

Abstract

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

Student: Bc. Ondřej Keresteš

Supervisor: PharmDr. Marta Kučerová, Ph.D.

Consultant: pplk. prof. RNDr. Miroslav Pohanka, Ph.D., DSc.

Title of diploma's thesis: Biosensors for testing of cholinesterases' inhibitors

Acetylcholinesterase (AChE, EC 3.1.1.7) cleaves acetylcholine in the nervous system. Binding of acetylcholine to the receptor and subsequent hydrolytic cleavage at central synapses or neuromuscular junction allows the repeated transmission of nerve impulses. This ensures controlled movement and optimal human cognitive abilities. In case of contact of AChE with the inhibitor, it becomes blocked, thus deteriorating cognitive functions and affects convulsions, with prolonged exposure death can occur.

The aim of this work was to test various approaches to measurement of the activity of cholinesterases exposed to two inhibitors, as well as the preparation of biosensors using selected immobilization techniques. Three immobilization procedures were tested. Two of them were using electrochemical detectors and the aim was to optimize the method of quantification activity of AChE by measuring the pH difference (AChE was anchored by the chitosan membrane) and chronoamperometrically (AChE was adsorbed on the printed electrode). The third procedure involved immobilization of AChE to magnetic particles and the analysis was performed spectrophotometrically by the optimized Ellman method. AChE from the electric eel was used, and in the case of printed devices the human recombinant AChE was used also.

Michaelis constants (K_M) and 50% inhibitory concentrations for the AChE were determined (IC_{50}).

Research is currently underway in the field of biosensorics, both for protection against nerve agents and for investigating the effect of inhibitors that are used for Alzheimer's disease therapy. Another promising application of the cholinesterase-based bioassay is in the field of environmental analysis and pesticides assay. Thus, biosensors have potentially a very broad applicability.