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# Miniaturization of microfluidic control systems for high-pressure chromatography

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### **Abstract**

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This thesis explores flow control and sensing in microfluidic chips for high-pressure applications. Sub-cm glass chips have been designed and fabricated with the aim of miniaturizing chemical analysis systems. Today, chemical analyses are performed worldwide for medical and environmental purposes. As more tests become available for a wider audience, the demand further increases. The large instruments that are typically used are expensive and have high chemical and power consumption. Miniaturizing components has, on the contrary, the ability to decrease volumes, costs, and environmental impacts. In addition to lower consumption, miniaturization carries several features: quick heat distribution, laminar flow, and higher pressure tolerances, to name a few. In this thesis, microfluidic chips are developed aiming to replace larger-scale instruments. Applications are centered around high-performance chromatography, which is a separation method used to separate and detect compounds in a sample. Different flow phenomena are also investigated, including fluid compressibility and capacitance, which become interesting when working at elevated pressures. Experiments have been made showing how this impacts the regulation of microfluidic flow. Thermal regulation of viscosity has been a centerpiece of this work. Controlling flow rate and pressure in a system by changing the viscosity of a fluid has proven effective for several applications. This was utilized to maintain back pressure at the end of a system as well as to control and stabilize flow at the beginning. It was also used to regulate composition and adjust parallel flows during experiments. Multiple chips were also connected to utilize several features and to get close to fully miniaturized and portable analysis systems. Apart from flow actuation, the microfluidic chips were also equipped with sensors for accurate sensing in close proximity.

*Keywords:* High pressure, Microfluidics, Thermal regulation, Chromatography, Fluid mechanics

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# List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I M. Andersson, **K. Svensson**, L. Klintberg, K. Hjort. *Microfluidic Control Board for High-Pressure Flow, Composition, and Relative Permittivity*, Analytical Chemistry. 2018 6;90:12601-8.
- II **K. Svensson**, S. Södergren, K. Hjort, *Thermally controlled microfluidic back pressure regulator*, Scientific Reports. 2022 12;12:569. (Shared first author)
- III S. Södergren, **K. Svensson**, K. Hjort, *Microfluidic active pressure and flow stabiliser*, Scientific Reports. 2021 18;11:22504. (Shared first author)
- IV **K. Svensson**, W. Chris, H. Westphal, S. Södergren, D. Belder, K. Hjort, *Coupling microchip pressure regulators with chipHPLC as a step toward fully portable analysis system*, Sensors and Actuators B: Chemical. 2023 15;385:133732.
- V **K. Svensson**, S. Södergren, K. Hjort, *Portable high-pressure pump system for HPLC combining pressurized gas and on-chip pressure regulation*, Sensors and Actuators A: Physical. 2024 1;371:115286.

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# Author's contributions

The Roman numerals correspond to the numbers in the list of papers.

- I Major part of experiments. Minor part of analysis and writing.
- II Most of experiments and analysis. Major part of concept, planning, design, fabrication, and writing.
- III Most of experiments and analysis. Major part of concept, planning, design, fabrication, and writing.
- IV Most of design and fabrication. Major part of concept, planning, experiments, analysis, and writing.
- V Most of concept, planning, experiments, analysis, and writing. Major part of design and fabrication.



# Abbreviations

BPR	Back Pressure Regulator
DAQ	Data Acquisition
HMDS	Hexamethyldisilazane
HF	Hydrogen Fluoride
HPLC	High Performance Liquid Chromatography
PAN	Phosphoric, Acetic and Nitric acids
PCB	Printed Circuit Board
PEEK	Polyether Ether Ketone
PID	Proportional Integral Derivation
UV	Ultraviolet
μTAS	micro Total Analysis System





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# 1. Introduction

Microfluidics is a topic of great interest currently undergoing extensive research as the field presents numerous benefits and opportunities. Making things smaller is not just about the ability to fabricate small yet functioning details. It also means entering a world with a new set of parameters to play with. Physical phenomena that are taken for granted in the larger, macroscopic world do not necessarily behave the same way on a microscale. The fields of physics and chemistry merge, and with many biological applications, microfluidics is truly an interdisciplinary research area.

In this thesis, several phenomena and design parameters in microfluidics will be discussed, along with their associated advantages and challenges. Improving one characteristic to its extent does not necessarily lead to better outcomes, and miniaturization is not unconditionally good or bad. To better control high-pressure microfluidics, a trade-off has to be made where characteristics like size, pressure, compressibility, and diffusion all impact each other. The key is to find features to work *with*, instead of working against them.

So why use microfluidics? Except for the interesting properties following downscaling, microfluidics have great potential to make improvements concerning both manufacturing and applications. Herein, the focus lies on microfluidics for chemical separation and analysis, but microfluidics is also used for *e.g.*, mixing [1], synthesis [2], and cell studies [3]. Chemical analysis is greatly impacting our society, and billions of analyses are made every year. For example, in health care, blood and urine analysis are crucial for diagnosing diseases, which improves and saves millions of lives. Environmental analysis is no different; dirty water is a deadly problem that spreads both infections and diarrhea [4]. Being able to test water, food, and soil can ensure quality and decrease suffering. Forensics is a third area where both biological and chemical traces are analyzed to gain valuable information [5].

Today's analytical instruments can facilitate many of the above-mentioned analyses, but there are still issues remaining. Some of these are size, power consumption, accessibility, lab requirements, sample volumes, and chemical waste. A way of keeping, and adding to, the benefits of the tests while also reducing their environmental impact is to miniaturize the instruments.

The size of a system directly influences the liquid volumes that are used in operation. Large sample volumes can be difficult to obtain when multiple tests are required or when the quantity is limited, such as for tests on neonates. The majority of the volume, however, comes from the chemicals used. These chemicals are often harmful, both for the person operating the instruments

and for the environment in terms of emissions. Although greener solvents are investigated [6], large volumes also increase costs for purchase, transport, and waste handling.

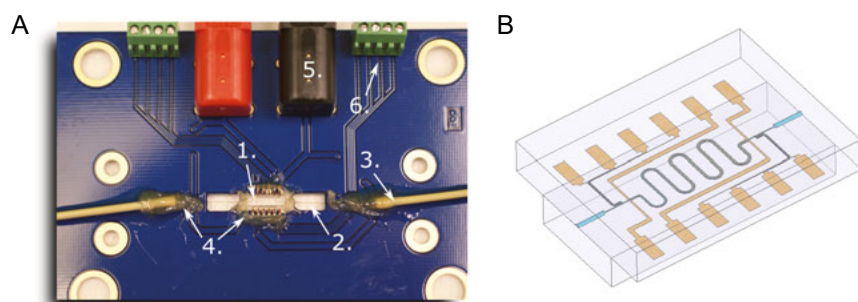
Making instruments smaller also positively impacts accessibility. Lack of access is partly a result of cost, both on an individual level and nationwide. Large component sizes also lead to stationary systems that cannot be moved or brought out to the field. This makes distance a factor, which especially affects people living in rural areas with insufficient infrastructure, and the elderly or sick who are too fragile to travel. For those managing to visit smaller hospitals, the samples might have to be transported to more advanced laboratories, which prolongs the result time. The administration and logistics also increase the risk of mistakes, such as samples getting lost or mixed up. Hence, portable instruments could greatly increase the accessibility of tests by being brought to patients' homes outside of hospitals. Today, there are several of these point-of-care (POC) tests available, where samples are taken and analyzed in the same place, and results are given within minutes [7, 8]. Except for the portable tests, a middle ground exists in the so-called bench-top instruments [9], which are smaller than regular industry instruments but more advanced than the, often single-use, POC tests. These bench-top instruments can be used in small hospitals or simple care centers and reduce the need to send samples to labs. In addition, for environmental testing, the instant results make it easier to decide when more samples are needed and where actions have to be taken.

To make smaller and more portable analysis systems, every required component within the system has to be compatible with the task, and, to be battery-driven, they should not be too power-consuming. Work is being conducted in developing microreactors [10], sensors [11], detectors [12, 13], valves [14], *etc.*, in smaller sizes while also investigating how downscaling affects physical properties and reactions. With the aim to bring instruments out of the lab environment, extended demands are also put on sensing and control to ensure reliable results.

In this work, the focus has been on keeping and improving control while miniaturizing important flow components. This has been achieved by designing and fabricating microfluidic chips in sizes below  $1\text{ cm}^2$ . The chips are made out of glass and contain fluidic channels with depths in the range of 10-100  $\mu\text{m}$ . Most of the chips also include integrated electrodes made of thin metal films. The metal reaches from the channels to the outer edge of the chips, where they are electrically connected to a printed circuit board (PCB). This electrical access makes it possible to perform both sensing and actuation directly at the channels. The approach resulted in high-quality microfluidic chips that were able to withstand high pressures in the range of 150-250 bar. An example of a chip is shown in Figure 1.1, where A shows a photo of a chip assembled on a PCB and B shows an illustration of the features inside the chip.

The papers presented in this thesis explore flow regulation on chip through thermal actuation. In **Paper I**, multiple chips were combined to control and measure a mixture of methanol and CO<sub>2</sub>. By heating the chips, and thereby the internal fluid, the viscosity of the fluids will change, which affects the pressure drop and flow rate. The temperature can be adjusted with resistive heating which were used with feedback regulation to control the flow in microfluidic systems. This technique was more thoroughly investigated in **Paper II** and **III**, where it was used for back pressure regulation and flow stabilization. The design of the chips was also further developed to improve the fabrication and assembly yield while holding smaller volumes and tolerating higher pressures.

In **Paper IV** and **V**, the microfluidic chips were used for pressure regulation, showing the performance in combination with high-performance liquid chromatography (HPLC). In **Paper IV**, the pressure regulation was used to improve the sample injection on a chip that included a chromatographic column (chipHPLC) and in **Paper V**, it was used together with pressurized gas to create a pump system used to drive an HPLC separation.



*Figure 1.1.* A typical microfluidic chip used in this thesis. A) A photo of a chip assembled on a PCB including 1. Chip, 2. Capillary, 3. PEEK tube, 4. Epoxy glue, 5. Heater connectors, 6. Temperature measurement connectors. B) A sketch of a chip with a microfluidic channel in blue and metal electrodes in gold.

## 1.1 Miniaturization

The aim of miniaturizing is either to reduce size or to gain desired physical properties or, of course, both. Miniaturization can be addressed in different ways: by shrinking the existing instruments, by replacing old technologies with new, or by combining and reducing the devices needed.

Miniaturization comes with some evident benefits, like the reduced volume needed for chemicals and samples, and the overall size of the system. But it also affects the physical properties of the system, something that has to be considered whether it is desired or not. One of the most apparent differences

is the surface-to-volume ratio. When decreasing the characteristic lengths by a factor ten, the volume scales with  $10^{-3}$  while the surface scales  $10^{-2}$ . This gives surface effects a larger impact which affects, for example, the system's inertia, pressure, and force. As weight is related to volume, the reduction reduces the impact of gravity which is often seen as a neglectable factor in miniaturized systems. This makes it possible to work with fluids with different densities in the same channel without them getting layered. For example, oil or gas can form bubbles in an aqueous solution that move along with the flow, without coalescing at the top as it would if the denser solution would sink to the bottom [15]. Particles with different densities and sizes may also be manipulated and sorted with microfluidics without getting sedimented.

Another distinct feature of miniaturized fluid structures is the movement of the flow. In microfluidics, laminar flow is a central property describing the stable and predictable streamlines. When laminar flow rules, two liquids flowing next to each other will stay side by side without getting mixed. The opposite is found in the macroworld, where turbulent flow creates chaotic streamlines and swirls that naturally mix the fluids.

Laminar flow is an excellent feature for separation applications. Particles can be separated either longitudinally along the fluid channel, as in chromatography, or across the channel area, as in acoustic [16] or inertial separation [17]. Two liquids can also be injected beside each other with the desire to transfer particles from one of the liquids to the other, without mixing the liquids themselves [18]. In both cases, laminar flow is essential for the particles to stay in their path without getting remixed along the flow.

Except for the microfluidic technology, laminar flow and low volumes are also evident in capillary analysis systems. The most common application is in capillary electrophoresis, where analytes are separated based on charge [19]. By applying a high voltage, the molecules start moving, and the higher the charge and the smaller the size, the faster they move. This benefits from a flat flow profile across the channel and simple construction using fused silica capillaries. However, the capillary system does not offer any wider range of design possibilities or integrated sensors or actuators, which is where microfluidics dominates [20, 21].

Even when laminar flow rules, it is not a guarantee that two liquids will always stay separated. With shrinking distances, the effect of diffusion plays a larger part. By adjusting the width and length of a channel, full mixing can be achieved with just the work of diffusion [22]. However, a long and narrow channel increases the pressure drop, which increases the demands on pressure tolerance. For quicker mixing, there are several techniques to use when designing microfluidic flow paths that include elaborated geometries and structures, such as herringbones [23].

In addition to molecules, shorter distances also speed up the transportation of heat. Low volumes reduce the energy needed to heat a medium and quickly reduce temperature gradients. This is useful when reactions need a certain

temperature [24] but can also be used to control the flow in the form of *e.g.*, thermally actuated pumps, valves [25], and filtration [26].

A challenge with miniaturized instruments is the function of moving parts. In the macro world, mixing and valving are easily done by stirring and mechanically moving components using gears and motors. These systems cannot be translated to the microworld by simply scaling down the dimensions. The high surface-to-volume ratio results in fragile structures that are difficult to fabricate and impossible to mount with screws or solder. Similarly, replacing or repairing broken or clogged units are difficult tasks. Therefore, strategies that involve manipulating flow without moving parts are promising and excellently suited for microfluidic systems, many of which are made on-chip. This includes elaborated geometries and appliance of *e.g.*, acoustics forces [27, 28], inertial forces [17], electric forces [19], and temperature changes [29].

## 1.2 Lab-on-a-chip or chip-in-a-lab

Lab-on-a-chip is a growing research field where an increasing number of applications are made on chips. The objective of this development is to achieve functional laboratories entirely on the chip. This development can be compared to the electronic world, where microchips with different functions are combined and electrically connected on PCBs, making up the foundation for *e.g.*, telephones and computers. Similarly, microfluidic chips with different properties are fabricated with channels that can be fluidically connected. As many of these chips also contain electrodes, PCBs can be utilized also here. Gradually, as more components are integrated into a chip, they can function as self-contained units and become compact enough for portability. Microfluidics have, however, not reached as far as electronics, and a lot of potential still remains unmet.

The lab-on-a-chip technology has advanced most in the analytical field, both research wise and commercially. Complete systems are commonly referred to as micro total analysis systems ( $\mu$ TAS) and can include *e.g.*, sample preparation, mixing, and separation. These systems allow for diagnostic testing using very small blood sample volumes in the microliter scale [30] and can be used to perform genome sequencing even from single cells [31]. In addition to pharmaceutical applications,  $\mu$ TAS are also used for environmental, food and beverage testing [32, 33].

Except for portable units, microfluidic chip systems typically consist of a single chip connected to multiple large instruments to form a functional circuit. Rather than a whole lab, only a sub-part of an experiment is actually performed on the chip. This can be called a chip-in-a-lab.

Making and connecting chips in the lab is a necessary step in the development of lab-on-a-chip, but it is also valuable on its own. As miniaturizing has several benefits except portability, chip technology provides many opportuni-

ties, and where labs with adequate equipment already exist, chips can easily be integrated to improve and provide new functions.

For instance, small channels enable controlled droplet formation, which opens up several opportunities, including single-cell analysis [3]. When working with cells, the chips can also be used in research to mimic desired environments by easily adjusting the temperature or creating body-like structures, as demonstrated in organ-on-chips [34]. Furthermore, precise process control is advantageous for microreactors, which can be used for separations or synthesis in the production of pharmaceutical chemicals [35]. In addition to chemical reactions, chips can also be used for sensing and controlling the flow, where one benefit is the reduced dead volume from connections [36].

### 1.3 HPLC

When talking about chemical analytics, both on and off chip, chromatography is often a preferred method, and here the main focus lies in high-performance liquid chromatography (HPLC). HPLC is the golden standard for many analyses and is used extensively in medicine and environmental laboratories. The aim of chromatography is to separate analytes in a sample to enable detection, either qualitatively or quantitatively. A separation column plays the main role, but several components are required to make a functioning system, including a pump, injection valve, detector, and sensor, Figure 1.2.

In chromatography, there are two main phases: a mobile phase and a stationary phase. The mobile phase acts as the fluid that transports the sample through the column. Typically, the mobile phase includes an aqueous buffer and a solvent, such as methanol, ethanol, or acetonitrile. The stationary phase refers to the material inside the column that consists of stacked particles that can vary in composition, charge, particle size, and porosity. The particles are often porous to increase the surface area and spherical to ease tight packing.

When a sample is injected into the column, its components will partly adhere to the stationary phase and partly flow with the mobile phase. Depending on the characteristics of an analyte, the ratio between the time it spends in the stationary phase and the mobile phase will vary. Hence, different analytes will elute, leave the column, at different times, and thereby be separated.

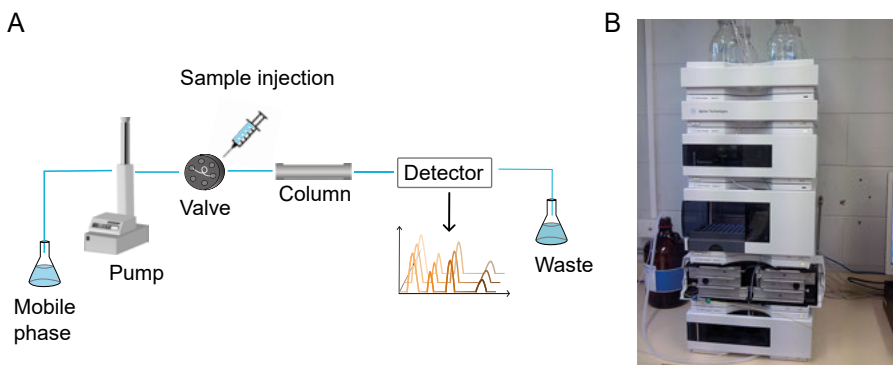
The separation is based on the difference between the stationary and mobile phase in relation to, *e.g.*, polarity, size, or charge [37]. In size-exclusion chromatography, the pore size of the stationary phase is set to trap smaller molecules that elute later than larger molecules that pass outside the pores. In ion-exchange chromatography, the stationary phase is charged to attract oppositely charged molecules. In normal-phase chromatography, the stationary phase is more polar than the mobile phase, making more polar analytes adhere for longer. In reversed-phase chromatography, used in **Paper IV-V**, the mo-



bile phase is the more polar phase and the stationary phase often consists of 3-5  $\mu\text{m}$ -sized silica particles with bonded carbon chains.

After the separation, the analytes should be detected, which can be done by different techniques, either quantitatively or qualitatively. Ultraviolet (UV) and visual spectroscopy is a common one; mass spectrometry, electrochemical, and fluorescence are others.

To get reproducible results, system control is an important factor, where the flow rate, pressure, temperature, and composition all impact the elution time of the analytes. For well-separated peaks, these parameters should also be tunable, and band broadening should be avoided. Band broadening is an effect of diffusion that can be reduced by minimizing dead volumes and shortening the distance to the detector. In contrast to ordinary liquid chromatography, HPLC has higher throughput, and with smaller dimensions, the pressure significantly increases. This places demands on the instruments used as well as all connectors and tubes to withstand the increased pressure without leakage.



*Figure 1.2.* A typical HPLC system. A) Sketch of different components included in the flow system. B) A photograph of an HPLC instrument. *Image reprinted from <https://commons.wikimedia.org/wiki/File:Agilent1200HPLC.jpg> with open access license CC BY-SA 3.0.*

## 1.4 High-pressure microfluidics

The chips presented in this thesis are all constructed to tolerate high pressure. When downscaling a system, the volume flow rate will decrease accordingly, lowering the overall throughput. To maintain a certain flow rate, the velocity has to increase which, in turn, significantly raises the pressure. An upside to this is that the force exposed on the chip from the fluidic pressure does not increase as much. Small devices can relatively easily be made to handle high pressure because of the relationship between size and force:  $F = P * A$ . By shrinking the cross-section area of a channel, the force will decrease pro-

portionally, and hence higher pressures can be tolerated. On the other hand, having a pressurized medium in a large container results in massive force and potential danger if it malfunctions. Especially when working with compressed gasses. If large volumes of gas expand in a room, it can cause direct respiratory danger. Hence, high pressure in combination with miniaturization is a great match.

While many applications and research are performed with low-pressure microfluidics, the high-pressure regime is a whole area in itself. High pressure comes with both requirements on the robustness and tolerance of the devices and offers opportunities in usable chemicals and applications.

A high initial pressure can be the outcome of several different situations: narrow geometries from miniaturization create a high pressure-drop for the desired flow rate; an application requires the initial parts to be performed at high pressure; or the whole experiment needs to be performed at high pressure regardless of pressure drop. For the latter, a restriction or back pressure regulator (**Paper II**), is needed in the end to hinder the pressure from dropping to atmospheric.

A common misbelief is that high pressure is a desirable feature in itself when it is often a side effect of reaching other goals. For example, in HPLC, P stands for performance but is often mixed up with pressure. The goal in HPLC is to get high throughput, *i.e.*, to fasten analysis time while keeping well-separated compounds, which means that the column should be tightly packed. This, together with making the channels smaller, inevitably leads to higher pressure, whether it is wanted or not. The reason for the focus on pressure is to address the demands it places on the equipment to be able to carry out these fast flows through narrow passages. We need pumps that can generate the high pressures, and sensors, actuators, tubes, and fittings that can withstand the pressures without leakage.

The reasons to operate the whole application at elevated pressure are related to the different behaviors of liquids and gasses at different pressures. Firstly, it determines if the fluid *is* a gas or a liquid. Or, if it is something in between, a supercritical fluid [38]. With increased pressure, fluids can be kept in a liquid state at high temperatures without boiling [39]. The gas and liquid phases of a certain fluid have different properties where the gas is more compressible and less viscous. High pressure also increases solubility, which means that it enables solvent compositions that cannot be used at atmospheric pressures. It also enables the dissolution of gasses into liquids [40], which makes it possible to make gas-enhanced liquid chromatography [41]. Even when gas inclusion is not the purpose, liquids often contain fractions of air. When the pressure lowers, air bubbles form which cause problems if they, for example, get stuck in the detector cell. Furthermore, high pressure can improve selectivity in liquid chromatography [42, 43], and can be used to simulate environments with increased pressures in a lab [44].

## 1.5 Environmental and ethical considerations

In today's society, the never-ending consumption is a problem that raises questions both about environmental sustainability and the morals of using materials and products produced in low-wage conflict areas. Developing new technology and creating more products will undoubtedly have an impact. High-pressure microfluidics necessitate fabrication in cleanroom, which demands high energy consumption to keep the standards of the room, and several lithography and cleaning steps using chemical baths and expensive equipment. The high-quality requirements of the chemicals, both in fabrication and operation, reduce the possibility of reusing any of the liquids. Waste chemicals must be properly managed during chip fabrication to prevent the release of any hazardous substances. Although chemicals and energy used in operations are the most visible and focused-on aspects, there are many steps in the life cycle of a product, including mining and fabrication, emissions, waste management, and landfill.

These factors have to be weighed against the potential benefits and potential side effects of compromises. For example, using less clean chemicals might result in dysfunctional devices and unreliable analysis results. While chemicals pose risks, so do inaccurately analyzed or contaminated blood samples for the patient which might be falsely diagnosed. With more countries reaching higher finances, the accessibility and consumption of new technology will increase. The advantages are that they can bypass some steps of large power-consuming instruments and hazardous emissions, while still gaining improved life quality.

The justification for energy and material resources used in this work is twofold: miniaturization and accessibility. Miniaturization leads to less chemicals and power used at operation, and portable devices also mean less transportation of samples. At a fabrication level, smaller devices mean less material needed for each device. Going from regular instruments to chip-based instruments greatly reduces consumption, while the size of the chips also matters. Herein, the chips are made on 4-inch glass wafers. Regardless of the number of chips on the wafer, the same amount of metal is used when the wafer is sputtered and the same volume of chemicals is used when the wafer is immersed. Making the chips smaller means more chips can be obtained from one wafer, which decreases the per-chip consumption.

The other part is accessibility, to let more people benefit from the materials we use. Small and inexpensive devices have the ability to increase the range of users. On a large scale, this means that more countries can get improved healthcare and quality assurance. On an individual level, portable instruments can be brought to places with insufficient infrastructure or to patients unable to travel.

## 2. Regulation in high pressure

### 2.1 Size and inertia

Inertia is an important parameter in regulation that gets highly affected when miniaturizing a system. Inertia is the resistance to change, which can be extracted from Newton's first law that states that an object or mass in motion will stay in motion if no forces act against it. A heavy mass traveling at high speed has a large inertia, and a significant force is required to stop or change its direction, hence inertia is inversely proportional to size. However, the term 'inertia' is also used more broadly to refer to resistance to change, such as in the case of thermal inertia.

In microfluidic calculations, inertia is often assumed to be negligible and momentum diffusion occurs instantaneously. This means that if the pressure changes in one part of the flow path, the new pressure will immediately spread throughout the entire system, an assumption that works best for incompressible fluids and low flow rates. Inertia can, however, also be utilized in microfluidics to *e.g.*, sort out particles of different sizes [45].

Low inertia makes a system sensitive to disturbances and a pressure fluctuation at the inlet will be noticeable throughout the whole system. But it also makes it easy to quickly compensate for this with active stabilization, as shown in **Paper III**. Low inertia is advantageous when parameters need to quickly be altered, for example during gradient experiments.

On the contrary, high inertia makes a system stable and less sensitive to small variations from the surrounding conditions, like pump pressure or ambient temperature. For a large bulky volume, the surface effects are reduced, which means less energy losses due to friction or temperature gradients at the surfaces. This comes to greatest use in systems where the same parameters are used constantly, *i.e.*, constant flow rate and temperature in, and between, experiments. However, changing flow parameters requires both extended waiting time and energy. When the flow rate of the pump changes, the pressure in the system will follow but it takes a long time before the new pressure conditions have stabilized in the entire system. This is connected to the fluid's compressibility, which is discussed further in section 2.3 *Compressibility*.

In addition to pressure and flow, size and inertia also affect the temperature. To keep a stable temperature, a large volume with a low surface area is preferable. It requires a lot of energy to be heated, but a lower continuous power to maintain the temperature. With smaller volumes and higher surface ratio, quick heating is possible although more heat losses will occur. The time

scale is important when handling fluids in motion as the time in the chip is often short. If the heating source is on the sides of a channel, there will be a gradient into the middle of the channel. With a high flow rate or large channel volume, the heating might be too slow to ever reach the medium in the middle before it flows past the heated area. Small volumes with high surface area are therefore preferred in a flow system to ensure sufficient heating.

This applies not only to the fluid volume but also to the chip material; a bulky material with poor thermal conductivity will retain the heat, while it will quickly dissipate in a thin chip with good thermal conductivity [29]. With fast heat dissipation, quick regulation can be obtained, and cooling can be achieved by simply turning off the heat. However, a lot of energy has to be constantly added to maintain a high temperature, which increases energy consumption. With high thermal inertia, less energy is needed to maintain an elevated temperature, but much is required for the initial heating. Additional cooling systems might also be needed, which increases both the size of the entire setup and its energy consumption. The optimal thermal conductivity is therefore dependent on the experiment variables to achieve the best combination of stability and energy efficiency.

Temperature alteration does not only apply to the time scale but also to the positions in the flow system. Different temperatures might be needed along the flow path to induce chemical interactions at specific locations or to spare sensitive materials. For these scenarios, low volumes reduce the need for extra cooling components to make the temperature reach ambient between these spots.

## 2.2 Thermal regulation of viscosity

To understand and regulate the flow rate or pressure drop along a flow path the Hagen-Poiseuille equation, Equation 1, is a good starting point. It describes the relation between pressure,  $P$ , and flow rate,  $Q$ , for an incompressible fluid moving in a flow pipe with laminar flow.

$$P = \frac{8Q\eta L}{\pi\left(\frac{D_h}{2}\right)^4} \quad (1)$$

To regulate a flow system, the most common way is to mechanically change the geometry, *i.e.*, the length,  $L$ , or the hydraulic diameter,  $D_h$ , in the equation. A longer or narrower flow path means that a higher pressure is needed for a certain flow rate. To add back pressure, a capillary is commonly used that is gradually shortened until the desired pressure is reached, hence adjusting the  $L$  in the equation. While in a manual pressure regulator, a knob is turned to throttle the flow, decreasing  $D_h$ . An alternative way to regulate flow without using mechanical moving parts is by changing the viscosity, the  $\eta$  in the equation.

The value of viscosity describes how thick or thin, or “easily flowing” a fluid is. For instance, water has a lower viscosity than syrup. In a liquid, the viscosity is caused by the friction between fluidic layers. Looking at a cross-section of a channel, the velocity of the fluid differs. This is easiest seen in the parabolic flow where the velocity is zero (in the absence of wall slip) closest to the wall and gradually increases to the middle of the channel. Dividing the fluid into layers, the layer closer to the middle will move faster than the layer closer to the wall, and thus give rise to friction between the layers. Viscosity is an important consideration when working with high-pressure microfluidics because of its high impact on the pressure drop. For example, ethanol could serve as a less toxic mobile phase than acetonitrile or methanol but its higher viscosity limits its area of use. Additionally, in gradient chromatography, the viscosity has to be considered as the mixture of alcohol and water has a higher viscosity than any of the pure liquids. However, viscosity can also be utilized for control in microfluidics.

The viscosity of a fluid depends on pressure and temperature, where the pressure affects both liquids and gasses in a similar way. With higher pressure, the molecules lay closer to each other which increases the frictional forces and hence the viscosity. This is most evident in gasses which have higher compressibility and thereby greater change in intermolecular distance with pressure. The temperature affects the kinetic energy of molecules which has opposite impact on the viscosity of gasses versus liquids. For gasses, molecules move freely with little molecular interaction. When temperature rises the molecules move faster which results in more collisions creating interactions that increase viscosity. Liquids, on the contrary, already have interaction between molecules and attractive forces must be overcome for one molecule to move away from another. By increasing temperature, the molecules gain more kinetic energy which makes it easier for them to move away from each other, and hence, viscosity decreases.

So how do we use viscosity for flow regulation? By connecting a microfluidic chip with a narrow channel in a flow system, we introduce a flow restriction. The impact this has on the flow depends on the position and restriction of the chip. If the chip is placed directly after a pump with a given pressure, it will reduce the flow rate and pressure for the components downstream of the chip. If the chip instead is placed at the end of the flow system it will contribute to withholding an elevated pressure throughout the whole system.

The restriction of the chip is regulated firstly by choosing the length and depth of the fluidic channel. Secondly, heaters are integrated to enable temperature regulation during experiments. By increasing the temperature of a liquid in the chip, the viscosity decreases, and hence does the pressure drop. The changes in viscosity with respect to temperature for commonly used solvents can be observed in Figure 2.1.

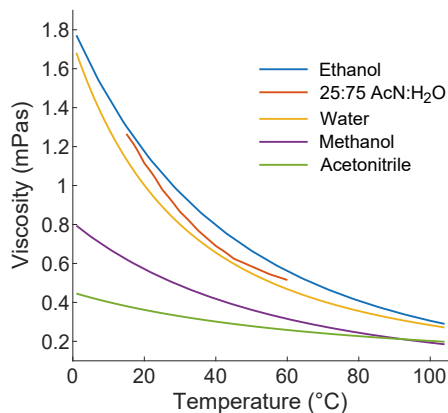


Figure 2.1. Viscosities at different temperatures for the commonly used solvents ethanol, water, methanol and acetonitrile [46], as well as for a mixture of water and acetonitrile [47]. Figure reprinted from **Paper V** with open access license CC BY 4.0.

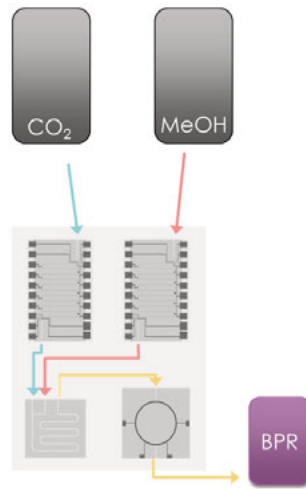
### 2.2.1 Regulation with CO<sub>2</sub>

Using thermal regulation of compressible fluids such as gasses and supercritical fluids is a more complex matter. As mentioned, higher temperatures result in higher viscosity for gasses. This can be used to regulate the flow rate by using a similar regulation system as for liquid but with an opposite feedback reaction - if the flow rate is too low, the heat should be reduced instead of increased in order to raise the flow rate. However, although the flow rate quickly changes, it takes longer time to change the pressure. For highly compressible fluids, an increased flow rate has to be maintained for a very long time before the pressure increases accordingly. This made pressure regulation of gasses too slow to incorporate in any of the presented systems.

The use of compressible fluids is nevertheless still interesting. The compressibility of N<sub>2</sub> and CO<sub>2</sub> was utilized in **Paper V** to store energy, while CO<sub>2</sub> was used with heat actuation for flow rate control in **Paper I**. When heating pressurized CO<sub>2</sub>, it is not just the viscosity that is affected but also the density. Above 63 bar, CO<sub>2</sub> is a liquid and above 74 bar and at 31 °C, it becomes a supercritical fluid. When reaching the supercritical state, a rising temperature makes the density decrease more than the viscosity decreases. In the Hagen-Poiseuille equation, Equation 1, the density is included in the volumetric flow rate as volume equals mass divided by density. A change in density or viscosity will thereby have an opposite effect on the pressure. When density-changes dominate, the pressure will rise with temperature and hence the same regulation previously used for liquids can be used. It is therefore important to decide which temperature region to operate in when working with CO<sub>2</sub> above its critical pressure.

The combination of regulating both methanol and CO<sub>2</sub> previous to mixing was also investigated in **Paper I**. Here, the fluids were regulated with one chip

each, which were coupled in parallel, Figure 2.2. Heating the chips affected the mixtures in different ways. By increasing the temperature and flow rate of methanol, the pressure at the mixing point increased, which reduced the CO<sub>2</sub> flow. This resulted in a higher methanol portion in the mixture but kept the overall flow rate stable. However, heating the CO<sub>2</sub> caused a different situation. By heating the CO<sub>2</sub> above 31 °C, the density decreases, which increases the volume flow rate from the chip but not the amount of CO<sub>2</sub> molecules in the resulting mixture. This left the pressure at the mixing unaffected, and therefore the methanol flow rate was maintained, although with less dense CO<sub>2</sub>, resulting in a decrease in the overall flow rate of the mixture.



*Figure 2.2.* Sketch of the setup used in **Paper I**. Four microfluidic chips are connected to a PCB. The two heat-actuating chips are coupled to a CO<sub>2</sub> and methanol pump. The fluids meet in a mixing chip before entering a permittivity-sensor chip connected to a back pressure regulator.

### 2.2.2 PID regulation

The flow control in the papers are all based on proportional-integral-derivative (PID) regulation, which is implemented in a Matlab script that controls the experiments. Although thermal effects are used to influence the flow, the temperature is not directly included in the regulation. The controlled parameters are composition (**Paper I**), pressure (**Paper II, III, and V**) and flow rate (**Paper III**), and the desired level of the chosen parameter is defined as the set value. The output used to reach this set value is the voltage which, in turn, affects the temperature. In PID regulation the set value is compared to a measured signal to calculate how big the error is, also known as feedback control [48].



PID regulation calculates the output value,  $V$ , as the sum of three parts seen in Equation 2. The proportional part, P-part, determines the magnitude to be applied in proportion to the size of the error,  $e$ . The contribution is calculated by multiplying the current error with a constant  $K$ . Using only the P-part results in stagnation at a plateau where both voltage and error are constant. To shift the plateau closer to the desired set value, an integrating part, I-part, is added. The integration accumulates the error building up to a change with time,  $t$ . The influence of the I-part is determined by the integral time constant  $T_i$  and is added to the equation as  $1/T_i$  multiplied by the integral of the error.

$$V(t) = K \times e(t) + \frac{1}{T_i} \times \int_0^t e(t) dt + T_d \times \frac{de}{dt} \quad (2)$$

If the system has a slow response, *i.e.*, high inertia,  $T_i$  should be large to avoid overshooting. Overshooting occurs when the regulation changes quicker than the system responds. If the pressure downstream of a chip is regulated and is currently too high, the voltage will decrease to compensate for this. When the pressure then decreases, so does the error and hence, the P-part. However, since the I-part adds up the errors, it keeps growing although the error decreases, until the error shifts from negative to positive, or vice versa. This can result in a high negative value, which will not have any direct effect on the system since the voltage cannot be negative. However, if the pressure gets too low and the voltage needs to increase again, the response time will be extended as the negative value of the I-part has to grow above zero before adding to the output.

To avoid overshooting, firstly, the integration time has to be set in proportion to the reaction time of the system and secondly, an anti-windup function can be used. An anti-windup function is made by defining and limiting the I-part to set maxima. The maximum value was set either as the maximum output from the power supply or as the voltage corresponding to power around 1 W to avoid overheating the chip. If the voltage exceeded the maximum it was redirected to this value. As the voltage cannot be negative, a minimum value was set zero and any value below this was redirected to zero.

Another approach to reduce overshooting is by the derivation part, D-part, which uses the derivation of the error to predict the future and dampen fluctuations. The parameter  $T_d$  is multiplied with the derivation and added to the equation. This was not always needed and therefore removed in the later papers as the regulator-induced fluctuations were neglectable.

The value of the regulation parameters,  $K$ ,  $T_i$ , and  $T_d$ , was set by manual tuning until satisfactory results were obtained. In an optimal regulation, the set value should be reached quickly without any overshoot and with minimum fluctuations. The optimal parameters differ, however, between experiments as they depend on the system's response to a unit change, and to the system inertia. This made it difficult to maintain optimal values for multiple experiments. Characteristics that affect the optimal parameters include:

*Heater resistance* The heating power is the voltage multiplied by the resistance of the heaters. The resistance varies with design and electrode material but also with position on the wafer because of the thickness gradient resulting from the sputtering process.

*Fluid medium* The viscosity's dependence on temperature differs between media. For instance, the viscosity of water changes more per degree Celsius than methanol.

*Flow rate* Higher flow rate means more cooling fluid running by and less time for the fluid to be heated in the channel. Hence, the highest temperatures might not be reached.

*Channel size* A longer channel offers more time to heat the fluid. A deep and wide channel increases the time in the chip but also increases the distances for the heat transport.

*Compressibility and volume* The flow rate changes quickly with temperature but pressure changes act slower since it includes mass compression. The amount of mass that has to be added or removed to reach a certain pressure level depends on the fluid's compressibility and the volume of the system.

*Sampling time* Short sampling times are preferable since changes or overshooting can quickly be detected and compensated for. In our case, Matlab's saving and plotting of data slowed down over time as more data was collected. A pause was therefore included in the script to prevent changes in the sample time during experiments.

*Dead time* The time it takes for a response to occur after the voltage has been changed is called dead time which slows down regulation. In **Paper I**, the fluids from the heating chips had to pass through a mixing chip before they reached the sensor chip which resulted in a delayed response.

*External cooling* When the heating is counteracted by cooling, more energy is required both to reach higher temperatures and to maintain them. Here, the cooling was run either with constant temperature, constant power, or altering power.

In the presented experiments, various cooling techniques were explored. In **Paper I-III**, the cooling was made by placing the chip on a water cooling board. The water was initially cooled to a few degrees and circulated to a water bath with Lauda heaters increasing the temperature to typically 8 °C, before it was sent to the cooling board. This setup provided a constant cooling temperature without any feedback. The large water bath offered high thermal inertia and quickly diluted the slightly heated water returning from the cool-

ing board. However, this solution was spacious and not suitable for a portable system. To first cool the water followed by heating to ensure a stable temperature also increases the power consumption. Therefore, another approach was explored in later papers.

In **Paper IV-V**, the cooling system was interchanged to a Peltier element and a fan. A Peltier element uses direct current to generate one warm and one cool side. The chip was placed upon the cool side, and the setup was placed in front of a small fan to remove excess heat, both from the warm side of the Peltier and from the chip heating. The Peltier was connected to a power supply that either used a constant power or was implemented in the Matlab script for alterations during the experiments.

## 2.3 Compressibility

Compressibility is a measure of how much a medium's density can be changed by pressure. Compressibility is most often used when talking about gasses where the molecules are sparse and there is a lot of space that can be compressed to fit more molecules. This is evident when, *e.g.*, pumping a tire. If you have a bike with a flat tire, it will be seen in the shape, it is flat. When you start pumping it, it returns to its rounded shape and looks full. However, if you press it with your hands, it will easily deform, the pressure is too low; there is still not enough air. You can thereafter keep pumping without the shape of the tire changing significantly, you are now compressing the air inside and increasing the pressure. To know if there is enough air, you can either look at a pressure gauge on the pump or press the tire with your hands to see how easily it deforms.

Liquids are generally said to be incompressible; the molecules lay closer to each other with less free space, which makes them harder to compress. At low pressures, compressibility is negligible; at atmospheric pressure, it is difficult to add more water to an already full bottle of water. However, as the pressure increases, this estimate is not as accurate and the compressibility is no longer negligible. This is especially evident in microfluidics, where even small volumes can make a big difference. However, this is often not taken into account, in this work the Hagen-Poiseuille equation is used which assumes incompressible fluids, although more elaborate equations exist [49].

In addition to pressure, compressibility also depends on temperature. When the temperature increases, the molecules move faster and collide more, making compression more difficult. However, for isothermal conditions, the coefficient of compressibility,  $\beta$ , is defined according to Equation 3, where  $V$  is the volume and  $P$  is the pressure.

$$\beta = -\frac{1}{V} \frac{\delta V}{\delta P} \quad (3)$$

To put this into context, one can look at water and methanol, which have a compressibility of 46 ppm/bar and 120 ppm/bar [50], respectively. If the volume,  $V$ , is 100 ml and we use pressure to add 1 ml more, the pressure will increase from 1 bar to 217 bar for water, and to 83 bar for methanol.

So what does this small compression matter in practice? The most well-known effect is in the prolonged stabilization time. For example, what happens if the flow rate increases from 100  $\mu\text{l}/\text{min}$  to 150  $\mu\text{l}/\text{min}$  in a 50 ml flow system with an initial pressure of 50 bar? As pressure is proportional to the flow rate, the new pressure should be 75 bar. But because of compressibility, a certain amount of fluid has to be added before that pressure can be reached. For water, the density changes from  $\rho(50 \text{ bar})=1.00057 \text{ g}/\text{cm}^3$  to  $\rho(75 \text{ bar})=1.0017 \text{ g}/\text{cm}^3$ , and with a 50 ml volume this corresponds to approximately 60  $\mu\text{l}$ . The added volume to the system can be calculated as the difference between the inlet flow rate and the outlet flow rate, starting at  $150-100=50 \mu\text{l}/\text{min}$ . When the pressure increases, so does the outlet flow rate which decreases this difference, resulting in an asymptotic behavior seen in Figure 2.3. Similarly, when decreasing the flow rate, the extra, compressed liquid has to flow out for the pressure to decrease accordingly. While this extra stabilization time can be annoying, it also creates an opportunity as a source of flow capacitance.

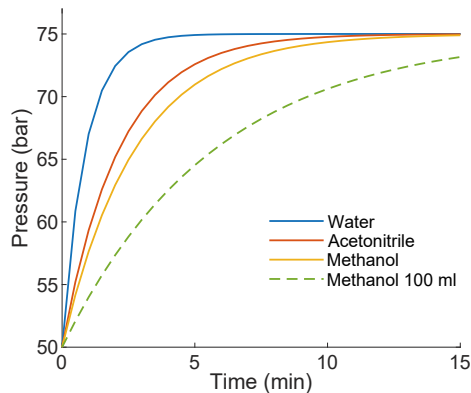


Figure 2.3. Theoretical pressure increase over time as a results of the flow rate changing from 100 to 150  $\mu\text{l}/\text{min}$  at  $t=0$  min. The calculations were performed using compressibility values of 46, 96, 120 ppm/bar for water, acetonitrile, and methanol [50], respectively. The upstream volume was 50 ml in all calculations except for the dashed line with 100 ml methanol.

## 2.4 Capacitance

Capacitance is often used in electronics where it describes a device's ability to store electric charge. This can be translated to the fluidic field as the ability to store volume or mass. When a pump in a flow system is turned off, there

is often a continued but decreasing outflow for a short period after, which is a result of stored fluid, called flow capacitance [51, 52]. Whether the flow capacitance is desired or not, it is important to know about and understand how it affects a system. The capacitance generally has two origins, compression of fluid at high pressures,  $P$ , or from varying volume,  $V$ , of the system. The capacitance,  $C$ , can be calculated with Equation 4.

$$C = \frac{\delta V}{\delta P} = -\beta V \quad (4)$$

In similarity to electronics, this capacitance could be used to store energy and dampen oscillations. A varying volume can be achieved through the integration of elastic membranes that undergo stretching during filling and subsequent retraction [53]. Elastic membranes are commonly utilized for lower pressures where *e.g.*, polydimethylsiloxane (PDMS) could be used [54]. These elastic membranes are, however, passive systems that cannot compensate for drift nor offer any further control possibilities. To make an active stabilizer, the pressure could instead be manipulated, which creates capacitance through the fluid's compressibility. When using compressible liquids, capacitance is not as simple as measure the fluid's ability to store volume. Since the volume of the system is not changed, the stored molecules rather add to the mass than the volume. However, when the pressure decreases together with the density, this extra mass results in increased volume.

Starting this work, the discussion about compressibility was centered on the use of CO<sub>2</sub> and other gasses. To minimize the stabilization time, the internal volume of components and connecting tubes were considered. Since then, two important realizations have been made:

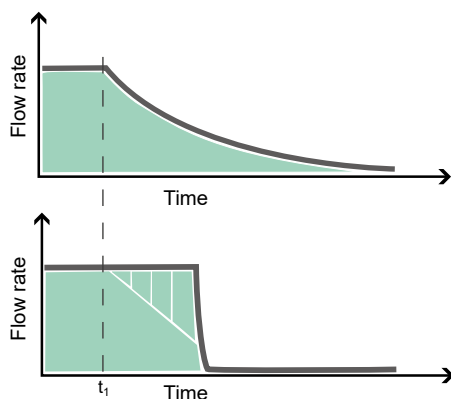
- Liquids are compressible.
- Pump volume matters.

The compressibility of liquids has already been discussed, but what about pump volume? The volume of a system is often counted as the volume in each component plus the connecting tubes, and to minimize the volume, tubes are shortened, and sensors and detectors are made smaller to reduce dead volumes. These volumes are important in the sense of *e.g.*, diffusion of samples after injection. But when discussing compression, the by far largest volume is usually found in the pump. If the pump is operated in constant flow rate mode, the pressure profile will differ depending on the volume in the system, as seen in Figure 2.3 (50 versus 100 ml methanol). Some pumps, like HPLC pumps, use liquid from a large reservoir but only pressurize about 1 ml at a time, which makes it easier to get reproducible results. Other pumps pressurize the whole volume at once, like syringe pumps. In the latter case it matters both how big the syringe/container is and how well-filled it is. A change in flow behavior can thereby occur over time depending on if the pump was recently filled or is almost running out, although the same instrument and settings are used.

As the compressibility is not generally thought of, different results can be obtained in different labs although all given parameters are the same, except the pump volume. This can become a problem when quick pressure changes or reproducible gradients are desired. In **Paper III**, a back pressure regulator (BPR) was developed using thermal regulation of viscosity. Although a BPR is mainly used to withhold a certain pressure, experiments were also made showing the time it took to reach different pressures depending on the pump volume.

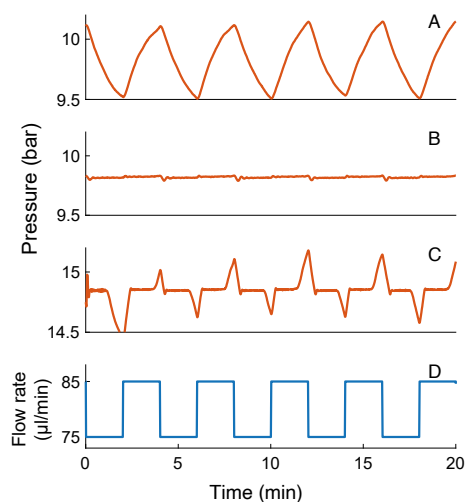
Now that this phenomenon had been further understood, we wanted to make use of it, which resulted in a microfluidic pressure and flow stabilizer, **Paper IV**. A stabilizer should keep the output flow constant although the incoming flow alters. This can be seen as the work of a buffer; the ability to keep conditions stable when affected by an external change. Hence, we introduced buffer capacitance. The buffer stabilizes the flow by storing liquid when the incoming rate is too high and releasing extra liquid to compensate when the incoming rate is too low. The size of the buffer capacitance is proportional to the compressibility and pressure change of the fluid, and the total volume upstream of the stabilizing chip including the pump.

The concept of buffer capacitance is visualized in Figure 2.4. As mentioned earlier: if the flow from the pump stops, the flow exiting the system gradually decreases, upper graph, which implies that there is stored fluid left in the system. By regulating the resistance of the system, in this case, through the chip, the stored fluid can be relocated, lower graph. This gives a longer time of correct and useful flow rate rather than a slowly decreasing one. The extra fluid can, as seen, only maintain the flow rate for a certain time period, but is used to dampen an altering flow, which often occurs in today's pumps.



*Figure 2.4.* A conceptual sketch of capacitance showing the eluting flow rate when the pump stops at  $t_1$ . In the upper graph, the resulting flow rate gradually decreases because of the fluid capacitance of the pressurized system. In the lower graph, this extra fluid is relocated to keep the initial flow rate for longer.

For demonstration, an intentionally altering flow rate was set from the pump, changing between 75 and 85  $\mu\text{l}/\text{min}$  every other minute, as seen in Figure 2.5D. The three upper graphs show how this affects the pressure in different situations. In A, the chip is not active, and the pressure changes with the flow rate without reaching stable plateaus. In the middle graphs, the stabilizing chip is active which reduces these pressure fluctuations, however, the pump volume differs which affects the buffer capacitance. In B, the pump is filled with 9 ml resulting in a sufficient capacitance to stabilize the current flow conditions. In C, the pump was filled with 4 ml and the capacitance was only enough for half of the time.



*Figure 2.5.* Comparison of the resulting pressure from a varying flow rate when the stabilizer is active or passive. A) The stabilizer chip is passive and the pressure fluctuates with the flow rate. B) The stabilizer chip is active and dampens the fluctuation. C) The stabilizer chip is active, but the low pump volume results in a too-low buffer capacitance to maintain the pressure the whole cycle. D) The set flow rate from the pump. *Figure made with data from Paper III.*

Gasses and supercritical fluids have magnitudes higher compressibility than liquids generating a higher fluidic capacitance. This means that they can store much energy, which provides opportunities in the making of portable devices. In **Paper V**, pressurized gas is used to replace the pump in an HPLC system. Here, the different characteristics of gasses and liquids are utilized where the gas stands for a large capacitance while the pressure regulation is performed with the liquid. By using a double-sided reactor with a separating piston, the gas and liquid phases can be separated. When the system is running, the pressurized gas expands and presses the piston that, in turn, presses the liquid creating a flow. As the gas expands, the pressure decreases which has to be compensated for to maintain a stable flow. This is made with a heat-actuated chip that starts in a cold state with high restriction. As the gas pressure de-

creases, the temperature increases to lower the restriction through the chip and thus withhold a constant pressure downstream, which is shown in Figure 4.1.



## 3. Chip Design and Fabrication

### 3.1 Design

There are numerous things to consider when designing and fabricating microfluidic chips, especially given the demands of withstanding high pressures. In this work, much effort was placed into developing the design of the chips and the fabrication processes. Both the robustness and the efficiency of the chips had to be considered, and trade-offs had to be made. Small changes, like the width of a channel, electrode material, or placement, often had consequences for further fabrication, and processes had to be refined, reordered, or exchanged. An example of a design for the BPR chip from **Paper II** is shown in Figure 1.1.

Starting with the microfluidic channel, a certain, but different, restriction was desired for the chips used in the papers. To reach this restriction, two parameters were considered: the length of the channel and its depth. The depth, or diameter, affects the robustness of the chips in two opposing ways. A smaller diameter gives a higher pressure tolerance because of the force-to-pressure ratio. On the other hand, narrower channels increase the risk of clogging, which can render the chips useless. Shallower channels also put higher demands on the accuracy of the etching; with a depth of around 10  $\mu\text{m}$ , an error of 1  $\mu\text{m}$  greatly changes the pressure drop through the channel.

To instead making the channel longer will increase both the channel volume and the chip size. To minimize the chip size, the channels are usually made in a meander shape. It is then important that there is enough space between the turns to create a sufficient bonding area for the glass. When working with low-viscosity mediums, like gasses, a longer channel is desired to reach the resistance without needing a too narrow channel diameter. In **Paper I**, a 7.8 cm long meander was used for the handling of  $\text{CO}_2$ , while in later work with liquids, the channel was only about 1 cm long. With the shorter channel, the chips could be made smaller and the outer dimensions were changed from 15 $\times$ 12 mm to 8 $\times$ 6 mm. This resulted in 130 chips per wafer, instead of previously 34 chips.

Deep channels are beneficial from a clogging point of view, but they also possess the highest risk of leakage and chip breaking. Usually, the largest structures with the highest risk of leaking are found at the chip's inlet and outlet [55]. When using isotropic wet etching on one of the wafers, the channel's width will be more than three times greater than the diameter of the connecting capillary. A technique to minimize the width is to etch half the depth on each

of two bonded wafers, requiring the width to be just 1.5 times larger, **Paper I**. However, that instead increases the demands on alignment. If the wafers are not properly aligned, it will not be possible to insert any capillaries at all.

Although several different structures and depths can be made in one wafer, every step in the fabrication comes with challenges and risk of lowering the yield. Contamination is one of these issues that can arise, not only from dust in the area but also from burned resist, metals, and contamination from chemical baths. Further cleaning procedures may be necessary to remove this, although that could imply harsh treatments that compromise small structures or metal films.

The integration of metal films poses several design questions. In this thesis, different alternatives have been explored: placing the films inside the channel in direct contact with the fluid, placing the films beside the channel in the glass at various distances, and using different designs and geometries of the electrodes.

In **Paper I**, both the sensors and heaters were placed inside the channel, while in the later papers, they were placed beside the channel. Having the electrodes inside the channel increased the risk of leakage through the electrodes as they created a path from the channel to the edge of the chip. This made the thickness of the electrodes crucial. In the fabrication, the metal is sputtered into trenches; if it gets too thin, the glass will not be able to completely close off the gap during bonding. If the electrodes instead are too thick and rise above the trenches, the glass cannot bond in the areas next to the metal. The sputtering process also resulted in a gradient thickness over the wafer, which imposes various pressure tolerances depending on the chip's placement on the wafer.

For increased pressure resistance, a design was made with the electrodes next to the channel instead. With a large-enough gap between the electrodes and channel, the fusion bonding makes the glass-glass interface form a bond that can be as strong as the bulk material [56]. In **Paper II**, electrodes were positioned 10  $\mu\text{m}$  away from the fluidic channel, which led to leakage still occurring through the electrodes at higher pressures of around 150 bar. When the electrodes were moved 65  $\mu\text{m}$  away from the channel, **Paper IV**, the chips could withstand a pressure of 250 bar. Another advantage of placing the metal outside the channel is that it will not risk any reaction with the fluid and metal flakes will not de-adhere and clog the channel.

The question is then how much this changed position affected the functionality of the electrodes. The quickest heating is, of course, obtained when the electrodes are placed in direct contact with the fluid in the channel. However, compared to macro world flows or external heating, tens of micrometers are practically negligible distances. A temperature response was seen immediately when starting the heater in **Paper II** where the sample time was 1.5 s. Having the temperature sensor in, or next to, the channel also did not give any measurable dead time. However, the sensors next to the channel did not

measure the highest or lowest absolute temperatures of the fluid, which is troublesome if the medium is heat-sensitive.

Connecting of the heaters also differed between the chips. In **Paper I**, 11 heaters were connected in parallel across the meander-shaped channel. In the later papers, only two heaters were connected in parallel, one on each side of the channel. In the case of uneven metal sputtering, many in parallel were preferred as the chip still worked even if one heater broke. This was especially relevant in the first paper where an older sputter was used, which resulted in a higher portion of broken electrodes and electrodes that got very thin and hence had increased resistance. Along with the temperature sensors, there were ten electrical connection pads on each side of the chip to be soldered to a PCB. In the later design, there were only six pads, which were also made smaller to increase the space between them. This adjustment made soldering easier and reduced the risk of short circuits between pads.

In **Paper III**, the width of the heaters was also updated, from a uniform width to a tapered design. Here, the heaters were narrow near the inlet and gradually widened towards the middle of the channel. Along the last half of the channel, the width stayed the same. This improved the heat distribution along the channel as more cooling is acting at the inlet where the room temperature fluid enters. By making the heater smaller here, the resistance increases, and a higher portion of the Joule heating acts there. This meant that higher power could be used to get a larger flow change without getting a higher maximum temperature. If the flow rate was too low, however, it got too warm at the inlet which occasionally led to corrosion in the glass. The optimal geometry of the heaters depends on the channel size, the flow rate, and if external cooling is used.

Unlike pressure and permittivity, the temperature could be measured both in direct contact with the fluid and in the chip next to it. The advantages of placing them on the side are that there is less risk of leakage through the electrodes and no risk of chemical interaction between the fluid and the metal. However, a more exact value is obtained when measuring in direct contact with the fluid.

## 3.2 Chip material

The bulk material of the chips consisted of borosilicate glass which is a widely used material in high-pressure microfluidics. Other commonly used materials are silicon and PDMS, where PDMS is mostly used in the low pressure regime. Silicon is used for high-pressure applications, often together with glass. Both materials are chemically inert, stable at elevated temperatures, and have well-known fabrication methods [57]. Another pressure-tolerant material found in microfluidics is stainless steel, it has however, limitations in terms of possible fabrication processes.

A significant property of glass is that it is optically transparent, which is beneficial both for detection and fabrication. Optically-based detection methods include absorbance spectroscopy, Raman, fluorescence, and chemiluminescence [58], which can all be used with glass chips. The see-through properties are also used in fabrication, for example, when inserting capillaries and when adding glue that has to ensure a tight seal without clogging the channel. The yield is rarely 100% in fabrication, and being able to sort out malfunctioning chips before assembling saves time. By optical inspection, broken electrodes can be found, as well as interference patterns arising from insufficiently bonded glass.

Additionally, glass is electrically insulating, which reduces the risk of leakage current and shortcuts between electrodes. Glass also has low thermal conductivity, which gives it high thermal inertia, which keeps the heat longer than *e.g.*, silicon. The amorphous structure of glass results in isotropic etching that gives rounded channels. This means that a channel will always be at least two times wider than it is deep, which limits the design possibilities. Another disadvantage of glass is that it can corrode at high temperatures in acidic environments.

The silicon wafers commonly used are thinner than glass wafers (0.5 mm versus 1.1 mm) and have high thermal conductivity, which results in quick heat distribution. This enables fast regulation but requires high power consumption to maintain an elevated temperature. Silicon is opaque but is mostly used in combination with glass, with the advantages of combining, for example, the structures easily made in silicon with the optical transparency of glass. Both glass and silicon are brittle in comparison to elastomers [59], which makes moving membranes fragile. Regarding fabrication, silicon can be both wet-etched with geometries that depend on the crystal orientation and dry-etched to get vertical walls. Instead of sputtering metal, electrical connections can be made directly into the silicon by doping the wafer.

### 3.3 Electrode material

In the glass chips, metal thin films were used to make electrically conductive electrodes used for sensing and heating. In **Paper I** and **Paper III-V**, the electrodes were made of platinum, while in **Paper II**, they were made of gold. The biggest difference between these materials lies in their electric resistivity, where platinum has  $1.06 \times 10^{-7}$  and gold has  $2.44 \times 10^{-8} \Omega\text{m}$  [60]. In the heating chips, a power of approximately 1 W was desired, and the preferred resistance of the electrodes depends on the maximum current and voltage of the power source. For platinum, approximately twice the voltage but half the current were needed to get the same power as for gold. For powers exceeding 1 W, the maximum voltage of 35 V from the power supply becomes the limiting factor, and hence more freedom is obtained using gold. With batteries,

the voltage can be increased by serial coupling, while the maximum current can be increased by coupling them in parallel. However, a too high current could cause malfunctioning and should be considered depending on the type of battery. The resistance of the electrodes is also adjusted by the thickness, as resistance is inversely proportional to the cross-section area. Here, the thickness was around 100-200 nm, with some variation across the wafer because of the sputtering gradient. A very thin film increases the risk of breakage, while a thicker film increases material consumption.

In addition to sufficient conductivity, the materials also need to withstand the remaining fabrication without being compromised. After the metal sputtering, there are several processing steps to be performed before the chips are finished. In all papers except **Paper II**, platinum was used because of its tolerance to chemicals and high temperatures [61]. Before bonding, the wafers are immersed in 80 °C nitric acid for 15 minutes. Gold could withstand these conditions initially, but if the time was overridden, the gold started to fall off. Likewise, the phosphoric-acetic-nitric acid (PAN) solution occasionally caused fall-outs. Another issue was that it easily self-adhered; when the resist covered with gold was removed, flakes of gold could redeposit on the gold electrodes and obstruct the bonding.

Except for the main material, there is also a need for an adhesive layer to make the metal stick to the glass. Here, tantalum was used instead of the commonly used chromium and titanium. The latter two did not withstand the remaining process steps, as they either detached in the nitric acid or diffused into the gold at high temperatures in the bonding furnace.

### 3.4 Fabrication

Here, a general fabrication of the glass chips will be described, containing commonly used processes. The exact fabrication parameters vary between the chips and can be found in respective paper.

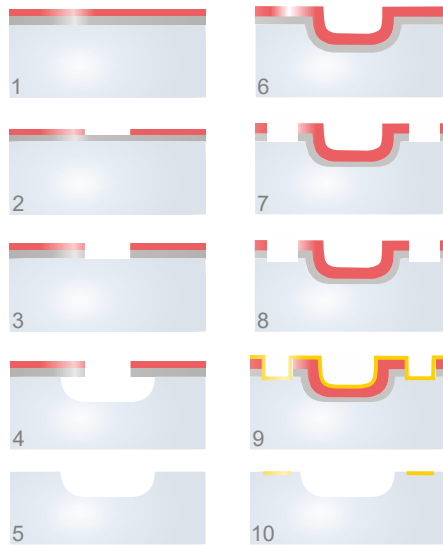
The glass wafers were first cleaned in a PAN solution followed by oxygen plasma to remove organic compounds. The designs were made in AutoCAD and transferred to the wafers via masks and photolithography, a step-by-step visualization is shown in Figure 3.1. Several masks were needed for each design, and all chips had different structures on the top and bottom wafers. To protect the areas that should not be etched, the wafers were sputtered with molybdenum and primed with hexamethyldisilazane (HMDS) for a hydrophobic surface with better adhesion to the resist. A positive photoresist was spun on the wafers, resulting in an approximately 1  $\mu\text{m}$  thick layer, Figure 3.1, step 1. The wafers were placed in a mask-aligner and exposed to UV light through a patterned mask. The UV light breaks the bonds in the resist, and the exposed resist dissolves in a developer. The pattern is thereby transferred to the resist layer, step 2. The exposed molybdenum was removed

by etching in the PAN solution until the glass was seen, step 3. To etch the main channel, the inlet, and the outlet, concentrated hydrogen fluoride (HF) was used, with an etch rate of around 6-7  $\mu\text{m}/\text{min}$ , step 4. When the glass etch was done, the resist was stripped with acetone, and the PAN solution was used to remove the rest of the molybdenum, step 5.

When the wafers were clean, the lithography could be repeated to make structures of different depths, step 6-7. In the process of making electrodes, the molybdenum under the resist served as a lift-off layer. If the wafer contained micrometer-deep structures, the resist-applying method had to be changed. Instead of spinning, the resist was diluted and sprayed onto the wafer. To ensure uniformity and coverage of channel walls, a thicker layer of 12  $\mu\text{m}$  was initially used, which worsened the possible resolution. To improve resolution, the resist was further diluted to get a 4  $\mu\text{m}$  layer and to keep the coverage of the channel walls, the wafer holder was preheated to 80 °C. A shallow channel was etched with buffered HF, step 8, before an adhesion layer and a conductive layer of metal were sputtered onto the wafer, step 9. For lift-off, the wafer was immersed first in acetone and then in the PAN solution, step 10.

When all channels and electrodes were made, the wafers were ready to be bonded. This was done with fusion bonding where either a structured wafer was bonded to an unprocessed wafer or two structured wafers were bonded to each other. Before bonding, the wafers were cleaned and activated in 80 °C nitric acid. They were then aligned, squeezed together, and placed in a furnace reaching 630 °C. In **Paper I**, the alignment was done manually, while in later papers, it was done using the bond aligner from the lithography, which enables very precise alignment without rotational errors.

The bonded wafers were diced into separate chips. For the heat-actuation chips, the electrode pads were exposed by dicing halfway through the wafers before the chips were completely separated.



*Figure 3.1.* Schematics of the wafer fabrication steps. A part of a glass wafer is shown in light blue, with molybdenum in gray and photoresist in red, the yellow parts in the last two steps symbolize the thin film metal.

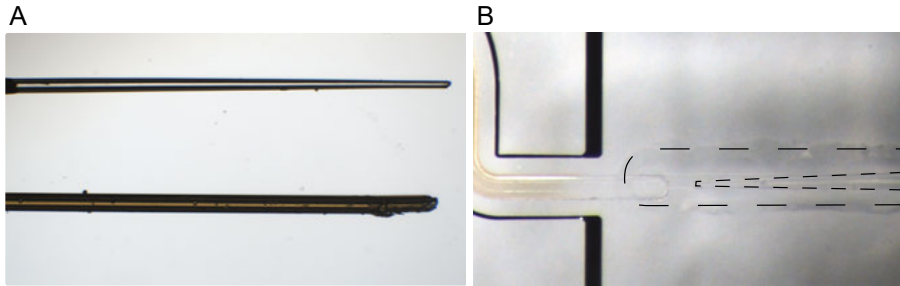
### 3.5 Assembly

To make a functioning system, the chips needed to have connections both to the electronic and fluidic interfaces. Customized PCBs were designed for the different chips and used for electrical connections. The open electrode pads on the heat-actuating chips were glued to the PCB with conductive glue while for the permittivity sensors, connections were made by inserting and gluing copper foils. Connectors and screw terminals were soldered to the PCB for connections with a power supply and a data acquisition unit (DAQ).

Glass capillaries were then manually inserted into the inlet and outlet of the chip and glued with epoxy. The viscosity of the glue was of high importance as it had to creep into the channel to completely seal the volume around the capillary for high-pressure tolerance and minimum dead volume. However, if it crept too far and reached the tip of the capillary, it quickly clogged it, leaving the chip useless. Therefore, tryouts were made with high- and low-viscosity epoxies and mixtures of them both. The curing of the epoxy also affected the creep, temperatures of 70 and 110 °C in the oven made curing quicker, but the heat temporarily lowered the viscosity and made it go further into the chip. To avoid clogging, some chips were therefore left to cure at room temperature overnight.

As mentioned in *3.1 Design*, the inlet and outlet are often the largest structures with the highest risk of leakage. In **Paper I**, these structures were etched on both the top and bottom wafers. In later papers, another method was inves-

tigated to keep the size down, where instead the capillaries were made smaller. Using a thinner capillary would increase the fluidic resistance significantly and removing the coating makes it too brittle to handle. Therefore, only the tip of the capillary was altered. By heating and pulling the capillaries, the polymer coating was burned off and a pointy end was obtained, Figure 3.2. This made it possible to have smaller inlets, while most of the capillary kept its properties.



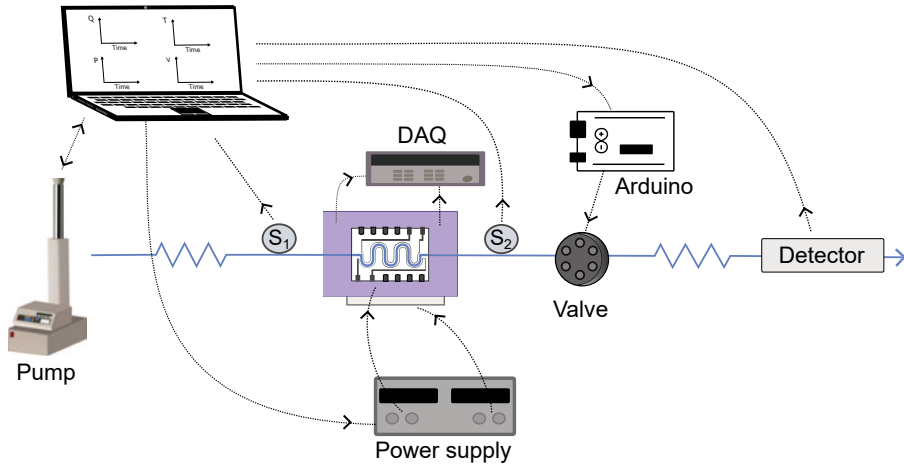
*Figure 3.2.* Glass capillaries used for fluidic connections in the chip. A) The tip of two capillaries used in the assembly. The upper one has been thermally pulled to remove the polyamide coating and to get a pointy end. The lower one is unaltered and has a constant diameter and consistent coating. B) A thermally pulled capillary inserted in a chip. The edges of the inlet channel and the capillary have been marked with dashed lines to improve visibility.

Finally, tubes of polyether ether ketone (PEEK) or steel were glued onto the capillaries using epoxy, an assembled chip is shown in Figure 1.1A. Thermal paste was added to the bottom of the mounted chip before the PCB was placed on a cooling system.

The experimental setup varied between the papers but an example with a heat-actuating chip is shown in Figure 3.3. The flow was initiated from a pump and measured with either pressure or flow rate sensors,  $S_1$  and  $S_2$ . The sensors were coupled to a computer and the signals were included in a Matlab script where they were used for feedback regulation. In **Paper I**, a permittivity sensor was also used, which was coupled to the computer via a network analyzer. The connectors on the PCB were coupled to a power supply that applied a certain voltage based on the feedback regulation. Screw terminals were coupled to a DAQ that measured the change in resistance with temperature. This could also be included in the Matlab script to set a maximum temperature in the regulation. When cooling of the chip was made with a Peltier element, it was coupled to another outlet on the power supply which was also included in the Matlab script. When a sample valve was used, it was coupled to an Arduino. By sending a pulse to the Arduino via the Matlab script, the injection time could be synced with the rest of the measured signals. When batteries were used instead of the power supply, these were coupled to the connectors



on the board with an Arduino and an NPN transistor for pulse width modulation.



*Figure 3.3.* Sketch of an experimental setup. The blue line shows the fluidic path, while the black lines show the electrical configurations.  $S_1$  and  $S_2$  symbolize sensors, and the purple rectangle is the PCB placed on a cooling element.

## 4. Applications

The chips presented in this thesis have been used to investigate phenomena like heat distribution, compressibility, and capacitance. But their main aim is to serve in applications as functioning devices. The function and area of use of the heat-actuating chips will be further described in this chapter, as will the different integrated sensors.

### 4.1 Heat actuation

The heat-actuating chips were used for various applications in this thesis. The flow-control properties, together with their extremely small volume and low power consumption, make them useful in a variety of fields.

In **Paper II**, a heat-actuated chip was used at the end of a system working as a BPR. BPRs are widely used in high-pressure microfluidics to ensure elevated pressure throughout the whole flow system. As mentioned in *1.4 High-pressure microfluidics*, some experiments are dependent on a certain pressure level, although the pressure drop through the system is not necessarily high. The reason for this can be to maintain a certain phase, composition, or solubility strength.

When choosing a BPR, there are several factors to consider. The size includes both the footprint of the whole device and the inner volume. A large dead volume increases waiting time and lowers resolution if the device is placed before a detector. The ability to control the pressure level also varies between different kinds of BPRs. Some have a set pressure level that cannot be changed, while others can be varied either digitally or analogously. The variation can be obtained by, *e.g.*, turning a knob or altering the length of a capillary. The working range and precision are also important factors. Here, an active BPR was made in the nanoscale volume, where the heat actuation enabled an active regulation with a variable pressure level and very high precision.

The excellent precision obtained by the heat actuation made it interesting also for stabilization, **Paper III**. Stabilizers are widely used in microfluidics and are placed at the beginning of a system after the pump. To get stable flow, expensive pumps are often needed, and even they cannot ensure fluctuation-free flows. Depending on the working principle of the pump, different fluctuation behaviors occur where a common one is a two-piston pump with a

pulsatile flow profile. Most stabilizers today are either big or passive. A passive stabilizer dampens noise but cannot handle drift. To be able to completely remove fluctuations in the pressure or flow rate, quick regulation in both directions is needed. The heat actuation made it possible to actively stabilize the flow, reducing both noise and fluctuation.

In addition to BPRs and stabilizers, the use of the heat-actuated chips was evaluated in three different flow systems. Mixtures or gradients are often desired in analysis for fast and efficient separation, which requires advanced pump systems. In **Paper I**, an alternative control board was presented that included two parallel-coupled thermal-actuated chips, one coupled to a methanol source and one coupled to a CO<sub>2</sub> source. The control board also included a mixing chip and a sensor chip that continuously measured the composition, Figure 2.2. This setup could be used to continuously mix a mobile phase for HPLC where the permittivity of the solution is of high interest. By varying the temperature, and hence the flow restriction, in either one or both of the chips, the flow rate and composition could be controlled. By integrating a gas container into the flow system, both mediums could be controlled using only one pump. This system showed the potential applications of using heat actuation for gas as well as the interesting effects of mixing gas and liquid.

In **Paper IV**, the chips were used for pressure regulation to make on-chip sample injection for chipHPLC. To make small and precise sample injections without band broadening, an on-chip technology is preferable. The injection is performed in two steps: first, the sample is loaded into the chip through a transversal channel; second, a part of the sample is injected into the main channel containing the column. In the first step, the pressure should be low to prevent passage through the column, while in the second step, it should be higher to force a portion of the sample through the column. Here, two chips were coupled in series at different positions in the flow system. One was placed upstream of the chipHPLC, which was heated when a higher flow rate was desired. The other one was placed downstream of the chip in the waste channel of the transversal channel. This one was initially heated to make a larger portion of the flow pass through and hence prevent it from entering the column channel.

In **Paper V**, a chip was used to make a portable pump system. Pumps are key elements in all flow systems and are often both large, expensive, and power-consuming. Instead of electricity, the stored energy in pressurized gas was utilized here. When the pressurized gas expands, it pushes a piston that transfers the movement to the mobile phase. The expansion makes the pressure of the gas decrease, which in turn slows down the movement of the piston and thereby the flow rate. To compensate for this, the heat-actuating chip was used. The chip was initially cooled to get a high flow resistance to match the high pressure of the gas. Along with the gas pressure decreasing, the chip is heated and the flow restriction is lowered. Thereby the pressure downstream of the chip can be kept stable, Figure 4.1.

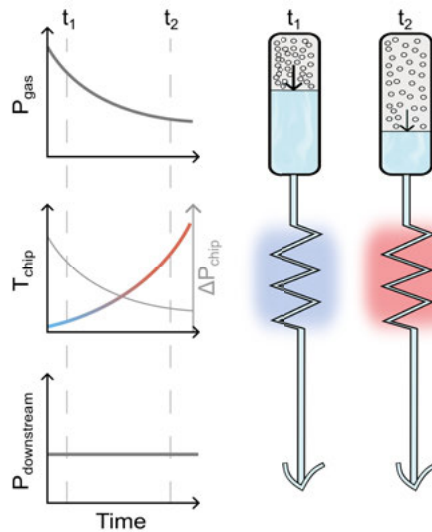


Figure 4.1. Conceptual sketch of the pump system using pressurized gas. The upper graph shows how the pressure of the gas decreases with time as it expands in the container, shown to the right. The temperature in the chip, middle graph, simultaneously rises, which makes the pressure drop through the chip decrease. The resulting pressure of the liquid downstream of the chip is stable, lower graph. *Figure reprinted from Paper V with open access license CC BY 4.0.*

## 4.2 Sensors

To ensure correct results and to enable accurate regulation, reliable and accurate sensors are needed, and as the system shrinks, so must the sensors. Often, sensors have a larger part containing electrical components and a part for fluidic connections, where the inner volume is minimized to avoid adding dead volume. There are multiple sensors on the market today including temperature, pressure, flow, and mass flow sensors, with volumes in the  $\mu\text{l}$ -ml scale. However, the sensors do not only need to be *small*, they also need to be *close*. As previously discussed, the small volumes associated with microfluidics make the heat quickly decrease, both on a time and distance scale. Making temperature measurements on the outside of a chip or in the fluid downstream does not provide relevant data. Similarly, a certain pressure can be set from the pump, but then tubes, sensors, and connections all affect the pressure before it reaches the actual application. Hence, although there are sensors with a small internal volume and great accuracy and precision, it is a waste of potential if the measurement is not close enough to the point of interest. Therefore, the integration of sensors into the microfluidic chip is crucial.

All papers presented in this thesis include chips with temperature sensors. These are made by 4-point resistivity measurement, where a higher temperature increases the resistance in an electrical resistor. The four measuring points

remove influences from the resistance in the connecting electrodes. Temperature sensors are, of course, included in the chips where thermal control is the aim, but they also have general importance. Working in different environments gives various surrounding temperatures, which is especially noticeable with portable devices that should be taken out in the field, but it also differs between labs. The fact that we are using "room temperature" as a regular expression in scientific experiments has caused problems over the years since the normal indoor temperature depends on the season and your position on the globe. How much the temperature affects an experiment depends on several factors, including the medium used, but it is one reason why different results can be obtained with seemingly the same experimental parameters. Hence, temperature measurement can be used both for quality control and problem-solving. There are also many applications where an elevated temperature is needed for a reaction to occur or to speed up the process.

In **Paper I**, a permittivity sensor was used to measure the ratio of methanol and CO<sub>2</sub>. Permittivity describes how much a medium polarizes when exposed to an electric field. Due to its symmetrical molecular structure, CO<sub>2</sub> has limited ability to polarize, resulting in low permittivity. Methanol, on the other hand, has an asymmetric structure with higher negativity around the oxygen atom and can therefore be more polarized. Here, the sensing chip worked as a parallel plate capacitor with electrodes both at the bottom and top of a channel. In contrast to detectors that detect analytes in a medium, the permittivity sensor is used to measure the bulk medium, like the mobile phase in an HPLC system. This is important to ensure correct composition, especially when working with gradients that are mixing during the run.

Measuring pressure is an inevitable part of high-pressure microfluidics. Pressure sensors are used to set parameters in pressure-dependent systems and for phase control. They can also be used to protect devices from pressures exceeding their tolerance levels. By integrating pressure sensors, more exact values at precise positions can be obtained with fewer instruments and extra connections. In parallel to the presented work, a microfluidic in-line pressure sensor was developed [62], where a piezoresistive pressure sensor was made in silicon and glass. Silicon was chosen to make a thin, flexible diaphragm. The force from the applied pressure creates stress tension in the diaphragm that changes the resistance. Since the chip was made with two pressure sensors separated by a flow restriction, the chip could also be used to obtain the flow rate by the Hagen-Poiseuille equation, Equation 1.

### 4.3 Multiple-chip systems

To realize portable lab-on-a-chip devices, several chips have to be combined. For chip-in-a-lab, maybe just one chip is needed, but why not combine more to utilize their advantages? To do this, two different strategies were employed

in this work. When combining fluidic units, connections and tubing have to be considered. While this is not the most important part of the macro world, the influence gets stronger when the components shrink. Most of the volume might actually be in the tubes, and when dealing with high pressures, tolerant connections are highly interesting, with several different solutions explored [63, 64].

The most noticeable difference between the presented multiple-chip systems is whether the chips are interchangeable or not. In **Paper I**, multiple chips were coupled on the same PCB, with capillaries in between. This resulted in a compact system with a small footprint. Using capillaries between the chips keeps the volume to a minimum and reduces the need for extra fittings. The compact design also reduces the distance between the chips, meaning that the connecting capillaries can be made shorter. In **Paper IV**, the chips were instead connected to separate PCBs and coupled together by regular tubes and fittings. This made the system less compact, resulting in a need for more connecting components. The benefit of this system is that the chips can be interchanged if one of them malfunctions, which was an issue in the control board that significantly lowered the yield. The capillaries are brittle, and if one broke or a chip clogged, the whole control board had to be discarded. The assembly also contains several manual steps, including gluing and soldering, that decrease the yield of fully functioning units.

The best approach to connecting multiple chips therefore depends on the yield of fabrication and assembly, as well as the life span of each chip.

## 5. Concluding remarks and future outlook

This work demonstrates flow control on glass chips for high-pressure microfluidics. By using integrated heaters, actuation was performed without any moving parts. The small size and possibility of battery drive make it a promising strategy for portable devices. Additionally, the integrated sensors enable precise control without adding extra volume.

The on-chip flow control could be used for further integration into microfluidic products. Combining several chips has already been shown in the papers in this work, but further combinations could also be made. To realize complete and portable HPLC systems, work is needed to combine the developed components, as well as connect additional devices. In this work, we have shown a pump system, flow regulation, sensors, and on-chip HPLC, which could all be connected and coupled to miniaturized detectors. This work is mainly made for proof of concept, and further optimizations are also needed for inclusion in complete products, *e.g.*, channel and heater design could be altered to better suit intended applications.

In addition to combining units, several functions could also be integrated into the same chip. Herein, temperature sensors are already integrated with other functions of the chip. Further integration could be made, *e.g.*, including sensors or actuation in chipHPLC. This would eliminate the need for multiple fittings and associated extra volumes. However, this would also complicate the fabrication and risk a lower yield, while using separate chips enables more flexible systems where different chips can easily be interchanged.

In this thesis, PID regulation was used to control the heat actuation, demonstrating excellent performance with both power supply and batteries using pulse width modulation. In the future, however, a more sophisticated PID system should be implemented to eliminate the need for manual adjustments of the parameters. Ideally, this would be a system that can change the parameters during long-term experiments to follow the system's changes in *e.g.*, pump volume. In the case of external cooling, this could also be implemented in the PID regulation to enhance stability and optimize energy consumption.

The microfluidic area is constantly growing, and this thesis presents a small part of the work made worldwide. Miniaturization of the flow system is forecast to grow even more as technical issues are overcome and new technology enables new fabrication processes and design parameters. This includes *e.g.*, the development of 3D-printed devices for microfluidics that can simplify fabrication. With today's great focus on environmental issues, microfluidic devices could also serve a place in large hospitals or laboratories, with the po-

tential to lower the high chemical consumption even when portability is not required.



# Sammanfattning

Kemiska och biologiska analyser spelar en avgörande roll i vårt samhälle idag. Vi kan ta blodprover för att kontrollera om vi är sjuka för att bli friska snabbare och vi kan ta prover på mat och vatten för att förhindra sjukdom i förväg. Det finns dock begränsningar i dessa analyser, såsom behovet av stationära och dyra instrument samt kravet på stora volymer av både kemikalier och prover. I den här avhandlingen undersöks möjligheterna att med design och tillverkning av så kallade mikrofluidala komponenter göra dessa instrument mindre, vilket har potential att förbättra analyserna, öka tillgängligheten och minska miljöpåverkan.

Vad är då mikrofluidik? Mikrofluidik handlar om flöden, vanligtvis med vätskor men också med gas, där flödeskanalerna är mycket små. Det gör att flödet får andra egenskaper jämfört med beteendet i större kanaler. Där den kanske tydligaste egenskapen är det så kallade laminära flödet. Det innebär att fluiden rör sig i kanalen i stabila flödeslinjer. Partiklar som ligger på kanten av kanalen kommer då ligga kvar på samma kant även när de rört sig en bit framåt. I större kanaler är flödet istället turbulent, som exempelvis i en fors där vatten virvlar fram i oförutsägbara rörelser som gör det svårt att beräkna var enskilda partiklar kommer hamna.

Mikrofluidik ger också flera fördelar när det kommer till behov av prov och förbrukning av kemikalier. När mindre provvolum behövs går det att köra flera tester från samma prov och det underlättar när det bara är möjligt att få små provvolymer, som exempelvis vid blodprov från bebisar. Det gör också att det behövs mindre mängder av de kemikalier som används vid analyserna. Dessa kemikalier är ofta skadliga för miljön och kan vara farliga att arbeta med samtidigt som de innebär en ökad kostnad både vid inköp, transport och avfallshantering. Att göra själva instrumenten mindre möjliggör även bärbara system med lägre energiförbrukning.

För att analysera ett blodprov används ibland kromatografi, vilket är en metod för att separera olika ämnen i provet. Provet injiceras först i en mobilfas, som är den vätska som flödar genom systemet, för att sedan passera genom en kolonn. Kolonnen innehåller oftast små partiklar med speciella ytegenskaper, porositet, storlek eller laddning. Beroende på storlek och kemi hos ämnena i provet kommer vissa att stanna kvar längre i kolonnen medan andra ämnen hellre stannar i mobilfasen och därmed flödar snabbare genom systemet. På så sätt kommer olika ämnen att lämna kolonnen efter olika lång tid.

För att få väl separerade ämnen bör kolonnen vara tätpackad med små partiklar. Det, tillsammans med små kanaler, gör att det krävs högre tryck för att

upprätthålla en hög flödes hastighet. Det gör att de komponenter vi tillverkar måste klara högt tryck, vilket utesluter användningen av silikon som annars ofta används i mikrofluidik vid lågt tryck.

I den här avhandlingen har små komponenter (chip) tillverkats av glas, ett högtryckståligt material som är genomskinligt och kemiskt inert. För att tillverka små strukturer som ska klara höga tryck krävs väldigt rena ytor och därför tillverkas de i ett så kallat renrum. Chippen är runt 1 cm<sup>2</sup> stora och innehåller flödeskanaler med djup på 10-100 µm. De flesta innehåller också elektroder, tunnfilmer av platina eller guld för elektriskt kontakt.

En svårighet med miniaturisering är tillverkningen av rörliga delar. Vid nedskalning ökar förhållandet mellan yta och volym, vilket gör att komponenter blir sköra och lätt går sönder. För att komma runt detta används här ett alternativt sätt att styra flöden på, nämligen genom att ändra temperaturen. Genom att lägga på en spänning över elektroderna i chippen uppstår resistiv värmning. Det är en passande metod för mikrofluidik då de små dimensionerna gör att uppvärmningen går snabbt och inte kräver så mycket energi.

Temperaturregleringen används för att förändra viskositeten på vätskan som passerar genom kanalen i chippet. Viskositet beskriver hur trögflytande en vätska är, exempelvis är sirap mer trögflytande och har därmed högre viskositet än vatten. Ju mer trögflytande en vätska är, desto högre tryck krävs för att få den igenom en tunn kanal, trycket är alltså proportionerligt mot viskositeten. När en vätska värms upp så sjunker dess viskositet, vilket gör att det krävs lägre tryck för att bibehålla samma flödes hastighet. Om flödes systemet innehåller en pump som ger ett konstant tryck kommer istället flödes hastigheten att öka. För gaser är det tvärtom, där ökar viskositeten med ökad temperatur. I artiklarna som ingår i den här avhandlingen har det undersökts hur termisk reglering av viskositet kan användas i olika flödes system.

I de flesta kemiska analyser med kromatografi används en av olika vätskor eller gaser som mobilfas, och i gradient-analyser ändras dessutom denna sammansättning under experimentets gång. I **papper I** kopplades fyra olika glaschip ihop för kontinuerlig blandning av en mobilfas. De två första chippen användes för termisk reglering där det ena var kopplat till metanol och det andra till koldioxid. I det tredje chippet mixades dessa fluider ihop för att till sist passera ett sensor-chip som mätte sammansättningen. Genom att ändra temperaturen på chippen och därmed fluiden i, så kunde både flödes hastigheten och kompositionen kontrolleras.

Som tidigare nämnt kan höga tryck vara ett resultat av flödes begränsningen i systemet. Men ibland är det önskvärt att hålla hela systemet under högt tryck, exempelvis för att bibehålla en viss fas eller lösnings förmåga. För att inte trycket ska nå atmosfärstryck för tidigt kan en flödes begränsare användas i slutet av systemet, en så kallad backtrycksregulator. I **papper II** användes ett chip som med hjälp av temperaturreglering kunde styra och bibehålla ett önskat backtryck, vilket i det här fallet var runt 80 bar. För att uppnå en

kraftig flödesbegränsning användes ett kanaldjup på 9  $\mu\text{m}$  vilket resulterade i en väldigt liten innervolym på bara 3 nl.

I den här avhandlingen användes återkopplande reglering för att styra flödesparametrarna, vilket betyder att den pålagda spänningen anpassas utifrån skillnaden mellan ett önskat värde och ett uppmätt värde. Temperaturen, och därmed viskositeten, ändras direkt med ändrad spänning men hur snabbt man kan reglera övriga flödesparametrarna varierar beroende på hur systemet ser ut. I **papper I** måste fluiderna från de temperaturreglerande chippen passera ett mixningschip innan de når sensorchipet. Det ger en viss fördröjning från att temperaturen ändras tills dess att en förändring upptäcks. När tryck- och flödessensorer istället användes var dessa placerade direkt innan, eller efter, regleringschipet vilket gjorde att en förändring sågs direkt. Hur lång tid det tog att uppnå ett önskat värde på trycket varierade dock beroende på fluidens kompressibilitet och systemets volym. Hur kompressibelt ett ämne är beskriver hur mycket det kan komprimeras, tryckas ihop, under ett pålagt tryck. Även om vatten och metanol har väldigt låg kompressibilitet var den tillräcklig för att påverka stabiliseringstiderna som ökade med ökad volym.

Förutom att introducera ett backtryck så bidrog temperaturregleringen även till att göra trycket mer stabilt, vilket utforskades vidare i **papper III**, där en flödesstabilisator togs fram. Att ha ett stabilt flöde är viktigt för att få korrekta och reproducerbara resultat, men är något som få pumpar kan erbjuda. De flesta pumpar har antingen en hög brusnivå, kontinuerliga fluktuationer eller drift (dvs. att flödet ändras långsamt under lång tid). I **papper III** användes ett temperaturreglerande chip som kopplades in direkt efter en pump för att stabilisera det utgående flödet. Hur stora fluktuationer som kan jämnas ut beror på hur mycket vätskan kan komprimeras, desto större volym desto större fluktuationer kan dämpas. Det som gav problem med långa stabiliseringstider för backtrycksregulatorn användes alltså här som en tillgång.

För att utvärdera temperaturregleringen tillsammans med kromatografi kopplades olika system ihop i de två sista artiklarna. **Papper IV** är ett samarbete med en forskargrupp i Leipzig, Tyskland, som även gör kromatografi på chip. De har tagit fram ett chip med ett så kallat injektionskors, den längsgående kanalen innehåller där en kolonn för separation medan en tvärgående kanal används för injicering av provet. För att få en så bra separation som möjligt vill man undvika den diffusion som sker av provet in i mobilfasen. Därför laddas provet först på chipet genom den tvärgående kanalen, och sedan injiceras en liten del av provladdningen in i kolonnen. För att undvika att provet går in i kolonnen direkt vid laddning användes ett kylt chip för att minska trycket i huvudkanalen och ett uppvärmt chip nedanför den tvärgående kanalen för att styra flödet dit. När provet sedan skulle injiceras ökades trycket i huvudkanalen genom att värma upp det förstnämnda chipet och kyla ner det andra chipet.

I **papper V** användes istället en kommersiell kromatografi-kolonn tillverkad av stålrör. För att driva flödet genom denna utvecklades ett alternativt pump-

system som använde trycksatt gas. Gaser är väldigt kompressibla, vilket ger stor inneboende energi som kan användas för att driva på ett flöde. Här används en behållare i form av ett rör uppdelat i två kammare med en kolv emellan. På ena sidan kolven finns den trycksatta gasen och andra sidan fylls med den önskade mobilfasen. När gasen expanderar rör sig kolven och pressar på vätskan. Trycket på gasen minskar när den expanderar vilket kompenseras för genom ett temperaturreglerande chip. Trycket på vätskan nedanför chippet kan beräknas som trycket på gasen minus tryckfallet över chippet. Genom att värma vätskan och därmed minska tryckfallet i samma takt om trycket minskar på gasen kan det resulterande trycket nedanför chippet hållas stabilt. På så sätt får vi ett pumpsystem som ger ett fluktationsfritt och stabilt flöde, som skalar väl med hur mycket volym som behövs och som kan göras bärbart då uppvärmningen kan drivas med batterier.

I många fall används idag ett mikrofluidalt chip i en större experimentuppställning där exempelvis en separation eller reaktion sker på chippet medan flödet styrs av större instrument. I det här arbetet har vi visat att även flödeskontrollen kan utföras på chip och att flera olika chip kan koppas ihop. Förutom att system kan bli så litet att det går att göra bärbart, har den termiska regleringen också visat flera fördelar i labbmiljö då den ger hög precision med extremt små inre volymer och låg energiförbrukning.

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