Abstract

Growing demands for competitiveness in the pharmaceutical industry tend towards continuous optimization of production procedures, as this could mean huge savings in the preparation of pharmaceutical ingredients. In the present doctoral thesis, the enzyme catalyzed reaction thus supported with the description of the reaction in the form of kinetic model was researched marking an important step towards the optimization of the whole process.

In the first part of the doctoral thesis the focus was given to the implementation of the reactions between the reactants acetyloxyacetaldehyde and acetaldehyde, and between the reactants chloroacetaldehyde and acetaldehyde in order to obtain experimental data base, which could then serve as an important tool in creating a kinetic model and obtaining kinetic parameters. Reactions were performed in batch and semi-batch reactors. Due to the known influence of reactants on the inactivation of the enzyme, the reactants were added to the reaction mixture in a way that their concentration in the reaction mixture would be as low as possible. The progress of the reaction was investigated by gas chromatography, while the time course of the concentration profiles observed for each spec were determined via a calibration curve. In studied enzyme catalyzed reaction the main product lactol is formed in a sequence reaction via intermediate and at the same time in parallel reaction also a side product known as 6-methyl-lactol is formed. In addition, other, undesired side products were formed, since the enzyme or the possible presence of microorganisms could catalyze alternative reactions due to the raw enzyme slurry.

In the second part of the doctoral thesis the obtained experimental data, with the assistance of the reaction scheme and knowledge about the process, were used in creating a kinetic model and obtaining the appropriate values of kinetic parameters. The kinetic model is based on the legality of enzymatic reactions, wherein it was presumed that binding of substrates to the enzyme follows the principle of random mechanism. The whole reaction was split in three associated steps of the reaction, namely the reaction of formation of the intermediate product, the reaction of formation of the main product, and the reaction of formation of the side product. At the stage of the mathematical modeling satisfactory agreement between experimental and calculated data was achieved. The validity of the model for measured activity of the enzyme was confirmed for the reaction acetyloxyacetaldehyde/acetaldehyde and chloroacetaldehyde/acetaldehyde as well.

In the third and final part of the doctoral thesis, the developed kinetic model with the corresponding kinetic parameters was used in the simulation of the reactions in the semi-batch, CSTR and PFR reactors. In doing so, the main goal was achieving the optimal concentration of the main product lactol and high yield of the reaction, while having other emerging species as low as possible. The limiting factor in achieving the mentioned goal was, in addition to the enzyme inactivation, the formation of undesired side products. On the basis of the simulation it was found that the most appropriate reactor for studied reaction was semi-batch reactor, however in the case of continuous operation the CSTR reactor is the most reasonable choice.

The thesis presents an important and original contribution to science, since there are no reliable works or literature dealing with the establishment of the kinetic model for the reaction with the enzyme catalyst 2-deoxyribose-5-phosphate aldolase, where the reaction product is pharmaceutically important statin intermediate.

Keywords: statins, enzymatic biocatalysis, 2-deoxyribose-5-phosphat aldolase, inactivation, kinetic model