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# Modeling, Realization and Characterization of Microreactors in Lab-on-a-Chip Devices

## Ph.D. Thesis Booklet

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# 1 Introduction

Gordon Moore's prediction on the development of integrated circuits became a standard measure in the technology progress of microsystems. This unbroken development in the recent 50 years led to a technology breakdown in the related fields such as microtechnology and precision engineering. Short after the first presented micro-electromechanical systems (MEMS) e.g. pressure sensors in the 70's, chip-scale manipulation of biological samples became reality, establishing the basics of Lab-on-a-Chip technology.
Microfluidics and Lab-on-a-Chip technology have its own role in chemical and especially in biochemical analysis and became even more significant in microreactor technology where small size makes enzymatic processes more effective and economical. Microreactors are usually defined as miniaturized reaction systems fabricated by using methods of microtechnology and precision engineering. The term 'microreactor' is the proposed name for a wide range of devices, having typically sub millimetre channel dimensions which can be further divided into submicron sized components e.g. micro and nanoparticle carriers [1].
Before the evolving of microreactor technology, the traditional way to conduct solution phase synthesis and analysis was the conventional batch mode in stationary reactors with stirring or shaking as the only means of mix reactants. Today, micro structured devices offer greatly enhanced performance compared to conventional batch systems due to effects arising from the microscale domain. *Time-resolved* processes carried out in flow microreactors are easier to scale up than *space-resolved* macroscopic batch processes therefore leading to accelerated process development. Microfluidic techniques provide better mixing of reagents, high surface to volume ratio provides more homogeneous temperature distribution therefore the process yield can be increased compared to the macroscopic realizations. In biocatalytic applications, the efficiency of the microreactor can be further improved by immobilization of enzymes on nanoscale carriers accommodated in the reactor volume. Re-usability of the biocatalyst makes the process economical and more environmental friendly. Further advantages of

microtechnology such as precision fluid handling provide outstanding repeatability and reproducibility.
Despite of the rapid development of enzymatic microreactors in the recent decade, important design questions still need to be answered.
Reaction kinetics is a key parameter of device design. Widely used kinetic parameters are deduced from the Michaelis-Menten model, which has a limited validity to batch reactions only. In flow systems the effects introduced by the flow itself should be also considered. Further complication in modelling can be expected from the immobilized enzymes. On one hand, immobilization may affect the kinetic parameters, on the other hand, the kinetic model should also be changed as the liquid and solid phases are moving related to each other.
Microreactors are built of a reaction chamber which may be filled by an appropriate carrier of the catalyst. The reproducible loading of this carrier is not always straightforward especially in micro scale. Even more challenging the determination of the actual loaded quantity of the carriers and biocatalysts
Long-time stability of the reactor and the reproducibility of the measurements may be affected by the flow rate, the substrate concentration, the immobilized biocatalyst morphology etc.
Micro scale may arise issues also in thermal design as the effects of axial heat conduction, viscous dissipation and low Reynolds numbers should be also considered.

## 2 Objectives

Thermal aspects of device design may play a key role in some bioMEMS devices such as droplet polymerase chain reaction (PCR) microreactors and nanocalorimeters. Approaches on thermal modelling have been demonstrated recently but no general solution has been presented so far for effective assistance of droplet based LoC devices design.
**Objective 1**: To construct a thermal compact model which provides direct input for a subsequent transient analysis and handles transient chemical

(e.g. enzyme) reactions taking place inside the droplets and resulting in a temperature field as output.

Possible applications of packed bed microreactors were demonstrated in biocatalysis and in enzyme screening, however, some questions still need to be clarified. An ever arising question is the accurate determination of the biocatalyst concentration in the reactor. Although several ad-hoc methods were already presented, there is no standardized method to measure the quantity of immobilized enzymes in micro chambers.
**Objective 2**: To implement a method for accurate, in-situ and on-line determination of the amount of biocatalyst particles in a microfluidic reaction chamber.

A layer built up of biocatalyst carriers (e.g. nanoparticles) may change its fine structure due to viscous effects caused by the flowing medium in the reactor. Changes in the layer structure may affect the activity of the biocatalyst.
**Objective 3**: To analyse the effects of structural changes in the layer structure on the biocatalytic activity. Furthermore, to create a measure to describe the structural changes and investigate the requirements of making reproducible measurements with enzyme biocatalysts.

Particle size and distribution undoubtedly affect the achievable enzyme activity in microreactors. However, the effect of different particle sizes or using a mixture of different particles have not been analysed before.
**Objective 4**: To investigate the effect of using different particle sizes on the enzymatic activity and on the possible loading capacity of the microchambers.

Due to their re-usability, working with enzymes in microreactors makes the process environmentally friendly and economical.
**Objective 5**: To analyse the kinetics of immobilized enzymes in a chip sized microreactor system and to present a method to carry out multi-parametric

measurements providing that the biocatalyst is reused during the measurements.

# 3 Summary of the scientific results

**Thesis 1** ***Thermal compact model for droplet microreactors***
*I established a novel thermal compact model for two phase flow microchannels. The model provides the time and space dependent temperature field of the microchannel. During the calculation the heat generated on the channel wall with a constant flux or inside the droplets and the velocity of the flowing medium are considered. The heat generated in the microreactor may be a consequence of a time dependent chemical reaction (e.g. enzymatic catalysis). [S1],[C1],[C2]*

1. A cell unit of the model is built up of heat conducting elements. Convective heat transfer is modelled by the switched capacitor's theorem. The amount of the heat stored in the capacitors is conducted through thermal conducting elements while the rest of the heat is switched towards the next capacitor at the rate of the fluid flow. The switching frequency depends on the flow velocity. The elements are aligned on the rectangular mesh of the channel geometry. I showed that the model can be realized either by finite 2D axisymmetric heat conduction elements aligned to the mesh or by an equivalent electrical network aligned to the mesh nodes. I showed that the two realizations are practically equivalent (the difference was smaller than $2.65\%$ in case of the investigated benchmark problems). The equivalent electrical network representation can be also calculated by industrial standard network solvers. I created a parser algorithm for the conversion between the two realizations.

2. I determined the range of Reynolds numbers where the model is valid. I provided an analytical relationship to calculate the upper limit of the Reynolds number. I found that the current model is valid under $Re = 7$. The results provided by the compact model were compared

to the results obtained by detailed CFD simulations of the identical problem. It was found that the relative error of the calculated temperature profiles remained below $7\%$ under $Re = 7$ and increased rapidly above that. The computational time required for the calculation of the compact model was reduced by two orders of magnitude compared to the detailed CFD model.

**Thesis 2** ***In-situ quantification of mangetic nanoparticles in a microchamber***

*I developed a novel method for the reproducible filling of the chambers of the chip sized reactor device with magnetic nanoparticles. The particles are separated and accumulated in the chambers by using permanent magnets. I developed an in-situ and on-line method for the quantification of magnetic nanoparticles located in a micro chamber. The measurement setup consists of a modified PDMS microfluidic structure encapsulating a resonant coil. With external passive components attached the arrangement forms a resonant coil magnetometer. I found that the resonance frequency of the magnetometer depends on the overall quantity of magnetic particles in the chamber.[S2], [C3]*

1. I measured the total mass of particles accumulated in the chamber of the microfluidic device using particles of $250\ \mathrm{nm}$ and $600\ \mathrm{nm}$ diameter, respectively. I found that the total mass of the accumulated particles were nearly identical in case of the two investigated particle types (difference was $2.7\%$). I verified, that the total mass of the $1:1$ mixture of two different sized particles ($250\ \mathrm{nm}$ and $600\ \mathrm{nm}$) accumulated in the chamber was significantly (by $17\%$) higher than the total mass of single sized particles in the same chamber.

**Thesis 3** ***Characterization of packed bed microreactors***
*I developed a Lab-on-a-Chip packed bed microreactor platform aiming the investigation of the reaction kinetics of enzymes immobilized onto magnetic nanoparticles. The platform enables the real time, optical (UV-VIS) in-line following of enzyme catalysed biotransformations taking place in the reaction chambers.[S3],[B1]*

1. I developed a method to perform multi-parameter enzyme catalysed reactions applicable in Lab-on-a-Chip devices. The method is based on subsequent measurement cycles where every cycle represent a different parameter setting. During the cycles the nanoparticles are magnetically trapped in the chambers so the biocatalyst load remains unchanged. The first phase of the cyclic operation is the release of the reagents by washing. The subsequent phase is the enzymatic reaction with the actual modified parameters.

2. I investigated the effect of the chamber filling on the reproducibility of the measurements. I found that the filling of the chambers with a given suspension of enzyme coated nanoparticles was resulted in more than $98\%$ of reproducibility related to the immobilized biocatalytic activity.

3. I experimentally characterized the repeatability of the enzymatic biotransformation from one parametrically identical cycle to another. I found that the repeatability of these measurements was more than $98\%$ related to the immobilized biocatalytic activity.

4. I developed a method for the optical inspection of the particle filled chambers. Two subsequent cycles were treated to be comparable only if the change of their normalized optical inspection values fell below $0.35$ on the range of $0-1$. I validated by measurements that every measurement cycles within a single experiment can be treated to be independent, if the difference of the measured biocatalytic activity between the first and last cycle was under $5\%$.

**Thesis 4** ***Kinetic characterization of microreactors***
*I characterized the reactions catalysed by the phenylalanine-ammonia-lyase (PAL) enzyme immobilized onto magnetic nanoparticles by using a multiple reaction chamber Lab-on-a-Chip device. Using this measurement set-up, I experimentally characterized the kinetic properties of the PAL-catalyzed reactions in micro-flow systems.[S3],[B1]*

1. I found that the biocatalytic activity was decreased by decreasing the particle diameter. For the experiments $600\,\mathrm{nm}$ and $250\,\mathrm{nm}$ diameter particles were used. I experimentally validated that the biocatalytic activity can be further increased by using a binary mixture ($250\,\mathrm{nm}$+$600\,\mathrm{nm}$) of the particles.

2. I characterized the biocatalytic activity of the ammonia elimination reaction of phenylalanine by PAL enzyme. Furthermore I characterized the effect of the substrate flow rate on the biocatalytic activity in the same reaction by multiparameter measurements. I validated that by increasing flow rate the biocatalytic activity increased until a saturation value of $20\,\mu\mathrm{l\,min^{-1}}$. I found that the viscous forces of higher flow rates do not have an effect on the enzymatic activity or on the reproducibility of the measurements.

3. I experimentally determined the kinetic constants of the above reaction conducted in flow microreactors at a fixed substrate flow rate of $20\,\mu\mathrm{l\,min^{-1}}$. I found that the reaction kinetic followed the Lilly-Hornby model and the biocatalytic activity was found to be $2.75$ fold higher, the $K_m$ value was found to be $3.64$ fold lower and the specificity constant was found to be $3.22$ fold higher than ones of the same reaction with identical parameters conducted in traditional shake vial.

# 4 Utilization of the results

**Compact model for the nutrient transport in blood capillary vessels** Modelling capillaries is among the deeply studied issues in many branches of physiology since with a reliable capillary model the capillary-organ interactions can be better understood. The tissues with higher rate of metabolism require higher density density of capillaries, therefore, understanding the capillary mass transfer is very important in cases of certain diseases such as cancer where the ever increasing malignant tumours require more and more nutriments. In droplet flow, internal mass circulations are developed which may affect the nutrient transport through the capillary wall. Developed on the basis of the thermal compact model for droplet microreactors, Márton et al. proposed a reduced order model for the description of mass transfer in capillary vessels. The model idea lies upon the similarity of the two phase droplet flow and the flow of red blood cells (RBC) in capillary vessels.[C4].

**Finding a new operation mechanism of PAL enzyme** By a reaction performed in the MagneChip device, Weiser et al. [S4] firstly demonstrated that PAL can catalyse the ammonia elimination from the acyclic dl-propargylglycine (PG) to yield (E)-pent-2-ene-4-ynoate indicating new opportunities to extend the MIO-enzyme toolbox towards acyclic substrates. Deamination of PG, being acyclic, cannot involve a Friedel-Crafts-type attack at an aromatic ring. Therefore a novel operation mechanism of the PAL enzyme was proved.
MagneChip, filled by PAL-MNPs, was used for the microscale biotransformation of L-propargylglycine in sodium carbonate-buffered $D_2O$. The device enabled to detect the formation of (E)-pent-2-en-4-ynoate at $242\ \mathrm{nm}$ and to produce measurable quantities of the product for recording $^1$H-NMR spectra without any work-up.

## Scientific Publications

[S1] Ferenc Ender, Márton Németh, Péter Pálovics, Andras Drozdy, and András Poppe. Thermal compact modeling approach of droplet microreactor based Lab-on-a-Chip devices. *Microelectronics Journal*, 45(12):1786–1794, 2014.

[S2] Ferenc Ender, Diána Weiser, András Vitéz, Gábor Sallai, Márton Németh, and László Poppe. In-situ measurement of magnetic nanoparticle quantity in a microfluidic device. *Microsystem Technologies*, 21(12), 2015.

[S3] Ferenc Ender, Diána Weiser, Botond Nagy, László Csaba Bencze, Csaba Paizs, Péter Pálovics, and László Poppe. Microfluidic multiple cell chip reactor filled with enzyme-coated magnetic nanoparticles - An efficient and flexible novel tool for enzyme catalyzed biotransformation. *Journal of Flow Chemistry*, Accepted, 2015.

[S4] Diána Weiser, László Csaba Bencze, Gergely Bánóczi, Ferenc Ender, Róbert Kiss, Eszter Kókai, András Szilágyi, Beáta G Vértessy, Ödön Farkas, Csaba Paizs, and László Poppe. Phenylalanine Ammonia-Lyase-Catalyzed Deamination of an Acyclic Amino Acid: Enzyme Mechanistic Studies Aided by a Novel Microreactor Filled with Magnetic Nanoparticles. *ChemBioChem*, 16(16):2283–2288, 2015.

## Conference Proceedings

[C1] Ferenc Ender. Modelling of heat transfer in Taylor flow in microchannels. In *Design, Test, Integration and Packaging of MEMS/MOEMS (DTIP), 2012 Symposium on*, pages 164–170. IEEE, 2012.

[C2] Ferenc Ender and Gusztav Hantos. Modelling of heat transfer in microdroplets as microreactors. In *18th International Workshop on THERMal INvestigation of ICs and Systems*, 2012.

[C3] Ferenc Ender, András Vitez, Gábor Sallai, Diána Weiser, and Márton Nemeth. In-situ measurement of nanoparticle quantity in microchambers. In *Design, Test, Integration and Packaging of MEMS/MOEMS (DTIP), 2015 Symposium on*, pages 1–6, apr 2015.

[C4] Márton Németh, Ferenc Ender, and András Poppe. Modeling of Circular Mass Transport of Nutrients in Capillary Vessels Using Microfluidic Approach. In *Proceedings of First European Biomedical Engineering Conference for Young Investigators: ENCY2015*, pages 102–105, Budapest, 2015. Springer Singapore.

## Edited Books

[B1] Ferenc Ender, Diána Weiser, and László Poppe. Microfluidic multiple cell chip reactor filled with enzyme-coated magnetic nanoparticles. In Margarita Stoytcheva, editor, *Lab on a Chip (in press)*. InTech Open, Rijeka, 2016.

## Unrelated Publications

[N1] Ferenc Ender, Gusztav Hantos, Dirk Schweitzer, and Peter Gabor Szabo. Thermal characterization of multichip structures. In *Thermal Investigations of ICs and Systems (THERMINIC), 2013 19th International Workshop on*, pages 319–322, sep 2013.

[N2] Ferenc Ender, Hunor Santha, and Vladimir Szekely. Optimization of microfluidic flow sensors for different flow ranges by FEM simulation. In *Electronics Technology (ISSE), 2010 33rd International Spring Seminar on*, pages 308–313, may 2010.

[N3] Ferenc Ender, Hunor Santha, and Vladimir Szekely. Flow sensor for microfluidic applications; Based on standard PWB technology. In *Electronics Technology, 2009. ISSE 2009. 32nd International Spring Seminar on*, pages 1–6, may 2009.

[N4] Ferenc Ender and Vladimir Szekely. Thermal transfer impedance variations by forced convective heat transfer in microchannels. In *Design, Test, Integration and Packaging of MEMS/MOEMS (DTIP), 2012 Symposium on*, pages 119–124, apr 2012.

[N5] Ferenc Ender, Gusztav Hantos, Andras Vitez, and Diana Weiser. In-situ thermal conductivity measurement of magnetic nanoparticle layers in Lab-on-a-Chip devices. In *Thermal Investigations of ICs and Systems (THERMINIC), 2014 20th International Workshop on*, pages 1–6, sep 2014.

[N6] Márton Németh, Ferenc Ender, and András Poppe. Heat and mass transfer reduced order modeling approach of droplet microreactor based Lab-on-a-Chip devices. *Microelectronics Journal*, 46(12):1152–1161, 2015.

[N7] Dirk Schweitzer, Ferenc Ender, Gusztáv Hantos, and Péter G. Szabó. Thermal transient characterization of semiconductor devices with multiple heat sources - Fundamentals for a new thermal standard. *Microelectronics Journal*, 46(2):174–182, 2015.

# References

[1] Wolfgang Ehrfeld, Volker Hessel, and Verena Haverkamp. *Microreactors*. WILEY-VCH Verlag GmbH, Weinheim, first edition, 2000.