Reg. No: 2022/45/N/NZ5/00953; Principal Investigator: mgr Justyna Weronika Głowacka

Selective determination of isoenzymatic lactate dehydrogenase activity in flow analysis format

Justyna Głowacka

Department of Chemistry, University of Warsaw

Lactate dehydrogenase (LDH, EC 1.1.1.27) belongs to the enzyme groups widely distributed in the cells of all living organisms, where catalyses the equilibrium conversion of pyruvate to lactate, using NADH/NAD^+ as coenzyme system (one of the stages of cellular respiration). Hence, its activity is considered as a valuable clinical parameter in the diagnosis of diseases such as anemia, myocardial infarction as well as various types of cancer. However, total LDH activity assay only allows for assessing general state of the internal organs or the early detection of organ failure. In order to obtain more precise information, including the localization of damaged cells, it would be necessary to estimate its isoenzymes composition whose proportions change depending on kind of disease. Currently, such an analysis, which is not routinely performed in the clinical laboratories, is done on demand using expensive, time-consuming and highly complex methods.

The main objective of presented project is to develop a bioanalytical flow system (based on the operation of microsolenoid devices to control the flow) for distinguishing and quantifying LDH isoenzymes activity. Such an approach should provide precise control of the incubation time of enzymatic reaction, maintenance of repeatable mixing and detection conditions as well as reduction of both reagents consumption and analysis time. In the course of research, LDH isoenzymes will be divided into three groups considering their location in the human body, namely LDH1 (heart muscle), LDH2 AND LDH3 (brain), and LDH4 and LDH5 (liver and muscle). The identification process of individual LDH isoenzyme fractions will be related to the inhibition or non-inhibition their activity by specific substances. The second goal of the project will be to improve a routine, low-sensitivity procedure of total LDH activity determination that involves the monitoring of NADH concentration increment at the wavelength of 340 nm. The proposed method is based on exploiting the strong reducing properties of NADH molecule, in other well-known redox system with photometric detection in visible range. In these studies, the detector role will be played by the photometric optoelectronic detector, consisting of two paired light-emitting diodes. Finally, the usefulness of developed flow system will be confirmed by the analysis of certified control sera and real human serum samples.