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Bi-enzyme sensor based on thick-film carbon electrode modified with electropolymerized tyramine

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Abstract

Bi-enzyme sensor based on thick-film epoxy-carbon electrode modified with polytyramine has been developed and examined for the determination of peroxidase substrates and cholinesterase inhibitors. Polytyramine was obtained on the electrode surface by repeated scanning of the potential from +600 to +1800 mV vs. Ag/AgCl in tyramine solution. The enzymes were immobilized in the polytyramine matrix by cross-linking with glutaraldehyde. The biosensor developed provides a reliable and inexpensive way for preliminary testing of common environmental pollutants with a single sensor in accordance with assumed toxic effect by the choice of appropriate substrate and measurement conditions. The bi-enzyme sensor makes it possible to determine substituted phenols and aromatic amines in the micromolar range of their concentrations and anticholinesterase pesticides with detection limits of 0.1 (Coumaphos) and 0.03 μmol l^{-1} (Chloropyrifos-methyl).

Keywords: Biosensor; Enzyme sensor; Electropolymerization; Polytyramine

1. Introduction

The increasingly stringent standards for the discharge of wastewaters call for the development of novel sensitive techniques for the preliminary estimation of quantities and toxic effects of the pollutants released in the environment. Electrochemical enzyme sensors are considered an alternative to the conventional spectrometric and chromatographic techniques for pollutant determination due to their low cost, simplified sample treatment, fast and sensitive response. Biosensors can be easily combined with conventional electrochemical equipment and implemented in the automated systems for the environmental monitoring or wastewater treatment control. Common pollutants present in the environment at ppm–ppb level affect key enzymes of metabolic pathways in living beings. This could be used for quantification of the pesticides, heavy metals, fluorides, cyanides, substituted phenols and thionic substances. The enzyme sensors based on cholinesterase [1–3], tyrosinase [4,5], urease [6,7] and peroxidase [5,8] have been described for this purpose. However, practical application of enzyme sensors is often limited by the problems of interpretation of their response in multicomponent media, e.g. wastewaters. The combination of several enzymes in a sensor array provides in some cases the identification of pollutants. Thus, cholinesterase, alkaline and acid phosphatases were proposed to use in three-electrode system developed for the determination of pesticides and heavy metals in one sample [2]. Similar pollutants were simultaneously determined in the biosensing system involving glassy capillaries covered with physically sorbed urease and cholinesterase [6]. However, the increase in the number of sensors in the array complicates the manufacture and the measurement protocol and hence increases the time necessary for sample testing and data interpretation. The immobilization of several enzymes on the same transducer is an alternative to the series of mono-enzyme sensors. In this work, we proposed to use for this purpose two enzymes, i.e. cholinesterase previously examined in testing industrial

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