

Review

# Enzyme inhibition-based biosensors for food safety and environmental monitoring

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Received 25 March 2005; received in revised form 23 June 2005; accepted 11 July 2005

Available online 25 August 2005

## Abstract

Analytical technology based on sensors is an extremely broad field which impacts on many major industrial sectors such as the pharmaceutical, healthcare, food, and agriculture industries as well as environmental monitoring. This review will highlight the research carried out during the last 5 years on biosensors that are based on enzyme inhibition for determination of pollutants and toxic compounds in a wide range of samples. Here the different enzymes implicated in the inhibition, different transducers forming the sensing devices, and the different contaminants analyzed are considered.

The general application of the various biosensors developed, with emphasis on food and environmental applications, is reviewed as well as the general approaches that have been used for enzyme immobilization, the enzyme catalysis, and the inhibition mechanism.

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**Keywords:** Enzyme; Biosensors; Environment; Food; Inhibitors

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## 1. Introduction

Because of their exceptional performance capabilities, which include high specificity and sensitivity, rapid response, low cost, relatively compact size and user-friendly operation, biosensors have become an important tool for detection of chemical and biological components for clinical, food and environmental monitoring. While electrochemical transducers combined with an enzyme as the biochemical component form the largest category, those biosensing systems that specifically depend on inhibition can be divided into three categories:

- Biosensors based on the immobilization of whole cells used as the biochemical component (Rekha et al., 2000; Durrieu et al., 2004; Chouteau et al., 2005). The use of this type of biosensor can increase the sensor stability and render the regeneration of the enzyme easier. However, such biosensors may suffer from side reactions due to the coexistence of several enzymes.
- Sensor devices coupled with reactors which contain an immobilized enzyme matrix. The inhibitor passes through the reactor and inhibits the enzyme (Lee et al., 2002). The residual activity of the enzyme is evaluated by measuring the enzymatic product before and after the inhibition.
- Biosensors based on direct enzyme immobilization on a transducer device. The enzyme and transducer elements are in close contact with each other and incorporated in a single unit. Some biosensors based on enzyme inhibition have been reported in the literature (Tran-Minh, 1985; Evtugyn et al., 1999; Luque de Castro and Herrera, 2003).

Inhibition-based biosensors have been the subject of several recent reviews. Biosensors based on ion sensitive field effect transistors (ISFETs) for the determination of some substrates and inhibitors were reviewed by Dzyadevych et al. (2003). Luque de Castro and Herrera (2003) have discussed the inhibition-based biosensors and biosensing systems. Other authors have reviewed the use of electrochemical enzyme-based biosensors for the determination of pesticides (Trojanowicz, 2002; Solé et al., 2003). Patel reported some specific applications of inhibition-based biosensors in the area of chemical and microbiological contaminant analysis implicated in food safety (Patel, 2002). Finally, some recent designs and developments relating to screen-printed carbon electrochemical sensors and biosensors for biomedical, envi-

ronmental, and industrial analyses have been reviewed by Hart et al. (2004).

In this review, we specifically provide an overview of the activity carried out since 2000 relative to biosensor systems which use an enzyme for inhibition-based analysis for food and environment safety. We also report the results from some studies in which the inhibited target enzyme is in solution while its product is detected using an enzyme-based biosensor.

Biosensors based on the principle of enzyme inhibition have by now been applied for a wide range of significant analytes such as organophosphorous pesticide (OP), organochlorine pesticides, derivatives of insecticides, heavy metals and glycoalkaloids. The choice of enzyme/analyte system is based on the fact that these toxic analytes inhibit normal enzyme function. In general, the development of these biosensing systems relies on a quantitative measurement of the enzyme activity before and after exposure to a target analyte. Typically the percentage of inhibited enzyme (%) that results after exposure to the inhibitor is quantitatively related to the inhibitor (i.e. analyte) concentration and the incubation time (Guerrieri et al., 2002; Ivanov et al., 2003). Consequently, the residual enzyme activity is inversely related to the inhibitor concentration.

## 2. Theoretical and practical considerations

### 2.1. Principle of the enzyme-based biosensor

Biosensors are analytical devices which tightly combine biorecognition elements and physical transducers for detection of the target compounds. In enzyme-based biosensors, the biological element is the enzyme which reacts selectively with its substrate (Guilbault et al., 2004).

It is well known that the response of a biosensor to the addition of a substrate is determined by the concentration of the product ( $P$ ) of the enzymatic reaction on the surface of the sensor. The reaction is controlled by the rate of two simultaneous processes, i.e. the enzymatic conversion of the substrate ( $S$ ) and the diffusion of the product from the enzyme layer. If there is a high enzyme activity, the decrease of the substrate concentration is not totally compensated by the transfer from the bulk solution due to the diffusion limitation, and because of this, only a fraction of the enzyme active centers is involved in the interaction with a substrate. In this case (diffusion

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