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Amperometric flow-through biosensor for the determination of cholinesterase inhibitors

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Abstract

An amperometric flow-through biosensor based on epoxy-carbon electrode and butyrylcholinesterase immobilised on nylon, cellulose nitrate or white tracing paper has been developed and examined for the determination of reversible and irreversible inhibitors. The analytical characteristics of inhibitor determination depend on the hydrophobicity of the membrane material. Flow-through biosensor with various enzymatic membranes makes it possible to determine fluoride in the concentration range 1×10^{-4}–25×10^{-4} mol l^{-1} and 3.5×10^{-5}–1×10^{-2} mol l^{-1} when cholinesterase solution is used. The analytical characteristics of fluoride determination do not differ significantly from those obtained in batch conditions. For diazinon the immobilisation of cholinesterase results in the decrease of detection limits from 5×10^{-9} mol l^{-1} (native enzyme) to 4×10^{-9} mol l^{-1} (nylon membrane) and 1.5×10^{-9} mol l^{-1} (cellulose nitrate membrane). The influence of membrane material on analytical characteristics of FIA determination of inhibitors is due to the non-stationary distribution of reagents (fluoride) or sorptional preconcentration of the inhibitor (diazinon) in membrane.

Keywords: Amperometry; Flow injection; Cholinesterase; Inhibitor detection

1. Introduction

Biosensor technologies are widely used for the detection and quantitative determination of biologically active compounds from complex biological and environmental samples [1,2]. A biosensor is most often described as a sensor incorporating a biological element such as enzyme, antibody, nucleic acid, microorganism or cell [3]. Starting from the seventies, biosensors had risen from the laboratory to commercial devices (glucose and urea determination [4–6]). At present the potentialities of biosensors in the field of environmental monitoring are discussed owing to the advantages they possess, i.e. high sensitivity towards hazardous pollutants and fast response [2,7]. In some instances biosensors are considered a real alternative to the conventional time-consuming and expensive techniques, i.e. liquid chromatography or mass spectrometry. Thus, cholinesterases are in particular described for determination of organophosphorus and carbamaic pesticides and heavy metal salts [8–20]. In the living beings these enzymes provide the saponification of the neurotransmitter acetylcholine in the synaptic membrane and play a key role in nerve function. The inhibitors prevent the enzyme activity either by competing with the substrate

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