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Immobilization of Candida Rugosa Lipase on Aluminosilicate Incorporated in a Polymeric Membrane for the Elaboration of an Impedimetric Biosensor

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Abstract: This work describes the development of a new biosensor for the detection of an organophosphate insecticide (Diazinon) in an aqueous medium. The method is based up on the immobilization of Candida Rugosa Lipase enzyme by adsorption on Aluminosilicate in order to incorporate it into a polymeric membrane. The electrical properties of the polymeric membrane have been characterized by electrochemical impedance spectroscopy (EIS). The linear range of the biosensor is from 10^{-8} M to 10^{-6} M with a detection limit of 10^{-8} M.

Keywords: Immobilization, Aluminosilicate, Biosensor, Diazinon, Impedance.

1. Introduction

Chemicals that pollute water come from fertilizers and phyto-sanitary products as insecticides, pesticides or fungicides widely used to eliminate the insects and protect plants. These products can be carried along by runoff waters and pollute the ground water, lakes, rivers, and seas. The monitoring of diseases caused by unhealthy water, and the outbreaks of mortality due to water contamination by toxic chemicals is a global priority by authorities and environmental controllers. Approximately 6000 children died each day because of diseases caused by polluted water. But humans are not the only ones who take the consequences of water pollution, fauna and flora are also victims. Toxic substances in polluted water can be stored by grown plants, whose subsequent consumption can cause digestive diseases, damages of liver and kidney [1]. Diazinon is an organophosphate insecticide widely used in agriculture, but this product is very dangerous because it kills by altering normal neurotransmission within the nervous system of target organisms. It inhibits the enzyme acetyl cholinesterase (AchE) that hydrolyses the neurotransmitter acetylcholine (Ach) in cholinergic and neuromuscular junctions. This results in abnormal accumulation of Ach in the nervous system [2]. This phenomenon is currently applied for the design of biosensors for the detection of organophosphorus pesticides [3].

Enzyme immobilization technology may be an effective means for enzyme reuse and for improving its utility and stability. Adsorption is still the most commonly used approach because of its easy use and being the least expensive. The forces between a support and the enzymes include hydrogen bonding, Van der Waals forces and hydrophobic interactions [4].

The present work focuses on the immobilization of Candida Rugosa Lipase enzyme by adsorption using a new carrier (Aluminosilicate) which is minerals composed of aluminum, silicon, and oxygen, plus counteractions. They are a major component of kaolin and other clay minerals. Candida Rugosa Lipase enzyme is characterized by its ability to catalyze the hydrolysis reactions of the ester [5]. So, we will exploit this to elaborate a biosensor by the incorporation of the immobilized enzyme into a polymeric membrane. It is then deposited on a gold electrode and electrochemical impedance spectroscopy is performed in order to detect Diazinon.

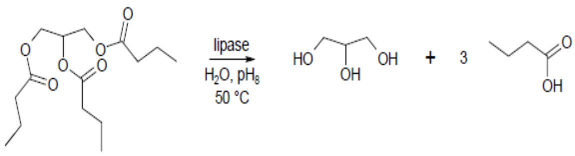

Fig. 1. The enzymatic reaction of an ester catalyzed by the lipase (hydrolysis).

2. Experimental

2.1. Immobilization of Lipase

Crude lipase AY (0.01–0.04 g) was dissolved in 3 ml of buffer solution (pH 7.0) and mixed with 0.1 g of Aluminosilicate. 15 ml of cold acetone was added, and mixture was stirred for 50 min at immobilization temperatures (20-30 °C). The immobilized lipase was collected by filtration and washed twice with buffer solution. The immobilized lipase was dried in a desiccator in the presence of silica gel until the stabilization of the mass.

2.2. Preparation of the Membrane

All chemical reagents were provided by SIGMA-ALDRICH (France). The Mixture composition used to obtain the membrane was 20 mg of immobilized Candida Rugosa Lipase (20 mg) as a bioreceptor and 100 mg of polychloroprene, these components were diluted in 1 ml cyclohexane.

50 µl of polymeric membrane was deposited on gold working electrode surface; the membrane was then evaporated at the ambient air (for 30 to 1 h). The sensors was then soaked for 1 h in 10^{-1} M (Diazinon) solution for its conditioning; the electrolyte chosen for these measurements was potassium monophosphate (KH_2PO_4) 10^{-1}M with pH 6.88.

2.3. Electrochemical Setup

Electrochemical impedance spectroscopy (EIS) measurements were carried out using an electrochemical cell consisting of three electrodes: the reference electrode was a saturated calomel electrode (ECS) used with a double junction; and a platinum wire of 1 mm diameter as the auxiliary electrode. The working electrode was a disc of gold whose surface was 0.19 cm^2.

The impedance experiments were performed using a Voltalab 40, model PGZ 301 impedance analyzer controlled by software (Voltamaster4) (Radiometer Analytical. S.A.) in the frequency range between 200 mHz-100 kHz. The experiments were performed in a Faraday cage in order to eliminate electrical interferences.

3. Results and Discussion

3.1. Optimization of Measurement Conditions

3.1.1. Applied dc Voltage

The effect of applied dc voltage is studied in order to define the optimum conditions for the impedimetric detection of Diazinon. The electrical behavior of gold/membrane/electrolyte interface is modeled with a classical Randles circuit. Polarization resistance Rp in parallel with capacitance Cp of the membrane are determined when the applied dc voltage varies. The lower value of Rp, 20.18 $k\Omega.cm^2$ is obtained at -900 mV.

3.1.2. Optimization of the Quantity of Incorporated Lipase Candida Rugosa

The optimization of the quantity of immobilized enzyme incorporated in the polymeric membrane was carried out. We observed that the value of the polarization resistance reaches a minimum value for a mass equal to 20 mg of immobilized enzyme.

3.2. Impedimetric Detection of Diazinon

The Nyquist impedance diagrams are plotted in the Nyquist plan after the injection of different diazinon concentrations (cf. Fig. 2). Approximate shape of half-circle is found, corresponding to a characteristic of a resistance parallel with a capacity. In addition, it is noticed that sizes of half-circles decrease with the increase in the diazinon, which corresponds to a decrease of the polarization resistance of the membrane. The Variation of polarization resistance was plotted as function of log concentration of diazinon (cf. Fig. 3) shows the calibration curve obtained with the biosensor, with a detection limit of 10^{-8}M. The linear range is from 10^{-8}M to 10^{-6}M.

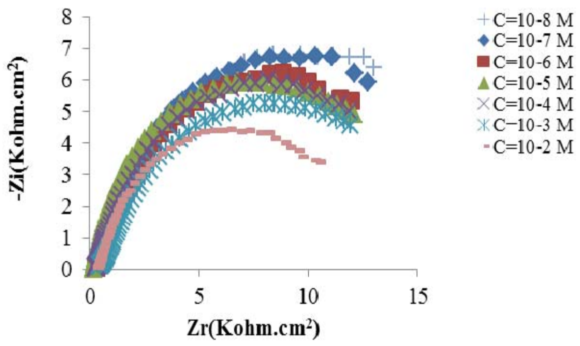

Fig. 2. Nyquist diagram in presence of different concentrations of diazinon.

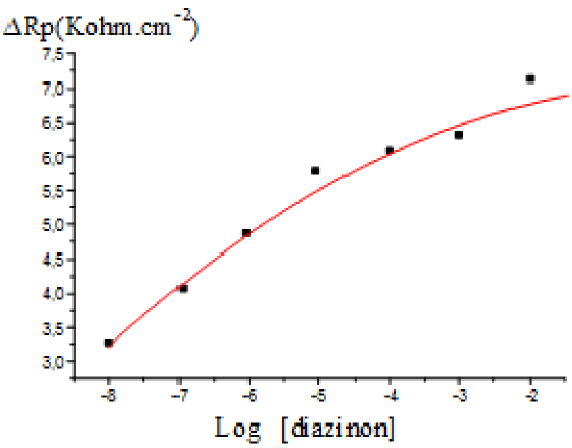

Fig. 3. Calibration curve of the biosensor $\Delta Rp = f \log[\text{diazinon}]$ obtained by impedimetric.

4. Conclusion

The development of a new impedimetric diazinon biosensor was accomplished and characterized by the immobilization of lipase enzyme on aluminosilicate with the use of a polymeric membrane.

The impedimetric enzyme electrode developed in this study provided linearity to diazinon in concentration range from 10^{-8}M to 10^{-6}M. This enzyme electrode exhibits a good sensitivity and fast response to diazinon.

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