

ABSTRACT

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Title of Doctoral Thesis **Sequential injection analysis with lab-on-valve module (SIA-LOV) technique in the analysis of biologically active substances.**

This dissertation is devoted to determination of biologically active substances by means of sequential injection analysis (SIA).

The theoretical part gives a recent overview of scientific knowledge concerning non-separative flow methods, focusing especially on SIA with Lab-on-Valve (LOV) module. Regarding applications of the SIA, problems of on-line sample treatment using solid phase extraction columns and of SIA methods employing enzyme reactions including sample pre treatment automation are dealt with. Theoretical background of enzymology, especially enzyme-substrate, enzyme-inhibitor and enzyme-inhibitor-reactivator interactions are discussed. Theory of cholinesterases is described in more detail, its role in human organism and its application as biomarker in environmental analysis is emphasized. An overview of detection techniques for determination of cholinesterase activity is also given.

The experimental part is split into two issues. The former one deals with automated sample treatment using combination of the SIA and the solid phase extraction columns, applied for drug assay in complex matrix. Amiloride in human urine was assayed using this technique.

The latter one is focused on the issue of automation of enzymologic studies of cholinesterase using SIA. Development of an automated analyser for nerve agents in the air samples is described. It comprised design of a special absorption unit and its connection to the microSIA-LOV analyser. Subsequently, possibility of automation of enzyme kinetics assay of cholinesterases using microSIA-LOV was tested. The applicability of SIA-LOV technique using enzyme inhibition in the field of pharmaceutical analysis was demonstrated on the SIA assay of neostigmine (a representative of quaternary ammonium carbamates) in tablets and injections. Further on, screening method for substances with anticholinesterase activity as potential anti Alzheimer disease drugs isolated from plants and for characterisation of their mechanism of action was devised. Finally automated SIA-LOV method for evaluation of biological effect of oxime reactivators of organophosphate-inhibited cholinesterase activity was developed.