12 ml respectively. The resulting isopropoxide-alcohol solution is slowly poured into the flask with nitric acid. The formation of a white precipitate was observed. Thereafter the temperature was increased to 80 °C, and the liquid was kept under constant stirring for 45 min. Finally, the heating was turned off, the foil was removed and the mixture was kept under constant stirring for further 5–7 days until the formation of the stable sol of titanium dioxide.

2.3. Production of titania ink

The sol prepared as described above was evaporated on a rotary evaporator to get xerogel. 0.4 g of solid (xerogel) sample was dissolved in 2 ml deionized water. Then 2 ml of the resulting sol was mixed with 6 ml of ethylene glycol, and the viscosity of the solution increased from 1.5 to 9.3 cP. To adjust the surface tension 6 μl of 1% solution of surfactant DX4010N was added to the resulting titania solution. The surface tension was reduced from 45.7 to 30 mN m^{-1}. Finished ink has been tested for printing reproducibility (latency test) by Dimatix material printer DMP 2831. The ink has latency time up then 5 min and jetting frequency from 1 to 5 KHz.

2.4. Production of bio-ink

Enzyme solutions were prepared using dry samples and certain buffers. 0.15 g of glucose oxidase (GOD) (15000 U/L) were dissolved in 50 ml TRIS buffer, pH 7.4 for glucose biosensor. 0.15 g of cholesterol oxidase (CHOD) (300 U/L) were dissolved in 50 ml potassium phosphate buffer, pH 6.8 for cholesterol biosensor. Using ethylene glycol, the viscosity of both enzymes solutions was adjusted to 10 cP.

2.5. Production of biosensor

For biosensor production we have used matte photo paper Lomond, gsm 230, which was subjected to several steps of printing applying few layers. Firstly, we printed on photo paper a specially prepared template with the boundary lines for the production of test strips and color chart for the comparison with the test area. The coating was applied to both sides of the paper by Xerox Phaser 8860 printer with Genuine Xerox Solid Ink. Thus, we created a hydrophobic coating on paper and a small limited area of a sensing element. Then using this pre-defined template with the hydrophobic coating, we applied titania ink into the sensing zone by the inkjet printer. For this purpose, we used the titania ink, which was prepared by the method described above. The ink was placed in Dimatix 10 pl cartridge and using the template printing was carried out only in the pattern areas where the hydrophobic coating was not applied. Thereby the positioning of the sensitive area was strictly on the paper. After printing, the sample was kept to dry completely. Finally, after drying the biosensor preform the recognizing element containing specific oxidases was placed on titania layer by the inkjet printer. Dimatix 10 pl cartridge was filled with bio-ink based on enzyme solutions made by the method described above. The printing was performed with the use of the same template and the ink was applied only on titania areas without hydrophobic coating. After the printing, the biosensor was kept under vacuum to dry and assayed for H_2O_2, glucose and cholesterol control solutions of different concentrations.

2.6. Enzyme activity detection and color intensity measurement

After printing and drying biosensors were cut into strips and tested for H_2O_2, glucose and cholesterol. For this purpose, 5 μl of the substrate was applied to the sensitive zone of the test strip, and in 2 min the color intensity was measured. Enzymatic activity was measured spectrophotometrically in terms of the absorbance increase compared to a control sample by the formation of the yellow colored complex of tetravalent titanium with hydrogen peroxide. Measurements were carried out in the range of 200–800 nm. In this case, we used Cary 60 UV–vis with Video Barrelino console for measuring diffuse reflectance. Peroxide aqueous solutions with concentrations from 0.0013 to 1% were applied for testing hydrogen peroxide sensitivity. Glucose aqueous solutions with concentrations from 3 to 100 mg/dL were used to test for glucose determination. Cholesterol alcohol solutions with concentrations from 6 to 200 mg/dL were employed for cholesterol detection testing.

2.7. Testing of reproducibility

To check the reproducibility and sensitivity of printing substrate we prepared 50 identical biosensor samples containing titania layer. 5 μl of 1% H_2O_2 was applied on each sample and in 2 min the reacted zone was measured by the adsorption method of diffuse reflection in the range from 200 to 800 nm. At the end of the experiment, the adsorption value of 50 samples was compared.