SUMMARY IN ENGLISH

This work is concerned on sequential injection analysis and its use in the field of pharmaceutical analysis. This flow method is a versatile, multi-purpose analytical system and for this is gaining more popularity in analytical chemistry.

Firstly the review based on pharmaceutical applications was published. The new enhanced techniques such lab-on-valve, micro-SIA, bead injection and sequential injection chromatography was discussed.

The experimental part is focused on the use of SIA for automation of long-lasting processes monitoring in pharmaceutical methods of analysis. Fully automated system for in vitro release testing of semisolid dosage forms was established. This test was based on Franz diffusion cell and its connection with SIA system. The system was tested for monitoring release profiles of three commercial preparations containing salicylic acid (Belosalic, Diprosalic, Triamcinolon S). The release profiles were statistically compared. The native fluorescence of salicylic acid was used for fluorimetric detection. Phosphate buffer pH 7.4 was the receptor medium, different membranes were tested. Samples were taken at 10 min intervals during 6 hours of the release test and each test was followed by calibration with five standard solutions.

Other work was based on the same fluorimetric determination of salicylic acid. The apparatus containing three Franz cells was set up. One-walled cells were used (in contrast with previous double-walled cell) and a water bath for temperature regulation. Sample application, sampling and all steps in SIA program were tested with the ointment Belosalic. The samples of the acceptor liquid (50 μl) were aspirated in 15 min intervals for the period of 6 hours from each of the 3 cells and dispensed to a fluorescence detector to determine the concentration of salicylic acid. The volume of the acceptor medium taken for analysis was automatically replenished after each measurement. The device allowed simultaneous monitoring of the release tests for up to six cells including automated computer-aided evaluation of the release profile parameters. The system had advantages of shortage of the analysis time and ability of implementation of two compared batches in the same run.

Other work concerned on separation and simultaneous determination of two active substances in topical pharmaceutical formulation composed of local anaesthetics lidocaine (L) and prilocaine (P). The methodology was based on the

sequential injection chromatography (SIC) with UV detection at 212 nm. Monolithic column Chromolith Flash RP-18, 25mm x 4.6mm (Merck, Germany) was used. Separation was performed using elution with binary mobile phase composed of acetonitrile-phosphate buffer 0.05 M (40:80, v/v) + 0.01% triethylamine (adjusted to pH 7.1 with H_3PO_4) at flow rate 0.6 ml.min-1. The analysis duration was less than 7 min. The SIC system was then coupled with double-walled Franz cell and fully automated system for in vitro release testing of semisolid dosage forms was developed. Simultaneous measurement of lidocain and prilocain release from semisolide preparation was done by this system. Samples (only 10 µl) were taken in 10.5 min intervals during 4 h of the release test. Each test was followed by calibration with five standard solutions, receiving medium was replenished automatically by the system.

All proposed systems can be favourably used for manufacturing process control, for monitoring of pre- and post-changes of product properties, batch uniformity monitoring etc. SIA system could be also used as a screening device in pre-formulation and product development.

Other aim was to automate sampling and quantification of the apparatus for combined determination of dissolution and permeation through Caco-2 monolayer by means of sequential injection analysis. USP apparatus 4 was used for dissolution measurement. Measurement was based on open systems for dissolution and permeation assessment. Dissolution and permeation of immediate release tablets with content of 10 mg of propranolol HCl was evaluated. Native fluorescence of propranolol HCl in Krebs Ringer Buffer (KRB) was used for quantification. Sampling was done at three different locations within the apparatus alternating at sampling port D (dissolution) and A (apical side of Caco-2 monolayer), intermitted by a measurement at sampling port B (basolateral part of Caco-2 monolayer, amount permeated) after every third loop of measurement at D and A. Sampling together with quantification took 0.7 min at sampling port D, 0.9 min at A and 1.3 min at B. Time for sampling at port B lasted longer because of the time needed for replenishing KRB. Sample volume differed for these different sampling ports and was for permeation monitoring 50 µl and for dissolution monitoring 25 µl. The obtained data was consistent with data obtained by manual sampling followed by HPLC analysis.

The SIA method is relatively simple, low cost and adaptable device. Easy automation of entire analysis and on-line sample pretreatment and evaluation are

benefits of this method. Automation decreases labor-intensive manual sampling and drop the high of HPLC measurement. Flexibility and versatility facilitate rapid sampling from different sampling ports with varying washing steps, varying aspiration volumes and enables automated replenishing of the withdrawn buffer. In addition, online measurement could reduce issues when working with unstable compounds.