ABSTRACT

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The aim of this work was to monitor kinetic profiles of permeation of fluorescent marker through cell monolayer (MDCKII-MDR) by sequential injection analysis. For this purpose, a new type of permeation module was prepared that could measure from 2 compartments – donor and acceptor.

The module was thermostated at 37°C during the experiment and liquid in the acceptor compartment was continuously agitated. Experiments were repeated to observe difference in interaction of fluorescent marker with cell membrane transporter. MDCKII-MDR monolayer cell inserts were replaced after the experiments.

The cell line used in this experiment include a genetically modified P-glycoprotein. Rhodamine 123, the fluorescent agent, was applied for on-line monitoring of extracellular transport.

The permeability kinetics of this marker was monitored using a fully automated sequential injection analysis system with five minutes sampling intervals for the testing period of 120 minutes. The sample was taken from the donor compartment in one cycle, and from the acceptor one in the next cycle.

Then, the effect of the P-glycoprotein inhibitor, which was verapamil in this work, was investigated. Its inhibition effect on the cell membrane transporter was proved in the resulting obtained kinetic profile.