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Nanomolar detection of methylparaben by a cost-effective hemoglobin-based biosensor

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ABSTRACT

This work describes the development of a new biosensor for methylparaben determination using electrocatalytic properties of hemoglobin in the presence of hydrogen peroxide. The voltammetric oxidation of methylparaben by the proposed biosensor in phosphate buffer (pH = 7.0), a physiological pH, was studied and it was confirmed that methylparaben undergoes a one electron-one proton reaction in a diffusion-controlled process. The biosensor was fabricated by carbon paste electrode modified with hemoglobin and multiwalled carbon nanotube. Based on the excellent electrochemical properties of the modified electrode, a sensitive voltammetric method was used for determination of methylparaben within a linear range from 0.1 to 13 μmol L^{-1} and detection limit of 25 nmol L^{-1}. The developed biosensor possessed accurate and rapid response to methylparaben and showed good sensitivity, stability, and repeatability. Finally, the applicability of the proposed biosensor was verified by methylparaben evaluation in various real samples.

1. Introduction

Parabens (alkyl esters of *p*-hydroxybenzoic acid) are widely used as preservatives to reduce microbial contamination since they exhibit a broad spectrum of antimicrobial activity (particularly useful against molds and yeasts), stability over a wide pH range, low price and sufficient solubility in water. Methylparaben (MP) is one member of a homologous series of parabens (Scheme 1) used singly or in combination to exploit the intended antimicrobial effect [1,2].

Parabens can have multiple biological effects, but it is generally considered that the inhibitory effects of parabens on membrane transport and mitochondrial function processes are keys for their microbiological activity [3,4]. One or more kinds of them are found in all types of cosmetic products. Since parabens are daily used in various human activities they are continuously released into the aquatic environment. They have been detected in river water and sewage treatment plant influents and effluents with the concentrations of up to 46 μg L^{-1} [5].

However, the recent studies verified that exposure to parabens disrupts or modulates the endocrine system and, therefore, they may have destructive subsequences on animal and human health [6]. Moreover, since parabens were detected in human breast tumors, the use of these preservatives in cosmetics is limited to a maximum content of 0.4% (*w*/w) for a single paraben and a maximum content of 0.8% (*w*/w) for total parabens by the European Economic Community (EEC). Therefore, selective, rapid and convenient determination of parabens is of great importance and has attracted particular attention in the last few years [7,8].

For the determination of parabens in different matrices, several analytical methods have been developed which are primarily included gas chromatography, high-performance liquid chromatography (HPLC), chemiluminescence and capillary electrophoresis [9–11]. Most of these methods are complicated since they need derivatization or combination with various detection methods. Also, they require expensive instrumentation with high operating costs, time-consuming extraction or preconcentration steps. On the other hand, electrochemical sensors offer many advantages over the mentioned determination methods such as high sensitivity and selectivity, low detection limit, low cost, rapid response, and convenient miniaturization [12]. Nevertheless, only a few electrochemical methods have been reported for the determination of parabens [13,14].

In our previous study, we introduced a novel biosensor for detection and determination of MP where the electrochemical determination of MP was carried out through the oxidation of phenolic hydroxyl by the catalytic effect of hemoglobin (Hb) in the presence of H_2O_2 [15]. With its known and documented structure, Hb is an ideal model molecule which possesses enzyme-like catalytic activity. However, Hb-based biosensors are more stable and inexpensive compared with enzyme-based biosensors. In this study we carried out further studies on the proposed

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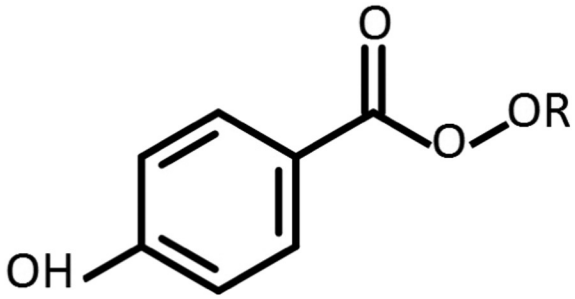

Scheme 1. General chemical structure of a paraben (where R = an alkyl group).

method for MP determination where, in order to investigate the selectivity of the prepared electrode for determination of MP, its performance in the presence of several potential interfering species was examined and the applicability of the fabricated biosensor by the determination of MP in different real samples was also examined. Sensor stability and repeatability as two important factors which representing the efficiency of a biosensor were also investigated in the present study.

2. Experimental

2.1. Materials and apparatus

All chemicals in this work were of analytical grade and used as received without further purification. MP was purchased from Sigma-Aldrich. The solutions were prepared in deionized water and deoxygenated by bubbling high purity (99.99%) nitrogen gas through them for 15 min prior to the experiments. The graphite powder with the particle size of smaller than 50 μm, 2.2 g cm^{-3} density and about 200–300 g L^{-1} bulk density (from Merck), and high-purity paraffin oil (from Sigma-Aldrich) were used for the preparation of the carbon pastes. Multiwalled carbon nanotubes (MWCNTs) with inner diameter: 2–6 nm; outer diameter: 5–20 nm; length: 1–10 μm; and 95% purity (from Plasmachem GmbH, Germany) were used to improve the surface area of the modified electrode.

Before fabrication of the modified electrode, MWCNTs were subjected to acid treatment in order to eliminate graphitic nanoparticles, metallic impurities, and also to improve the electron transfer properties. In a typical experiment, 500 mg of the MWCNTs was dried at 400 °C for 2 h in a nitrogen atmosphere. Then MWCNTs were dispersed in 50 mL of 6.0 mol L^{-1} HCl for 4 h under constant agitation in a nitrogen atmosphere. The dispersed MWCNTs were allowed to cool down to room temperature. Then they were filtered and the residue was washed several times with deionized water until neutral pH was attained and finally the residue was dried.

All electrochemical experiments were performed in phosphate buffer (PB) solutions at room temperature using a computer-controlled μ-Autolab modular electrochemical system, PGSTAT 101(Netherlands), driven with NOVA software (upgrade 1.10). The electrochemical cell was assembled with a conventional three-electrode set-up, including the carbon paste electrode (CPE) modified by MWCNTs and Hb (MWCNTs/Hb/CPE) as working electrode, an Ag/AgCl electrode (from Metrohm) as a reference, and a Pt foil as the counter electrode. Hb which is an inexpensive and abundant peroxidase alternative for determination of phenolic compounds was used as an electrocatalyst for MP

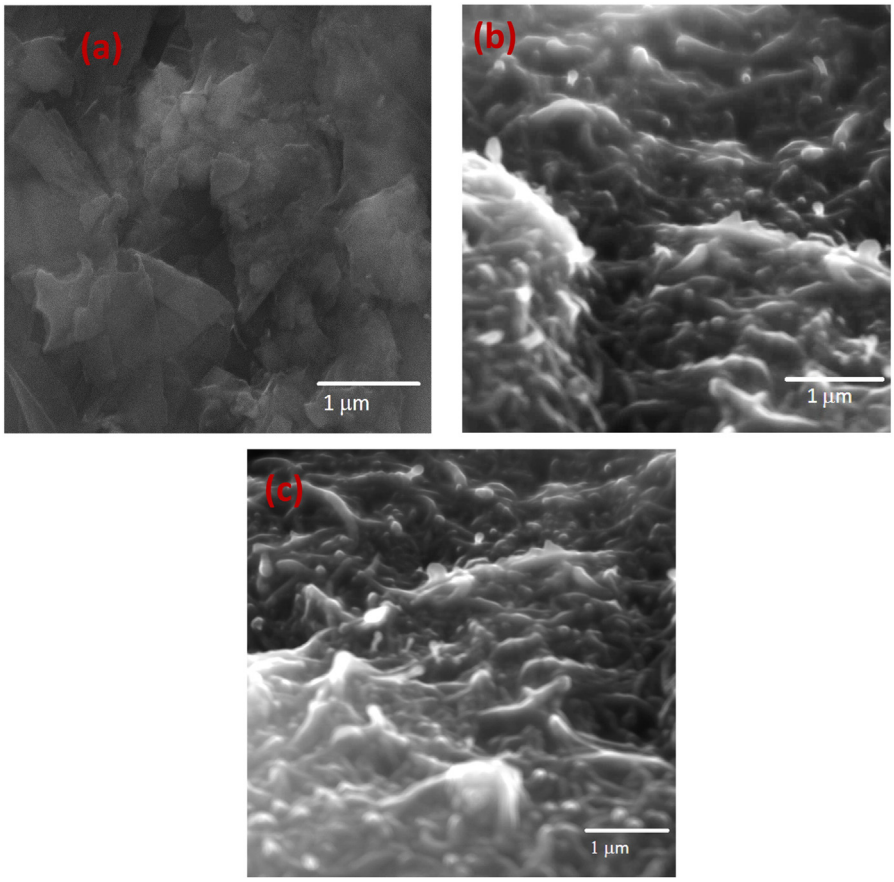

Fig. 1. SEM micrographs of the surface of (a) CPE, (b) CPE/MWCNTs, and (c) MWCNTs/Hb/CPE.