Automated permeability assessment of drugs formulated as solid dosage forms by means of sequential injection technique (SIA)

Stephan A. Motz[a], Jana Klimundová[c], Ulrich F. Schaefer[a], Stefan Balbach[b], Thomas Eichinger[b], Petr Solich[c], Claus-Michael Lehr[a]

[a]*Saarland University, Biopharmaceutics and Pharmaceutical Technology, Im Stadtwald, Building A4.1, P.O. Box 15 11 50, 66041 Saarbrücken, Germany*

[b]*Sanofi-Aventis Pharma Deutschland GmbH, Analytical Sciences Department, D-65926 Frankfurt/Main, Germany*

[c]*Laboratory of Flow Analysis, Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05, Hradec Králové, Czech Republic*

Objectives

To implement an entire automation for an apparatus assessing permeability of solid oral dosage formulations based on a Caco-2 monolayer and a flow through dissolution cell as described by Motz et al. [Motz et al., Eur J Pharm Biopharm, 2006, accepted] in order to widen the bottleneck formed by time and labor consumptive sampling and quantification.

Experimental Methods

Buffer for dissolution and permeation was KRB, pH 7.4. Test tablets contained 10 mg of propranolol HCl. Caco-2 cells were cultivated according to routine cell culture protocols (TEER>350 Ω•cm). Flow in the dissolution module (Apparatus 4, USP) was 6.5 ml·min^{-1} and 1.0 ml·min^{-1} in the permeation module; both modules were connected via a stream splitter. For quantification of dissolved and permeated propranolol HCl, a FIAlab 3500 system together with a FIAlab fluorescence detector PMT-FL (λ_{EXC} 260 nm) was used. It was shown that none of the excipients interfered with the analysis.

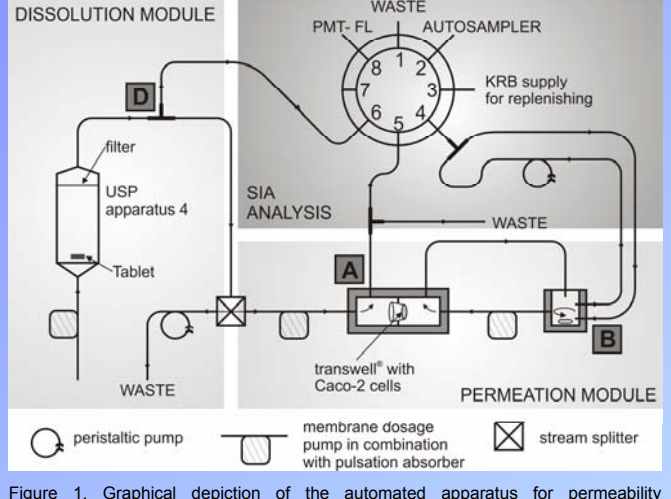

Figure 1. Graphical depiction of the automated apparatus for permeability assessment of solid dosage forms.

Results and Discussion

Since the concentrations to be assessed with SIA analysis are differing strongly, two independent calibrations have been performed. For relative high concentrations at sampling port D and A, 25 µl of sample were injected into the fluorescence detector. and therefore, calibration was done via of first order polynomial equation (Figure 2) (R^2>0.9999). For the lower concentrations at sampling port B, 50 µl of sample were injected. Here, limit of quantification was 40 ng·ml^{-1} (10σ). Lower injection volumes (25 µl) yielded corresponding results. Measurement in sextuple showed a standard deviation for the lowest standard (0.05 µg·ml^{-1}) 2.35 % and for the highest standard (80 µg·ml^{-1}) 1.63 %; regression line exhibited an R^2 > 0.999.

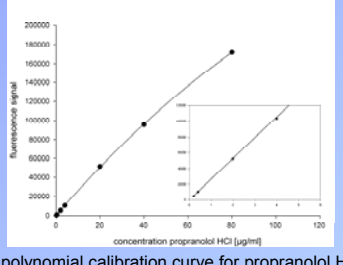

Figure 2. First order polynomial calibration curve for propranolol HCl in KRB; injection volume 25 µl for quantification at D and A.

Dissolution measurement revealed complete dissolution of the 10 mg propranolol HCl tablets (98.7 % ± 4.0%, n = 7) after 120 min (Figure 3). Caco-2 monolayers retained their integrity throughout the experiments.

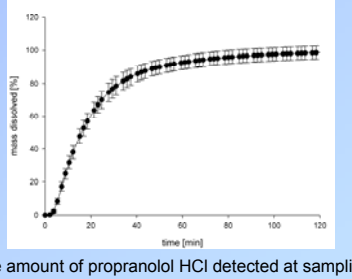

Figure 3. Cumulative amount of propranolol HCl detected at sampling port D.

Both data sets from manual and automated measurement are summarized in Figure 4. At this sampling port B, 4.07 ± 0.37 µg of propranolol HCl have been detected after 120 min. In comparison, previous experiments with manual sampling followed by analysis with HPLC led to a permeated amount of 4.23 ± 0.45 µg.

Also at sampling port D and A only minor differences have been detected. Since the tubing has been optimized in automated setup (upper plot, Figure 4), peaks appear to remain sharper throughout the passage of the apparatus.

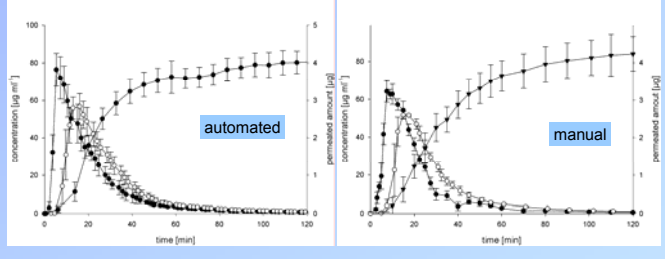

Figure 4. Comparison of the concentration time trends of the automated (upper plot) and the manual sampling and analysis (lower plot).
●: sampling port **D**issolution
○: sampling port **A**pical
▾: sampling port **B**asolateral

For a more detailed study of this topic, the reader is referred to S. Motz et al., Analytica Chimica Acta, 2006, doi:10.1016/j.aca.2006.07.080

Conclusion

Automation by means of SIA successfully yielded the required results.

1) Number of data points was increased by factor two, costs for manpower, sampling, and analysis were tremendously decreased.

2) The automated apparatus yielded comparable results to those obtained with manual sampling (similar dissolution and permeation profile).

Therefore, an important step has been done order to investigate the new tool for assessing permeability of solid dosage forms.

Acknowledgements

The authors (JK and PS) gratefully acknowledge the financial support of the Grant Agency of the MSMT of the Czech Republic, Research Project MSMT 0021620822.

Norbert Ochs is thanked for his kind support in designing and constructing the custom made parts of the apparatus.