Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1195–1200

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Electroanalytical properties of a novel biosensor modified with zirconium alcoxide porous gels for the detection of acetaminophen

Veronica Sima[a], Cecilia Cristea[a], Florina Lăpăduş[a], I.O. Marian[b], Ana Marian[b], R. Săndulescu[a,*]

[a] *Analytical Chemistry and Instrumental Analysis Department, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 4 Pasteur Street, 400 349 Cluj-Napoca, Romania*
[b] *Physical Chemistry Department, Faculty of Chemistry and Chemical Engineering, "Babeş-Bolyai" University, 11 Arany Janos Street, 400 028 Cluj-Napoca, Romania*

ARTICLE INFO

Article history:
Received 31 March 2008
Received in revised form 28 July 2008
Accepted 3 August 2008
Available online 23 August 2008

Keywords:
Alcoxide porous film
Zirconium oxide
Poly(ethyleneimine)
Acetaminophen

ABSTRACT

The development of composite electrodes for biosensors construction based on HRP and zirconium alcoxide film for acetaminophen detection and finally, acetaminophen determination in pharmaceutical products is described. The enzyme immobilization is performed by retention in a polyetilenimine and zirconium alcoxide porous gel film, technique that offers a good entrapping and in the mean times a "protective" environment for the biocomponent.

The operation principle of the biosensor is based on monitoring the amperometric signal generated by reduction at the electrode surface of the enzymatically generated quinoneimine from acetaminophen. The resulting biosensor shows a linear response towards acetaminophen with a linear range of 1.96×10^{-5} M and 2.55×10^{-4} M and a limit of detection of 1.17×10^{-7} M. The proposed biosensor shows long term stability and good reproducibility.

1. Introduction

Biosensors applied in the control of drug release and concentration from pharmaceutical forms and metabolites detection represent a convenient alternative to other analytical methods. Generally biosensors are analytical devices sensitive and selective those associate a biocomponent to a transducer.

The enzymes are considered useful in the fabrication of the biosensors. Besides the problems relied to the entrapment of the enzymes at the surface of electrode, another challenge is to preserve the microenvironment of the enzyme and hence the lifetime of the biosensor. Several methods were used to immobilize the enzyme at the electrode surface like adsorption [1], cross linking [2], covalent binding [3], biological membranes [4], magnetic microparticles [5], entrapment in sol–gel [6] etc. The immobilization into an electrochemical polymer or polymerizable matrices were successfully used in the development of the amperometric biosensors, due to the fact that the procedure is effective and simple and the enzyme is less affected than during other methods of entrapment [7,8].

Lately many approaches used inorganic zirconium to immobilize enzyme during polymerization or electro-deposition [9,10]. According to the literature, nano-sized zirconium gel or thin film were used to immobilize hemoglobin [9], DNA [10], myoglobin [11] and HRP at gold electrode [12].

Zirconium oxide nanoporous gels were used to entrap the biomolecules due to their biocompatibility. The zirconium oxide nanogel was recently used for the entrapment of hemoglobin and myoglobin [13] and the protein ZrO_2 film preserve their bioactivity and show a good electrocatalytic behavior towards the reduction of H_2O_2. The analytical characteristics of the developed biosensor proved that the nanogel preserved catalytic activity and a good hydration microenvironment for the enzyme. Due to its lack of toxicity, good conductivity, affinity for groups containing oxygen, the ZrO_2 nanogels became attractive for the construction of biosensors.

A novel amperometric biosensor is described for the detection of acetaminophen (*N*-acetyl-*p*-aminophenol) as model compound. Acetaminophen is widely used as analgesic antipyretic drug having actions similar to aspirin. It is a suitable alternative for the patients who are sensitive to aspirin and safe up to therapeutic doses [14]. The large scale therapeutic use of that drug generated the need for the development of rapid and reliable methods for the determination of acetaminophen. Current methods for the analysis of acetaminophen include spectrophotometric [15], chro-

* Corresponding author. Tel.: +40 264 59 31 18; fax: +40 264 59 26 57.
E-mail address: rsandulescu@umfcluj.ro (R. Săndulescu).

0731-7085/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jpba.2008.08.018

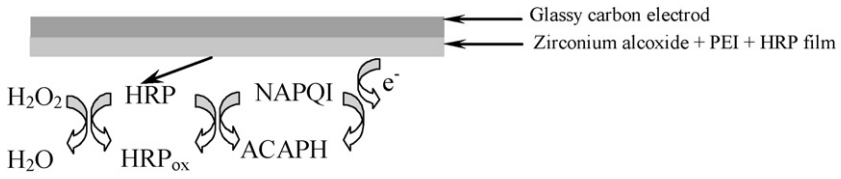

Fig. 1. The mechanism of biocatalytic peroxidation of the acetaminophen by the HRP immobilized at the surface of the electrode.

matographic [16] and electrochemical approaches [17–19]. The use of nanoporous magnetic nanoparticles for the construction of amperometric HRP immobilized biosensor was done with a good linearity range [5,20].

In the human body the acetaminophen is metabolized to *N*-acetylbenzoquinonimine (NAPQI) [5] in a similar way as the HRP is doing in the presence of hydrogen peroxide.

The novel HRP biosensor for the detection of acetaminophen uses the poly(ethyleneimine) (PEI) to entrap the enzyme and the zirconium alcoxide. Poly(ethyleneimine) is a linear polymeric cation and kept during several weeks a good permeability. It was used by various authors as entrapment material in the development of biosensors [21] and showed a good rate of electron transfer between the biocomponent and the electrode [22].

The enzyme (horse radish peroxidase, HRP) was entrapped into a porous alcoxide gel of zirconium and poly(ethyleneimine) at the surface of a glassy carbon electrode. The obtained configuration was used to study the biocatalytic oxidation of acetaminophen in the presence of the hydrogen peroxide. The biosensor was applied to the assay of acetaminophen in drug formulation (Perdolan® and Generic drug formulation) by the standard addition method.

The mechanism of action of the HRP biosensor is presented below (Fig. 1).

2. Materials and methods

2.1. Chemicals

The horse radish peroxidase enzyme (1.11.1.7 type II, 180 U/mg) was provided by Sigma.

Acetaminophen was provided by Merck.

Composition of Perdolan® declared by the producer is 200 mg acetylsalicylic acid, 200 mg acetaminophen, 46 mg caffeine, talc, maize starch, microcrystalline cellulose, polyvidone acetate.

Composition of generic drug formulation per tablet is 200 mg acetylsalicylic acid, 200 mg acetaminophen, 46 mg caffeine, 5.6 mg talc, 45 mg maize starch, 44.9 mg microcrystalline cellulose, 22.5 mg polyvidone acetate and was made in our department.

All reagents were of analytical grade, used as received.

The zirconium alcoxide was prepared according to the literature [23] and was completely characterized.

The alcoxide nanogel was prepared by solving the ZrO_2 powder in ethanol for 2 h and mixing it for 4 h.

Two different zirconium alcoxide gels have been prepared, starting from different amounts of zirconium salt: 0.25 M and 0.4 M alcoholic solutions by refluxing for 2 h at 90 °C then allowed to cool at room temperature.

Poly(ethyleneimine) (MW 60000) from Aldrich was used without purification.

The stock solution of the acetaminophen (10^{-2} M) was dissolved in phosphate buffer and kept in the refrigerator. Fresh solutions of zirconium alcoxide and poly(ethyleneimine) were prepared before the assays in phosphate buffer pH 7.4.

2.2. Film preparation

5 mg PEI with 125 μl ethanol and 120 μl distillated water were mixed for 15 min with vortex. 6.5 μl porous gel were added and mixed another 15 min with vortex.

2.3. Enzyme immobilization

Two solutions containing 0.03 mg/ml and 0.06 mg/ml HRP in phosphate buffer 0.1 M pH 7.4 have been prepared.

Equal amounts of the enzyme solution and the above described alcoxide gel were mixed for 15 min and 20 μl of the resulting mixture was deposited on the glassy electrode surface and left over night at 4 °C for drying. For another 24 h it was left at 4 °C in 5 ml phosphate buffer for hydratation.

2.4. Electrochemical methods

Amperometry and cyclic voltammetry were performed in a conventional three electrodes setup: modified glassy carbon electrode (working electrode), platinum (auxiliary electrode), Ag/AgCl 3 M KCl (reference electrode), under stirring conditions. All the cyclic voltammetry experiments were recorded at 100 mV s^{-1}.

During amperometry, the biosensor potential was kept at 0 V. The working temperature was room temperature (25 °C).

The glassy carbon electrodes used as working electrodes were provided by BAS Inc. (West Lafayette, USA) and were carefully washed with demineralized water and polished with diamond paste (BAS Inc.).

The experiments were achieved by using an AUTOLAB PGSTAT 30 (Ecochemie, The Netherlands) equipped with GPES and FRA2 software.

All experiments were performed in phosphate buffer pH 7.4 in the presence of hydrogen peroxide 0.1 mM. The pH of the solution was controlled by a ChemCadet pH-meter.

During the amperometry studies the working potential was imposed and the background was allowed to arrive at a steady-state value. Different amounts of acetaminophen standard solution or hydrogen peroxide were added into the stirred electrochemical cell and the current was recorded as a function of time.

2.5. Microscopy and surface studies

For the surface analysis a JEOL JSM-5600LV electron microscope was used.

3. Results and discussions

3.1. Film characterization

In order to test the permeability of the film, cyclic voltammograms were recorded in the presence of the redox system model, Ferro and ferric potassium cyanide. Experiments performed at GCE–Zr alcoxide 0.25 M and 0.4 M showed similar behavior with