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**Title:** Automated microfluidic systems for performing physical, chemical and biological experiments in FEP/PTFE microtubings

The development of microfluidic techniques has qualitatively transformed the understanding and approach to many problems in physics, chemistry, and biology. One of the goals of microfluidics is to design devices that integrate one or several laboratory protocols on a single integrated fluidic circuit. Such systems enable precise handling of liquids that allows, e.g., sample dispensing, microbial encapsulation, liquids transport, merging, mixing, and splitting of reaction compounds, etc. The flexibility of integrating multiple functionalities in space and time enables designing microfluidic platforms tailored to the project's specific requirements. In many applications and protocols, automatization of flow and processes realized by microfluidic circuitry is required to ensure satisfying function throughput and robustness against random disruptions. Automation offers experimental solutions that can be used to verify theories that need thousands of experiments with different parameters, find optimum chemical reaction conditions to maximize its yield or monitor the growth of bacteria in hundreds of micro bioreactors. Herein, three automated microfluidic systems performing physical, biological, and chemical studies are presented.

In the first chapter of the thesis, the theory behind the physics of flow in a microliter scale in designing integrated and automated microfluidic platforms is described. In the beginning, the notions of viscosity, surface tension, diffusion, and dimensionless numbers are introduced. Their implementation is essential in understanding limits and advantages while transferring standard laboratory protocols into a microliter scale. Furthermore, the fundamental physical laws of motion of viscous fluids are introduced. The Hagen-Poiseuille equation and Kirchhoff's laws for fluidic circuits are necessary while designing integrated microfluidic networks to predict the flow of fluids within the fluidic channels. Finally, different microfluidic approaches for the automation of biochemical assays are presented. Fundamental principles of selected techniques are described, and rationales behind the choice of a pressure-driven system in this work are provided.

In the second chapter, an automated microfluidic system capable of studying the influence of viscosity and interfacial tension of a non-wetting droplet on a relative mobility difference in a channel with a circular cross-section is described. The system was implemented to investigate the dependence of capillary number and ratio of viscosity coefficient (between aqueous and continuous phase) on the relative mobility difference. Furthermore, changes in physical parameters were monitored during the growth of bacteria culture in a micro bioreactor. Additionally, a presented automated microfluidic system was validated to study the mobility of a non-wetting droplet in a capillary with a circular cross-section.

In the third chapter, a tubing-based automated microfluidic cell culturing system capable of measuring optical density (OD), oxygen concentration, and pH in droplets is presented. Presented studies were performed in two stages where at the beginning, only oxygen-sensitive nanoparticle sensors were used. Determination of absolute concentration of oxygen level compared to an optical density (OD) in droplets during the cultivation of *E. coli* and *M. smegmatis* was obtained. Additionally, oxygen transfer rate (OTR) from the carrier oil phase to droplets and through the tubing wall of a microfluidic platform was investigated. In the second stage of the project, previously used nanosensors were additionally embedded with pH-sensitive dye. Firstly, the feasibility of measuring pH in micro droplets was validated. Further, the attempt to simultaneously monitor optical density, oxygen concentration, and pH in droplets seeded with bacteria was performed. The main goal of the experiment was to differentiate pathogenic strain *S. aureus* from non-pathogenic strain *S. epidermidis* based on the pH changes due to their metabolic profile in the selective medium.

Finally, in the fourth chapter, an automated fluidic platform conjugated with a benchtop NMR is introduced. The system was capable of studying multiple chemical reactions with precise control of the ratio of reagents and in-line measurement of the $^{1}$H NMR spectrum. The main goal of this work was to build the capability to conduct multiple chemical reactions which require longer residence time (24 hours) with online measurement of the reaction yield. Therefore, for the very first time for the fluidic system conjugated with a benchtop NMR, the idea of segmented flow was introduced where the cross-talk between experimental segments with well-defined chemical composition was minimized by additional spacers implemented. This work consists of a description of i) designing process where using of segmented flow in the platform conjugated with the benchtop NMR was validated, ii) optimization of volume for experimental and spacing segments, iii) selection of a compatible fluid for the spacer, and iv) determination of the design of the flow cell which compromises the reliability of the NMR measurement and requirements related to physics of flow within the entire fluidic network. Finally, as a proof of concept, the reaction of benzylamine with acetic anhydride was performed, and the reaction yield was calculated.

Projects presented in the following thesis are examples of microfluidics combined with automation which introduces a qualitative change in ability to tackle both fundamental questions and rapid optimization methods in physics, chemistry and biology. Capability of such platforms to perform tedious, time consuming and systematic experiments allows developing of innovative techniques and methodologies to monitor different phenomena, e.g. measurement of growth of bacteria without using commonly used fluorescent labels or measurement of dispersed light. Moreover, the methodology used to design presented microfluidic networks from tubes and fluidic connectors is universal and provides great flexibility to be implemented in wide variety of different applications. It can be especially attractive if rapid prototyping is required or more advanced fabrication methods such as soft lithography or injection moulding are unavailable.