Effective use of enzyme microreactors -

thermal, kinetic, and ethical guidelines.

Promotoren
Prof. dr. ir. R.M. Boom
Hoogleraar Levensmiddelenproceskunde
Wageningen Universiteit en Researchcentrum

Prof. dr. M.J.J.A.A. Korthals
Hoogleraar Toegepaste Filosofie
Wageningen Universiteit en Researchcentrum

Co-promotor
Dr. ir. A.E.M. Janssen
Universitair Docent, leerstoelgroep Levensmiddelenproceskunde
Wageningen Universiteit en Researchcentrum

Promotiecommissie
Prof. dr. ir. J.E. Gardeniers
Technische Universiteit Twente

Prof. dr. J.C.M. van Hest
Radboud Universiteit

Prof. dr. ir. H. Gruppen
Wageningen Universiteit en Researchcentrum

Prof. dr. M.J. van den Hoven
Technische Universiteit Delft

Dit onderzoek is uitgevoerd binnen de onderzoekschool VLAG.

Effective use of enzyme microreactors -

thermal, kinetic, and ethical guidelines.

Jan W. Swarts

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M.J. Kropff,
in het openbaar te verdedigen
op woensdag 10 juni 2009
des middags te half twee in de aula.

Swarts, Jan W.

Effective use of enzyme microreactors - thermal, kinetic, and ethical guidelines

Thesis Wageningen University, the Netherlands, 2009 - with summary in Dutch and English

ISBN: 978-90-8585-373-2

Contents

Chapter 1

Introduction

The work in this thesis was carried out within the Euregion Rijn-Waal INTERREG IIIA program of 'Microreactors: Small devices for expanding markets'. In this program process engineers and organic chemists from Wageningen University and Research Centre, organic chemists from Radboud University in Nijmegen, and micro electronic and software engineers from Fraunhofer Institute IMS in Duisburg worked together.

The aim of the program was to develop a (bio-) chemical micro reaction/extraction system. This system consists of hardware (microchips, pumps, control systems, analysis systems) and software (control software, analysis read-out systems). This system could be used as a reaction and/or extraction condition screening method for stochiometry, temperature, residence time etc. (Koch et al., 2008a,b; Tetala et al., 2007a,b). Operation of the system should be as simple as possible. Ideally, the system should be able to work autonomously.

Microreactor technology as used in the (bio-)chemical micro reaction/extraction system has many reported benefits, such as reduced enviromental, financial, and safety burdens when compared to processing on a larger scale. Despite these claims only a few examples of microtechology application outside the laboratory are known. This program aimed to present a practical implementation of microreactor technology through tackling some of the hurdles in a fundamental manner.

1.1 What is microreactor technology?

In recent decades, techniques to make ever-smaller electric circuits have found application in chemical engineering. The photolithographic techniques used in micro

electronic chip manufacturing are now applied in the fabrication of reaction vessels, pipes, mixers, and many other lab-equipment on the scale of a human hair. With increasing technical possibilities, the possibilities have become better (smaller and more accurate experimenting) and wider (more applications, such as in biology and medicine) (Auroux et al., 2002; Jensen, 2001; Reyes et al., 2002).

The term microtechnology generally includes micro electronics. In this thesis we made use of microfluidic technology, which is microtechnology with at least one fluid inside the system. The prefix 'micro' is used when at least two relevant dimensions of the system are in the range of 0.1 to 100 micrometer. Typical volumes for microfluidic channels are in the sub-microliter scale. Due to these small dimensions, flow in these channels is typically laminar. When we further restrict this microfluidic technology to systems in which reactions occur in the fluid the term microreactor technology is used.

1.2 Why use microreactor technology?

Microreactor technology is reported to have many benefits over regular chemical methods. The first benefits often mentioned are the high heat and mass transfer rates. Due to the small dimensions over which temperature and concentration gradients can exist mass and heat transfer can be very quick. This could minimize the time needed for heating and mixing. This in turn could enable high reaction rates due to the absence of diffusion limitation. A second benefit is the improved control over such processes. A very low fluid to chip volume ratio could facilitate a very stable fluid temperature.

A third reported benefit is the reduced need for materials and energy. Due to the miniaturized reaction environment and highly sensitive analysis methods, only minute amounts of reagents are needed. Reagents that are either very expensive, environmentally unfriendly, or poorly available can more easily be tested at this scale. Furthermore, the reacting components are usually added continuously. This means that their concentrations or relative flow rates can be adjusted during the process. A fourth and last benefit often mentioned is the increased safety. Due to the small volumes, highly toxic or explosive materials can be dealt with in relative safety. In case of one faulty microreactor, only a small amount of energy or chemicals could be released, while the other (parallel) microreactors would continue working.

Some of the current problems with microprocessing include the lack of a single standard for processing equipment, and the low throughput of a single chip. Solutions, such as standardization and off-the-shelf numbering-up methods, still need to be developed.

While working on the microreactor system as discussed in the first paragraphs of this chapter, we came across some hurdles that needed to be solved. As we wanted to use enzymes in the microsystem, we needed to know how to use the data from microreactor results. Can findings from microsystems be used in large scale processing, and when does diffusion limitation play a role? Furthermore, the intended microsystem consisted of a multi-use chipholder and discardable microchips. The thermal behavour of this type of microsystems is not extensively covered in literature. The thermal design and use of such microsystems needed to be investigated.

1.3 Enzymes in microreactors

In the late 90s, microreactors were first used for enzyme assays (Cohen et al., 1999; Hadd et al., 1997). Often, enzyme microreactors were used to demonstrate analysis techniques. These techniques included on-chip product separation with fluorescence measurements of protein kinase A (Cohen et al., 1999), on-chip amperometric and fluorescent detection for the study of immobilized phosphatase (Mao et al., 2002b; Wang et al., 2001), and the use of Thermal Lens Microscopy for determining the effect of heating on the activity of peroxidase (Tanaka et al., 2000). Enzymatic microreactors have also been used to investigate the effect of mixing in droplets (Song and Ismagilov, 2003), the possibilities of multi-enzyme or cascade reactions (Lee et al., 2003; Mao et al., 2002b; Wang et al., 2001) and the effect of higher temperatures on activity (Arata et al., 2005).

To see whether microreactors would increase or decrease enzyme activity, or leave it unaffected, kinetic parameters determined with microreactors have been compared to off-chip results. On-chip kinetic parameters were found to be similar to off-chip parameters for soybean peroxidase and galactosidase. However, limited data sets were used for such comparisons (Hadd et al., 1997; Lee et al., 2003; Srinivasan et al., 2002) and no unambiguous conclusion could be drawn as also increased enzyme activity in a microreactor was claimed for trypsin, glucosidase, and horseradish peroxidase (Kanno et al., 2002; Miyazaki et al., 2001; Tanaka et al., 2001). These increases were attributed to an increased diffusion of enzyme molecules and the fluid velocity profiles

in microchannels.

Whether faster or slower diffusion of any of the components contributes to a higher or lower enzyme activity in microreactors was discussed in some papers. Diffusion limitation on the effective enzyme activity was hinted on by Kanno et al. (2002), but not investigated. Maruyama et al. (2003) did investigate the effect of diffusion, but used an excess of enzyme to make diffusion dominant. More recently, Ristenpart et al. (2008) investigated enzyme kinetics in a microsystem with mixed diffusion and reaction limitation. This article demonstrates a method for rapid extraction of kinetic data from experimental results.

1.4 Temperature control in microreactors

Higher temperatures lead to a higher enzyme activity, but also inactivate enzymes at too high temperatures. Rapid heating and cooling has proven to be effective for on-chip PCR, but the use of microchips for the investigation of temperature effects on enzymes has been rather limited. Increased enzyme activity in heated microreactors was found for β-galactosidase and α-amylase (Arata et al., 2005; Melander et al., 2006). The effect of temperature was more thoroughly studied by determining the activation energies of phosphatase and β-glucoside hydrolase on-chip (Mao et al., 2002c; Thomson and Nidetzky, 2008).

Many methods to heat a microfluidic chip to a single temperature or to create a temperature gradient have been proposed. Temperature control can be achieved by means of in-chip heating devices (Keuren et al., 2005; Lao et al., 2000; Mao et al., 2002c; Wijngaards et al., 2004), out-of-chip heating devices (Kopp et al., 1998; Mao et al., 2002a), and non-contact heating (or cooling) (Oda et al., 1998; Slyadnev et al., 2001; Sundaresan et al., 2005). As the intended microsystem uses discardable microchips, the most commonly described type, in-chip heating, is uneconomic.

A multi-use chipholder that can provide a wide range of temperatures under a wide range of processing conditions could facilitate a robust and economically viable microreactor environment. Such a multi-use chipholder should contain all necessary electronics, sensors, and actuators, while the (discardable) chip should be as simple as possible. The effect of design and use of such discardable chips and a single multi-use heater has received little attention in literature.

1.5 Research aim

The goal of this thesis research was to investigate the effect of the use of microreactors on enzyme kinetics and the effect of the design and use of a microsystem on the temperature of fluids inside the chip. This included the comparison of different enzymatic reactions on micro and bench scales and the study of diffusion limitation on the enzyme reaction rates in microsystems. Diffusion effects could further be investigated using multiphase micro extraction systems. To use microsystems effectively the temperature of fluids in a microsystem in use should be stable and predictable. This could be investigated by combining computer models and practical experiments. Furthermore, successful adoption of microreactor technology in practice requires an understanding of social and ethical aspects of microreactor technology applications.

1.6 Thesis outline

The microsystem in this thesis was used to test a wide range of experimental conditions. The effect of design and practical use of the microsystem on fluid temperature should be considered before using it with temperature sensitive processes. Given the temperature dependence of enzyme activity, we investigated the effect of experimental conditions on fluid temperature using the temperature dependent fluorescent dye Rhodamine B. **Chapter 2** describes the effect of fluid flow through the chip and air flow over the chip at practically normal flow rates on the internal fluid temperature. Also, the effect of the fluid flow rate on transient heat up and cool down was investigated.

To control temperature in the chip, we used a PEEK chipholder with a resistance heater covering only a third of the chip. In **chapter 3** we look at the effect of the thermal properties and the design of the chipholder, and the movement of air on chip temperature using computational fluid dymamics. The focus was on the temperature distribution in the whole chip at the level where the microchannels were located. The models included steady state and transient calculations.

Once the temperature profile in a chip is known and the design is adapted to the user's needs, the microfluidic chip can be used for enzyme reactions. **Chapter 4** describes the esterification of 1-butanol and propionic acid by *Candida antarctica* Lipase B in a water-decane system. The reaction rate at various combinations of substrate concentrations was described with a Ping Pong Bi Bi model with alcohol

inhibition. The effect of temperature on activation and first-order inactivation was investigated with Arrhenius relations.

After the fairly slow esterification reaction, **chapter 5** describes a faster hydrolysis reaction. *Ortho*-nitrohenyl-β-D-galactopyranoside was split into *ortho*-nitrophenol and galactose by β-galactosidase. This reaction was quenched on-chip by increasing the pH. The concentration of *ortho*-nitrophenol in the reaction mixture was measured with in-line spectroscopy. The effect of fluid residence time and corresponding diffusion time on the reaction was investigated experimentally and with computational fluid dynamics models.

When products using microreactor technology have been developed successfully, they can start to enter the professional or consumer market. **Chapter 6** explores possible social and ethical aspects of applied microreactor technology. We explore some of the possible applications and their associated benefits and costs. Furthermore, the necessary communication related to the introduction of such a new technology is discussed.

Finally, **chapter 7** deals with some general points of discussion. It holds some examples of two and three phase extraction systems and microchips with membranes, and shows how computer models could be used to explore further pathways for the use of microfluidic chips for extraction purposes. This chapter further frames the findings of this thesis in the consequetive choice, design, use, and wider application of microreactor technology.

References

Arata, Hideyuki F., Yannick Rondelez, Hiroyuki Noji, and Hiroyuki Fujita. 2005. Temperature alteration by an on-chip microheater to reveal enzymatic activity of β-galactosidase at high temperatures. *Analytical Chemistry* 77(15):4810–4814.

Auroux, Pierre-Alain, Dimitri Iossifidis, Darwin R. Reyes, and Andreas Manz. 2002. Micro total analysis systems. 2. Analytical standard operation and applications. *Analytical Chemistry* 74(12):2637–2652.

Cohen, Claudia B., Emily Chin-Dixon, Sang Jeong, and Theo T. Nikiforov. 1999. A microchip-based enzyme assay for protein kinase A. *Analytical Biochemistry* 273:89–97.

Hadd, Andrew G., Daniel E. Raymond, John W. Halliwell, Stephen C. Jacobson, and J. Michael Ramsey. 1997. Microchip device for performing enzyme assays. *Analytical Chemistry* 69:3407–3412.

Jensen, Klavs F. 2001. Microreaction engineering - is small better? *Chemical Engineering Science* 56:293–303.

Kanno, Ken-ichi, Hideaki Maeda, Showta Izumo, Masakazu Ikuno, Kazuhito Takeshita, Asuka Tashiro, and Masayuki Fuji. 2002. Rapid enzymatic transglycosylation and oligosaccharide synthesis in a microchip. *Lab on a Chip* 2(1):15–18.

Keuren, Edward van, Mark Cheng, Oliver Albertini, Cheng Luo, John Currie, and Makarand Paranjape. 2005. Temperature profiles of microheaters using fluorescence microthermal imaging. *Sensors and Materials* 17(1):1–6.

Koch, K., R.J.F. van den Berg, P.J. Nieuwland, R. Wijtmans, H.E. Schoemaker, J.C.M. van Hest, and F.P.J.T. Rutjes. 2008a. Enzymatic enantioselective C-C-bond formation in microreactors. *Biotechnology and Bioengineering* 99(4):1028–1033.

Koch, Kaspar, Rutger J. F. van den Berg, Pieter J. Nieuwland, Roel Wijtmans, Marcel G. Wubbolts, Hans E. Schoemaker, Floris P.J.T. Rutjes, and Jan C.M. van Hest. 2008b. Enzymatic synthesis of optically pure cyanohydrins in microchannels using a crude cell lyate. *Chemical Engineering Journal* 135S:S89–S92.

Kopp, Martin U., Andrew J. de Mello, and Andreas Manz. 1998. Chemical amplification: continuous-flow PCR on a chip. *Science* 280:1046–1048.

Lao, Alex I.K., Thomas M.H. Lee, I-Ming Hsing, and Nancy Y. Ip. 2000. Precise temperature control of microfluidic chamber for gas and liquid phase reactions. *Sensors and Actuators A: Physical* 84:11–17.

Lee, Moo-Yeal, Aravind Srinivasan, Bosung Ku, and Jonathan S. Dordick. 2003. Multienzyme catalysis in microfluidic biochips. *Biotechnology and Bioengineering* 83(1):20–28.

Mao, Hanbin, Matthew A. H Olden, Min You, and Paul S. Cremer. 2002a. Reusable platforms for high-throughput on-chip temperature gradient assays. *Analytical Chemistry* 74:5071–5075.

Mao, Hanbin, Tinglu Yang, and Paul S. Cremer. 2002b. Design and characterization of immobilized enzymes in microfluidic systems. *Analytical Chemistry* 74(2):379–385.

———. 2002c. A microfluidic device with a linear temperature gradient for parallel and combinatorial measurement. *Journal of the American Chemical Society* 124:4432–4435.

Maruyama, Tatsuo, Jun-ichi Uchida, Tomohiro Ohkawa, Toru Futami, Koji Katayama, Kei-ichiro Nischizawa, Ken-ichiro Sotowa, Fukiko Kutoba, Noriho Kamiya, and Masahiro Goto. 2003. Enzymatic degradation of *p*-chlorophenol in a two-phase flow microchannel system. *Lab on a Chip* 3:308–312.

Melander, Claes, Wiebke Tüting, Martin Bengtsson, Thomas Laurell, Petra Mischnick, and Lo Gorton. 2006. Hydrolysis of maltoheptaose in flow through silicon wafer microreactors containing immobilised α-amylase and glycoamylase. *Starch/Stärke* 58:231–242.

Miyazaki, Masaya, Hiroyuki Nakamura, and Hideaki Maeda. 2001. Improved yield of enzyme reaction in microchannel reactor. *Chemistry Letters* 30(5):442–443.

Oda, R.P., M.A. Strausbauch, A.F.R. Huhmer, N. Borson, S.R. Jurrens, J. Craighead, P.J. Wettstein, B. Eckloff, B. Kline, and J.P. Landers. 1998. Infrared-mediated thermocycling for ultrafast polymerase chain reaction amplification of dna. *Analytical Chemistry* 70:4361–4368.

Reyes, Darwin R., Dimitri Iossifidis, Pierre-Alain Auroux, and Andreas Manz. 2002. Micro total analysis systems. 1. Introduction, theory, and technology. *Analytical Chemistry* 74(12):2623–2636.

Ristenpart, William D., Jiandi Wan, and Howard A. Stone. 2008. Enzymatic reactions in microfluidic devices: Michealis-Menten kinetics. *Analytical Chemistry* 80(9):3270–3276.

Slyadnev, Maxim N., Yuki Tanaka, Manabu Tokeshi, and Takehiko Kitamori. 2001. Photothermal temperature control of a chemical reaction on a microchip using an infrared diode laser. *Analytical Chemistry* 73(16):4037–4044.

Song, Helen, and Rustem F. Ismagilov. 2003. Millisecond kinetics on a microfluidic chip using nanoliters of reagents. *Journal of the American Chemical Society* 125(47):14,613–14,619.

Srinivasan, Aravind, Xiaoqiu Wu, Moo-Yeal Lee, and Jonathan S. Dordick. 2002. Microfluidic peroxidase biochip for polyphenol synthesis. *Biotechnology and Bioengineeing* 81(5):563–569.

Sundaresan, S.G., B.J. Polk, D.R. Reyes, M.V. Rao, and M. Gaitan. 2005. Temperature control of microfluidic systems by microwave heating. In *Micro Total Analysis Systems 2005*, eds. Klavs Jensen, Jongyoon Han, D. Jed Harrison, and Joel Voldman, vol. 1, 657–659. Boston (MA), USA.

Tanaka, Yuki, Maxim N. Slyadnev, Akihide Hibara, Manabu Tokeshi, and Takehiko Kitamori. 2000. Non-contact photothermal controle of enzyme reactions on a microchip by using a compact diode laser. *Journal of Chromatography A* 894:45–51.

Tanaka, Yuki, Maxim N. Slyadnev, Kiichi Sato, Manabu Tokeshi, Haeng-Boo Kim, and Takehiko Kitamori. 2001. Acceleration of an enzymatic reaction in a microchip. *Analytical Sciences* 17:809–810.

Tetala, K. Kishore R., Bo Chen, Gerben M. Visser, Audrius Maruška, Olga Kornyšova, Teris A. van Beek, and Ernst Sudhölter. 2007a. Preparation of a monolithic capillary column with immobilized α-mannose for affinity chromatography of lectins. *Journal of Biochemical and Biophysical Methods* 70:63–69.

Tetala, Kishore K.R., Bo Chen, Gerben M. Visser, and Teris A. van Beek. 2007b. Single step synthesis of carbohydrate monolithic capillary columns for affinity chromatography of lectins. *Journal of Separation Science* 30:2828–2835.

Thomson, M.S., and B. Nidetzky. 2008. Microfluidic reactor for continous flow biotransformations with immobilized enzymes: the example of lactose hydrolysis by a hyperthermophylic β-glycoside hydrolase. *Engineering in Life Sciences* 8(1):40–48.

Wang, Joseph, Madhu Prakash Chatrathi, and Baomin Tian. 2001. Microseparation chips for performing multienzymatic dehydrogenase/oxidase assays: Simultaneous electrochemical measurement of ethanol and glucose. *Analytical Chemistry* 73(6):1296–1300.

Wijngaards, D. D. L., G. de Graaf, and R. F. Wolffenbuttel. 2004. Single-chip micro-thermostat applying both active heating and active cooling. *Sensors and Actuators A: Physical* 110(1-3):187–195.

Chapter 2

Temperature effects during practical operation of microfluidic chips

Abstract

The temperature dependent fluorescence of Rhodamine B was used to investigate the temperature effect of several system parameters in a microfluidic chip. This was combined with computational fluid dynamics calculations. Limited air movement over the chip had no significant effect on the temperature of the fluid running through the chip. Also, fluid flow through the channels at 0.01 to 8 μl min^{-1} had no effect on the chip temperature or heating and cooling dynamics. The temperature varied greatly over the length of the chip. During transient operation of the chip, the heat up and cool down rates varied over the chip, and were dependent on the distance to the heater. The thermal time constant for heat up was four to five times lower than for cool down. The results can be used as tools for operating a temperature controlled microfluidic chip.

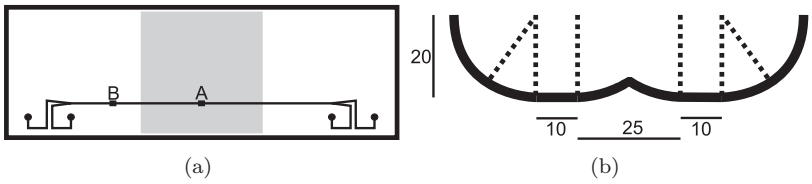
This chapter has been published as Swarts, Jan W.; Janssen, Anja E.M.; Boom, Remko M. 2008. Temperature effects during practical operation of microfluidic chips. *Chemical Engineering Science* 63:5252-5257

2.1 Introduction

Temperature is one of the most important parameters in all chemical reactions. Some biochemical reactions can only be carried out under mild conditions. Other reactions are only possible at very high temperatures. Lastly, some exothermic reactions create heat and could induce a run-away reaction. To control the reaction, one has to control the temperature. Nowadays, many reactions are performed using microtechnology; where only very small amounts of reactants are used. This leads to several advantages in temperature control. Because of the small volumes, only small amounts of energy are required for heat up or cool down. Furthermore, these small reaction vessels have high surface area to volume ratios. This large surface area can be used to control temperature and thus reduce inhomogeneity problems (Auroux et al., 2002; Jensen, 2001; Reyes et al., 2002).

Thermal control consists of two parts: sensors and actuators. The sensor measures the temperature and supplies the actuator with this data. The actuator compares this value to a predefined value and can take action, i.e. heating or cooling. Therefore, accurate temperature control is only ensured by including accurate temperature sensing. Temperature sensing of fluids running through a microfluidic chip can be done directly or indirectly. With direct temperature measurements, the temperature of the fluid itself is measured, while with indirect sensing, the temperature of a component in close contact with the fluid is determined. An example of a direct method is the use of temperature dependent fluorescent dyes such as Fluorescein (Chabinyc et al., 2001; Mao et al., 2002a; Sinton and Li, 2003) and Rhodamine B (Erickson et al., 2003; Guijt et al., 2003; Ross et al., 2001). Indirect methods include temperature measurements of the exterior of the chip, such as with a Polydimethylsiloxane/Rhodamine B thin film (Samy et al., 2008).

In this research, Rhodamine B was used to determine the temperature of fluids running through glass microchips. The method discussed by Guijt et al. (2003) and Ross et al. (2001) was used in an adapted form to investigate the steady state temperature effect of some system parameters, such as air moving over the chip and fluids running through the channels. Transient experiments were performed to learn more about the heat up and cool down behavior of the microfluidic chip. The results from practical experiments are compared to those from computer simulations. These results can be used as tools for operating a temperature controlled microfluidic chip.



(a) (b)

Figure 2.1: Chip design a) chip layout with channels (lines), inlet and outlet holes (circles), position of heater (grey), and positions of camera (rectangles, A and B) and b) cross section of double channel, all sizes in micrometers.

2.2 Materials and Methods

2.2.1 Microchip set-up

A microchip set-up was used with aqueous fluids running through the channels. These fluids contained Rhodamine B (Merck, Germany). As the temperature rises, the molecule's fluorescent intensity decreases. From literature, this temperature effect on intensity is estimated to be 1.7 - 2.4 % K^{-1} (Keuren et al., 2005; Mao et al., 2002b; Ross et al., 2001).

In the microfluidic set-up, flow was pressure driven. Syringes, mounted on syringe pumps, supplied the fluid through fused silica capillaries to a glass microchip. This microfluidic chip had HF-etched channels and access-through-holes for connecting capillaries (Micronit, The Netherlands). For drainage, fused silica capillaries were connected to the microchip at the exits of the microchannel. A schematic drawing of the channel design and cross section of the double channel is presented in figures 2.1a and b. The microfluidic chip was placed in a chipholder, which was equipped with a resistance heater (with a 14 x 14 mm copper plate) pressed against the chip and a 22 mm fan above the chip. A Pt-1000 sensor was placed at the heater to have a continuous temperature feedback loop. These actuators and sensor were connected to a control device that could be operated from a computer. This set-up allows a user to set the desired temperature for the microchip and to monitor the temperature measured by the sensor.

To obtain quantitative information about the fluorescent intensity, the chipholder with chip was placed under a Zeiss Axioplan 2 imaging microscope with a 10x/0.30 Plan-Neofluor objective and a Texas Red filter set. This filter set consists of an exciter filter (540 - 580 nm), an long pass dichroic mirror filter (585 nm), and an emitter filter

(600 - 660 nm) for detection. A mercury lamp was used to illuminate the chip and to excite the Rhodamine B. A Zeiss AxiocamMRc camera was used to take pictures of the chip, with an integration time of 1.17 s for steady state and 0.5 s for transient experiments. These pictures showed a red fluorescent signal in the channels against a near-black background. The red intensity was calculated using image analysis in Matlab 7.04 (The Mathworks, USA). Outliers were determined with 95% confidence according to Dixon (1953) and subsequently excluded from calculations.

The experiments were designed to resemble normal operation. During normal operation, two on-chip single channels are connected (fig. 2.1a and b) to form one double channel. A solution of 0.1 mM Rhodamine B in a HEPES buffer was pumped through both channels. This buffer consisted of 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 99.9%, Merck, Germany), 16 mM NaCL, corrected to pH 7.4 using NaOH. Rhodamine B was stored in the dark at -80°C until dilution and the experiments were performed in a dimly lit room to prevent photo-bleaching.

2.2.2 Microchip experiments

Calibration curves were constructed to correlate fluorescence intensity to temperature. The heater and cooler were used to reach chip temperatures that were set in the Graphical User Interface of the control box. During a heat up sequence, the temperature was increased from room temperature to 25°C and then in 5°C increments to 90°C. After each increment, when the temperature sensor readout was stable within a 0.3°C margin from the set temperature for 5 minutes, the mercury lamp was unblocked and the measurements started directly. This prevented photo-bleaching of the Rhodamine B or heating of the chip by the lamp. For each measurement ten pictures were taken and stored as 1300 by 1030 pixels, 16-bit tiff images.

These images were then analyzed using Matlab. The RGB pictures were loaded and split by color. Only the red color matrix was used. All pixels with a red intensity above a certain cut-off value were included in the evaluation. All other pixels were assumed to be in the background and not used in further calculations. The most stable cut-off value was determined by varying this value and minimizing the sensitivity for the number of included pixels. This was done by fitting an equation for the varied cut-off values to the resulting number of included pixels. This equation's zero-values for the second order derivative were calculated. At different positions on the chip, the background is different. Therefore, a new cut-off value was calculated for each set of

pictures taken at a certain position. The calculated cut-off value was used to include or exclude each individual pixel. The average red intensity of the included pixels (I) was used in further calculations.

Rhodamine B fluorescence intensity decreases exponentially with increasing temperature (Kolodner and Tyson, 1982, 1983). Therefore, the natural logarithm of the ratio of the intensity (I) to the intensity at a reference temperature (I_0) should be linear with temperature (T). This is shown in Eq. (2.1).

$$ln\frac{I}{I_0} = ln(I_0) - c_l \cdot T \tag{2.1}$$

The linear correlation parameter (c_l) is found from heat up and cool down calibration curves. In all experiments the reference temperature is the ambient temperature of 22°C.

Steady state experiments covered the variation of three parameters. Firstly, the effect of air movement was investigated. The chipholder with microscope was shielded from outside air movement and calibration experiments were repeated. Secondly, the influence of different flow rates was tested. Water at room temperature entered the chip which is at 50°C and the flow rate was varied from 0 to 8 μl min^{-1}. Thirdly, the possible temperature gradient over the chip was investigated by repeating the calibration experiments, not directly above the heater, but at some distance (position B, shown in figure 2.1a).

The chip was also tested under transient operation. Calibration, heat up, and cool down experiments were performed under different flow rates. During heat up experiments, the chip was heated from 30 to 90°C. Cool down experiments ran from 90 to 30°C. Pictures were taken every three seconds. From the exponential part of the heat up curves the thermal time constant, τ, was calculated. The lumped capacitance theory states that in such a case fluid will heat up exponentially according to Eq. (2.2) (Incropera and DeWitt, 2002).

$$\frac{T - T_\infty}{T_0 - T_\infty} = e^{-t/\tau} \tag{2.2}$$

In this equation, T is the temperature (K), with subscripts 0 for initial and ∞ for steady state, t is the time (s). Because of the low Biot number, the lumped capacitance theory is valid in the one-diminsional vertical direction from heater, through glass to

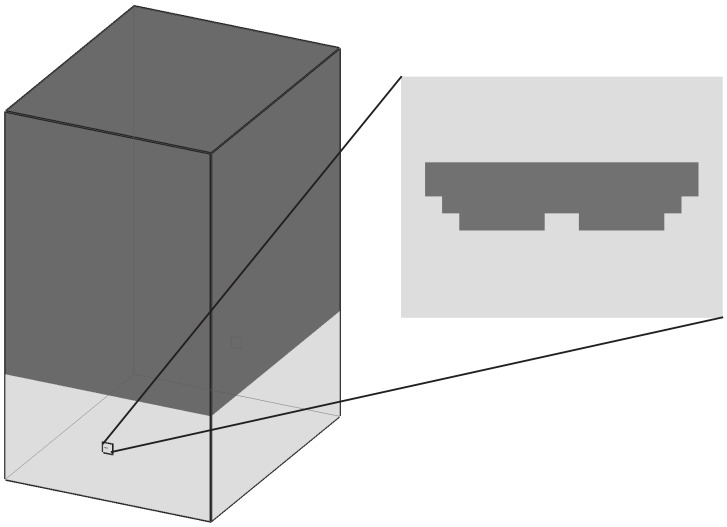


Figure 2.2: Model lay-out, with glass part (bottom, lighter color), air (upper, darker color) and a channel running from front (insert) to back.

air. In this article, the thermal time constant will be used to compare the heat up rate in the early exponential phase.

2.2.3 Computer simulations

The results from the experiments were compared to results from models, which were constructed and run using STAR-CD 3.26 (CD adapco, UK), a finite volume computational fluid dynamics code. The models were meshed using cubes. Calculations were done on Intel Xeon 2.8GHz systems.

The constructed model (fig. 2.2) is a 4 mm wide by 5 mm long detail from the 2.2 mm high chip. The top right tip of the double channel is positioned in the middle of the slice. In the glass, the double channel is presented as a set of fluid cells (see insert figure 2.2). The cross sectional shape is approximated with square cells. The bottom of the chip has a fixed temperature. This corresponds to the practical case, where the heater is a flat copper plate with a controlled temperature. The fluid cells have two types of boundary conditions: an inflow with a fixed velocity and a free outflow at atmospheric conditions. The total volume of air above the chip has four vertical faces and one top side. One side face has an open inflow condition. The other four

sides have atmospheric open outflow conditions. The air and fluid are assumed to be at ambient temperature (22°C). Initially, the glass chip is assumed to be 30°C.

2.3 Results and Discussion

In this section, the results from experimental work and computer simulations are combined. Firstly, the validity of the experimental method is discussed. Secondly, the steady state effect of some process parameters is investigated by experimental work and simulations. Lastly, the microfluidic chip is heated up and cooled down under different conditions. The practical experiments of all three of these aspects under investigation were performed at two positions of the chip. They are both discussed in each section.

2.3.1 Method validation

At two positions on the chip (A and B, see fig. 2.1a), temperature dependent fluorescence pictures were taken after stepwise increase and decrease of the temperature. Figure 2.3a shows the correlation between heater temperature and the fluorescence intensity, expressed as $ln(I/I_0)$. This steady state measurements graph shows a linear correlation parameter of 2.0 % K^{-1} (linear regression, $R^2 > 0.99$), in line with the literature (Kim et al., 2000; Mao et al., 2002b; Sakakibara and Adrian, 1999). The same procedure was followed for heat up and cool down sequences and nearly the same correlation was found. This shows that the time taken to assume steady state was sufficiently long. In additional experiments, the correlation between heater temperature and $ln(I/I_0)$ was investigated when a picture integration time of 0.5 in stead of 1.17 seconds was used. The same linear correlation parameters were found; therefore a picture integration time of 0.5 seconds can be used in transient experiments. Furthermore, the 95% error bars is most cases are so small that they are partially blocked by the graph symbols (dots) Only at 90°C, the error bars are significant. The temperature should be too low for boiling and no other artifacts were observed to explain this behavior.

The linear correlation coefficient of 2.0 % K^{-1} is used to calculate the fluid temperature at position B (see figure 2.3b). Again, the 95% error bars are very small. Figure 2.3b shows that the fluid temperature at a small distance from the heater is much lower than would be expected from the read-out of the temperature sensor. At position B, the fluid temperature rises by only half of the temperature increase

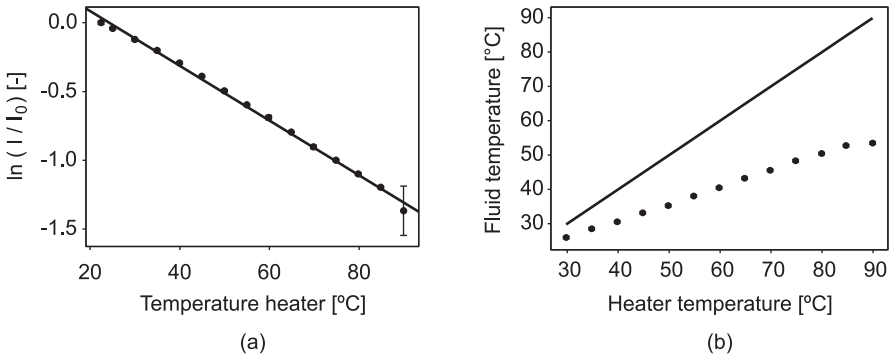


Figure 2.3: a) Temperature relations between heater temperature and $ln\ (I/I_0)$ at position A (calibration curve), b) temperature calibration at position B (•) vs. heater temperature. Error bars indicate 95% confidence intervals.

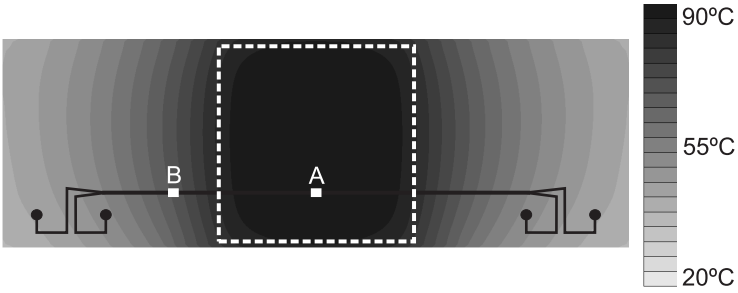


Figure 2.4: Result of computer simulation showing the temperature over the chip at fluid level, including channels (black lines), inlet and outlet holes (black circles), positions of camera (white rectangles), heating element below the chip (dashed white box), and symmetry plane (black dotted line).

reported by the sensor.

The simulation result in figure 2.4 shows the temperature gradient over half the chip with a symmetry plane on the dotted line. The temperature in the channel above the heater is close to 90°C, but drops dramatically with increasing distance from this heater. This steep temperature drop in the simulations corresponds to the experimental findings in this research (fig. 2.3b). The simulations showed that exact temperature profiles are determined by the exact geometrical shape and materials of the chip and chipholder and the connecting interfaces. Deviations between experimental and model results (up to several degrees at position B) are the topic of a present study in our lab.

2.3.2 Air flow velocity variation

During normal operation, microchip set-ups are used in a closed space. As a result, only low velocity movement air, less than 1 m s^{-1}, is expected. To check for the effect of forced convection a shielded set-up with only natural convection is tested. The results from the shielded set up showed a linear correlation parameter of 2.0 % K^{-1}, for heat up, and 1.9 % K^{-1}, for a cool down sequence. This corresponds to the results from the unshielded set-up (see section 2.3.1).

The effect of air velocity was also investigated by means of computer simulations. In the simulations, the chip is heated to 50°C, the initial temperature of the fluid, and the air is 22°C. The fluid flows through the channels with an average flow rate of 1 μl min^{-1}, which is commonly used in practical operation. Figure 2.5 shows the effect of three different air flow velocities on the temperature of the fluid in the channel. An air flow velocity of 0 or 1 m.s^{-1} has negligible effect on the temperature of the fluid. At 1 m s^{-1} the fluid heats up to 48°C after flowing through 3 mm of channel. At 5 mm from the point of entrance, the temperature is nearly 49°C, which is only 1°C from the set temperature. When there is no forced convection, the temperature effect is even smaller. In the case of strong forced convection (at 10 m s^{-1}), the effect is much stronger. At 5 mm from the entrance, the temperature is still almost 4°C below the set temperature.

The results from both practical experiments and computer simulations show that limited forced convection has little effect on the fluid temperature. Fluid enters the microfluidic chip through two single channels that meet to form a double channel after

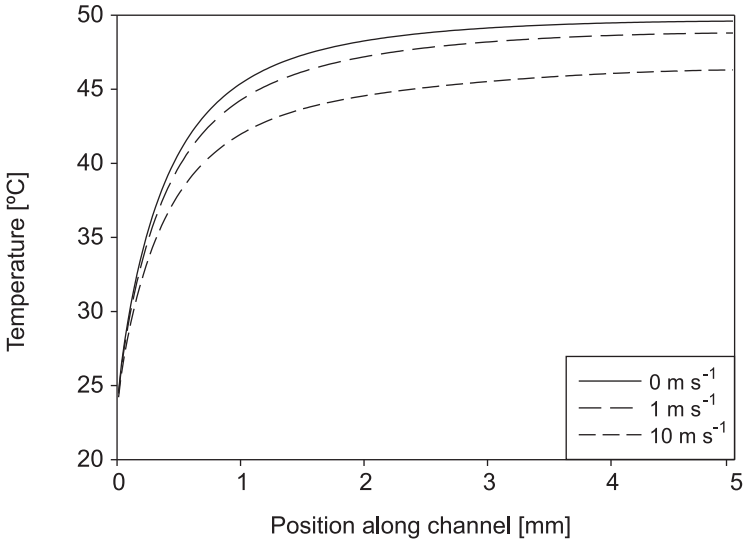


Figure 2.5: Effect of air flow velocities from 0 to 10 m s^{-1} on the fluid temperature (computer simulations). Initial temperature is 22°C and set temperature is 50°C.

a few millimeters (see fig. 2.1a and b). Chip functionality usually starts after the joining of the two channels. Therefore, the first millimeters are less important. Results show that the temperature, after a few millimeters, is close to the set temperature as long as there is no strong convection.

2.3.3 Fluid flow rate variation

The effect of flow rate variation on the fluid temperature was tested (see figures 2.6 and 2.7). Figure 2.6 shows the fluid temperatures on position A and B at selected flow rates. In constructing this graph, we assumed the lowest flow rate (0.01 μl min^{-1}) had no effect on the temperature which was dictated by the known temperature profiles. All other temperatures were based on this first value. Figure 2.6 shows that all flow rates had little effect on the temperature at location A. The maximum difference between the highest and the lowest value is in the same order of magnitude as the 95% confidence interval. Furthermore, no clear trend is visible.

The temperature behavior of the fluid at position B deviates somewhat more from the set temperature and does show a trend. Figure 2.6 shows that at higher flow rates the temperature at position B is somewhat lower. This lower temperature could

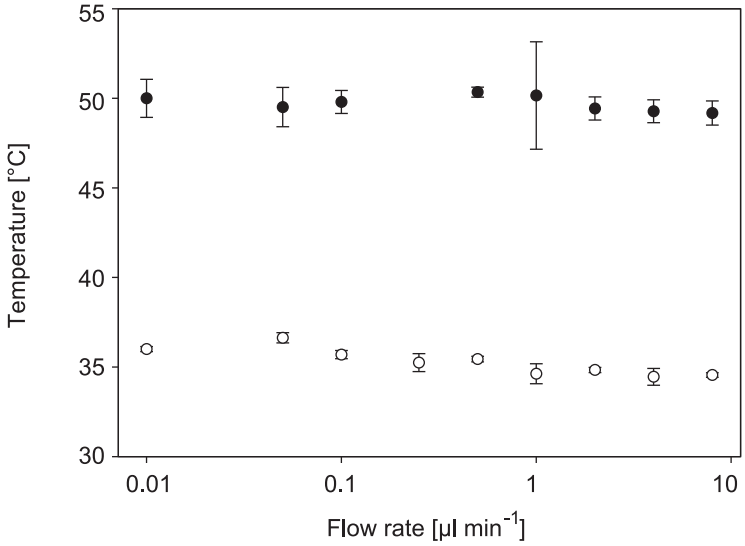


Figure 2.6: Effect of different flow rates on fluid temperature at positions A (•) and B (◦). Data obtained from microchip experiments.

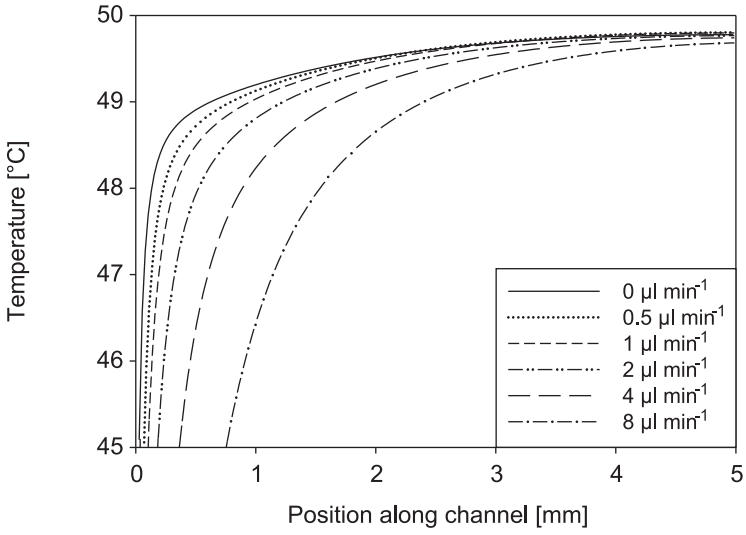


Figure 2.7: Effect of different flow rates of water on fluid temperature as a function of position along the channel. Positions A and B are not shown, as they are further along the channel. Data obtained from computer simulations.

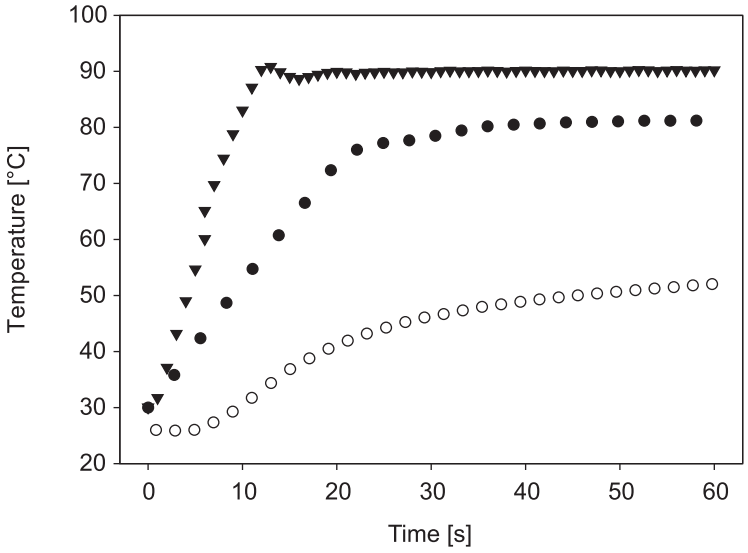


Figure 2.8: Fluid temperature at position A (●), B (○), and heater temperature (▼) during heat up of chip.

have two reasons. Firstly, the residence time of the fluid in the chip could have been too short to ensure heat up (i.e. a temperature gradient in the fluid). Secondly, the larger fluid volume entering the heated chip could have cooled down the chip (i.e. a temperature gradient in the chip).

Computer simulations (fig. 2.7) show that with increasing flow rate, the fluid indeed remains at a lower temperature in a larger portion of the channel. This is most clear within the first millimeter of the channel. At 5 millimeters into the channel the effect of flow rate is less than 0.5°C. This finding confirms the results from the practical experiments, which were conducted a few centimeters into the channel.

2.3.4 Transient operation

Figure 2.8 shows the change in temperature over time at positions A and B in the chip, and the heater temperature (90°C). The heater temperature rises faster than the temperature at position A, while the temperature at the position B rises slowest. Furthermore, the rise in temperature does not start at the same moment. Again, the heater temperature reacts faster than the temperature at position A, while the temperature at position B reacts even later. Finally, the final temperature at the

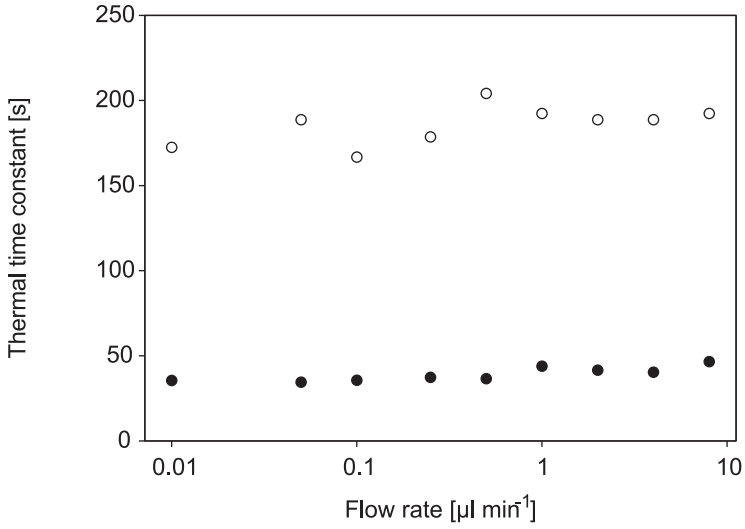


Figure 2.9: Effect of flow rate on thermal time constant for heat up (•) and cool down (○). Measurements from position B.

different positions does not seem to be the same, which is in agreement with the steady state results.

The delay in the start of heat up is caused by the distance from the heater to the position of temperature measurement. This delay is in accordance with penetration theory calculations. The distance to the heater also influences the heating rate. Parts of the chipholder drain energy from parts of the chip, resulting in a temperature gradient over the chip. This determines the steady state temperature at each location. The magnitude of this possible heat drain is determined by the thermal properties and the size of both the chipholder and the chip and by the size and position of the contact area between the chipholder and the chip.

Heat up (such as fig. 2.8) and cool down curves were measured for different flow rates. From these curves we determined the maximum heat up and cool down rate. The effect of flow rate is expected to be strongest in the part of channel before it reaches the heater. For this reason pictures were taken at position B. With the assumption that the channel wall dictates the fluid temperature, the thermal time constants were calculated. Figure 2.9 shows that the thermal time constant for heat up is lower than for cool down. In the case of heating, the chip is heated directly by

a hot plate. Because of the relatively large heating power, varying the flow rate has no effect on the speed of heat up. The chip is solely cooled by forced convection of air over the heater. Figure 2.9 also shows that different flow rates have no effect on the cool down rate. This is line with the expectation that the heat capacity of the air is the limiting factor.

2.4 Conclusions

During this research a glass microchip in a PEEK chipholder was heated with a local and external resistance heater. The chip was cooled with forced air convection. The effect of several practical operation parameters on the temperature was tested. Steady state temperatures varied greatly over the length of the chip. This spatial variation also applied for heat up and cool down rates. Furthermore, when the chip was operated under moderate circumstances, the air flow velocity (< 1 m s^{-1}) and fluid flow rate (< 8 μl min^{-1}) had no effect on the fluid temperature. These results from practical experiments were confirmed by results from computer simulations. The results in this article outline the limitations and the possibilities of a microfluidic chip's thermal behavior. The conclusions can be used in operating a temperature controlled microfluidic chip.

Nomenclature

c_l	linear correlation parameter, (K^{-1})
I	fluorescence intensity, $(-)$
I_0	reference fluorescence intensity, $(-)$
t	time, (s)
T	temperature, (K)
T_0	reference temperature, (K)
T_∞	temperature at infinite time, (K)
τ	thermal time constant, (s)

References

Auroux, Pierre-Alain, Dimitri Iossifidis, Darwin R. Reyes, and Andreas Manz. 2002. Micro total analysis systems. 2. Analytical standard operation and applications. *Analytical Chemistry* 74(12):2637–2652.

Chabinyc, Michael L., Daniel T. Chiu, J. Cooper McDonald, Abraham D. Stroock, James F. Christian, Arieh M. Karger, and George M. Whitesides. 2001. An integrated fluorescence detection system in poly(dimethylsiloxane) for microfluidic applications. *Analytical Chemistry* 73:4491–4498.

Dixon, W.J. 1953. Processing data for outliers. *Biometrics* 9(1):74–89.

Erickson, David, David Sinton, and Dongqing Li. 2003. Joule heating and heat transfer in poly(dimethylsiloxane) microfluidic systems. *Lab on a Chip* 3:141–149.

Guijt, Rosanne M., Arash Dodge, Gijs W.K. van Dedem, Nico F. de Rooij, and Elisabeth Verpoorte. 2003. Chemical and physical processes for integrated temperature control in microfluidic devices. *Lab on a Chip* 3:1–4.

Incropera, Frank P., and David P. DeWitt. 2002. *Fundamentals of heat and mass transfer - 5th edition*. New York: John Wiley & Sons, Inc.

Jensen, Klavs F. 2001. Microreaction engineering - is small better? *Chemical Engineering Science* 56:293–303.

Keuren, Edward van, Mark Cheng, Oliver Albertini, Cheng Luo, John Currie, and Makarand Paranjape. 2005. Temperature profiles of microheaters using fluorescence microthermal imaging. *Sensors and Materials* 17(1):1–6.

Kim, Haeng-Boo, Kousei Eueno, Masahiro Chiba, Osamu Kogi, and Noboru Kitamura. 2000. Spatially-resolved fluorescence spectroscopy study on liquid/liquid extraction processes in polymer microchannels. *Analytical Sciences* 16:871–876.

Kolodner, Paul, and J. Anthony Tyson. 1982. Microscopic fluorescent imaging of surface temperature profiles with 0.01°C resolution. *Applied Physics Letters* 40(9):782–784.

———. 1983. Remote thermal imaging with 0.7-μm spatial resolution using temperature-dependent fluorescent thin films. *Applied Physics Letters* 42(1):117–119.

Mao, Hanbin, Matthew A. H Olden, Min You, and Paul S. Cremer. 2002a. Reusable platforms for high-throughput on-chip temperature gradient assays. *Analytical Chemistry* 74:5071–5075.

Mao, Hanbin, Tinglu Yang, and Paul S. Cremer. 2002b. A microfluidic device with a linear temperature gradient for parallel and combinatorial measurement. *Journal of the American Chemical Society* 124:4432–4435.

Reyes, Darwin R., Dimitri Iossifidis, Pierre-Alain Auroux, and Andreas Manz. 2002. Micro total analysis systems. 1. Introduction, theory, and technology. *Analytical Chemistry* 74(12):2623–2636.

Ross, David, Micheal Gaitan, and Laurie E. Locascio. 2001. Temperature measurement in microfluidic systems using a temperature-dependent fluorescent dye. *Analytical Chemistry* 73:4117–4123.

Sakakibara, J., and R.J. Adrian. 1999. Whole field measurement of temperature in water using two color laser induced fluorescence. *Experiments in Fluids* 26:7–15.

Samy, Razim, Tomasz Glawdel, and Carolyn L. Ren. 2008. Method for microfluidic whole-chip temperature measurement using thin-film poly(dimethylsiloxane)/Rhodamine B. *Analytical Chemistry* 80(2):369–375.

Sinton, David A., and Dongqing Li. 2003. Microfluidic velocimetry with near-wall resolution. *International Journal of Thermal Sciences* 42:847–855.

Chapter 3

Modelling thermal interactions in a heated microsystem

Abstract

Computational fluid dynamics models were used to estimate the effect of some parameters on the temperature of a heated microsystem. The system consisted of a PEEK chipholder, with a relatively small heater, and a glass chip, surrounded by air. The material and design of the chipholder had a dominant effect on the temperature. Bringing the heater from 22 to 80°C resulted in a temperature gradient of over 40°C over the length of the chip at fluid level. Due to slow heating, quick switching of temperature is not possible. The steady state temperature profile at fluid level can be changed by adapting the geometry and material of the chipholder. By including the system's thermal properties in microfluidic system design desired temperature profiles can be obtained. Computer models, such as described in this paper can be used to design a system which thermal behavior matches the process requirements for the intended use.

This chapter has been submitted for publication as Swarts, Jan W.; Janssen, Anja E.M.; Boom, Remko M. Modelling thermal interactions in a heated microsystem.

3.1 Introduction

Over the past decades the use of microtechnology in chemical, pharmaceutical and biotechnological areas has become more and more commonplace (Auroux et al., 2002; Jensen, 2001; Reyes et al., 2002). In many of these areas temperature control is of great importance, for instance in maintaining mild conditions for bio-chemical reactions (DeWitt, 1999; Eijkel et al., 1998; Fletcher et al., 2002; Jähnisch et al., 2004). Temperature control can be achieved by means of in-chip heating devices (Keuren et al., 2005; Lao et al., 2000; Mao et al., 2002b; Wijngaards et al., 2004), out-of-chip heating devices (Kopp et al., 1998; Mao et al., 2002a; Swarts et al., 2008), and non-contact heating (or cooling) (Oda et al., 1998; Slyadnev et al., 2001; Sundaresan et al., 2005).

In-chip heating is very fast because of the short distance between heater and fluids. It also reduces the response time from sensor to actuator (i.e. heater or cooler). In-chip heating is most frequently described in literature, however it has the disadvantage that it needs heaters in every microchip. Out-of-chip heating devices and non-contact heating or cooling systems can make use of one multiple-use heating device and many simpler discardable microchips. As these microchips have no need for electrodes or other temperature actuators embedded in the chip, chip-fabrication can be kept relatively simple.

In this paper the temperature profile in a chipholder with a relatively small out-of-chip heater and a microfluidic chip is investigated. The out-of-chip heater covered only part of the chip, which has consequences for the temperature distribution over the chip. The goal of this research is to understand the temperature distribution in the chip and the contribution of all relevant parameters of the system. Two-dimensional numerical calculations were done to estimate the contribution of some of the parameters. Next, a three-dimensional model is used to assess the steady state and transient temperature profiles. Some parameters in this three-dimensional model were changed to investigate the chip and chipholder temperature with variations to the original chipholder design. The results from these numerical models can be used to predict the effect of design and engineering choices on the temperature profile in the chip.

3.2 Materials and Methods

The numerical models in this article are constructed to resemble physical geometries, that are used in practice. Both the physical geometry and the numerical methods will be discussed.

3.2.1 Physical geometries

The set-up consists of a microfluidic chip, a chipholder with a heater, air flowing over the chip, and fluids flowing through the chip. The microfluidic chips are made of borofloat glass, and are produced by Micronit (Enschede, NL). The chips are 15 mm wide, 45 mm long and 2.2 mm high. This glass chip is transparent for visible light and chemically inert. Other physical properties of this glass can be found in table 3.1. The chip has wet-etched channels at the middle of its height (1.1 mm from the bottom). A part of the chip's lay-out is schematically displayed in figure 3.1. The channels are two single channels combining into a double channel (C in fig. 3.1). The double channel is 85 μm wide and 20 μm deep. The total length is approximately 5 cm. The total channel volume is about 0.09 μl. The chip has powderblasted holes which provide outside access to the channels (B in fig. 3.1). In figure 3.1, the dashed black line in the middle indicates the position of the cross section, shown in figure 3.2a. As the whole system (chip, chipholder, air) is symmetrical in this plane, only half of the system needs to be modelled.

Table 3.1: Physical properties of materials

Material	density kg m^{-3}	specific heat kJ kg^{-1} K^{-1}	thermal conductivity W K^{-1} m^{-1}
Borofloat glass	$2.2{\cdot}10^3$	0.9	1.2
PEEK	$1.3{\cdot}10^3$	1.34	0.25
Air	1.205	1.006	$26.4{\cdot}10^{-3}$
Aluminium	$2.702{\cdot}10^3$	0.903	237
Stainless steel	$8.055{\cdot}10^3$	0.48	15.1

As is shown in fig. 3.2b, the chipholder is a two piece construction which clamps the glass chip (D in fig. 3.2a) between a top part (B in fig. 3.2) and a bottom part with heater (F in fig. 3.2a and c). The chipholder is made from Poly-Ether-

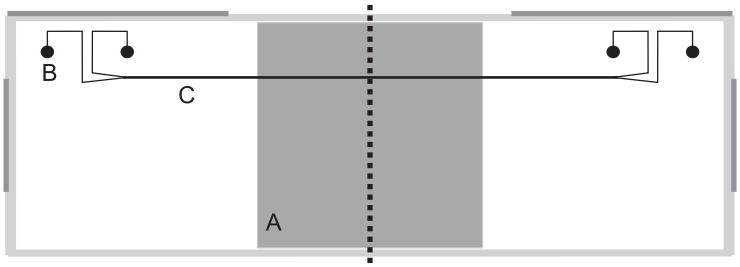


Figure 3.1: Top view of chip lay-out with heater (A), access holes (B), and fluid channel (C).

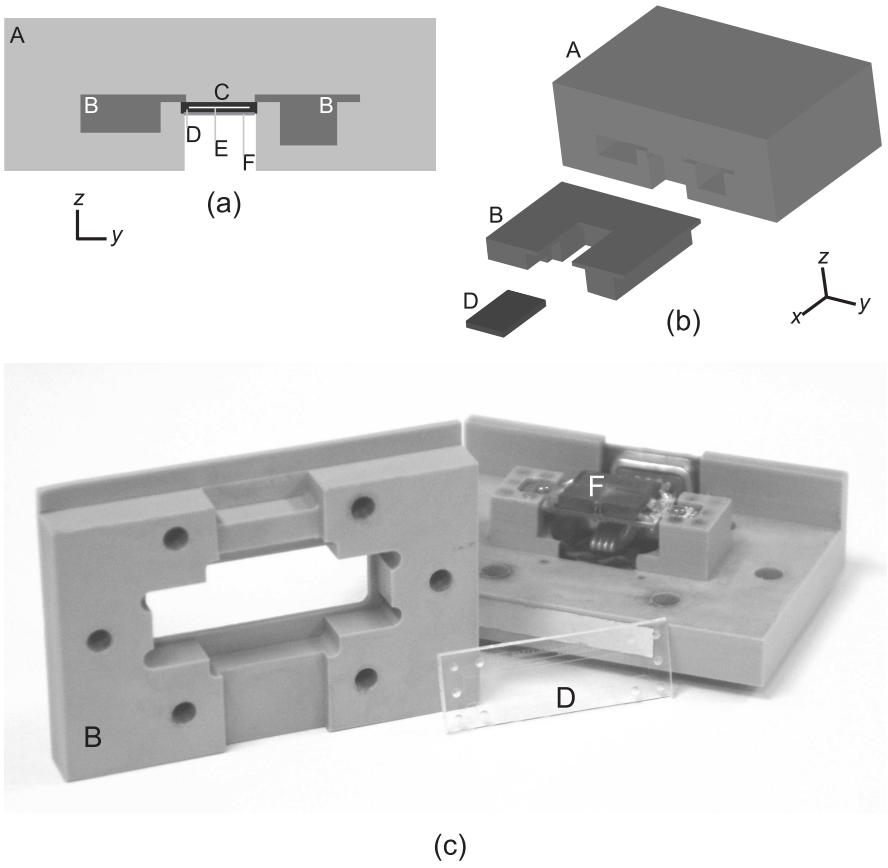


Figure 3.2: 2D (a) and 3D (b) model geometries of chipholder (B) with optical inspection window (C), heater (F), holding a microchip (D) with fluid channels (E) in ambient air (A), c) photograph of the microsystem.

Ether-Ketone (PEEK), which was chosen for its chemical inertness and durability. Relevant thermal properties of PEEK are presented in table 3.1. The two parts of the chipholder are pressed together with metal screws and bolts, which are at a sufficient distance from the chip not to have an effect on the temperature. The chipholder is designed to enable visual inspection of the chip from the top (C in fig. 3.2a) and ensure structural rigidity.

The top part of the chipholder (left and right, B in fig. 3.2a) touches the top part of the chip along its entire perimeter. This contact is approximately 1 mm wide (varies with position), shown as a gray perimeter in figure 3.1. Furthermore, at some positions the side part of the chip is in contact with the chipholder (darker gray in fig. 3.1, not visible in fig 3.2a). The opening of the chipholder in which the chip is placed is too wide to touch both long sides of the chip, so only one side is in contact with the PEEK (darker gray lines in fig. 3.1).

The bottom part of the chipholder (white area under F in fig. 3.2a) consists of PEEK, printed circuitboard, electrical wiring, springs, and copper plates on the top. The chip only touches the top part of the chipholder and the 14 x 14 mm copper plates (A in fig. 3.1, F in fig. 3.2a). During heating, current runs through a resistance heater, connected to the copper plates. The temperature is measured by sensors on the heater. Both the copper plates and the glass are very smooth so full thermal contact is assumed. There is no additional resistance between these two components. By assuming that the heater is a wall with a fixed temperature on the glass chip, its physical properties are of no importance. The heater's properties are therefore not included in the table.

The last component included in the models is the air (A in fig. 3.2a) which is in contact with the chip and chipholder. When the air above the chip is heated or cooled, buoyancy effects could influence the chip temperature. Again, the relevant physical properties can be found in table 3.1. For all buoyancy calculations air is considered to be an ideal gas. The dynamic viscosity is described using the Sutherland equation with a dynamic viscosity of $1.716 \cdot 10^{-5}$ kg m^{-1} s^{-1} at 0°C and a Sutherland constant of 116.

The fluid channels (C in fig. 3.1 and E in fig. 3.2a) are not included in the calculations. During normal operation, only a small volume of fluid runs through the chip (typically 1 mg sec^{-1} through a 3 gram chip). As we have shown in earlier

research, these moderate flow rates should have no effect on the temperature of the chip (Swarts et al., 2008).

3.2.2 Numerical methods

Two-dimensional models

Figure 3.2a shows the 2D geometry which was used for calculations. The 2D structure is actually a thin slice of the 3D set-up (fig. 3.2b). This pseudo 2D (slice of 3D) model is considered to have symmetry planes on the y-z planes (x-direction is perpendicular to the visible plane). The heater under the glass is considered to have no physical presence other than its projection on the glass chip. Because of this, a horizontal wall under the chip is used as a heater boundary. This boundary has a fixed temperature with no heat resistance to the chip. As the heater acts as a fixed temperature wall, the material under the heater does not influence the temperature of the chip and is subsequently not included in calculations. The bottom of the model has a wall boundary condition at room temperature. This is the bottom part of the chipholder which can only be heated indirectly by radition from the top part and heated air. These effects are expected to be minimal. The hole under the heater is a complex structure of several parts, but is not expected to have a strong temperature effect. It is modelled to be an adiabatic wall. All other boundaries are considered to be 'pressure' boundaries, including mass transfer (buoyancy effects) and heat transfer (convection through buoyancy, conduction, and radiation). The chip and chipholder are modelled to radiate to a 20°C wall outside the model, with an emissivity of 0.2 and a thermal reflectivity of 0.8.

The total geometry is 85 mm wide and 30 mm high. This includes a 10 mm layer of air on the sides, a 5 mm layer on the bottom, and a 15 mm layer of air on the top of the chipholder. The total geometry was meshed evenly into even 0.25 mm sized cubes in the inner core on the model comprising of the chip, chipholder and an air layer of at least 3 mm. To minimize the required number of cells, the air outside this model inner core was meshed to 0.5 mm cubes. The 2D geometry was 0.5 mm deep (perpendicular to screen) and fluid flow in the flat x-direction was not calculated.

In this model no forced convection is assumed, so all air movement is caused by buoyancy. In order to investigate the effect of freely flowing air, buoyancy was swithed on and off. Furthermore, we investigate the effect of the thermal properties of PEEK. To do this the chipholder with its thermal properties (specific heat, thermal conducti-

vity etc.) were eliminated from the model. The interface between the PEEK and the air or chip is then replaced by a 'wall' condition with constant ambient temperature. The chipholder functions as a heatsink in that configuration.

Three-dimensional models

A three-dimensional model of the system in figure 3.2b was constructed. The part right from the black line in fig. 3.1 was replaced by a symmetry plane. This symmetry plane is located on front faces in the x-y plane of fig. 3.2b and is the same as the front side of the 2D model (fig. 3.2a). The total structure measures 55 x 85 x 30 mm (l x w x h) and consists of cubic 0.25 mm cells in the core and 0.5 mm cells on the outside, similar to the 2D geometry.

The model has three types of boundaries: wall, symmetry and pressure boundaries. First, the heater was positioned as shown in fig. 3.1 and 3.2a and was fixed at 80°C. No heat resistance was assumed. There was a wall boundary on the bottom of the geometry fixed at 22°C, and an adiabatic wall around the hole under the heater. Second, the right side of the model was given a symmetry boundary. Third, the outside of model (all air) is given an atmospheric pressure boundary condition. The PEEK chipholder was included with all of its thermal properties. All parts had an initial temperature of 22°C. Full thermal contact between air and either chip or chipholder was assumed. The radiation properties were the same as for the two-dimensional case.

Variations on chipholder design

The three-dimensional model was adapted to simulate different design choices that could be made. The model remained unchanged with the exception of one of the following variations. First, the chipholder was modelled not to be made from PEEK, but from aluminium. Second, the chipholder material was changed to stainless steel. The properties of these materials can be found in table 3.1. Third, the 23 x 13.5 mm (11.5 x 13.5 mm with symmetry plane) see-through hole in the top of the PEEK chipholder was elimated (C in fig. 3.2a). This means that the top of the chipholder was completely closed. These three-dimensional models were run in steady state with no buoyancy effects.

Computational fluid dynamics code

The numerical models described in this article were constructed and run using a commercial computational fluid dynamics code (STAR-CD 3.26, CD-adapco, London UK). This code solves the geometry with initial and boundary conditions using a finite volume method (CD adapco Group, 2004). The geometries here were meshed in a highly structured way using regular hexahedrons. Calculations to normalized residuals below 10^{-6} were done on Intel Xeon 2.8GHz CPUs and took between 1 minute (simple steady state situations) to 100+ hours (long running transient models).

3.3 Results and discussion

The effect of some parameters such as buoyancy and thermal properties of the chip-holder was investigated. First, 2D steady state calculations were done to estimate the effect of buoyant air and to assess the thermal effect of PEEK. Second, some 3D simulations were done to investigate the temperature gradient over the whole chip in steady state and transient mode. Third, the 3D steady state models were used for variation of some design parameters. Finally, we give some design guidelines based on the results.

3.3.1 Two-dimensional models

Effect of movement of air on the temperature of the chip

Figure 3.3 shows the steady state temperature distribution in the cross section of the chip with (fig. 3.3a) and without air movement (fig. 3.3b). The lowest temperatures are found in the corner of the glass, where the chip touches the PEEK chipholder. The temperature is highest near the heater. On the possible positions of the channels (indicated by the white line in fig. 3.3) we find a lowest temperature of above 79°C. Figure 3.3b shows the temperature distribution when air is buoyant. The movement of air above the chip is limited ($v \ll 1$ m s^{-1}). The temperature profile is similar to the case without air movement. The lowest temperature found at possible channel positions is approximately 79°C. The maximum difference in temperature is only a few tenths of a degree and this suggests that buoyancy can be neglected in this model.

Figure 3.3: 2D cross section of chip, temperature profile when a) excluding and b) including air movement. White line indicates possible position of fluid channels. Legend is cut short, temperatures below 70°C are indicated as 70°C. Model geometry is part of fig. 3.2a.

Effect of thermal properties of PEEK on the temperature of the chip

Another aspect under investigation is the effect of the thermal properties of the PEEK chipholder. At full 'heatsink capacity' the component has such thermal properties, that it will remain at the initial temperature and that it will cool down all components connected to it (when $T_{heater} > T_{ambient}$). Figure 3.4 shows the temperature distribution in the whole system for PEEK as a heatsink (fig. 3.4a) and PEEK with its thermal properties (fig. 3.4b).

When the chipholder is considered to be an ideal heatsink (shown in fig. 3.4a), the largest temperature gradients can be found in the air. The inset shows the temperature in the chip, which is similar to fig. 3.3. The lowest temperatures are found in the top corners, at the ambient temperature boundary condition. The channel has a lowest temperature of approximately 65°C.

Figure 3.4b shows the temperature distribution when all thermal properties of PEEK are included in the calculation. As the PEEK takes up thermal energy and its temperature rises, this heated PEEK heats the air. In this way, the temperature profile in the whole system is affected. The chipholder also has a strong direct effect on the temperature of the chip. In the chip part near the top corners, the temperature is much higher than in fig. 3.4a. The temperature of the chip at the possible channel locations is over 77°C (fig 3.3a reveals it is over 79°C). The temperature at possible fluid position can differ over 13°C depending on the in- or exclusion of the materials thermal properties. This difference can have an impact for many applications of the system.

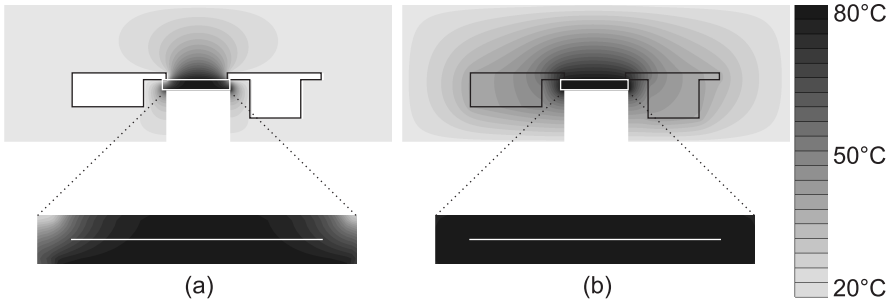


Figure 3.4: Temperature profile in 2D cross section of chip, chipholder, and air, when a) PEEK is a heatsink and b) PEEK has all thermal properties. The chip is shown enlarged as an inset.

3.3.2 Three-dimensional models

Steady state model

In the previous paragraphs, air flow by buoyancy was shown to have a small effect. Therefore, the model in three dimensions does not include buoyancy effects. Since the inclusion of all thermal properties of PEEK did have an effect on temperature, the chipholder is fully included in the 3D models. As in all earlier models the system was initially at a room temperature of 22°C. The heater is set to 80°C. The system is relaxed to steady state. Results from this simulation are presented in fig. 3.5.

Figure 3.5 shows top views of the chip, at a height just above the heater (fig. 3.5a), and at fluid level (fig. 3.5b). The temperature profiles in figure 3.5 show a steep gradient in the length direction of the chip (horizontal in figure). This gradient is the result of the short distance between the fixed high temperature of the heater and the low temperature of the chipholder. Lower temperatures are found at fluid level (1.1 mm above the bottom), when compared to the bottom (adjacent to heater). In steady state, the main temperature gradient is positioned a) in the air above the chip, directly above the heater and b) in the glass, away from the heater (results not shown here).

There is a difference between the top part of the figures (partial side-contact with chipholder) and the bottom part of the figures (no side-contact with the chipholder). This difference is visible but not very large. Apparently this partial contact is not very important and is not investigated any further.

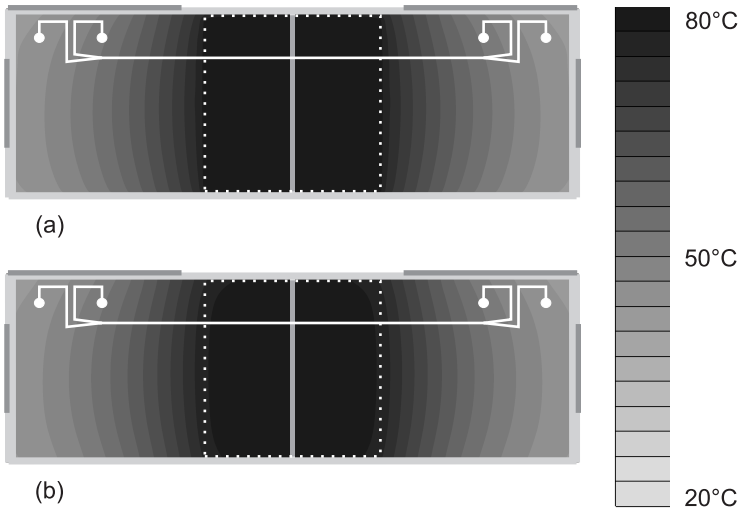


Figure 3.5: Top view of chip, temperature profile with chipholder effects at a) bottom of chip and b) fluid level. White lines indicate possible position of channels. White dashed rectangular indicates position of heater. Results from 3D models.

Transient model

The steady state three-dimensional model was used for transient calculations. The chip with chipholder and air were heated by the heater with a set temperature of 80°C. The whole system was initially at 22°C. The heating process was followed for 2000 seconds. Figure 3.6 shows the results of these models as the temperature in the chip at fluid level. Only half of the chip is shown; the right hand side of the chip is a symmetry plane.

Figure 3.6 shows that temperature development is rapid in the first few seconds, but as time progresses, the profile becomes more and more stable. To give an indication of the temperature development, one point in the chip is followed in time. In normal chip design, the area available for channels is about 80% in both length as width direction. Within this area, the lowest temperatures can be found in the corner, at the position furthest from the heater. This position can be found in in the chip, at 4.5 mm away from the edge in the length of the chip and at 2.5 mm from the edge in the width direction. After 300 seconds the total temperature increase (from ambient to steady state) at this position is at 81%. The temperature is not stable, but changes rather slowly. After 1600 seconds, the temperature is at less than 0.1°C from the

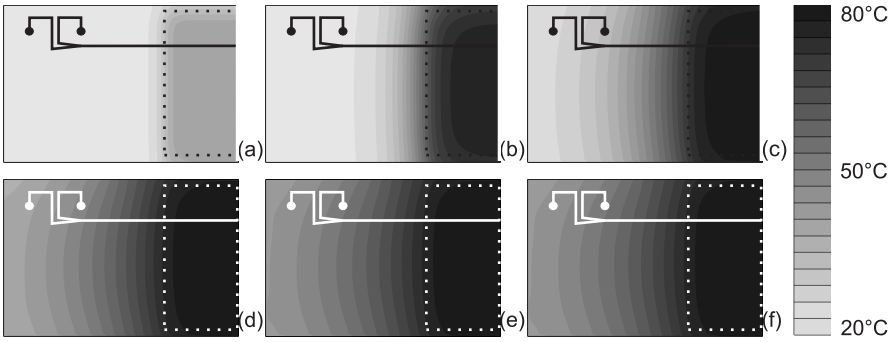


Figure 3.6: Chip temperature at fluid level (top view) after a) 1 second; b) 10 seconds; c) 50 second; d) 300 seconds; e) 1600 seconds; and f) reaching steady state. Results from three dimensional models with symmetry plane at right side. Black and white lines indicate position of channels and heater (dotted).

steady state ($< 0.3\%$ of total temperature increase).

This information can be used to estimate what time it would take to reach steady state in practical experiments. After 1600 seconds the temperature profile is nearly the same as in steady state (< 0.1°C). This period can be assumed to suffice for steady state experiments. In a system with a relatively small heater and a large chip, some parts of the chip are too far from the heater to quickly respond to a change in heater temperature. Therefore, temperature control with a short response time is not possible.

Some effects of material choice and chip design

Figure 3.7 shows effect of choice of material for the chipholder. The top picture (fig. 3.7a) shows that the temperature profile in the chip at fluid level changes dramatically when the chipholder is made from aluminum instead of PEEK. The temperature at the long edges (left in the picture, also shown in fig. 3.7d) now stays above 50°C. This is a difference of at least 10°C when compared to the original case. Furthermore, in the middle of the chip (near the symmetry plane at the right side of the picture) the temperature drops towards the top and bottom of the picture. This temperature drop was not visible with the PEEK chipholder.

Figure 3.7b shows the temperature profile in the case of a stainless steel chipholder. This profile is similar to the profile with the aluminum chipholder. The temperature

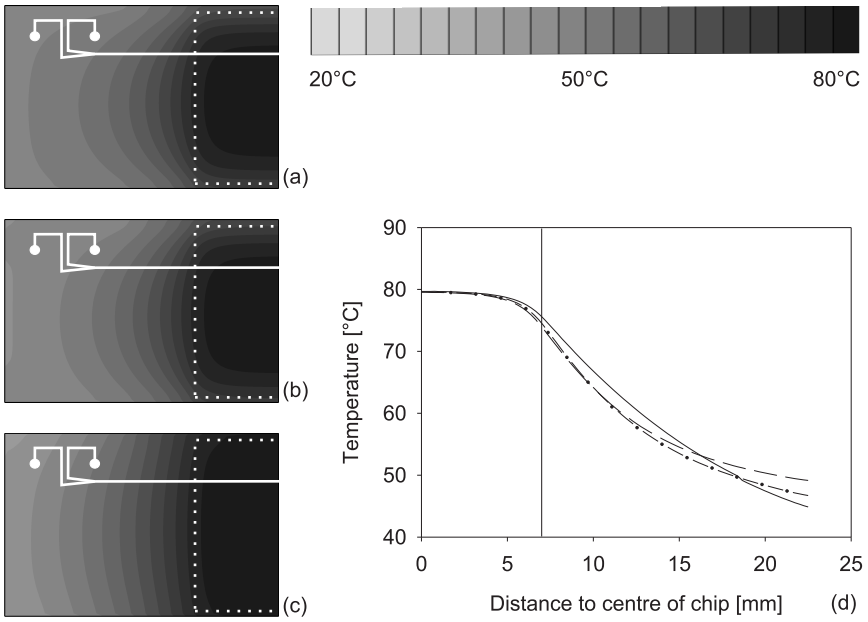


Figure 3.7: Steady state chip temperature at fluid level (top view), variations to standard model; a) aluminium chipholder, b) stainless steel chipholder, and c) PEEK chipholder without optical inspection window. Results from three dimensional models with symmetry plane at right side. White lines indicate position of channels and heater (dotted). Dotted circle in a) indicates corner position of fluid channel area. The temperature at fluid level in the middle of chip (horizontal right to left) is presented in d) with a dashed line for aluminium, dash-dot-dash line for stainless steel, and drawn line for PEEK chipholder.

near the long end of the chip is just below 50°C (also visible in fig. 3.7d) and the temperature drop in the middle of the channel towards the top and bottom of the picture is less steep as compared to fig. 3.7a. This means that the temperature profile of the stainless steel chipholder is in between the profiles of the aluminum and PEEK chipholders.

The bottom picture (fig. 3.7c) shows the profile when a chipholder is modelled without the inspection window in the top. The area above the heater is fully in the 76 - 80°C range (more clearly shown in fig. 3.7d). The temperature drop near the top and bottom of the picture in the middle of the chip is not observed as it was in the previous two variations. The temperature drop in the length direction (horizontal in picture) away from the heater position is steeper than in the aluminum and stainless steel case. In general, the 'closed-top' chipholder's temperature profile looks more

one-dimensional in the length direction of the chip.

When we review the results with the aluminum and stainless steel chipholders, we observe two things. First, the overall temperature is more even over the entire chip. The lowest temperature found at fluid level is about 10°C closer to the heater temperature than in the original PEEK case. This suggests that such a chipholder would allow a larger part of the chip to be isothermal. Second, the temperature near the heater, especially near the edges of the chip (top and bottom of pictures), drops dramatically over a short distance. This indicates that the fluid channels should not be too close to the sides of the chip.

The elimination of the window from the PEEK chipholder has an effect on the temperature profile. The temperature at the long end remains a few degrees higher than in the original chipholder with inspection window. The temperature at the top of fig. 3.7c is slightly lower than at the bottom the picture. This is similar to the case of the original chipholder. The top part of the pictures shows the temperature at the long side of the chip which is in contact with the chipholder on both the top and the side part of the chip. The bottom of picture shows the side of the chip that only touches the chip on the top. The additional contact area at the top side of the picture resulted in a lower temperature.

These two types of profiles (fig. 3.7a and b vs. 3.7c) are caused by the thermal properties in the former cases and the contact area between the chip and chipholder in the latter case. Both aluminum and stainless steel have a much higher thermal conductivity (respectivelly 950 and 60 times higher) than PEEK. With a PEEK chipholder the air will more quickly extract heat from the system, compared to the aluminum and stainless steel cases. The PEEK will function more as as insulator and remain at a lower temperature. The aluminum and stainless steel will more rapidly transport the heat away from the chip and attain a much higher temperature. The volumetric specific heat (specific heat divided by density) of aluminium and stainless steel is lower than PEEK. This is not expected to have a large effect on the steady state situation, though it could affect the transient behaviour, as the increased amount of energy needed to heat up the chipholder will have to be supplied by the same heater.

The effect of adding more contact area between the chip and chipholder by filling up the inspection window can affect the temperature of the chip. Even in the case of

a poor conductor such as PEEK, the effect of having side contact between chip and chipholder or closing the top is clearly visible.

Design guidelines

Based on these findings some design guidelines can be set up. With a temperature-sensitive process on a chip, designing its microfluidic set-up should have thermal behaviour as one of its key design parameters. Its starting-point should be the desired temperature of the fluid in the chip. From this starting point either the chip or entire set-up should be designed. Often the chipholder is a fixed object. In these cases, the chip could be designed as such that the fluid channels are positioned in the correct temperature area in the chip. Computer models as described in this paper could be used to find out which non-isothermal areas within the chip should be avoided.

When the entire system can be thermally designed from scratch, the position and size of the heater, the chipholder material, and the contact areas between chip and chipholder are crucial. First, the heater (or cooler) is the source of the temperature difference between the system and the ambient environment. By positioning the fluid channels close to this heater and establishing a thermal barrier between these channels and the ambient environment, the fluids can most easily be kept at the desired temperature. This thermal barrier could be the part of the chip above fluid level, part of the chipholder or both.

Second, the choice of material for the chipholder will determine its thermal insulative or conductive properties. With a large heater, an insulating material could result in a more uniform temperature profile, while with a smaller heater a more conducting material is suitable. When the objective is not a stable temperature, but rather a stable gradient, similar reasoning could be applied. Conduction or insulation can generate stable temperature gradients with a single heater of cooler. However, working at high temperatures with a highly conducting chipholder might pose some problems for handling.

Third, the size and position of contact areas between chip and chipholder can be used to generate these stable temperatures or temperature gradients. The contact area will determine what part of the chip will be subject to the heat flow intended to obtain the desired temperature profile. By smart design of the chipholder's geometry a single heater can be used to generate a more complex temperature profile.

The original three dimensional model is an approximation of a real system which was used in experiments (Swarts et al., 2008). Practical results have shown that at a small distance from the heater the temperature of the fluid is dramatically lower than the set temperature of the heater. The results from this paper confirm the experimental findings (Swarts et al., 2008). although similar conclusions can be drawn, temperature differences between the results from experiments and models were up to 10°C. This could be attributed to differences between the experimental and model parameters such as the thermal properties of the type of PEEK, the approximation of the geometry, and some additional resistance between the heater and the chip. In the end, the chip's application determines the allowable margin of error. The combined effort of heater, chipholder, and chip will determine whether these margins are met.

3.4 Conclusions

With the chipholder described in this paper, the effect of buoyancy on the fluid temperature inside the chip is small. The effect of chipholder design and material is much greater. The temperature varied greatly over the chip. The temperature of fluid in the chip depends on its proximity to the heater and the chipholder, and the thermal properties of the chipholder's material. Furthermore, the chipholder in this article is incapable of rapid switching of temperature. It takes several minutes for the parts of the chip at a distance from the heater heat up.

While building a microsystem for temperature sensitive processes, the thermal requirements of the process, such as a stable temperature or a temperature gradient, should be kept in mind during the design phase. Key design parameters are the size and position of the heater, the thermal properties of the chip and chipholder's material, and the position and size of the contact areas between the chip and chipholder. Computer models, as described in this paper, can be used to design or use an appropriate microsystem for thermally sensitive processes.

References

Auroux, Pierre-Alain, Dimitri Iossifidis, Darwin R. Reyes, and Andreas Manz. 2002. Micro total analysis systems. 2. Analytical standard operation and applications. *Analytical Chemistry* 74(12):2637–2652.

CD adapco Group. 2004. *STAR-CD version 3.20 - methodology.*

DeWitt, Sheila H. 1999. Microreactors for chemical synthesis. *Current Opinion in Chemical Biology* 3:350–356.

Eijkel, Jan C.T., Albert Prak, Simon Cowen, Derek H. Craston, and Andreas Manz. 1998. Micromachined heated chemical reactor for pre-column derivatisation. *Journal of Chromatography A* 815:265–271.

Fletcher, Paul D. I., Stephen J. Haswell, Esteban Pombo-Villar, Brian H. Warrington, Paul Watts, Stephanie Y. F. Wong, and Xunli Zhang. 2002. Micro reactors: prinicples and applications in organic synthesis. *Tetrahedron* 58:4735–4757.

Jähnisch, Klaus, Volker Hessel, Holger Löwe, and Manfred Baerns. 2004. Chemistry in microstructured reactors. *Angewandte Chemie International Edition* 43:406–446.

Jensen, Klavs F. 2001. Microreaction engineering - is small better? *Chemical Engineering Science* 56:293–303.

Keuren, Edward van, Mark Cheng, Oliver Albertini, Cheng Luo, John Currie, and Makarand Paranjape. 2005. Temperature profiles of microheaters using fluorescence microthermal imaging. *Sensors and Materials* 17(1):1–6.

Kopp, Martin U., Andrew J. de Mello, and Andreas Manz. 1998. Chemical amplification: continuous-flow PCR on a chip. *Science* 280:1046–1048.

Lao, Alex I.K., Thomas M.H. Lee, I-Ming Hsing, and Nancy Y. Ip. 2000. Precise temperature control of microfluidic chamber for gas and liquid phase reactions. *Sensors and Actuators A: Physical* 84:11–17.

Mao, Hanbin, Matthew A. H Olden, Min You, and Paul S. Cremer. 2002a. Reusable platforms for high-throughput on-chip temperature gradient assays. *Analytical Chemistry* 74:5071–5075.

Mao, Hanbin, Tinglu Yang, and Paul S. Cremer. 2002b. A microfluidic device with a linear temperature gradient for parallel and combinatorial measurement. *Journal of the American Chemical Society* 124:4432–4435.

Oda, R.P., M.A. Strausbauch, A.F.R. Huhmer, N. Borson, S.R. Jurrens, J. Craighead, P.J. Wettstein, B. Eckloff, B. Kline, and J.P. Landers. 1998. Infrared-mediated thermocycling for ultrafast polymerase chain reaction amplification of dna. *Analytical Chemistry* 70:4361–4368.

Reyes, Darwin R., Dimitri Iossifidis, Pierre-Alain Auroux, and Andreas Manz. 2002. Micro total analysis systems. 1. Introduction, theory, and technology. *Analytical Chemistry* 74(12):2623–2636.

Slyadnev, Maxim N., Yuki Tanaka, Manabu Tokeshi, and Takehiko Kitamori. 2001. Photothermal temperature control of a chemical reaction on a microchip using an infrared diode laser. *Analytical Chemistry* 73(16):4037–4044.

Sundaresan, S.G., B.J. Polk, D.R. Reyes, M.V. Rao, and M. Gaitan. 2005. Temperature control of microfluidic systems by microwave heating. In *Micro Total Analysis Systems 2005*, eds. Klavs Jensen, Jongyoon Han, D. Jed Harrison, and Joel Voldman, vol. 1, 657–659. Boston (MA), USA.

Swarts, Jan W., Anja E.M. Janssen, and Remko M. Boom. 2008. Temperature effects during practical operation of microfluidic chips. *Chemical Engineering Science* 63(21):5252–5257.

Wijngaards, D. D. L., G. de Graaf, and R. F. Wolffenbuttel. 2004. Single-chip micro-thermostat applying both active heating and active cooling. *Sensors and Actuators A: Physical* 110(1-3):187–195.

Chapter 4

Comparison of two-phase lipase-catalyzed esterification on micro and bench scale

Abstract

Lipase type B from *Candida antarctica* was used to catalyze the esterification of propionic acid and 1-butanol in a water/*n*-decane two phase system on micro scale and on bench scale. The reaction was described by a Ping Pong Bi Bi mechanism with alcohol inhibition. The kinetic parameters on micro and bench scale were compared; no significant differences were found. Furthermore, effects of temperature on activation and inactivation of the enzyme were found to be similar on micro and bench scale. Therefore, parameters found on either scale can be used for the other scale. Enzyme kinetic parameters can be determined on a micro scale, with very low consumption of reagents and catalyst, and then be applied to bench scale. This can reduce the cost of optimizing enzyme processes by downscaling.

This chapter has been published as Swarts, Jan W.; Vossenberg, Petra; Meerman, Marieke H.; Janssen, Anja E.M.; Boom, Remko M. 2008. Comparision of two-phase lipase catalyzed esterification on micro and bench scale. *Biotechnology and Bioengineering* 99:855-861.

4.1 Introduction

In recent years, the use of enzymes in microfluidic systems, e.g. microreactors, has grown considerably. The limited use of reagents and the limited need for enzymes for these experiments can result in reduced costs and time. Examples of microscale applications are the screening of enzymes with microarrays (Zheng and Ismagilov, 2005), the exploration of cascaded enzyme reactions (Lee et al., 2003), and the determination of enzyme kinetic parameters (Urban et al., 2006).

The channels in micro chips are usually very narrow (100 μm and smaller). This reduces the distances for mass diffusion, thus increasing mass transfer rates. Due to the microreactor's small dimensions, not only mass transfer, but also heat transfer can be fast. The internal fluidic volume in a microreactor is usually very small compared to the whole chip and chipholder. Temperature can therefore be much better controlled even at relatively high fluid velocities of centimeters per second. Fluids in a microfluidic chip can thus be subjected to a well-controlled temperature profile.

In this research, we investigated the esterification of 1-butanol and propionic acid in a two phase system consisting of water and *n*-decane. Both substrates favor presence in the aqueous phase. Butyl-propionate, which is formed as the product, has a partitioning coefficient favoring the organic phase. Selective extraction of the ester from the aqueous phase prevents the reaction to reach equilibrium. Multiphase flow in a microfluidic chip can facilitate the rapid extraction of the product by reducing the diffusional distance and increasing the interfacial area (Brody and Yager, 1997; Weigl and Yager, 1999). The continuous extraction and consequently low concentration of product near the enzyme can result in a higher reaction rate and a higher total conversion.

The goal of this research is to compare an enzymatic synthesis reaction in a microfluidic device to this reaction on bench scale. The focus is on two research goals. First, the kinetic parameters from bench and micro scale experiments are compared. Second, the effect of temperature on the enzyme is investigated. The results from this research show how temperature controlled microreactors can be used with enzymatic synthesis reactions and how they behave as compared to bench scale reactors.

4.2 Materials and Methods

4.2.1 Chemicals

Lyophilized lipase type B from *Candida antarctica* (CalB) was purchased from Biocatalytics (Pasadena, USA). Propionic acid (99.5%), butyl-propionate (99%), and acetonitrile (99.9%, HPLC grade) were obtained from Sigma-Aldrich (Milwaukee, USA). *n*-Decane (95%) was obtained from Fluka (Steinheim, Germany). 1-Butanol (99.5%) was purchased from Riedel-de Haën (Seelze, Germany). Trifluoroacetic acid (99.8%) was purchased from Merck (Darmstadt, Germany). Milli-Q water (Millipore, Billerica, USA) was used for the experiments in this paper.

4.2.2 Experiments micro scale

The enzymatic reaction was performed at room temperature (approximately 22°C), in microfluidic chips from Micronit (Enschede, The Netherlands). The two phases merged on-chip in an etched channel that was 90 μm wide and 40 μm deep (isotropic etching). The total channel volume on-chip was 3.5 μl. The chip was placed in a PEEK chipholder, which was constructed in-house. To supply the chip with fluids, Harvard Apparatus Pico Plus 11 syringe pumps (Holliston, USA) were equipped with SGE 1 ml luer lock syringes (Austen, USA). These syringes were connected to the chip with fused silica capillaries from Bester (Amstelveen, The Netherlands) with an internal diameter of 50 μm. The reaction mixture left the chip through a single fused silica capillary with an internal volume of 0.2 μl.

The enzyme solution (approximately 6 g l^{-1}) was prepared with milliQ and loaded into one syringe. The other syringe was loaded with decane in which both substrates were dissolved. Initial substrate concentrations were varied from 0.18 - 1.21 M for propionic acid and 0.09 - 1.21 M for butanol. These concentrations were calculated as the overall concentration over the combined two phase reaction mixture in the microreactor. The flow rate of fluid in the chip was varied, but the ratio between the flow rate of the aqueous and organic phase was always kept at unity. The flow rate was checked by weighing the sample after collection. Typical errors in the flow rate were found to be less than 5%. Whenever a new experiment was started (i.e. new substrate concentrations, new flow rate) the system was left to stabilize for 15 minutes. This would ensure equilibration over the channel length in terms of concentrations, flow rates, and reaction conditions. Also, all fluid from earlier settings would have left the system completely. At all flow rates the fluid's Reynolds number was approximately

unity. Therefore, laminar flow could be assumed.

The samples were collected at -12°C in 0.5 ml tubes, pre-filled with 175 μl of decane. Typical sample size was 60 μl. The sample was centrifuged to separate the two phases. The top layer (the decane phase) was analyzed by HPLC.

Micro scale experiments at higher temperatures were performed by placing the chipholder with chip in a water bath, which was set to 40, 45, 55, 60, or 61°C. The feed and exit capillaries were in the water bath for only a very short length, giving a residence time of less than 1% of that in the chip. No other changes were made to the procedure.

4.2.3 Experiments bench scale

A mixture of 720 μl enzyme solution (6 g l^{-1}) and 720 μl *n*-decane was prepared and butanol and propionic acid were added. Their concentrations were varied from 0.18 - 1.12 M. The reaction mixtures were mixed at room temperature by end-over-end incubation at 80 rpm. At fixed intervals, a sample was taken and immediately centrifuged to separate the two phases. The top layer of decane was analyzed by HPLC.

Enzyme inactivation was investigated by adding aqueous enzyme stock solution (12 g l^{-1}) to water, which was preheated to the desired inactivation temperature. The resulting 6 g l^{-1} enzyme solution was mixed in a heated water bath and subsequently placed in a preheated oven. The samples were mixed end-over-end for an incubation period of 1 - 120 minutes. After this incubation period, the tubes were cooled in ice. The residual enzyme activity was determined with the esterification reaction at concentrations of 0.18 M for both butanol and propionic acid at 25°C. The ester formation was followed over time (0 - 40 minutes) and the initial activity was determined from the slope of the linear part of the ester production curve.

4.2.4 Sample analysis

HPLC analysis was performed on a 4.6 x 250 mm Prevail C18 RP column from Alltech (Deerfield, USA), packed with 5 μm particles. Acetonitrile (70%) and water with 0.1% TriFluoroAcetic acid (30%) was used as eluent at 1.5 ml min^{-1}. The column was kept at 40°C. Ester concentrations were determined with UV detection at 210 nm.

4.2.5 Kinetic models

The kinetics of esterification with lipases are known to follow the Ping Pong Bi Bi mechanism. Alcohol is known to have a competitive inhibiting effect on the esterification rate (Chulalaksananukul et al., 1990; Janssen et al., 1999; Rizzi et al., 1992). Equation 4.1 shows the initial reaction rate equation in absence of the reaction products (Segel, 1975).

$$v_0 = \frac{V_{max}}{1 + \frac{K_A}{[A]} \cdot \left(1 + \frac{[B]}{K_{iB}}\right) + \frac{K_B}{[B]}} \tag{4.1}$$

In this equation v_0 is the initial velocity (mmole s^{-1} gram $enzyme^{-1}$), V_{max} is the maximum velocity (mmole s^{-1} gram $enzyme^{-1}$), K_A is the Ping Pong constant for propionic acid (M), K_B is the Ping Pong constant for butanol (M), K_{iB} is the inhibition constant for butanol (M), $[A]$ and $[B]$ are the concentrations of propionic acid and butanol (M). The amount of water formed during esterification is negligible compared to the amount of water in the water phase. Thus, all kinetic parameters are apparent parameters in abundance of water.

When we assume no accumulation in the micro reactor, an increase of product quantity in a section of the channel must be due to enzymatic production. The mass balance over this section can be concentration- and volume-integrated and an expression for residence time can be found. Equation 4.2 can be used to correlate the residence time to the concentration of the outgoing product. This equation is valid for both batch (bench scale) and continuous/plug flow systems (micro scale). The derivation of this equation can be found in appendix 4-I.

$$\begin{aligned} \tau \quad = \quad & \frac{K_{iB} + K_A}{V_{max} \cdot K_{iB}} \cdot [P]_{out} + \frac{K_A}{V_{max} \cdot K_{iB}} \cdot ([A]_{in} - [B]_{in} - K_{iB}) \cdot ln\left(1 - \frac{[P]_{out}}{[A]_{in}}\right) \\ & - \frac{K_B}{V_{max}} \cdot ln\left(1 - \frac{[P]_{out}}{[B]_{in}}\right) \end{aligned} \tag{4.2}$$

In this equation, τ is the residence time (seconds), $[P]$ is the concentration of ester (M), the subscript 'in' denotes the concentration before the reaction, and 'out' denotes the concentration after the reaction. Equation 4.2 assumes steady state (no product or enzyme accumulates), and that the concentration of both substrates is equal to their respective initial concentration reduced by the concentration of the product at that position/time. Furthermore, it is assumed that the flow in the micro channel is a constant plug flow and that the flow rate of the aqueous phase is equal to the organic phase. The equation is not valid for higher degrees of conversion.

The catalytic activity as well as the inactivation of the enzyme is temperature dependent. The parameters involved are coupled to temperature by the Arrhenius relation. Higher temperatures will result in a higher esterification rate and therefore in a higher production of ester (per volume or time). By contrast, these higher temperatures will also increase the rate of inactivation of the enzyme, decreasing the ester production. The balance between the catalytic and the inactivation processes will determine what temperature is most suitable for running esterification processes at given conditions.

4.2.6 Computational analysis

For fitting the initial activity data to equation 4.1, Tablecurve 3D v1.04 (Jandel Scientific, USA) was used. For additional estimation of the confidence intervals nonlinear regression was calculated in Mathcad v11.0 (Mathsoft, USA).

4.3 Results and Discussion

4.3.1 Experiments in a microreactor

By using an enzyme reaction in a microreactor set-up one has to deal with stopping the enzyme reaction post-chip. On bench scale, the enzyme reaction is stopped by centrifuging the sample and taking part of the decane for analysis. This decane contains no enzyme, so no new ester will be formed. As it took at least 10 minutes to collect the sample on micro scale, this method could not be used to stop the reaction, directly after passage through the microreactor. The reaction should be stopped or sufficiently slowed down during collection to eliminate post-micro system reactions. No chemical quenching could be used, since this might disturb the partitioning of the reactants. For our system we have chosen to collect the samples at -12°C. The reaction rate was measured to be much lower at -12°C than mixed at room temperature (results not shown here). By having a maximum time for sample collection of one hour, the error of post-chip reaction could be reduced to 10 - 15% in extreme cases and 5% in most cases.

The stability of the enzyme is affected by the presence of (high concentrations of) propionic acid. Moreover, the presence of butanol leads to flocculation of the enzyme under some conditions (not shown in this article). Therefore we have chosen to add both substrates to the organic phase. This results in spontaneous ester formation, for which we corrected the results. The spontaneous ester formation in the syringe, before

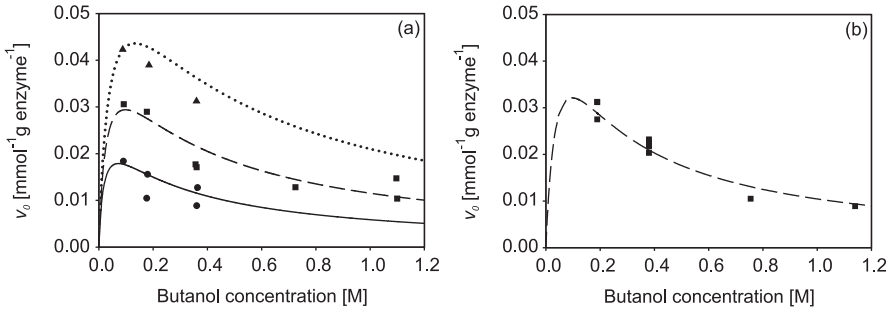


Figure 4.1: Initial enzyme activity as function of the butanol concentration on (a) micro scale and (b) bench scale. The propionic acid concentration was constant at 0.18 M (● for experiments, line for fitted model), 0.37 M (■ for experiments, dashed line for fitted model), and 0.73 M (▲ for experiments, dotted line for fitted model).

contact with the enzyme was linear with time and typically amounted to less than 10% of the total ester concentration (results not shown). The spontaneous formation mainly occurred in the syringes on the syringe pump. The same syringe charge was used to test different flow rates, so the total residence time could be as much as several hours. The residence time in the syringe was noted for each experiment and the results were corrected for the spontaneous esterification.

4.3.2 Enzyme kinetics

Kinetic experiments were conducted at room temperature on both micro and bench scale. Graphs were made of the ester concentrations in the organic phase versus the residence time. The initial enzyme activity was based on the linear part of the slopes of the obtained curves. The kinetic parameters were fitted to these initial activities according to equation 4.1. Figure 4.1 shows the initial activities from experiments and the fitted model as a function of the initial butanol concentration on micro scale (fig. 4.1a) and on bench scale (fig. 4.1b). The kinetic parameters found from both scales are shown in table I.

Figure 4.1 shows an initial increase in activity with increasing butanol concentrations on the micro as well as bench scale. The results show a subsequent decrease in activity at butanol concentrations above 0.1 M. Table 4.1 shows that the values of K_B and K_{iB} seem lower than that of K_A. Even though the confidence intervals are quite large, this probably implies that the affinity of lipase type B from *Candida antarctica* for butanol is higher than for propionic acid. The enzyme is thus more

Table 4.1: Kinetic parameters and 95% confidence intervals determined from micro scale and bench scale experiments

Parameter	Micro scale	Bench scale
V_{max}	0.15 ± 0.16 $mmol\ s^{-1} g\ enz.^{-1}$	0.11 ± 0.068 $mmol\ s^{-1} g\ enz.^{-1}$
K_A	0.86 ± 1.1 M	0.29 ± 0.33 M
K_B	0.088 ± 0.19 M	0.086 ± 0.18 M
K_{iB}	0.24 ± 0.24 M	0.086 ± 0.11 M

resistant to higher relative concentrations of propionic acid and will be more active at these concentrations. The low values for K_{iB} indicate that inhibition of the lipase by butanol is important at all concentrations. The values found for K_B and K_{iB} on bench scale were determined with measurements at relatively high concentrations (see fig. 4.1). This makes the exact concentration at which the inhibiting effect of butanol becomes important less reliable. A somewhat wider range of concentrations was used in the micro scale experiments, and the values determined with the micro scale experiments are therefore somewhat more reliable. The confidence intervals are rather wide because the fitted parameters are linked and determining them independently is extremely difficult. Furthermore, the limited number of measurements (17 sets for micro scale and 16 sets for bench scale) and the relatively large number of parameters that were fitted could affect the size of the confidence intervals.

By comparing the kinetic parameters on bench and micro scale the value of both is within the 95% confidence interval of the other. We conclude that there is no significant difference between the kinetic parameters of the esterification reaction on bench and micro scale.

Figure 4.2 shows the correlation between the experimentally found ester concentrations and those calculated from equation 4.2 using fitted parameters from micro scale experimental results (fig. 4.2a) and from bench scale experimental results (fig. 4.2b). Equation 4.2 can only be used at low conversion since the equilibrium constant is not included. For this reason, only results with substrate conversions under 40% were used. Although the results from micro scale experiments with short residence times resulted in low conversion rates and the bench scale experiments with long residence times resulted in higher conversion rates they are both adequately described by the model.

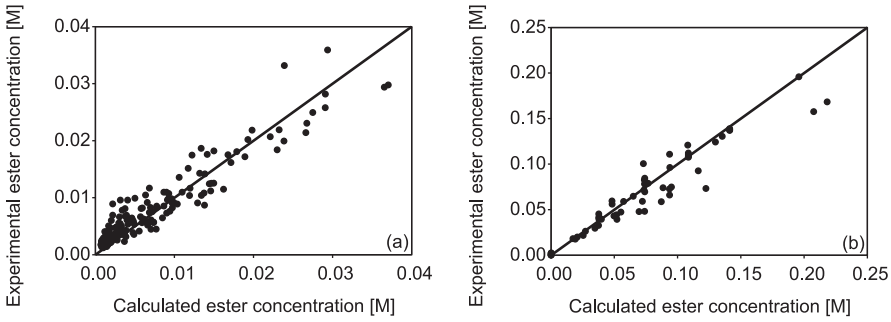


Figure 4.2: Parity plots of the experimental ester concentrations and those calculated from equation 4.2 using the parameters from table 4.1. Results from (a) micro scale and (b) bench scale.

When we assume that the enzyme works the same on both scales, the molecular affinity parameters should be the same. However, possible differences in mass transfer limitation could lead to a difference in V_{max}. With the application of the kinetic affinity parameters from the micro scale experiments to bench scale results and fitting the V_{max}, no significant changes are found. The newly found V_{max} is 0.15 mmole s^{-1} gram $enzyme^{-1}$; the 95% confidence intervals remain roughly the same. This supports the conclusion that there is no significant difference between the enzyme kinetics on micro and on bench scale.

With bench scale experiments, the style and speed of mixing determines the droplet size and corresponding diffusional distance. More vigorous stirring could have reduced the droplet size but this does not influence the reaction rate (results not shown here). The moderate style of end-over-end mixing at 80 rpm results is sufficient to prevent diffusion limitation. The droplet size and corresponding average diffusional distance on bench scale could not be determined, due to the instability of the emulsion after sampling. The average diffusional distance in the microreactor was estimated to be 41 μm by assuming stratified flow with the line of symmetry in the centre of the channel.

For high flow rates, the flow remains parallel for most of the channel length. However, at lower flow rates the two phases flow in parallel for a few centimeters (from a total length of 1.18 m), then break up into droplets. This droplet formation has been linked to increased mass transfer due to internal circulation in and between the droplets. This would mean that at lower flow rates and corresponding longer residence times, the reaction rate could be higher than at higher flow rates when no droplets

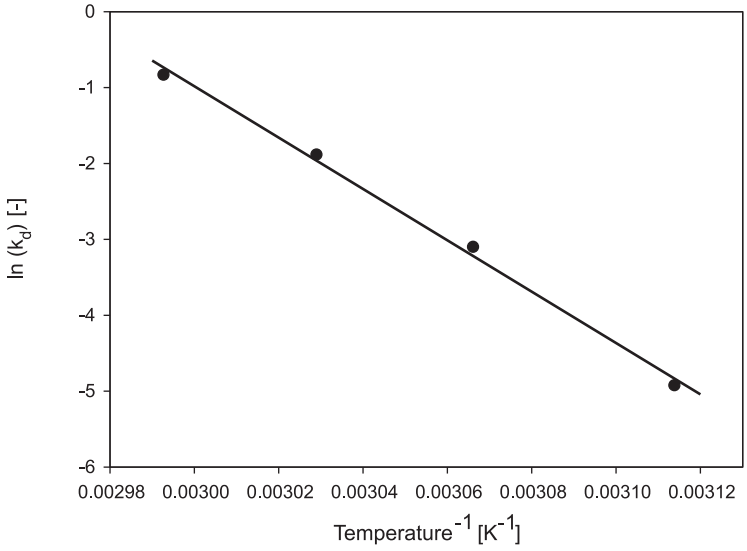


Figure 4.3: Arrhenius plot for inactivation on bench scale.

were observed. As there is no difference between bench and micro scale experiments, the mass transfer once more was shown not to be a limiting factor. However, it is likely that the change in flow profile has an impact on the experimental outcome. In most cases the highest flow rates resulted in a higher than expected conversion. Whether this is due to droplet internal mixing, variation in residence time distribution (Trachsel et al., 2005), or another physical process, is not clear at this moment.

4.3.3 Thermal effect on kinetics of CalB

The residual enzyme activity after incubating the enzyme at higher temperatures on bench scale was calculated and used to estimate the enzyme inactivation parameters. A first-order mechanism was assumed to describe the enzyme inactivation. The natural logarithm of the inactivation (k_d) was plotted against the inverse of the temperature in an Arrhenius plot, see figure 4.3. The activation energy of the inactivation process was found to be 280 kJ mole^{-1} ($R^2 > 0.99$). This agrees well with literature values for lipase B from *Candida rugosa*, where activation energies of 260 kJ mole^{-1} (Shnyrov et al., 1999) and 201 kJ mole^{-1} (Lee and Choo, 1988) are reported.

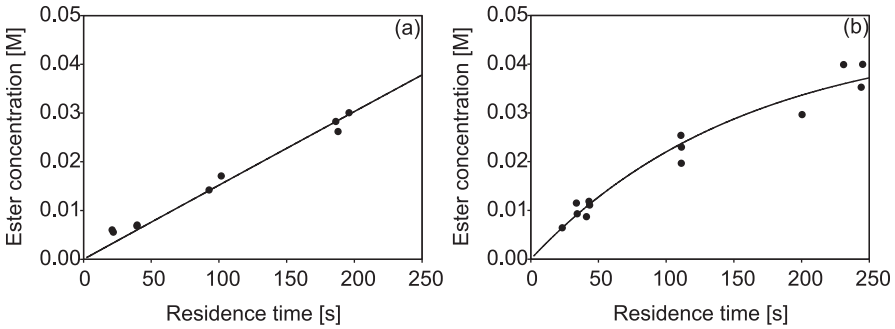


Figure 4.4: Predicted (line) and measured (•) overall ester concentration on micro scale at (a) 45°C and (b) 60°C as function of residence time.

On micro scale, enzyme experiments were conducted at temperatures varying from 40 to 61°C. Ester concentrations were measured at different residence times. Figure 4.4 shows two examples of these results. The activation energy for inactivation found on bench scale was used in a first order inactivation mechanism and combined with equation 4.1 to fit the micro scale results. The initial enzyme activity (v_0) was varied and fitted to the experimental results. Figure 4.4a shows that at relatively low temperatures (45°C) inactivation is not a significant factor within the range of used residence times. The linearity over 250 s indicates that the enzyme activity is still constant.

Figure 4.4b shows that at 60°C the line quickly loses its linearity. After 250 seconds, the enzyme has lost over 70% of its activity. The difference between the lines in figures 4.4a and b shows the inactivation effect of a modest temperature increase of 15°C. Unfortunately, microreactor experiments at temperatures above 60°C at longer residence times resulted in enzyme denaturation and blocking of the microchannels. At this temperature micro scale experiments could only be conducted with residence times up to approximately 45 s. Very high flow rates resulted in pressure gradients that were too high for the connections in the system. Using microfluidic chips with shorter channels and/or wider channels might help to reduce blocking by denatured enzyme. However, complete prevention of such blocking at higher temperatures is not possible.

In fitting the data of figure 4.4, the effect of continued enzyme reaction in the exiting capillary was taken into account. The reaction mixture exits the micro chip

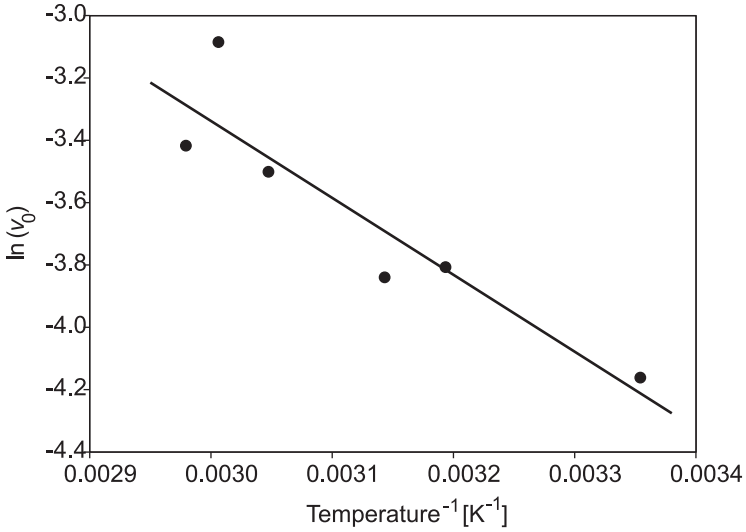


Figure 4.5: Arrhenius plot for initial enzyme activity on micro scale.

and cools down to room temperature within 1 second. The enzyme in this mixture was assumed to continue catalyzing the reaction at room temperature. The reaction rate at room temperature (0.016 mmol s^{-1} g $enzyme^{-1}$) was calculated from the previously determined kinetic parameters. The fraction of active enzyme was assumed equal to the active fraction at the exit of the micro chip.

The enzyme activity at different temperatures was used to construct an Arrhenius plot (figure 4.5). The six data points (25 to 61°C) resulted in a moderately good fit ($R^2 = 0.83$) and an activation energy of 22 kJ $mole^{-1}$. This is in the same order of magnitude as values found in literature. Using Novozyme 435 (lipase from *Candida antarctica*) for the esterification of ketoprofen with *n*-propanol in dipropyl ether activation energies of 44.0 to 55.5 kJ $mole^{-1}$ were found (Duan et al., 1997).

Figure 4.4 shows that the model using inactivation data from bench scale experiments corresponds well to the results of micro scale experiments. The inactivation found on bench scale can thus be applied on micro scale. This affirms the correlation between micro and bench scale processes. Experiments in micro scale can therefore be used to predict denaturation in bench scale processes. Furthermore, on bench scale it is nearly impossible to instantly heat and cool the reaction mixture. Doing

enzyme experiments on these temperatures with residence times under a minute is only possible in these micro structured reactors. Heating up and cooling down the mixture at high flow rates to the desired temperature (± 1.5°C) could be done in 1 second.

4.4 Conclusions

The kinetics of the type B lipase from *Candida antarctica* catalyzed esterification of propionic acid and 1-butanol in a water *n*-decane two phase system was described by a Ping Pong Bi Bi mechanism with alcohol inhibition. No significant differences were found between the parameters found on micro scale and those found on bench scale. Mass transfer limitation does not appear to play an important role in this reaction. As the kinetics on both scales are similar, parameters found on either scale can be used for the other scale.

The activation energy of the first-order inactivation process was 280 kJ mole^{-1}, based on bench scale experiments. This inactivation energy was used to describe inactivation on micro scale. Using the bench scale results, the activation energy for the esterification reaction was determined to be 22 kJ mole^{-1}. Inactivation and activation seemed to behave similarly on micro and bench scale.

Enzyme kinetic parameters can be determined on a micro scale, with very low consumption of reagents and catalyst, and then be applied to bench scale. This can reduce the cost of optimizing enzyme processes by downscaling. Furthermore, experiments requiring short (< 1 minute) block-shaped heat up or cool down curves can better be performed on micro scale compared to bench scale.

Nomenclature

$[A]$	concentration of propionic acid, (M)
$[A]_{in}$	initial concentration of propionic acid, (M)
$[B]$	concentration of 1-butanol, (M)
$[B]_{in}$	initial concentration of 1-butanol, (M)
k_d	inactivation constant, $\left(K^{-1}\right)$
K_A	Ping Pong constant for propionic acid, (M)
K_B	Ping Pong constant for 1-butanol, (M)

K_{iB}	inhibition constant for 1-butanol, (M)
$[P]_{out}$	concentration of ester after reaction, (M)
V_{max}	maximum velocity, $\left(mmol\ s^{-1} gram\ enzyme^{-1}\right)$
τ	residence time, $(seconds)$
v_0	initial velocity, $\left(mmol\ s^{-1} gram\ enzyme^{-1}\right)$

References

Brody, James P., and Paul Yager. 1997. Diffusion-based extraction in a microfabricated device. *Sensors and Actuators A: Physical* 58:13–18.

Chulalaksananukul, W., J.S. Condoret, P. Delorme, and R.M. Willemot. 1990. Kinetic study of esterification by immobilized lipase in *n*-hexane. *FEBS Letters* 276(1,2):181–184.

Duan, G., C. B. Ching, E. Lim, and C. H. Ang. 1997. Kinetic study of enantioselective esterification of ketoprofen with *n*-propanol catalysed by a lipase in an organic medium. *Biotechnology Letters* 19(11):1051–1055.

Janssen, Anja E.M., Birte J. Sjursnes, Alexander V. Vakurov, and Peter J. Halling. 1999. Kinetics of lipase-catalyzed esterification in organic media: Correct model and solvent effects on parameters. *Enzyme and Microbial Technology* 24:463–470.

Lee, Moo-Yeal, Aravind Srinivasan, Bosung Ku, and Jonathan S. Dordick. 2003. Multienzyme catalysis in microfluidic biochips. *Biotechnology and Bioengineering* 83(1):20–28.

Lee, Yuan-Kun, and Choon-Liang Choo. 1988. The kinetics and mechanisms of shear inactivation of lipase from *Candida antarctica*. *Biotechnology and Bioengineering* 33:183–190.

Rizzi, M., P. Stylos, A. Riek, and M. Reuss. 1992. A kinetic study of immobilized lipase catalysing the synthesis of isoamyl acetate by transesterification of isoamyl acetate by transesterification in *n*-hexane. *Enzyme and Microbial Technology* 14:709–714.

Segel, Irwin H. 1975. *Enzyme Kinetics*. New York: John Wiley & Sons.

Shnyrov, Valery L., Luis Diez Martínez, Manuel G. Roig, Arkady E. Lyubarev, Boris I. Kurganov, and Enrique Villar. 1999. Irreversible thermal denaturation of lipase B from *Candida rugosa*. *Thermochimica Acta* 325:143–149.

Trachsel, Franz, Axel Günther, Saif Khan, and Klavs F. Jensen. 2005. Measurement of residence time distribution in microfluidic systems. *Chemical Engineering Science* 60:5729–5737.

Urban, Pawel L., David M. Goodall, and Neil C. Bruce. 2006. Enzymatic microreactors in chemical analysis and kinetic studies. *Biotechnology Advances* 24:42–57.

Weigl, Bernhard H., and Paul Yager. 1999. Microfluidics: Microfluidic diffusion-based separation and detection. *Science* 283(5400):346–347.

Zheng, Bo, and Rustem F. Ismagilov. 2005. A microfluidic approach for screening submicroliter volumes against multiple reagents by using preformed arrays of nanoliter plugs in a three-phase liquid/liquid/gas flow. *Angewandte Chemie International Edition* 44:2520–2523.

Appendix 4-I
Derivation of the concentration- and volume-integrated rate equation

For a plug flow reactor with no accumulation or axial dispersion a mass balance for a section dV can be set up. For a batch reactor, the same derivation can be used.

$$0 = \phi_V \cdot [i] - \phi_V \cdot ([i] + d[i]) + r_i \cdot dV \tag{A-1}$$

Where ϕ_V is the volumetric flow rate ($m^3\ s^{-1}$), $[i]$ is the concentration of component i (mol m^{-3}), and r_i is the reaction rate involving component i (mol $m^{-3}\ s^{-1}$). Splitting the terms results in;

$$\frac{d[i]}{r_i} = \frac{dV}{\phi_V} \tag{A-2}$$

For the product mass balance this equation can be integrated.

$$\int_{[P]_{in}}^{[P]_{out}} \frac{d[P]}{r_P} = \int_0^V \frac{dV}{\phi_V} \tag{A-3}$$

The right side of this equation can be reduced to; $\frac{V}{\phi_V} = \tau$, where τ is residence time (s). The reaction rate can be described by a Ping Pong Bi Bi mechanism with alcohol inhibition;

$$r_P = \frac{V_{\max}}{1 + \frac{K_A}{[A]} \cdot \left(1 + \frac{[B]}{K_{iB}}\right) + \frac{K_B}{[B]}} \tag{A-4}$$

$$\begin{aligned} \tau &= \int_{[P]_{in}}^{[P]_{out}} \frac{1 + \frac{K_A}{[A]} \cdot \left(1 + \frac{[B]}{K_{iB}}\right) + \frac{K_B}{[B]}}{V_{\max}} \cdot d[P] \\ &= \int_{[P]_{in}}^{[P]_{out}} \left(\frac{1}{V_{\max}} + \frac{K_A}{V_{\max}} \cdot \frac{1}{[A]} \cdot \left(1 + \frac{1}{K_{iB}} \cdot [B]\right) + \frac{K_B}{V_{\max}} \cdot \frac{1}{[B]}\right) \cdot d[P] \end{aligned} \tag{A-5}$$

If we assume that $V_{org} = V_{aq}$, then $r_A = r_B = -r_P$ and $[A] = [A]_{in} - [P]$ and $[B] = [B]_{in} - [P]$. All concentrations are overall concentrations (over two phases).

Substituting;

$$\tau = \int_{[P]_{in}}^{[P]_{out}} \left(\frac{1}{V_{\max}} + \frac{K_A}{V_{\max}} \cdot \frac{1}{([A]_{in} - [P])} \cdot \left(1 + \frac{1}{K_{iB}} \cdot ([B]_{in} - [P]) \right) + \frac{K_B}{V_{\max}} \cdot \frac{1}{([B]_{in} - [P])} \right) \cdot d[P] \tag{A-6}$$

Separating terms;

$$\tau = \int_{[P]_{in}}^{[P]_{out}} \left(\frac{1}{V_{\max}} + \frac{K_A}{V_{\max}} \cdot \frac{1}{([A]_{in} - [P])} + \frac{K_A}{V_{\max}} \cdot \frac{1}{([A]_{in} - [P])} \cdot \frac{1}{K_{iB}} \cdot ([B]_{in} - [P]) + \frac{K_B}{V_{\max}} \cdot \frac{1}{([B]_{in} - [P])} \right) \cdot d[P] \tag{A-7}$$

$$\tau = \int_{[P]_{in}}^{[P]_{out}} \left(\frac{1}{V_{\max}} + \frac{K_A}{V_{\max}} \cdot \frac{1}{([A]_{in} - [P])} + \frac{K_A}{V_{\max} \cdot K_{iB}} \cdot \frac{([B]_{in} - [P])}{([A]_{in} - [P])} + \frac{K_B}{V_{\max}} \cdot \frac{1}{([B]_{in} - [P])} \right) \cdot d[P] \tag{A-8}$$

Further separation to find all terms with variable $[P]$;

$$\tau = \int_{[P]_{in}}^{[P]_{out}} \left(\frac{1}{V_{\max}} + \frac{K_A}{V_{\max}} \cdot \frac{1}{([A]_{in} - [P])} + \frac{K_A \cdot [B]_{in}}{V_{\max} \cdot K_{iB}} \cdot \frac{1}{([A]_{in} - [P])} - \frac{K_A}{V_{\max} \cdot K_{iB}} \cdot \frac{[P]}{([A]_{in} - [P])} + \frac{K_B}{V_{\max}} \cdot \frac{1}{([B]_{in} - [P])} \right) \cdot d[P] \tag{A-9}$$

Integration results in;

$$\tau = \left[\frac{[P]}{V_{\max}} - \frac{K_A}{V_{\max}} \cdot \ln([A]_{in} - [P]) - \frac{K_A \cdot [B]_{in}}{V_{\max} \cdot K_{iB}} \cdot \ln([A]_{in} - [P]) - \frac{K_A}{V_{\max} \cdot K_{iB}} \cdot (-[P] - [A]_{in} \cdot \ln(-[A]_{in} + [P])) - \frac{K_B}{V_{\max}} \cdot \ln([B]_{in} - [P]) \right]_{[P]_{in}}^{[P]_{out}} \tag{A-10}$$

Separating into different terms with $[P]$;

$$\tau = \left[\frac{K_{iB} + K_A}{V_{\max} \cdot K_{iB}} \cdot [P] - \frac{K_A \cdot (K_{iB} + [B]_{in})}{V_{\max} \cdot K_{iB}} \cdot \ln\left([A]_{in} - [P]\right) + \frac{K_A \cdot [A]_{in}}{V_{\max} \cdot K_{iB}} \cdot \ln\left([P] - [A]_{in}\right) - \frac{K_B}{V_{\max}} \cdot \ln\left([B]_{in} - [P]\right) \right]_{[P]_{in}}^{[P]_{out}} \tag{A-11}$$

$$\tau = \frac{K_{iB} + K_A}{V_{\max} \cdot K_{iB}} \cdot \left([P]_{out} - [P]_{in}\right) - \frac{K_A \cdot (K_{iB} + [B]_{in})}{V_{\max} \cdot K_{iB}} \cdot \ln \frac{([A]_{in} - [P]_{out})}{([A]_{in} - [P]_{in})} + \frac{K_A \cdot [A]_{in}}{V_{\max} \cdot K_{iB}} \cdot \ln\left(\frac{[P]_{out} - [A]_{in}}{[P]_{in} - [A]_{in}}\right) - \frac{K_B}{V_{\max}} \cdot \ln\left(\frac{[B]_{in} - [P]_{out}}{[B]_{in} - [P]_{in}}\right) \tag{A-12}$$

We can assume that $[P]_{in} = 0$;

$$\tau = \frac{K_{iB} + K_A}{V_{\max} \cdot K_{iB}} \cdot [P]_{out} - \frac{K_A \cdot (K_{iB} + [B]_{in})}{V_{\max} \cdot K_{iB}} \cdot \ln\left(1 - \frac{[P]_{out}}{[A]_{in}}\right) + \frac{K_A \cdot [A]_{in}}{V_{\max} \cdot K_{iB}} \cdot \ln\left(1 - \frac{[P]_{out}}{[A]_{in}}\right) - \frac{K_B}{V_{\max}} \cdot \ln\left(1 - \frac{[P]_{out}}{[B]_{in}}\right) \tag{A-13}$$

And we can conclude that;

$$\tau = \frac{K_{iB} + K_A}{V_{\max} \cdot K_{iB}} \cdot [P]_{out} + \frac{K_A}{V_{\max} \cdot K_{iB}} \cdot ([A]_{in} - [B]_{in} - K_{iB}) \cdot \ln\left(1 - \frac{[P]_{out}}{[A]_{in}}\right) - \frac{K_B}{V_{\max}} \cdot \ln\left(1 - \frac{[P]_{out}}{[B]_{in}}\right) \tag{A-14}$$

Chapter 5

Diffusion limitation on enzyme activity in a microreactor

Abstract

The effect of diffusion limitation on enzyme activity in a microreactor was studied using the hydrolysis of ortho-nitrophenyl-β-D-galactopyranoside catalyzed by β-galactosidase from *Kluyveromyces lactis* as a model system. The Michaelis-Menten kinetic parameters on micro and bench scale were similar. With residence times below a few seconds, diffusion effects limited the reaction rate and therefore reduced the conversion per volume of enzyme microreactor. The critical residence time where diffusion limits the conversion increased quadratically with channel width, increased with enzyme concentration, and decreased with substrate concentration. These general rules can be used in choosing parameters when setting up an enzyme microreactor system. When an enzyme microreactor should be used efficiently, diffusion effects should not be overlooked.

This chapter will be submitted for publication as Swarts, Jan W.; Kolfschoten, Ruben C.; Jansen, Mieke C.A.A.; Janssen, Anja E.M.; Boom, Remko M. Diffusion limitation on enzyme activity in a microreactor.

5.1 Introduction

Over the years microreactors have been credited with many advantages over conventional systems. Due to the small internal dimensions, diffusion of heat and mass can be very rapid. Furthermore, the limited use of chemicals and energy could reduce financial and environmental burdens.

Enzymes have been used in microfluidics systems to catalyze the production of very specific molecules. In the 1990s, enzyme microreactors were first used for enzyme assays (Cohen et al., 1999; Hadd et al., 1997). Enzyme microfluidic systems were further used for the determination of enzyme kinetics (Lee et al., 2003; Ristenpart et al., 2008; Srinivasan et al., 2002; Swarts et al., 2008), the screening of enzymes in droplets (Song and Ismagilov, 2003; Zheng and Ismagilov, 2005), the investigation of temperature effects on enzyme activity (Arata et al., 2005; Mao et al., 2002b), and the study of cascaded enzyme reactions (Lee et al., 2003; Mao et al., 2002a; Wang et al., 2001).

The reaction rate of an enzyme is determined by its activity and the availability of the substrate at the enzyme's active site. In a system where the substrate has to bridge a large distance to the active site, the effective reaction rate could be limited. Due to the small dimensions, typically 10 to 100 μm, microreactors could reduce these diffusional limitations of enzymes.

The effect of diffusion on enzyme activity was studied as early as in the 1960s (Hornby et al., 1968; Lilly and Hornby, 1966; Sharp et al., 1969). That work was focused on the effect of diffusion on the enzyme activity of β-galactosidase and ficin immobilized on the membranes. The diffusion limitation on the effective enzyme activity was hinted on by Kanno et al. (2002) but not investigated. Maruyama et al. (2003) did investigate the effect of diffusion, but used an excess of enzyme to make diffusion dominant. More recently, Ristenpart et al. (2008) investigated enzyme kinetics in a microsystem with both diffusion and reaction limitation. This article focuses on rapid extraction of kinetic data from experimental results, rather than estimating the effect of limitation.

In this research the effect of diffusion limitation on the β-galactosidase catalyzed cleavage rate of *ortho*-nitrophenyl-β-D-galactopyranoside (*o*-NPG) in a microreactor is investigated. This reaction is chosen as a model system and results in the yellow

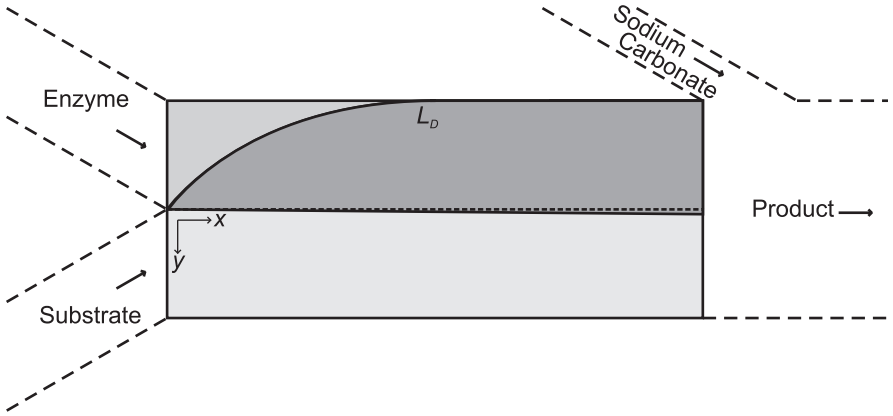


Figure 5.1: Schematic diffusion profiles of substrate (bottom to top) and enzyme (top to bottom) in the microreactor. The dotted line indicates the original position of interface, L_D indicates position in x-direction where diffusion of substrate is complete. Dashed lined indicate the supplying and exiting channels not taken into consideration for calculations.

product *ortho*-nitrophenol (*o*-NP) and the colorless galactose. First, the kinetics of this reaction are determined on bench and micro scale and are compared. Second, the reaction is tested under diffusion limiting circumstances in a microreactor. Finally, the effect of diffusion combined with a reaction is further studied using computer models and theoretical parameters. The results from this research demonstrate under which conditions the short diffusion paths in microreactors eliminate diffusion limitation in an enzymatic reaction.

5.2 Theory

In the enzyme microreactor discussed in this paper a Y-shaped junction brings a substrate and an enzyme solution into one laminar flow reaction channel. Figure 5.1 depicts this reaction channel schematically. A flow with the enzyme and a flow with the substrate enter the left side of the rectangle. Even though the two laminar streams in the microchannel will not mix by convection, there will be molecular diffusion between them. Substrate and enzyme start to diffuse over the boundary between the two (aqueous) flows. Since the substrate is a smaller molecule, it diffuses more quickly into the enzyme stream. The enzyme diffuses much more slowly into the substrate stream. The curved lines indicate the theoretical fronts of the diffusing molecules.

The reaction will take place at those locations where both substrate and enzyme are present, indicated by the dark gray area in figure 5.1. From position L_D onwards in the x-direction, the substrate has distributed more or less evenly over both streams and the enzyme reaction should be at its kinetically determined rate over the whole width the channel. The position of L_D relative to the total reaction channel length is an indication of the importance of diffusion limitation resulting in a significantly lower product concentration at the exit of the channel. In the extreme case that diffusion is very fast compared to the other processes (i.e. the reaction mixture is ideally mixed at $t = 0$ s), the analytical solution, equation 5-1, should apply.

$$K_m \cdot \ln\left(\frac{[S]_0}{([S]_0 - [P]_t)}\right) + [P]_t = V_{\max} \cdot [E] \cdot t \tag{5-1}$$

In this equation, K_m is the Michaelis-Menten constant (mM), $[S]_0$ is the initial substrate concentration (mM), $[P]_t$ is the product concentration at residence time t (mM), V_{max} is the maximum enzyme reaction rate (μmol s^{-1} g enzyme^{-1}), $[E]$ is the enzyme concentration (g enzyme m^{-3}), and t is the residence time (s).

A dimensionless number often used to express the ratio of diffusion time to reaction time is the second Damköhler number (Da_{II}). Although the use of this number has been proposed for enzyme microreactor systems (Kockmann et al., 2004), it is not used extensively.

$$Da_{II} = \frac{t_D}{t_r} = \frac{y^2}{D}\frac{v_0 \cdot [E]}{[S]_0} \tag{5-2}$$

$$t_D = {y^2}/{D} \tag{5-3a}$$

$$t_r = \frac{[S]_0}{v_0 \cdot [E]} \tag{5-3b}$$

In equation 5-2 , t_D is the characteristic time needed for diffusion (s), t_r is the characteristic reaction time (s), y is the diffusion distance (m), v_0 is the initial reaction rate (μmol s^{-1} g enzyme^{-1}), and D is the diffusion coefficient (m^2 s^{-1}). Equation 5-2 is composed of the two parts of equations 5-3a and 5-3b. Equation 5-2 assumes that the reaction will continue at a zero-order initial rate. By inserting the Michaelis-Menten kinetic equation in the Damköhler number we obtain:

$$Da_{II} = \frac{t_D}{t_r} = \frac{y^2}{D} \cdot \frac{V_{\max} \cdot [S] \cdot [E]}{(K_m + [S]) \cdot [S]} = \frac{y^2}{D} \cdot \frac{V_{\max} \cdot [E]}{(K_m + [S])} \tag{5-4}$$

Equation 5-4 is again built up from the characteristic diffusion and reaction times.

$$t_D = {y^2}/{D} \tag{5-5a}$$

$$t_r = \frac{(K_m + [S])}{V_{\max} \cdot [E]} \tag{5-5b}$$

The calculations of the charactaristic diffusion (eq. 5-5a) and reaction (eq. 5-5b) time indicates how parameters influence the ratio between the two. For instance, a high enzyme concentration will lead to a low characteristic reaction time and consequently to less kinetic limitation.

Over the course of a reaction, conversion progresses and the substrate concentration decreases. The Da_{II} from eq. 5-4 is not constant as from eq. 5-2. With very low substrate concentrations, the reaction rate approaches 0. A full conversion as assumed in the Da_{II} number will only be reached after an infinitely long time.

To avoid complication due to the 100% conversion assumption, we propose a critical time, as an alternative to the Da_{II} number. We calculate the ratio of the product concentration exiting the microreactor including diffusion (from numerical models) to the concentration without diffusion limitation (from eq. 5-1) at various residence times. The residence time at which this ratio is 0.9 is chosen to be the critical time. This critical time is a measure for the effect of diffusion on the reactor efficiency.

5.3 Materials and Methods

5.3.1 Chemicals

The β-galactosidase from *Kluyveromyces lactis* (in solution, $\geq$ 3000 U ml^{-1}), *ortho*-nitrophenol (*o*-NP, 98%), potassium phosphate (99%), and sodium carbonate (99%) were purchased from Sigma-Aldrich (Milwaukee, WI). The *ortho*-nitrophenyl-β-D-galactopyranoside (*o*-NPG) was obtained from Fluka (Steinheim, Germany). The cobalt (II) chloride (hexahydrate) was purchased from ICN Biomedicals Inc. (Aurora, OH). Milli-Q water (Millipore, Billerica, MA) was used for the experiments in this article.

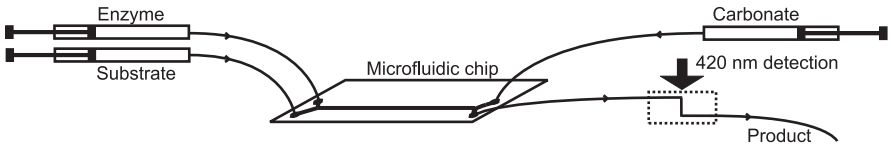


Figure 5.2: Schematic representation of the experimental set-up; enzyme and substrate are combined on-chip, at the end of the reaction channel, sodium carbonate is added to stop the reaction. All fluids exit the channel and pass through a z-shaped cell for detection at 420 nm.

5.3.2 Experiments on bench scale

At 23°C, two 150 ml buffer solutions were mixed in a 500 ml stirred vessel with baffles and a three-blade propeller type stirrer at 350 rpm. Both buffers contain 25 mM of sodium phosphate, 15 μM cobalt chloride and were set to pH 7.3 using sodium hydroxide. One buffer contained β-galactosidase at a concentration of 0.2 g l^{-1}. The other buffer contained o-NPG at concentrations varying from 1 to 20 mM. Over the first 2 to 4 minutes, 0.5 mL samples were taken and mixed with 0.5 ml 1% (w/w) sodium carbonate. Addition of sodium carbonate resulted in a hundredfold lower activity (results not shown), which ensured no significant reaction after sampling. The concentration of o-NP was measured with a spectrophotometer at 420 nm. The linear part of the o-NP concentration vs. time plots (9 to 11 samples, $R^2 > 0.995$) was used to calculate the initial enzyme activity.

5.3.3 Experiments on micro scale

The micro scale enzymatic reaction was carried out at room temperature (20 - 22°C). The two aqueous streams were combined on-chip. The microchannels were on average 83 μm wide and 40 μm deep and were isotropically etched in a microchip from Micronit (Enschede, The Netherlands). A schematic view of the chip is presented in figure 5.2. The total microchannel volume on-chip was 0.113 μl. An in-house constructed PEEK chipholder facilitated the connections between the chip and capillaries which supplied the fluids. These were fused silica capillaries from Bester (Amstelveen, the Netherlands) had a 50 μm diameter and were connected to SGE 1 ml luer lock syringes (Austin, TX). The syringes were placed in Harvard Apparatus Pico Plus 11 syringe pumps (Holliston, MA).

The enzyme solution (approximately 0.2 g l^{-1}) and the substrate solution (o-NPG concentration varying from 1 to 20 mM) were prepared with a 25 mM potassium

phosphate buffer with 15 μM Co^{2+} set to pH 7.3. The two solutions were placed on the same syringe pump and consequently pumped at the same rate. The solutions (shown left in fig. 5.2) were combined at a 1:1 volumetric ratio. Just before exiting the chip, this stream was joined by a 1% (w/w) sodium carbonate in Milli-Q stream (top-right in fig. 5.2). The combined enzyme and substrate flow was matched with this carbonate solution in a 1:1 volumetric ratio. Due to the inhibiting effect of sodium carbonate (even at fairly low concentrations) and the relatively fast diffusion (shorter diffusion distance due the compressing of streams and the small molecule), reaction stoppage was assumed to be instantaneous.

In most cases, the fluid's Reynolds number was below unity. Depending on the flow rates and position in the system, it varied from approximately 0.02 to 25. With these values, laminar flow could be assumed. Therefore, the dominant type of mixing was by means of molecular diffusion.

The reaction mixture left the chip through a fused silica capillary which was connected to a LC Packings U-Z View capillary flow cell (Sunnydale, CA). The total post-chip volume until detection was 0.8 to 1.2 μl. This flow cell was placed in an UltiMate UV-VIS detector from Dionex (Sunnydale, CA) and had a 10 mm light path for accurate measurements. The measured absorbance at 420 nm was correlated to the *o*-NP concentration. The *o*-NP concentration was plotted against the residence time (inversely proportional to the flow rate) and from the linear part of this graph the initial activity was calculated.

Two methods were employed to investigate the effect of diffusion limitation on enzyme reactions practically. In the first method the original 83 μm wide micro channel was used, but both the enzyme concentration and the flow rates were increased by a factor of 10. A second method to determine the effect of diffusion limitation was to increase the time needed for diffusion, by using a wider microchannel. The same fluids as with the kinetic experiments were pumped through microchannels with effective channel widths of 183 and 283 μm.

5.3.4 Computer models

Two-dimensional numerical models were constructed to calculate the concentration of all components at any position in the channel. The models were constructed in COMSOL Multiphysics from COMSOL (Burlington, MA). Similar to figure 5.1, two

rectangular shapes sharing one long side were used as a representation of the domains of the two aqueous streams. As both streams are laminar and have the same flow rate, we can assume that the fluids will stay in their initial domains. All other components (*o*-NPG, *o*-NP, galactose, and β-galactosidase) are free to diffuse over the interface between the two domains.

The two rectangular fluid domains were 41.5 μm wide (equal to real effective diffusion distance) and 2 mm long. The real length of the channel was 34 mm. Assuming constant volumetic flow rate, the superficial fluid velocity was scaled proportionally to the ratio between the real length and the model length.The diffusion coefficients D of all diffusing components in water were calculated using the Wilke-Chang equation (Wilke and Chang, 1955). These coefficients were $0.64{\cdot}10^{-9}$ m^2 s^{-1} for *o*-NPG, $0.94{\cdot}10^{-9}$ m^2 s^{-1} for *o*-NP, $0.85{\cdot}10^{-9}$ m^2 s^{-1} for galactose, and $0.047{\cdot}10^{-9}$ m^2 s^{-1} for β-galactosidase.

The reaction was modeled using Michaelis-Menten kinetics and parameters from micro scale experimental results. Combinations of substrate concentration and initial activity were fitted to Michaelis-Menten kinetics using Athena Visual Studio V12.0 (Athena Visual Studio, Naperville, IL).

5.4 Results and Discussion

5.4.1 Enzyme kinetics

Kinetic experiments were conducted at room temperature on both bench and micro scale. Figure 5.3 shows the initial activities from experiments and the fitted model on micro-scale (fig. 5.3a) and bench scale (fig. 5.3b). The activity is expressed as the number of micromoles produced per second per gram of the original enzyme solution. The kinetic parameters obtained from these fitted models are summarized in table 5.1.

Figure 5.3 shows that for increasing substrate concentrations, the enzyme activity increases. The initial steep slope of the activity vs. substrate curve and the subsequent leveling off is consistent with Michealis-Menten kinetics. The values for K_m are somewhat lower but in the same range as those found in literature: Cavaille and Combes (1995) and Dickson et al. (1979) both reported a K_m of about 1.7 mM. The values for the kinetic parameters as presented in table 5.1 are very close for both

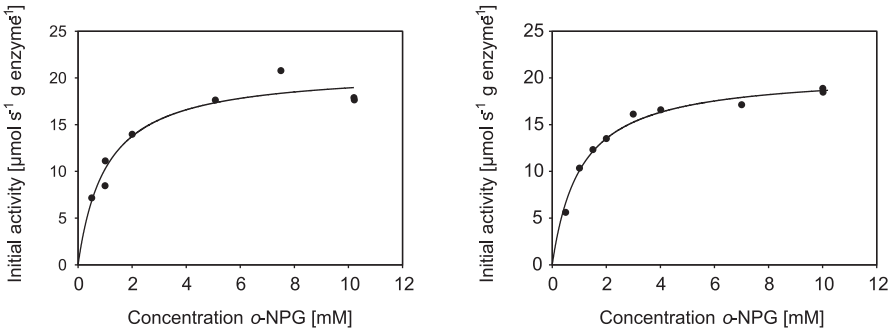


Figure 5.3: Initial β-galactosidase activity as function of the o-NPG concentration on (a) micro scale and (b) bench scale, symbols indicate experimental findings, drawn lines indicate the model based on fitted kinetic parameters.

Table 5.1: Kinetic parameters and 95% confidence interval determined from micro scale and bench scale experimental results.

Parameter	Micro scale	Bench scale
V_{max}	$20.9 \pm 2.3\ \mu mol\ s^{-1} g\ enz.^{-1}$	$20.6 \pm 1.0\ \mu mol\ s^{-1} g\ enz.^{-1}$
K_m	$1.04 \pm 0.45\ mM$	$1.05 \pm 0.21\ mM$

experimental scales.

Figure 5.4 shows the product concentration in the microchannel with increasing residence times. The results from the numerical COMSOL model are compared to the analytical solution of equation 5-1. Figure 5.4 shows that the numerical results generally correspond to the analytical solution. With increasing residence times, the product concentration increases. Eventually, the *o*-NP concentration levels off at 3 mM, which corresponds to 100% conversion. Only at residence times below 10 s, do the two models give different results, due to diffusion limitation. The results from practical experiments in figure 5.3a, to determine the kinetic parameters, were typically obtained at residence times of around 30 seconds. Figure 5.4 shows that ignoring the effect of diffusion limitation at these concentrations yields a 2% overestimation of the conversion. At these residence times, this overestimation is negligible, but it becomes significant at higher flow rates (i.e., smaller residence times).

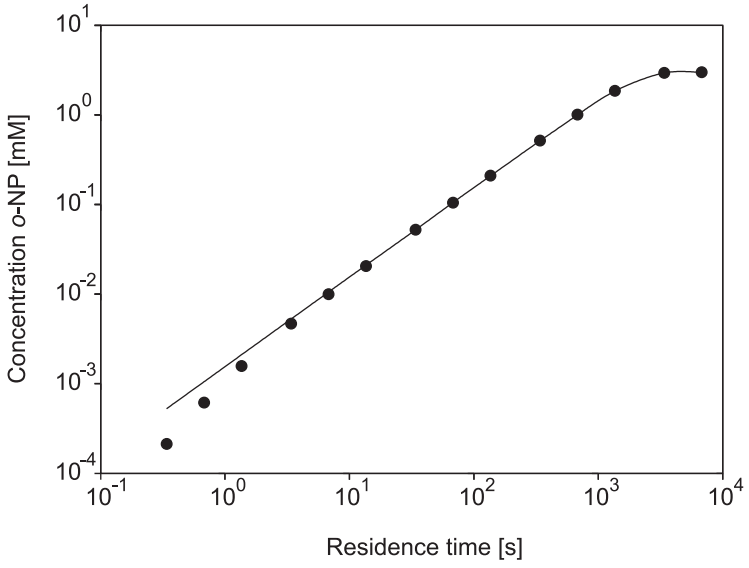


Figure 5.4: Concentration of *o*-NP from the numerical COMSOL model (symbols) and the analytical equation at different residence times (line).

5.4.2 Diffusion limitation in enzyme microreactors

Two methods were employed to investigate the effect of diffusion on product concentration; the use of wider micro channels and the combination of a high enzyme concentration and high flow rates. Figure 5.5 shows the effect of micro channels of 183 μm (fig. 5.5a) and 283 μm (fig. 5.5b). Figures 5.5a and b show the increasing product concentration with increasing residence times, as obtained from the analytical solution (eq. 5-1), the numerical model, and experiments. The analytical solution gives much higher product concentrations than either the experiments or the numerical solution, indicating diffusion limitation in the latter two cases. The numerical and experimental results are in good agreement, indicating that the lower concentrations are indeed caused by diffusion limitation. The models can therefore be used to further investigate the effect of diffusion.

As shown in figure 5.5a and b, the diffusion limitation clearly increases with wider channels, as would be expected with diffusion limitation. The maximum diffusion distance in this microchannel is half the channel width. By increasing the channel width from 83 μm to 183 and 283 μm, the characteristic diffusion time increases by

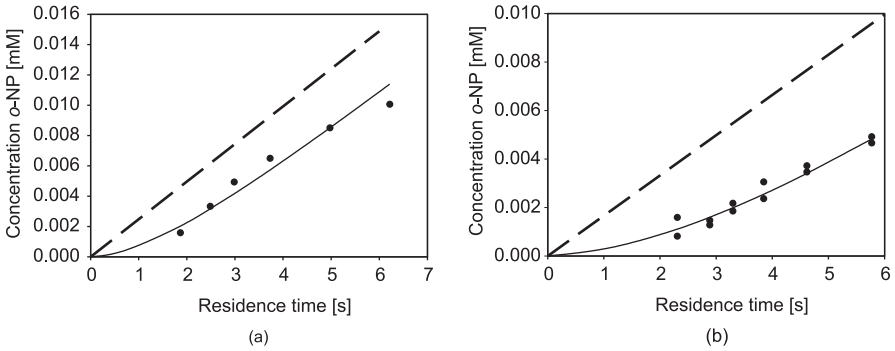


Figure 5.5: The *o*-NP (product) concentration as function of the residence time; the dashed line represents the analytical solution, the solid line the numerical solution, and the symbols are the experimental results. The widths of the micro-channels are a) 183 μm and b) 283 μm.

a factor of 4.9 and 11.6 (quadratically according to equation 5-3a), while the total reaction volume scales linearly and increases by a factor of 2.2 and 3.4 respectively.

Similar results are obtained by using the original 83 μm wide channels with a tenfold higher flow rate and enzyme concentration. This means that in the right hand term of equation 5-1, V_{max} is constant, $[E]$ is tenfold higher, and t is tenfold lower. In absence of diffusion limitation, the product concentration should be the same. However, figure 5.6 shows that diffusion limitation is important at all residence times (albeit relatively more important at lower residence times) The effect is well predicted by the numerical model which shows that diffusion limitation indeed is the cause of the reduction in product concentration.

The numerical models correspond well to experimental results as was shown in figures 5.5 and 5.6. We can therefore use these models to study the effect of the system parameters on the critical time, where diffusion limitation causes a 10% reduction in product concentration. The enzyme reaction was kept the same, but parameters, as channel width and enzyme and substrate concentrations were varied to investigate the contribution of diffusion under these circumstances.

Figure 5.7a shows the efficiency of the system (the ratio from numerical and analytical calculations, which is a measure for diffusion limitation). At a residence time approaching 0 seconds, the substrate and enzyme are completely separated, and the efficiency is 0. At longer times, a uniform distribution of all components is obtained

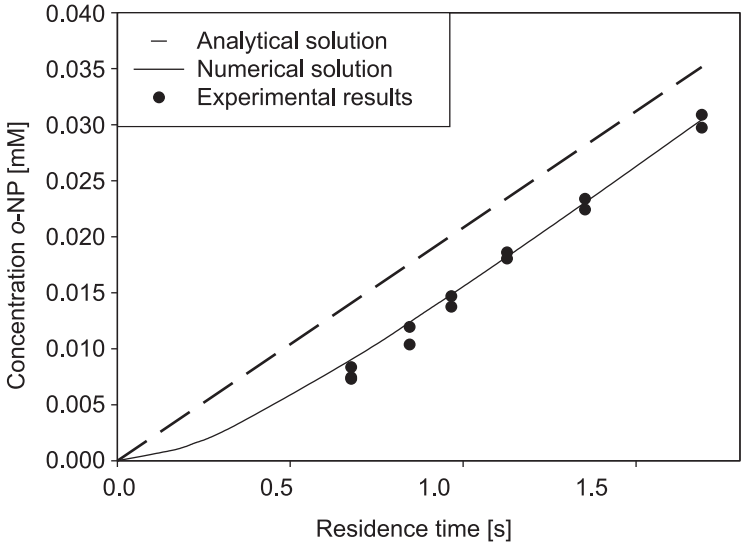


Figure 5.6: The *o*-NP (product) concentration as function of residence time; dashed line for analytical solution, solid line for numerical solution, and symbols for experimental results. Microchannel with a width of 83 μm. The enzyme concentration is a factor of 10 higher than in the experiments of fig. 5.4 and 5.5.

and the practical reaction rate becomes equal to the intrinsic reaction rate. The efficiency will thus approach 1.

Figure 5.7a shows that with increasing channel widths it takes longer to approach the analytical result. To illustrate this, figure 5.7b shows the critical time, at which the numerical result is 90% of the analytical result, shown as a gray dotted line in figure 5.7a. Here, diffusion effects cause a 10% limitation on the effective reaction rate. This critical time is plotted against the maximum diffusion distance in the micro channel, which is the distance the substrate has to travel to the enzyme (equal to half the channel width).

The effect of a wide range of enzyme concentrations on the critical time is shown figure 5.7c. The critical time is fairly constant at 3.5 to 4 seconds up to an enzyme concentration of 1 g l^{-1}. At higher enzyme concentrations the critical time slightly increases to about 6 seconds. It seems that at low enzyme concentrations ($<$ 1 g l^{-1}) diffusion is fast enough to supply the enzyme with substrate. At higher enzyme concentrations, the reaction is faster, leading to local depletion of substrate. At very

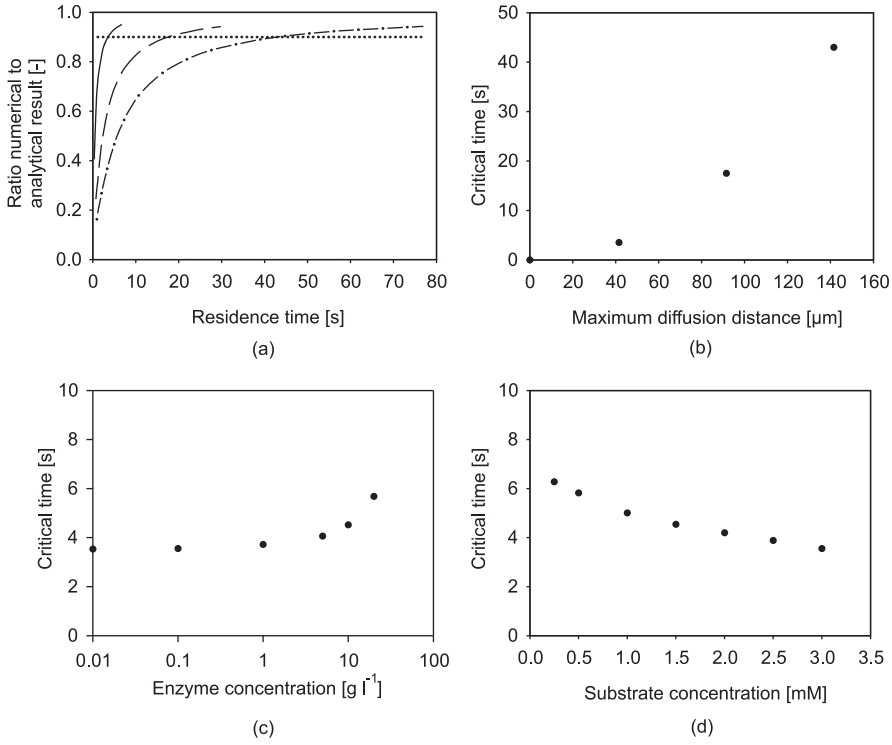


Figure 5.7: a) Effect of different channel widths; the ratio of product concentration from numerical models to analytical solution is plotted against the residence time for different channel widths (solid line is 83 μm, dashed line is 183 μm, dash-dot-dashed line is 283 μm) , b) effect of different channel widths; critical time at which this ratio is 0.9 vs. maximum diffusion distance (equal to half the channel width), c) critical time vs. enzyme concentration on a logarithmic scale, and d) critical time vs. substrate concentration.

high enzyme concentrations (100 g l^{-1} and higher) any substrate would be converted very fast. The analytical solution gives a 99% conversion in just over 2 seconds. The critical time would no longer be an expression of diffusion limitation.

Figure 5.7d shows the effect of substrate concentration on the critical time. At low substrate concentrations the critical time is high; with increasing concentrations the critical time decreases. When we only look at diffusion of the substrate, different substrate concentrations do not change the shape of the theoretical diffusion front from figure 5.2. At the front the concentration starts to become non-zero, reaching the bulk concentration towards the original substrate channel. This concentration

profile is similar to an error function. When we include reaction, the shape of the front will change, depending on this reaction.

Comparing the results of figure 5.7b-d with the Da_{II} number from equation 5-2, and its corresponding characteristic diffusion and reaction time of eq. 5-5a and 5-5b, we can see similarities and differences. Fig. 5.7b shows that with increasing diffusion distances, the critical time increases quadratically, as does the Da_{II} number due to the increased diffusion time (eq. 5-5a). The Da_{II} number thus also predicts more diffusion limitation with an increased channel width.

According to eq. 5-5b, the enzyme concentration should correspond inversely to the extent of diffusion limitation. However, numerical studies (fig. 5.7c) showed an increase of the critical time, i.e. an increase of diffusion limitation, after an initial near constant level. Initially, the enzyme concentration and the total reaction rate is so low, that the critical time is purely diffusion driven. With increasing enzyme concentrations, substrate will be depleted around the enzyme and cause a lower reaction rate per gram of enzyme due to Michaelis-Menten kinetics.

Again, according to equation 5-4, the Da_{II} should scale inversely with the term $(K_m+[S])$. A low $[S]$ in eq. 5-5b will which results in a lower limit the characteristic reaction time. When $[S]$ is significant compared to relative to K_m, the characteristic reaction reaction time will be higher. Thus, diffusion limitation at higher substrate concentrations is less likely. Similarly, the critical time from numerical studies (fig. 5.7d) decreased with increasing substrate concentrations.

As the Da_{II} number assumes zero-order kinetics and one-dimensional Fickian diffusion we can expect deviations with more complex cases. The enzyme we use follows Michealis-Menten kinetics and diffusion results in a concentration gradient rather than a propagating substrate front with a uniform substrate concentration behind it. The effect of varying parameters on the Da_{II} number correspond to our numerical findings with respect to the channel width and the substrate concentration, but differ with the enzyme concentration. The numerical model is a valuable tool added to the use of dimensionless numbers as it can deal with non-ideal systems, demonstated by the variation of the enzyme concentration.

Even though this study focuses on Michaelis-Menten kinetics, and some of the relations are only valid for this type of kinetics, the approach can be adapted to

other kinetics. Furthermore, we studied a microreactor under laminar (creep) flow conditions which implies that mixing by convection does not take place. Many mixing methods have been proposed in microfluidic technology which would enhance mass transfer. We can however extract general lessons from figures 5.7b-d.

When the goal of using enzyme microreactor is converting the substrate to products, diffusion limitation is not desired as it reduces volumetric productivity (conversion per volume). When the residence time is much higher than the critical time given in this paper the reduction in the efficiency is very small. In contrast, when the residence time is lower than the critical time the reduction in reactor efficiency is significant ($> 10\%$). Such a significant efficiency reduction is most likely to occur with wide microchannels (fig. 5.7b), high enzyme concentrations (fig. 5.7c), and low substrate concentrations (fig. 5.7d).

5.5 Conclusions

The hydrolysis of *ortho*-nitrophenyl-β-D-galactopyranoside catalyzed by β-galactosidase from *Kluyveromyces lactis* was shown to follow Michaelis-Menten kinetics on bench and micro scale. The kinetic parameters on both scales were the same. With the time scales applied during the experiments the reaction seems not to be affected by diffusion limitation. Diffusion limitation could be observed with experimental residence times below a few seconds. At these short residence times the volumetric efficiency of the enzyme microreactor (conversion per volume) decreased. The critical residence time where diffusion limits the conversion significantly increases quadratically with channel width, increases with enzyme concentration, and decreases with substrate concentration. Estimations based on numerical calculations rather than the Da_{II} number can used in wider range of conditions as it can be used in non-ideal situations. An enzyme microreactor can be run most efficiently when these factors receive appropriate attention.

Nomenclature

D	Diffusion coefficient, $\left(m^2\ s^{-1}\right)$
Da_{II}	Second Damköhler number, $(-)$
$[E]$	Enzyme concentration, $\left(g\ l^{-1}\right)$
K_m	Michaelis-Menten concentratrion, (mM)

L_D	Distance at which diffusion is theoretically complete, (m)
$[P]$	Product concentratrion, (mM)
$[P]_t$	Product concentration at time = t, (mM)
$[S]_0$	Initial substrate concentration, (mM)
t	Time, (s)
t_D	Characteristic diffusion time, (s)
t_r	Characteristic reaction time, (s)
v_0	Initial enzyme reaction rate, $\left(\mu mol\ s^{-1} g\ enzyme^{-1}\right)$
V_{max}	Maximum enzyme reaction rate, $\left(\mu mol\ s^{-1} g\ enzyme^{-1}\right)$
x	Distance in flow direction, (m)
y	Diffusion distance, (m)

References

Arata, Hideyuki F., Yannick Rondelez, Hiroyuki Noji, and Hiroyuki Fujita. 2005. Temperature alteration by an on-chip microheater to reveal enzymatic activity of β-galactosidase at high temperatures. *Analytical Chemistry* 77(15):4810–4814.

Cavaille, Dominique, and Didier Combes. 1995. Characterization of β-galactosidase from *Kluyveromyces lactis*. *Biotechnology and Applied Biochemistry* 22(1):55–64.

Cohen, Claudia B., Emily Chin-Dixon, Sang Jeong, and Theo T. Nikiforov. 1999. A microchip-based enzyme assay for protein kinase A. *Analytical Biochemistry* 273:89–97.

Dickson, Robert, Lesley R. Dickson, and Jennifer S. Markin. 1979. Purification and properties of an inducible β-galactosidase isolated from the yeast *Kluyveromyces lactis*. *Journal of Bacteriology* 137(1):51–61.

Hadd, Andrew G., Daniel E. Raymond, John W. Halliwell, Stephen C. Jacobson, and J. Michael Ramsey. 1997. Microchip device for performing enzyme assays. *Analytical Chemistry* 69:3407–3412.

Hornby, W.E., M.D. Lilly, and E.M. Crook. 1968. Some changes in the reactivity of enzymes resulting from their chemical attachment to water-insoluble derivatives of cellulose. *Biochemical Journal* 107:669–674.

Kanno, Ken-ichi, Hideaki Maeda, Showta Izumo, Masakazu Ikuno, Kazuhito Takeshita, Asuka Tashiro, and Masayuki Fuji. 2002. Rapid enzymatic transglycosylation and oligosaccharide synthesis in a microchip. *Lab on a Chip* 2(1):15–18.

Kockmann, Norbert, Michael Engler, and Peter Woias. 2004. Theoretische und experimentelle Untersuchungen der Mischvorgänge in T-förmigen Mikroreaktoren - Teil 3: Konvektives Mischen und chemische Reaktionen. *Chemie Ingenieur Technik* 76(12):1777–1783.

Lee, Moo-Yeal, Aravind Srinivasan, Bosung Ku, and Jonathan S. Dordick. 2003. Multienzyme catalysis in microfluidic biochips. *Biotechnology and Bioengineering* 83(1):20–28.

Lilly, M.D., and W.E. Hornby. 1966. The kinetics of carboxymethylcellulose-ficin in packed beds. *The Journal of Biochemistry* 100:718–723.

Mao, Hanbin, Tinglu Yang, and Paul S. Cremer. 2002a. Design and characterization of immobilized enzymes in microfluidic systems. *Analytical Chemistry* 74(2):379–385.

———. 2002b. A microfluidic device with a linear temperature gradient for parallel and combinatorial measurement. *Journal of the American Chemical Society* 124:4432–4435.

Maruyama, Tatsuo, Jun-ichi Uchida, Tomohiro Ohkawa, Toru Futami, Koji Katayama, Kei-ichiro Nischizawa, Ken-ichiro Sotowa, Fukiko Kutoba, Noriho Kamiya, and Masahiro Goto. 2003. Enzymatic degradation of *p*-chlorophenol in a two-phase flow microchannel system. *Lab on a Chip* 3:308–312.

Ristenpart, William D., Jiandi Wan, and Howard A. Stone. 2008. Enzymatic reactions in microfluidic devices: Michealis-Menten kinetics. *Analytical Chemistry* 80(9):3270–3276.

Sharp, A.K., G. Kay, and M.D. Lilly. 1969. The kinetics of β-galactosidase attached to porous cellulose sheets. *Biotechnology and Bioengineering* 11:363–380.

Song, Helen, and Rustem F. Ismagilov. 2003. Millisecond kinetics on a microfluidic chip using nanoliters of reagents. *Journal of the American Chemical Society* 125(47):14,613–14,619.

Srinivasan, Aravind, Xiaoqiu Wu, Moo-Yeal Lee, and Jonathan S. Dordick. 2002. Microfluidic peroxidase biochip for polyphenol synthesis. *Biotechnology and Bioengineeing* 81(5):563–569.

Swarts, Jan W., Petra Vossenberg, Marieke H. Meerman, Anja E.M. Janssen, and Remko M. Boom. 2008. Comparision of two-phase lipase catalyzed esterification on micro and bench scale. *Biotechnology and Bioengineering* 99(4):855–861.

Wang, Joseph, Madhu Prakash Chatrathi, and Baomin Tian. 2001. Microseparation chips for performing multienzymatic dehydrogenase/oxidase assays: Simultaneous electrochemical measurement of ethanol and glucose. *Analytical Chemistry* 73(6):1296–1300.

Wilke, C.R., and Pin Chang. 1955. Correlation of diffusion coefficients in dilute solutions. *AIChE Journal* 1(2):264–270.

Zheng, Bo, and Rustem F. Ismagilov. 2005. A microfluidic approach for screening submicroliter volumes against multiple reagents by using preformed arrays of nanoliter plugs in a three-phase liquid/liquid/gas flow. *Angewandte Chemie International Edition* 44:2520–2523.

Chapter 6

Social and ethical issues of microreactor technology

Abstract

This article is the first systematic investigation of social and ethical issues that the introduction of microtechnology applications in the market might bring about. Through interviews with experts from academia, industry, policy and control, and NGOs, three examples of microreactor technology applications in nutrition, in medicine, and in energy carrier supply are investigated. The benefits and costs, and their distribution are discussed for these examples. Furthermore, possible strategies of communication surrounding a public introduction of such a novel technology are considered. The applications proposed in this paper are only three out of an infinite number of possibilities. However, the discussion of these examples can be used as a framework for discussing future applications as they might be developed in the future. Timely discussions of relevant issues might prevent societal problems as encountered with the GMO-scare in 1990s. Appropriate communication of these novel applications may further improve their chances of success in the market.

6.1 Introduction

Scientific and technological advances in one field can spark unexpected revolutions in another. The development of micro-electronics has led to entertainment such as radio and television, home-computing, and the internet as an important source of information for a large portion of Western society. Techniques to produce ever-smaller electronic circuits have also found application in chemical engineering. The same photolithographic techniques applied in micro-electronic chip manufacturing are used to create reaction vessels, pipes, mixers, and many other pieces of lab equipment on the scale of a human hair. With increasing technical knowledge, these laboratory possibilities have become deeper (smaller and more accurate fabrication) and wider (more applications, such as in biology and medicine) (Auroux et al., 2002; Jensen, 2001; Reyes et al., 2002).

Current micro chemical systems are mainly used in academic and commercial research and are tested to prove their robustness and applicability in the portfolio of chemical synthesis methods. Considering the rapid technological evolution in the last years, it is likely that these novel synthesis methods will be improved as the technology matures. In the near future, a robust and user-friendly bench top system could be used as a stand-alone synthesis environment. This would enable distributed on-demand chemical production. The future will bring more applications of microreactor technology, some of which cannot be predicted. By extrapolating the current technology and research, we can come up with some examples that could become a reality over the next decades.

While nanotechnology is being scrutinized, social and ethical issues surrounding microreactor technology have not yet been systematically investigated. This paper offers a first general discussion on the subject but does not try to answer all questions on or discuss all possibilities for microreactor technology. Rather, this paper offers a simple framework to discuss some social and ethical issues in selecting possible future microreactor applications. In this article we intend to answer three connecting questions. First, what type of applications might come from microreactor technology, and what would exemplify its possibilities? Second, what could be the social and ethical issues involved in such future applications? Third, how should microreactor technology applications be communicated to the public?

This paper draws upon social, ethical, technical, and popular information sources for references to the current state of affairs. To predict future applications and some of their social and ethical issues, the authors interviewed experts from technical universities, industry, policy agencies, and critical NGOs. Appendix 6-I lists the items and questions discussed during these interviews. Appendix 6-II contains an anonymized list of the interviewees, including the abbreviations used with the quotes in this chapter. The quotes are often translated and adapted linguistically by the author, as indicated by the "JS". Finally, the selection of interviewees is not intended to be a numerically proportional representation of those involved, but rather to cover a broad range of opinions.

6.2 Microreactor technology

6.2.1 Current microreactor technology

The technique of transferring patterns and etching materials had been around for decades before it was introduced to the natural sciences of chemistry and biology in the form of microfluidic devices. With the development of DNA chips and more lab-on-a-chip devices in the early 1990s, the application of these techniques gained momentum. The most important applications of microfluidic technology can be categorized by their goals: to analyze (Huikko et al., 2003; Janasek et al., 2006; Kling, 2006; Service, 1998a; Talary et al., 1998; Walt, 2005; Yager et al., 2006), to do research in (Folch et al., 1999; Jönsson et al., 2004; Köhler and Henkel, 2005; Lee and Lee, 2004), to produce energy (Dyer, 2002; Service, 2002), and to create molecules. This fourth and final group of microfluidic applications is the focus of this paper.

Microreactor technology has been used to perform many types of reactions, catalyzed or non-catalyzed, aimed at building or breaking molecules in simple one-step reactions or complex cascaded reactions. The objective of the system can also be to investigate the reaction (kinetics), to investigate multiple reactions routes, or to produce a known chemical substance by a known route. The first microreactor technology products have entered the market. Future products could include small scale reaction environments for educational purposes, automated reaction conditions testing system, or small scale chemical production plants (Belder et al., 2006; Burns, 2002; deMello, 2006; Kikutani and Kitamori, 2004; Service, 1998b).

6.2.2 Future microreactor technology

Predicting the future is a highly uncertain business. Microreactor technology has still not made a public breakthrough. The world is still waiting for the 'killer application' (Blow, 2007). The products that are proposed in this article as possible future developments are based on interviews with experts from different fields.

The interviewees suggested several possible microreactor technology applications that could become a reality in future decades, but this paper focuses only on the three most discussed applications in the interviews: microreactor technology with personalized nutrition, microreactor technology in medicine supply, and microreactor technology in energy carrier supply.

Application 1: microreactor technology with personalized nutrition

A first application of microfluidic technology could be found in retail, supplying consumers with *personalized nutrition*. Personal food choice can be based on short-term personal preference (taste) of long-term considerations (health). In the latter case, personalized nutrition based on DNA profiles is a possibility (Korthals, 2007). A consumer would communicate his wishes regarding a food product to the front-end of the microreactor application (a machine-interface or a shop clerk). The product could be a carton of milk with more fat (taste), more iron (genetic disposition for low blood iron), and a specific oligosaccharide (improving colonic bacterial population).

In the back-end of the application, the base product ('blank' milk) would be mixed with ingredients (fat and iron). A microfluidic system can take care of this instantly. It can also supply the very specific oligosaccharides by producing these on-site and on-demand. Because many consumers have many different wishes regarding the product, it is nearly impossible to store all the ingredients to produce everyone's milk on-demand. The use of microreactor technology would allow a limited number of ingredients to be stored and on-site, on-demand (bio-) chemical production of specialty components. The application thus goes beyond old-fashioned cutting and mixing and acts as a small-scale production site. However, not all products could be adapted to personal preferences. For example, liquid and emulsion-based food products are more likely to allow last minute individual accommodation than solid products such as bread and meat.

Application 2: microreactor technology in the supply of medicine

A second application of microreactor technology could be found in the *supply of medicine*. Pharmaceutical companies currently produce large amounts of medicines that are distributed through chemists and doctors. The chemist checks whether the combination of medicines or the patient's medical history conflicts with the use of the new medicine. The chemist might also prepare some medicine by diluting the active ingredient to the right dosage, either in a fluid or in a controlled release polymer. Again, microreactor technology could take care of the mixing, diluting, and the fabrication of active components. An appliance to manufacture these medicines could also be placed in hospitals or with a general practitioner.

With microreactor technology, part of the medicine production would move from the pharmaceutical companies to the chemist, reducing the number of stock keeping units for chemists. Active Pharmaceutical Ingredients (APIs) with similar chemical structures, for instance, could be stored in a precursor form (an inactive base structure). Chemical groups can be attached to or detached from the precursor medicine until the active component is obtained. On-demand and on-site, the desired component could be produced in the desired dosage.

Another possibility could be to move part of the dosing and fabrication of medicine to the home of the chronically ill patient. This semi-domestic appliance could be like a coffee machine, in which standardized cartridges of medicines or precursors are fitted. The appliance supplies the right combination of medicines in the right dosage at the right moment to the patient. This would eliminate having to swallow combinations of pills and variations of the dose in time. The appliance would indicate when to ingest the freshly prepared cup of water with all medicine dissolved at the right dosage. It is even possible to combine this system with an analysis. The patient could have an at-home analysis system to test blood or saliva (similar to self-administered tests for diabetes patients) and the dosage of medicine could be adapted to the need of the patient.

Application 3: microreactor technology in energy carrier supply

The third application of microreactor technology could be found in *energy carrier supply*. This includes the production of energy-carrying molecules and the (bio-) transformation of energy-carrying molecules to molecules of a more appropriate form. At the current rate of increasing oil prices and the increased attention for CO_2 emissions from fossil fuels, the call for different energy sources is becoming louder and louder. Microreactor technology can play a role in many processes in this area. With highly efficient catalytic micro scale reactions, transformation processes of CO and H_2 to hydrocarbons with the Fischer-Tropsch process, steam reforming of propane to form hydrogen, and soybean oil to biodiesel conversion could be done with microreactor technology in a highly scalable manner. A microreactor plant could also convert solar electricity to energy carriers such as hydrogen.

These applications could be found in different sizes. The smallest size would be at the domestic appliance level. The largest application size could be imagined at national or subnational levels. These large applications could be used in developing countries with increasing energy needs and little existing infrastructure, on the one hand, and a potential for biomass production (e.g., sugar in Brazil, or algae around the equator), on the other.

6.3 Social and ethical issues of microreactor technology

Microreactor technology can be evaluated by the rules that govern other science and technology. Universal concepts such as fairness, equity, justice, and power determine whether an issue is ethical. Like the 'nano' case (Grunwald, 2004, 2005; Lewenstein, 2005; Swierstra and Rip, 2007), microreactor technology also probably has no need for a specialized 'ethics' as this was shown for the 'nano' case. Both nano and micro technology are enabling technologies that can make the usual ethical issues more acute, but not change their nature. These two technologies could be dealt with according to the New and Emerging Science and Technology ethics, with certain recurring ethical argumentative patterns (Swierstra and Rip, 2007). This article will not describe these patterns, but will instead focus on the universal concepts.

In this section of the article, we discuss why microreactor technology would be used. What are the goals of using the technology, and why this is a good thing? Then we will discuss the risks, or costs (effectual risks), associated with this new technology. Finally, we will look at the distribution of these costs and benefits. These issues are first discussed in theory and then applied to the proposed applications.

6.3.1 Theoretical social and ethical issues

The good life and scripts

When evaluating risks and benefits of any technology, the underlying preconceptions of what is 'good' or 'bad' have to be understood. Such preconceptions are laid down in views of *the good life*. These views are centered around values that are considered to be most important, for instance, healthy living, economic wealth, or intellectual development. These values and their corresponding views of the good life are embedded in the science or technology itself and the ensuing applications. The inventor of such applications has certain assumptions about the direction of morality, technology, and society. These assumptions can be, for instance, that food is a means to being healthy and that personal choice in consumption of foods is directed at decreasing the chances of disease. As such, the design of a microsystem to personalize food products at home or at a distribution point (supermarket) implicitly assumes that people can and will use such instruments in the way the innovator intended them. The good life in this case can be a healthy life, where people strive to minimize chances of disease by consuming health-promoting foods on a personal level.

Preconceived notions of what technology might bring forth (functionally and socially) are 'inscribed' into the innovations. Such notions can be called 'scripts'. Extracting such scripts from innovations can reveal the values behind them and the normative assumptions with regard to these values (Akrich, 1992; Komduur et al., 2008). In addition to promoting the good life, scripts are also used to counter anticipated risks.

Acceptability of risks is determined by the view of the good life: according to certain views of the good life, as few as possible or zero risks should be taken in life, while others think that risks are acceptable up to a certain level.

Risks

As with any human activity, chemical industry is not risk free. A catastrophic event like the Bhopal accident in 1984 shows the magnitude of destruction that large scale chemical processing combined with inadequate safety regulations can cause (Broughton, 2005). One claim of microreactor technology is that it reduces if not eliminates risks with proper operation (Jensen, 2001). However, no technology is 100% safe. The risks involved with microreactor technology come in many shades of harm and likelihood.

Risks can be direct or indirect. Direct risks are most clearly identified. For example, in construction work working at great heights comes with the risk of falling The annual number of injuries could be an expression of costs. In addition to such clear risks, there are many more types of risk that are less easily identified. This decreased identifiability can be due to an unclear cause and effect relation. This unclarity can be caused by a time delay (smoking leading to cancer), by a lack of physical result (more administration leading to less privacy), or by a network of causes and effects (CO_2 emissions leading to global weather effects).

Assessing or quantifying risks and costs can be a difficult matter. When low level risk occurs fairly frequently, calculations can be rather simple. For example, the risk of using cheap pens in an office leads to frequent replacements because the cheap ones do not usually last as long as their more expensive counterpart. This risk calculation is fairly straightforward. When the risk involves catastrophic effects (nuclear meltdown) or emotional or physical harm to people (less freedom or a decreased life expectancy), calculation of both probability and harm is much more difficult. As people are dealing with new technology and new applications in a new environment, both the uncertainties and possible stakes are extremely high. This post-normal situation makes controlling the future nearly impossible (Funtowicz and Ravetz, 1993). While assessing and controlling the risk may not be possible, discussing them and dealing with them is crucial.

Currently, literature dealing with risks of microreactor technology in a broad sense is available. Some papers deal with the effects of medical diagnostics on privacy and safety (Räikkä, 1998; Renegar et al., 2006). Others deal with the safety and distribution of nanotechnology embedded in microfluidic devices (Grunwald, 2004). These technologies apply microreactor technology, but the social and ethical issues are fo-

cused on the medical or nano-applications and not on microreactor technology. Some attention is being paid to possible misuse of microreactors with chemical weapons, though. (Löwe et al., 2002). However, until now articles that discuss the social and ethical issues of microreactor technology have been few and far between.

Balancing benefits and costs

As risk is unavoidable, any progress or even status quo involves some cost. Cost is therefore not the only important factor in evaluating new technologies. Costs, however, should come with appropriate benefits. When the costs of an action, i.e. the effectual risks, outweigh the probable benefits, this action is not advisable. When the benefits are greater than the costs, the distribution of costs and benefits should not be overlooked. A misdistribution of cost and benefits within one society or between societies could deem a new technology unwelcome.

From a *utilitarian* viewpoint, the introduction of a new technology or group of applications is morally right when the total use of this change brings about a net positive contribution. The principle of utility should result in 'the greatest happiness for the greatest number' (Bentham, 1948). Answering ethical questions is a matter of calculating the net balance of benefit and harm. This holds true even when one greater group reaps the benefits while another smaller is faced with the costs. The groups that are divided by the cost/benefit line can be based on different criteria such as geography, time, wealth, and power.

With the first criterion, geography, different areas, mainly nations, block other areas from the benefits, while the costs are more distributed. For example, a country could block a river going to another country to generate hydroelectricity. Second, a clear example of cost/benefit misdistribution over time is the sustainability issue. Can the world continue producing and consuming on the same scale while maintaining the same level of prospects for generations to come? What will be the effect of today's benefits (use of energy and raw materials) on tomorrow's risk?

The third and fourth criteria, wealth and power, work in much the same way. Those who can afford the new technology will benefit, while others will not. The 'old-haves' can first and foremost benefit from new technology. Those who have power are inclined to maintain or improve their status. This widens the gap between the wealthy and the poor. Other technological examples of unequal wealth and power

distribution are the digital divide and the nanotechnological gap (Barker et al., 2005; Grunwald, 2004). This misdistribution is maintained by structures like the protection of intellectual property and the required financial investment for high-tech research.

We could apply the utilitarian view to the subgroups divided by the cost/benefit line. If those who are now confronted with the costs (the poor, the powerless etc.) received at least as many benefits as the rich, and the costs were distributed fairly, this would be morally right. Moreover, the morality is dependent on scale. What is morally right on the scale of the subgroup level could be morally wrong on a sub-subgroup or individual level.

Other ethical positions could also be applied. When we turn to the medical adagium *'primum non nocere'* (*'First, do no harm'*), new risks are avoided. However, possible benefits are also discarded. Proponents of environmental safety movements often favour this *precautionary principle*. According to the precautionary principle, technology should only be developed or applied when it is scientifically guaranteed that no harm will come to the environment or humankind. The burden of proof lies with the advocates of the new technology (Raffensperger, 1998, 2007). These advocates retort that implementation of the precautionary principle not only hampers technological progress, but also prevents improvement of the human condition in cases where any risk is involved. Though the precautionary principle is popular at times, a very strict adherence to it seems to be scientifically unfeasible as it demands to prove that something (i.e. risk) does not exist (Belt, 2003).

6.3.2 Applied social and ethical issues

The good life of microreactor technology

The proposed applications of microreactor technology are aimed at fulfilling present or perceived future needs associated with different views of the good life. This wide variety of goals can be reduced to a smaller set of ideas of what people want or need. During the interviews with the experts, four aspects of the good life were most frequently and extensively discussed. These were *autonomy*, *profit*, *health*, and *sustainability*. This categorisation is very similar to the win-win-win or Triple-P bottom line of People, Profit and Planet as proposed by Elkington (1994) and used, for instance, by Shell (1998, 1999). Discussing all four aspects in detail would be too excessive and go beyond the scope of this paper. As such, we will only focus on *autonomy*, both in the sense of being able to and being allowed to choose what you

want (individuality and independence), as autonomy is the most unique and defining characteristic of microreactor technology. The other three aspects will be touched upon only briefly.

Application 1: microreactor technology with personalized nutrition. The use of microreactor technology in food dispensers would allow consumers to decide what characteristics some of their food products should have. Instead of a selection of products the producers and retailers offer, products can be suited to an exact personal preference. Food can thus suit a person's wants and needs. When choosing the exact make-up of personalized nutrition, different food roles are invoked. Food can be regarded as a means to add physical fuel (energy source) and mental fuel (taste, well-being) to the human body. When consumers can choose what caloric content (for fuel), what fat content (for fuel and taste), or what specific flavours (taste) their mayonnaise will have, they are more free to adjust the products to their wishes. Consumers are then no longer subject to product-push from the producers and distributors. This autonomy could also be interpreted as independence; consumers and smaller producers could become less dependent on large scale producers as the economies of scale in food supply will diminish.

(...) There could be the effect that production, the addition of value to a product, will no longer be exclusive to large producers. Also, small players could become active in this area. (...) When primary producers can add value to their agricultural produce, they can make a better living. (...) (University1)

Food could furthermore be a means to instil health either by adding or removing ingredients. Health is viewed as the absence of sickness and the presence of fitness. Adding 'healthy' or removing 'unhealthy' ingredients aims to reduce the chances of sickness and increase the chances of physical fitness through food. The way to achieve these goals is through medical and food science. By interpreting data from epidemiological research and personal DNA-profiles, science can predict what harm is likely to befall an individual and can help avoid it. From research in nutritional and food science, science can then reason what buttons to push to make an individual 'as healthy as you can be'.

To make sure the consumer gets what he want or needs, the dispensing system will probably be connected to an information system. In this system, information on prior consumer experiences (taste evaluation by this consumer, like-minded individuals, or

consumers, in general) could be stored and communicated to the consumer at home or at the microreactor food dispensing unit. The system could also contain information on the genetic make-up and current personal health status of the consumer or society at large. The system could communicate that the consumer should not consume a certain food product construction, as it contains gluten, and suggest consuming another low fat product (because of a registered weight loss program) with additional vitamin C (because of the current influenza epidemic).

The use of such an information system is an example of a technical inscription. The developers' intent to use food for 'health' is expressed in their view that the 'health' role of food is dominant and the inclusion of healthy components in food will make people more healthy. The combination of information on the consumer's physical state and possible effects of food choice on the individual and displaying this information at the moment of purchase facilitates a 'healthy' food choice.

Application 2: microreactor technology in medicine supply. With microreactor technology enabled medicine supply, people can receive exactly what they need and when they need it. Furthermore, the number of doctor visits for check-ups and adjustment of the prescribed drug could be reduced. This could give people more freedom by eliminating time consuming trips to the doctor. Because individuality is not an expression of personal choice in medicine; a person's body, combined with diagnostic science and pharmaceutical technology, will determine what medicine the individual will get.

Taking medicine is no small matter. The active ingredient can be invasive and aims to change the body's functioning. Over and undermedication can have serious effects. It is therefore critically important to supply a patient with an exact amount of Active Product Ingredient. Using microreactor technology to produce and/or to dose this ingredient will allow for more accurate medication. The combination with a microdiagnostic system could even shorten the feedback loop of diagnosis and medication, giving patients exactly what they need when they need it.

(...) [A patient (JS)] takes pills for high blood pressure. Of course, the patient does not always have high blood pressure; it fluctuates. When blood pressure can be automatically monitored, sending a signal to take a pill or not, or to take a different dose, [taking medication (JS)] would be (...) more convenient to the patient. Now,

he goes to check-ups and his prescription is adjusted only once in a while, with uncertainty in between. (...) (University2)

The governing view of the good life is that health is the absence of sickness and that sickness can be combated or controlled with medication. The more accurate the medication is, the more accurately the disease can be fought. As the severity of diseases varies between patients and over time, it is important to be able to vary the dosage of the medicine. When diagnostic and pharmaceutical tools speed up the diagnosis-medication feedback loop, the patient will be healthier. This could be inscribed by a technical combination of a microreactor dispenser and a diagnosis system.

Individuality, or more specifically independence, could also be supported by replacing doctor and chemist visits with home diagnosis and medicine dosage. Patients with chronic disease will be less dependent on those frequent visits and will be more free to plan their time. Combined doses of multiple medicines in one convenient administration can further replace complex systems of timed intake of different drugs.

Application 3: microreactor technology energy carrier supply. With microreactor technology as part of the energy supply, the case could be made that people can choose what energy to use. They could opt for standard grid power and natural gas from the pipe system. With easy and cheap transition from one source to the other, users could disconnect themselves from the grid and choose to buy their power somewhere else. When companies or individuals are offered the chance to choose freely the energy source they use, they can consciously do so. This choice could be based on price, sustainability, or independence.

On a larger scale, countries or provinces could start providing for their energy needs. In doing so, they would become less dependent on large oil and oil infrastructure companies. This is especially the case for countries like Brazil, with vast quantities of biofuel resources, or countries around the equator with plenty of sunlight, which could be used directly or through the farming of algae and subsequent transformation into a relevant energy carrier. Due to the flexible applicability and scalability, the system can be easily introduced in many underdeveloped countries.

(...) [Developing countries choose (JS)] not to build an old-fashioned installation. Such an installation [has to be bought (JS)] from the developed world for a lot of

money and with the associated dependency. A microreactor installation could be built gradually [and users wouldn't (JS)] have to invest a 100 million [dollars (JS)] (...) (Industry1)

This new shape of global energy supply is rooted in the idea that countries want to be independent from other regions. On a more technical level, countries could express their autonomy by introducing their own brand of energy supply system. With each region developing its own system with its own specifications, the microreactor technology energy systems market could grow quite diverse.

Other drivers. In addition to *autonomy*, the interviewees frequently mentioned three other aspects: *profit*, *health* and *sustainability* to describe the benefits of microreactor technology. The benefits from these aspects are often entwined with autonomy and with each other. The *profit* of any product is important for any company or individual. Using microreactor technology could give an individual a competitive advantage or allow him to produce or consume more efficiently. Because reducing expenses would allow him to reallocate scarce means in other directions, microreactor technology could enable him to reach more goals. *Health* could be one such goal. As explained in the individuality section, microreactor technology could supply the food components or drugs that a person more specifically needs. This would improve personal health, as a person's specific health problem will be dealt with more appropriately. Increased health could be attained at lower cost due to earlier intervention or prevention, both of which tie this benefit to profit. A fourth and final driver that was mentioned is *sustainability*. From a nature conservation standpoint, in an ideal world people would all choose the production or consumption route that has the lowest environmental impact. The use of microreactor technology to produce medicine or energy carriers could reduce the burden of production on natural resources and the quality of the natural environment. Increasing oil prices and governmental price instruments could tip the economic balance for greener production and consumption.

Risks of microreactor technology

The risks of microreactor technology can be direct (physical harm) or indirect (unintended side effects or ill-considered perceptions of the good life). For the purpose of continuity, we will first separately discuss the autonomy-associated risks of the three applications. Within such a discussion of an application, the application's specific risks are also discussed. Later, we will touch upon risks associated with the other

three drivers.

Application 1: microreactor technology with personalized nutrition. The individualisation of food could reduce it to its fuel and health roles and overlook its natural, cultural, and communal functions. With microreactor technology, food is possibly no longer meant to feel connected to nature, to express cultural belonging, or to be an instrument of group behaviour (eating and living together); food is reduced to a choice that people make as individuals and is based on their preferences, either towards taste or personal health. The choice of such a food stuff is further channelled to one person, in that consumption of his food could conflict with other people's needs. For instance, based on an individual's DNA, he should consume more iron to prevent deficiencies, but he prefers to eat sweeter food. However, this meal could conflict with a fellow diner's diabetic and iron allergenic nature.

(...) The [DNA-profile (JS)] is made for one person. What if someone else eats from [this DNA-based specific food (JS)]? ... One cannot assumed that the use will exclusively be the intended use. (...) (Policy and Control1)

By allowing an individual to choose very accurately the food he wants or needs, he excludes other people's needs. This could further be inscribed in the appliance by making one person meals to prevent another from eating the 'wrong food'.

It is doubtful whether all people would like these choices. Standing in front of the microreactor food dispenser in the supermarket, will people know what they want? Will people know what to choose and why? Will people all not find themselves in Michael Pollan's Omnivore's Dilemma?

(...) The cornucopia of the American supermarket has thrown us back on a bewildering food landscape where we once again have to worry that some of those tasty-looking morsels might kill us. (Perhaps not as quickly as a poisonous mushroom, but just as surely.) Certainly the extraordinary abundance of food in America complicates the whole problem of choice. (...) (Pollan, 2006)

(...) In these types of situations, it is also important to know whether consumers can make the analysis of what is good for them. (...) (NGO2)

Furthermore, when food is individualized, the information on individual food choices will be stored, either for that individual's personal future reference, for health documentation, or for commercial purposes. Even today many people surrender part of their privacy in exchange for a price reduction in using customer cards. Details on shopping patterns are stored and can be used for targeted commercial activities. Depending on privacy laws and the terms of contract signed by the consumer, stored personal data could also become available to third parties, such as a health insurance company.

Due to rising health care costs, more attention is currently being paid to disease prevention than in the past. Promoting healthy living and eating is already part of the policies of many health insurers. Healthy living could be further promoted by sponsoring the use of microreactor technology food dispensers in supermarkets. When people eat the right things, they will get a reduction on their insurance premiums. In addition to health insurers, governments could also start helping people with their food choices. Personalizing food in this case means eating what individuals should eat, not what they want to eat.

Another risk specifically associated with microreactor food dispensers is food safety. Current centralized food production allows for a specifically trained staff and an extensive set of rules and safety precautions for producing large quantities of a small set of products. Many tools are available to monitor quality and safety. In the case of an extremely distributed production of food, risk of microbial contamination or other food hazards will have to be re-evaluated in a new context.

Application 2: microreactor technology in medicine supply. With home medicating, especially with a connected home-analysis appliance, the health level will be determined by a value from an analysis or by the concentration of an active component in the medicine. This reduced information set does not show other unrelated health problems. During a face-to-face consultation, a doctor could discover much more detail, not measured or catered for by the appliance, and may therefore prevent further health problems for the patient. In addition, the question of who should be measuring remains uncertain. Should people with minor physical and or mental disabilities become their own nurses or chemists?

(...) The patient is not an objective observer. [In the case of (JS)] lithium measurements [in blood (JS)], which is a medicine for manically depressed patients, the patient

is not mentally fit. Should this patient check his own lithium? (...) (University2)

Independence from doctor visits further assumes that the visit to the doctor is a burden rather than a social event. The application of home diagnosis (possibly through microtechnology) and home-medicating inscribes this assumption. Some people like to visit the doctor and see and talk to fellow-patients or want to visit the doctor to inquire about something else that has been physically bothering them. These people are not included in this view of the good life.

In addition to risks directly associated with the autonomy aspect of microreactor technology, more safety concerns could arise with the advent of microreactor enabled medicine supply. In administering medicine, it is important, above all, that the patient receives what he needs. The drugs administered should be of the right type, purity, and quantity. A foolproof system, combining hardware and safety precautions, is essential for a successful introduction to the market. In the case of mixing and diluting, the Active Product Ingredient is present in a high concentration, and this high concentration should be rigorously protected from system error and misuse.

Misuse of the application is a topic that requires some further study. When people have a machine in their house that is capable of high tech chemistry in a controlled manner, many other types of misuse could arise. The first and most innocent version of misuse is the unwittingly faulty operation of the system, resulting in production and intake of the wrong drug or the wrong concentration. In a wider context, the production or handling of chemicals can be dangerous for those around the work area. Currently, only a small group of trained staff work with toxic chemicals. Although microreactor technology is fairly safe due to its small volumes, the sheer number of microreactor systems and their distribution into inhabited areas has to be taken into account when evaluating the possibility of mishaps.

Overusing the machine for its intended purpose, producing drugs, and selling these is a second possibility of misuse. Third, more extreme types of misuse such as the wilful use of illegal modifications, for criminal or terrorist purposes, are not entirely impossible. Preventing such possibilities need to be considered when designing a system of hardware, distribution, and regulation. It must be added that it is questionable whether these risks are entirely new and unique to microreactor technology.

Application 3: microreactor technology energy carrier supply. A home fuel or energy converter will increase people's freedom to choose their energy source. Introducing such a system assumes that people care about the kind of power they get, either to become less dependent on one source, to choose the cheapest option available, or to increase sustainability. The current one-size-fits-all solutions for electricity and gas might lose importance. As more people become detached from the grid, who will maintain and pay for the social infrastructure for power for all? Energy, along with water supply and sanitation, has been an early part of public services. Removing the energy supply from the collective domain could be a step towards full self-sufficiency, with less regard to the socially weak.

On a much larger scale, countries or regions could also become more independent. This could reduce the international level of connectedness (international cooperation), or at least shift the balance of power. Powerful energy nations could then loose power and powerless nations could gain power. Connecting different energy systems from different regions could also become a problem. A similar situation can be found where different countries have different plugs and sockets for electricity. There is not one standard plug that works around the world, as could become the case with a diversified microsystems market. When different regions insist on their independence and energy system, interoperability could become a problem.

(...) It could also be a disadvantage when [a country (JS)] runs into trouble and [that country (JS)] makes something nobody else can supply. (...) The moment its own factory stops running it cannot go to the neighbours and borrow something. It doesn't fit. (...) (Industry1)

Other drivers. Moving away from *autonomy* to the three other drivers, *profit* often becomes a dominant driver in a capitalist society. When using microreactor technology in health improvement (in medicine or food) can result in reduced health care costs, the personal freedom of people to eat and do what they like could be at risk. The possible health benefit becomes subject to a market economy and limits personal freedom. Additionally, the assumption that *health* can be controlled through diet and medicine could yield undesired results. In the past, science has greatly helped to enhance the length and quality of living through sanitation and improved food quality. In Western society, most 'simple' problems are now solved. Further improvement of health through food is focused on improving eating patterns (difficult to realise) and the effect of micro-components (effects still elusive). Trying to steer people towards

'eating right' is therefore extremely difficult and could result in unwanted results.

(...) There is a trade-off. Anything that works (...) carries with them an increased risk, in effect they become like medicines. (...) If the foods generated by this machine become clinically effective, then they carry potential risks and the need for regulatory oversight of a kind that you might get for a medicine for example. (...) If they aren't effective, then you're cheating. (...) (NGO1)

A single future microreactor application compared to its present day counterpart could have an improved *sustainability*. This reduced impact (and often associated cost) of a single product or use could induce increased use or consumption (Terpstra, 2008). For instance, over time the automotive industry has improved the efficiency of the internal combustion engine, which has allowed cars to go further on less gas. However, due to increased safety and comfort demands, the car's weight and energy consumption have increased. These have cancelled out most of the improved mileage. As a result, the total US car fleet mileage in 2004 was 25.0 miles per gallon, the same that it was in 1984 (U.S. Department of Transportation, 2004).

Balancing benefits and costs with microreactor technology

In the previous two sections of this paper, some of the benefits and costs (effectual risks) of the use of microreactor technology were discussed. Ideally, we could attribute utilistical weights and probabilities to these discussions or prioritize one over the other. However, this is not yet possible because the effects and likelihoods of microreactor technology are still quite uncertain. In this section, we will try to position benefits and risks and consider the effects of their distribution over different groups. Since we address only a small selection of arguments on this subject, this discussion will only be an example of how such positions could be discussed. The aim is not to come to a judgment but to consider different viewpoints. We will discuss the three applications, followed by a general discussion.

Application 1: microreactor technology with personalized nutrition. The main issue with personalized nutrition is the freedom to choose. Microreactor technology gives people the opportunity to choose what they like or what they need and allows them to choose what goals they strive for. At the same time, freedom to choose may not be absolute. Will people know what to choose and will they be allowed to do so by government or health care organisations? What price will they have to

pay for this freedom? Possibly, those who can afford it can choose what they want. Meanwhile, less privileged individuals might be persuaded to eat what is right for them in exchange for a lower VAT or health insurance premium. When microreactor food is more expensive and not sponsored, those who can afford it can eat what is specifically right for them. Lower income individuals might still be eating the mass produced food. In any case, the risks and benefits will not be equally distributed over different levels of wealth.

(...) They are generally marketed in a way that you will have to pay some kind of premium for the functional food product, or in this case the service of producing one. That means that in effect you are marketing to the sort of the 'healthy wealthy', who are the sort of people who don't need to eat them in public health terms. (...) (NGO1)

The use of microreactor technology could help break the law of economy of scale, bringing more suppliers and producers into the market place. This could reduce the power of large production companies in production and distribution. Smaller companies and different parties in the distribution chain could fill an important role in what people can buy and eat. On the other hand, it is uncertain whether a few large companies will dominate or a more dynamic and diverse, multi-company business will arise to dominate the production of microreactor technology. Will those now in power allow the emergence of manifold new producers? In other words, will power block the benefits for the powerless?

The focus on the health and fuel roles of food through personalized nutrition will be determined by the extent of microreactor technology use. Given the limited range of products, it is unlikely that whole meals will be prepared on a regular basis with microreactor technology. The roles of food have changed over the years as a result of changing social structures. The advent of personalized food portions could facilitate further change in these roles, but it seems unlikely that it will be a dominant, steering force.

Two other risks of microreactor technology mentioned earlier are the use of personal information (privacy) and food safety. These risks are not unique to microreactor food supply. Current safety and privacy laws can be adapted to new technology. This is an ongoing process that needs attention, but it should not result in insurmountable problems.

Application 2: microreactor technology in medicine supply. The key criterion for using or not using microreactor medicine is what is best for the patient. With personalized medicine, the patient will get what he needs when he needs it. Diagnosis-medication feedback loops can be shortened to reduce both under and overmedication. On the other hand, reducing diagnosis to a small number of measured health parameters and reducing the number of doctor examinations might reduce the chances of spotting symptoms that are not being looked for. In many cases, automated or personal distant diagnosis and medication will be right for the patient. The problem lies with the group of patients that need new expert input from an actual doctor. In all probability, the personal supply of medication at home will only cater to those with a chronic disease, which follows a known course and has known indicators of illness. Using microreactor technology in medicine supply will not eliminate visiting a doctor for the initial diagnosis and treatment.

It is not yet clear who will benefit from microreactor technology. As health care is an enormous industry with widely established institutions, profit will be an important factor in the chances of success. How this will turn out is unsure. Will producers be able to produce more cheaply, with less waste, or will consumers be paying less for the product? Will all patients receive better care or will variation in health care supply favour the rich with choice and handicap the poor with force? Whether cost and benefit for both producer and consumer will be divided along the lines of wealth and power will be determined by discussion in society and the consequences of this discussion in the political debate.

The costs of distributed chemical production systems, either by poor operation, or by intentional misuse, could be addressed by either technical or policy countermeasures. A home appliance should be designed to be technically foolproof. Its handling should be easier than operating a microwave or a coffee-maker. It should work correctly or not at all. Any tampering with the system should result in failure to work. Other possibilities are that any production is pre- and/or post-approved through automated contact with the producer or medical institute, or that the appliance has an onboard analysis system for a chemical check. In the case of medicine production at a chemist's or at a doctor's, limitations could be less strict.

In addition to technical means, policy measures could also reduce risks. A first measure could be the limited release of the appliances. By allowing only chemists, doctors, and hospitals to own and operate such a system, monitoring would be much

easier. A system of permits, purchase and production registration, and regular checks would allow the technology to be more widely adopted. The chemical cartridges could also be subject to such a policy system. Finally, when skilled operation is necessary, a mandatory training program could be installed for all operators.

Application 3: microreactor technology energy carrier supply. The possible costs and benefits for the individual household and the large regions are similar. The microreactor energy system could cater to an individual and allow him the freedom to choose his own source. For example, the environmentally minded will try to reduce their footprint, while farmers might want to use their animal or plant waste. Moreover, regions with a large amount of sunlight might want to grow algae or use the sunlight directly. Other regions could use their sugar production capacity for ethanol of hydrogen production. By greening the energy supply, the cost and benefit distribution over time could improve as energy became more sustainable.

For decades, those who could supply energy had political power. With rising oil prices, many are looking for new energy sources. For those with enough wealth or power, the future supply of energy can be ensured by negotiation, collaboration, purchase or force. The ability to be a future energy supplier seems to be determined by geography, i.e. sunlight, farming acreage, etc. Benefits seem to reside with those areas, whereas the cost (energy security and price) lie with those areas that cannot provide for their energy needs. It is remarkable to notice that with the relative ease of implementing microreactor technology in large scale energy supply, the current underdeveloped countries around the equator could benefit more than the traditionally richer countries.

The importance of energy in combination with rising prices leads to nervous markets. For an individual to maintain his level of activity as a person, household, or region, he has to ensure his energy supply. This means protecting what he has and trying to obtain what he needs. In turn, this could result in a more aggressive protection of those who have access to energy. This could then lead to a situation in which a multitude of parties or regions develop their own microreactor system to supply their power. As a result, the balance of power will shift from the known situation to a situation with more groups who all have their own brand of energy supply. The world needs international agreement on trade and standards to ensure interoperability and market stability. In doing so, the risk of market and political turbulence could be averted and the transition from a small group to a multitude of energy suppliers

Table 6.4: A selection of key aspects, benefits, and costs associated with the autonomy driver of microreactor technology applications.

Key aspect	Benefit	Cost
Technological advances	Functionally better products	Safety and uncertainty
Choice	Freedom to choose	Forced to choose, inequality
Independence	Self-determination	Loss of coherence

could enrich the portfolio of energy supply possibilities.

General discussion. When we now group the costs and benefits, we can connect them by key aspects. Table 1 lists these key aspects, benefits, and possible costs. The first aspect, safety, can be addressed most easily. As discussed earlier, safety concerns could be countered by adequate technical and policy provisions. The success of any application depends on recognising such concerns in an early phase of development and dealing with them appropriately. This includes doing what is necessary to remedy the problems and communicating these actions. With choice and independence, the two other aspects, technical solutions are near impossible. The success or failure of an application lies in the public's acceptance of the application and the rules and regulations accompanying its introduction and use. As it will take many years before the proposed products will reach the market, people have enough time to prepare; the applications will not come into being within a day. This means that before entering the market, the technology and its applications will undergo a long process of incremental change.

During this process, drivers, key aspects, benefits, and costs can be discussed in learned societies, political circles, and the public domain. To facilitate a fair and balanced discussion, all aspects of the application or technology should be communicated in an adequate way otherwise the application might fail as shown by the introduction of GMO products. This technology communication will be discussed more extensively in the next section.

6.4 Technology communication

Not only effectual risk but also fear of risk can be damaging. The person or organization responsible for a potential risk also involuntarily creates fear of this risk (Schummer, 2001). This aspect has not always appropriately been dealt with. New technology or its applications are sometimes put into the market because they can be produced (technology-push). Even when research shows that risks are minimal, attention should be paid to the public and its perception of a changing technological environment. This subsection will deal with risk perception and ways of communicating novel technologies.

6.4.1 Theoretical technology communication

The public concern with risks from technological advances has existed for a number of years. Think of Mary Shelley's 1818 book, *Frankenstein*. In the real world, scientific study has reported the public's perception of risk for decades (Gurabardhi et al., 2004). How risk is perceived depends heavily on the person perceiving. Experts often express risks by *'(...) estimates of annual fatalities(...)'* (Slovic, 1987); risks are quantified by a number. When the uncertainty of risk increases, the range of numbers grows. However, there will still be a number that can be compared with other numbers indicating probable costs or risk. The general public has a different view of risk. Public perception depends on factors such as uncertainty, dread-factor, possible catastrophes, and the freedom to take or avoid a risk (Groth III, 2001; Slovic, 1987).

In addition to these factors, risk perception can be based on views of the good life. A certain view of what is desired is always accompanied by a view of what is not desired or perceived as risk-bearing (Korthals, 2004). In addition to the intended benefits, the perceived risks are also inscribed in products. An example of the good life could be that safe transportation is the most important benefit of driving a car. This is inscribed by installing seat belts to maximize safety. A perceived risk could be people not wearing a seat belt. This could be countered by having an audio or visual alarm system for not wearing a seat belt.

The discrepancy between expert and public perception of risk has not gone unnoticed. The fact that not communicating about the same issues is not helping any of them is equally understood by engineers (Borchelt, 2001; The Royal Academy of

Engineering, 2003, 2004), risk scientists (Wynne, 2006), and consumers (Groth III, 2001). Answering the questions why and how to communicate is more difficult.

Different reasons for communicating could be to inform (*'(...) to enable them to understand (...)'*), or to educate (*'(...) to enhance the level of scientific(...) literacy(...)'*) the public (Borchelt, 2001). Others state that communication can be oriented towards persuading the public with (technical) content, informing them in the process (participating), or even fully integrating the public (partnering) (Chess, 2001; Fischoff, 1995). Historically, the most common strategy has been to show the scientific evidence for the benefits of the new technologies. Public mistrust of science has been continually perceived as a lack of information (deficit model) and can be overcome by filling that knowledge gap (Wynne, 2006). Vast amounts of scientific information have been fed to the public through printed mass media, radio, television, and the internet. However, such a steady flow of information does not drastically change the public's opinion (Hornig, 1995). Incidents portrayed in the media can influence people's opinion in a more direct manner (Chess, 2001; McInerney et al., 2004; Slovic, 1987).

Different types of information come from different sources that interact with each other as Schummer has shown for nanotechnology (Schummer, 2005). On the one hand, consumer activist groups (ETC group, 2003) and science fiction, such as Michael Crichton's 2002 book *Prey* on nanotechnology, present an apocalyptic nightmare of technology. However, at the other end of the spectrum, those who stand to benefit from the popularization of the technology: companies and research institutes who have invested time, money, and their good name, will paint a more utopian picture of what could be achieved with the new technology (Gordijn, 2005). Both extreme visions of technology are mostly based on simplified views and information and will be judged by the majority of the public accordingly.

In recent years, the deficit model as the basis for communicating with the public has encountered opposition (Borchelt, 2001; The Royal Academy of Engineering, 2003; Wynne, 2006). However, the literature shows several reasons for abandoning this model. First, the public feels it is not taken seriously by science and governmental institutions. It has seen examples of science and government unable to deal with technology and governance gone awry (e.g., Chernobyl or mad cow disease). The public has grown to mistrust them (Macoubrie, 2005; The Royal Academy of Engineering, 2004; Wynne, 2006). Second, the scientific world is faced with problems

that are very complex. It is unable to anticipate completely the physical or social outcome of its inventions (global warming, GMO) and, as such, it cannot predict or communicate the consequences of these inventions (Funtowicz and Ravetz, 1993; Wynne, 2006). Third, scientists form a group with its own set of conventions and assumptions. Their paradigm sometimes prevents them from going beyond what they can measure but what could be relevant (Funtowicz and Ravetz, 1993; Slovic, 1987; Wynne, 2006).

Recognizing that a more interactive form of communication will be beneficial, many have embraced the concept of the two-way dialog (Better Regulation Task Force, 2003; Borchelt, 2001; Funtowicz and Ravetz, 1993; Groth III, 2001; The Royal Academy of Engineering, 2003, 2004; Wynne, 2006). However, this two-way dialog is interpreted in different ways. We have grouped these ways into four categories. These categories are based on the degree of influence that the public has on the outcome: the *very weak*, the *weak*, the *strong*, and the *very strong interaction*. This categorization is very similar to Sandler and Kay's example of social and ethical issues with nanotechnology (Sandler and Kay, 2006).

The very weak interaction: This is the classic case of the deficit model. For instance, science determines what is most important and distributes this message. The scientific community is the neutral gatekeeper because it is financially independent of the research outcome. By keeping a good overview of all relevant aspects and not being distracted by momentary hypes, the public will be informed in an appropriate, well-balanced, and reliable manner. Any interference from the public, industry, or the government would damage these qualities of information. Similar reasoning could be applied to communication from politics ('we are the people, so we know what they want to know') or industry ('we have the latest and newest information, and are closest to practice').

The weak interaction: In this case, the public is still informed by 'those-who-know', such as the scientific community, politicians, or industry. However, weak interaction is two-way communication. The primary communicator listens to the public and finds out what they want to know. What are their wishes, fears, and perceived risks? How do they want to be informed? The process behind the communication (doing research for science, making laws for politicians, producing products for industry) is not directly affected by this interaction with the public. Merely the manner in which they communicate and the content of this communication is negotiable. Borchelt

(2001) gives an example of this in the third finding in his article 'Communicating the future: report of the research roadmap panel for public communication of science and technology in the twenty-first century.'. In short, the experts freely choose their direction of action, within the boundaries of law.

The strong interaction: In the case of strong interaction, the public is not only informed of the findings, but also plays an indirect role in the decision making. They are involved in identifying problems and defining their boundaries. They could be consulted on defining the tests to measure risks and included in feedback discussions on the results. In more extreme cases of risks, the public can even block new technology. The effect of the public on the expert actions is indirect; its power will mostly be reactive through NGOs and elected government (Better Regulation Task Force, 2003; Groth III, 2001). The public will not have additional new tools to stop or promote new technology.

The very strong interaction: Now, public interaction becomes public control. The public has control over what is allowed and what is not. Publicly appointed committees have to pre-approve the scientific production of knowledge, its distribution, and its use. Some NGOs such as the ETC group ETC group (2003) and, to a lesser extent, some risk scientists (Funtowicz and Ravetz, 1993; Wynne, 2006) support this view.

6.4.2 Applied technology communication

The market introduction of novel technology is rarely a goal in itself. The introduction should be aimed at maximizing benefits, minimizing costs, and finding an optimal balance in doing so. Differences in perception of these aspects between different groups could be a problem in wide adoption of new technology. It is crucial to bring together the views of such groups through communication and, more importantly, discussion. The unidirectional communication from the past was aimed at filling an information deficit. Most now agree that this type of information flow is insufficient for settling differences of opinion in society. Engineers, scientists, and consumers (Borchelt, 2001; Groth III, 2001; The Royal Academy of Engineering, 2003, 2004; Wynne, 2006) have stated that discussions should be more open and multidirectional.

Discussions between groups that are passionate about their different views can be a difficult matter. It will take a joint effort to overcome the respective paradigms. These

paradigms are not necessarily confined to a certain group such as science, industry, public, or government; paradigms include not only the respective viewpoints of the groups, but also the perception of views of other groups. For example, new technology is an opportunity and costs can be reduced only by technical means (perceived view for science and industry), new technology is a risk and should be avoided (perceived view for critical groups and consumers), and new technology's costs can be controlled by rules (perceived view for government).

It is never easy for an individual or group to see beyond its own ideas, especially when personal interests are at stake. This emphasises the need for timely discussion, when the stakes are not too high. The introduction of GMO food products in the 1990s demonstrated the effect of ineffective and late interaction between the different groups. The backlash against GMOs resulted in technological, economic, and social damage (Sandler and Kay, 2006). The situation with microreactor technology now is not the same as it was with GMOs, but the history of the GMOs illustrates what could happen when no lessons are learnt. Timely discussions of social and ethical issues as mentioned in this paper could ease tensions before they run too high as well as avoid economic and technological damage for the technology's supporters in science and industry.

With discussion on technological development in the early phases, those who might oppose the technology will not feel overrun with a development that they cannot influence. On the other hand, those that propose the new technology have not invested all they have in fixed directions or products. Discussions in which both parties do not yet have a fixed position on the outcome (based on either promises to the organisation's rank and file or based on the vested financial interests) could prevent a trench war with no winners. During these discussions the participants should be willing to dig deeper than the arguments and reasoning common to their own background. Exploring the basic elements of costs, benefits, assumptions, and scripts as discussed in this paper could help to level the playing field. By agreeing on a common set of goals and measuring tools, future discussions can be facilitated with measured expressions of actualized benefits and costs (The Royal Academy of Engineering, 2003).

What power the public should have to promote, block or steer new technology is much debated, as was shown in the previous section. When we look at the examples from the recent past, the public is not entirely powerless. Public uproar over GMOs, in general, and GM food, in particular, brought about EU rules on the allowance

of GM to the EU market (Regulation (EC) No. 1829/2003) and labelling of GM foods (Regulation (EC) No. 1830/2003). Public concern with radiation from cellular phones and GSM or UMTS base stations has made some Dutch municipalities reluctant to install base stations. In different regions of the world, governments prohibit human embryo research and the construction of nuclear power plants because of public concern. These examples demonstrate that the public can affect technology and its application predominantly through political channels. Currently, a large number of critical organisations are becoming very proficient in influencing industry. A good example is Greenpeace, which used public opinion to stop the sea disposal of the Brent Spar (Talary et al., 1998) and motivates electronics producers to reduce the amounts of toxins in their consumer appliances (Apple, 2008; Greenpeace International, 2008). The more participative discussion that is proposed demands an approach from the NGOs that is markedly different than their current methods.

Proponents of the new technology stand to benefit from a more favourable public opinion. To avoid technological and financial damage due to public backlashes, new technology proponents must start the debate and begin discussing what they are doing and plan to do. The public, facilitated by democratic power and an active range of critical organisations, is waiting. When a novel technology may face sizable public resistance, discussions early in the process and subsequent corrections in the direction of research could result in reduced damage for industry and research. These corrections could include more extensive research of a risk or a particular niche of interest. As it is in the interests of the proponents, it would be logical that they (e.g., research organisations and trade organisations) initiate these discussions. Governmental organisations could facilitate these initiatives.

The different groups will not always be able to agree on all subjects. Their first objective should be getting a clear picture of all the views of the good life, associated scripts in applications, and arguments. They can then try to understand each other's positions to find a common ground, to find a way to work around problems, or to agree to disagree. A common ground could be found by all parties agreeing to either a specific application or a specific research direction. The proposed direction of technology could also be so broad that parties with different views of the good life see something of their interest in the development. The discussion should not simply be directed at a go or no-go decision for a certain application; it could question the direction of the research or the type of applications that are being proposed. This could help find a way around difficult subjects or possible future applications. When

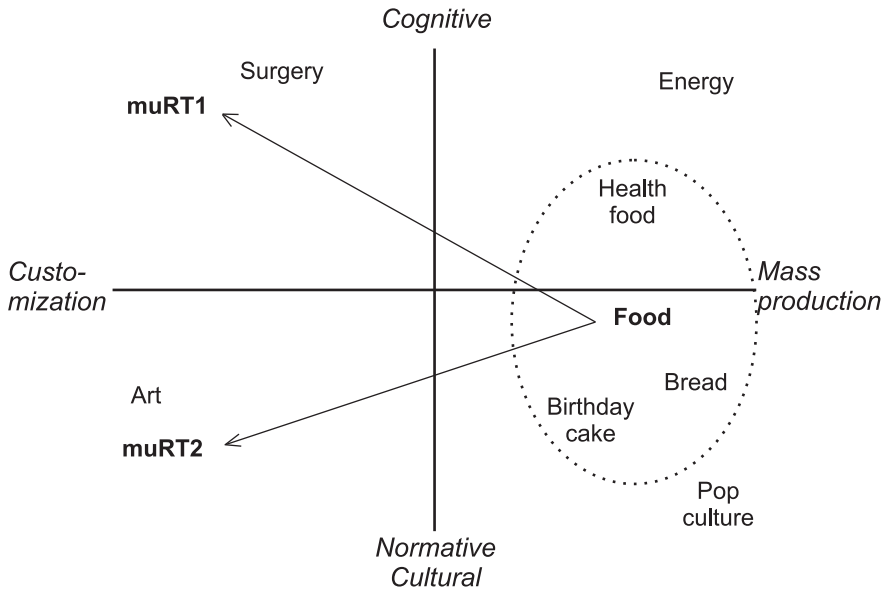


Figure 6.1: Location of different consumption products positioned by their degree of customization-mass production and cognitive-normative/cultural consumption.

no agreement is reached, existing political and legal means could be addressed to make a decision.

Over the past decades, we have seen some examples of increasing public power over industry (for instance, Greenpeace, as discussed earlier), especially through the professionalization of NGOs. Given the power the public currently has, it seems that a more formal power is not needed as suggested in the *very strong interaction*. A separate, new body of power that could officiate technological developments by handing out yellow and red penalty cards would need a new set of legal instruments. This very strong interaction would cripple technological progress by instituting a bureaucratic machine to review all proposed research and application. Very few would support such a system, especially as the current system results in very few catastrophes.

By using the system of *strong* interaction, the public can exercise its democratic right to prevent what it perceives as unwanted developments. However, the current system is not ideal. As broad discussions from earlier phases in research have been rare until now, the interaction has been reactive at best. Nanotechnology could prove one

of the first cautiously positive examples. To reach the goals of maximizing benefits, minimizing risks, and taking care of their distribution, a committed communication from all stakeholders in the early phases is necessary.

6.4.3 Microreactor technology entering society

Microreactor technology is a novel technology that has the potential of broad application. The examples mentioned in this paper are merely an indication of what could be expected based on our current views of the technology, the market, and society. Over the next decades, the impact of microreactor technology on our lives will become clear. During these decades the debate of what could be and should be developed can run parallel to the actual technical development. The development of microreactor technology has just past the proof of concept phase. The first professional applications are entering the research and clinical market. This is a good moment to start discussing the possible directions the technology could take. Discussions between industry and science are abundant. Including the critical consumer organisations and policy and control agencies is the logical next step.

During this discussion, the aspects from this paper, such as autonomy, profit, health, and sustainability could be discussed. The tools used in this paper could serve as a basis for extracting relevant views and preconceptions from beliefs and questions. Taking autonomy as an example, we now demonstrate how conflicting views could be resolved.

Let us regard two opposing views on autonomy in relation to microreactor technology's use in the production of individualized food. The technical view of individualized food supply could be that people can eat better through informed and personalized nutrition. Based on a known medical and/or genetic disposition, science can predict what an individual should eat to be healthier and therefore have an improved life. An opposing view could be that food should not be subject to medical, industrial or governmental supervision. Food and eating are a part of people's personal lives. People base their choices in food on much more than just health. Clearly, conflicting ideas exist on the role of food. The former view assumes the cognitive position that food is a means to be healthy. Food has functional roles as fuel (food composition) and medicine (vitamin content). The latter view assumes a more normative or cultural role of food. Eating is what people do to feel good. Food choice can be based on taste (high sugar content), cultural belonging (turkey with Christ-

mas), or applicability in a group function (beer while watching a football match). Furthermore, the ability of third parties to look into an individual's shopping basket, through information technology is undesirable.

Enforcing the views of science or industry will diminish a product's chances of success in the market. Inscribing the view that food is a medicine into the application, enforcing or strongly suggesting healthy choices at the microreactor food dispenser, will conflict with other views of food. This conflict might damage the application's chances of wide adoption. Taking other views of food and privacy into account and understanding an individual's inscriptions, the microreactor food dispenser could be designed differently. A first step could be the elimination of an obligatory identification. When the consumer can receive and pay for his individualized product anonymously, privacy is better guarded. Furthermore, the microreactor dispenser interface could be more neutral in its lay-out. Healthy choice options could be proposed when the consumer indicates this. By making the options and product information neutral, the consumer can decide for himself what he wants to eat.

Visualizing different ideas about the directions of a new technology can help understanding between groups. Figure 6.1 shows the position of some consumption products when they are evaluated on production type and consumption type. Products that are mass produced are at the right end of the spectrum, while products from highly customized production are on the left. Products that are consumed or purchased based on cognitive arguments can be found at the top, those based on normative or cultural motives are at the bottom. The two arrows represent the two directions that microreactor technology could take. While a microreactor food dispenser for cognitive health purposes is located in the top-left quadrant (muRT1), a dispenser that facilitates a completely free choice could also cover the bottom-left quadrant (muRT2) and thus supply both ends of the vertical spectrum. Similar graphs could illustrate the different positions that the various groups envision for novel technologies.

This is one example of exploring the benefits, costs, and preconceptions. Benefits such as more freedom to choose food, and the ability to adapt it to personal needs are maximized. Costs such as privacy issues and pressure to choose what is 'right' are decreased. At the same time, preconceived inscriptions about what is 'right' are recognized and can be avoided. The example shows only one aspect with one possible conclusion. Many more aspects can be discussed and each with a wide variety of potential outcomes. The discussions might not always result in full agreement, but

could at least prevent social, technological, and economical failure.

6.5 Conclusions

Although attention for social and ethical issues with microreactor technology has been rather low in the literature, many aspects of microreactor technology deserve ethical discussions. In this paper, we discussed three possible future applications of microreactor technology, analyzed their benefits and costs, and tried to find a balance between them. Out of a group of key aspects, *autonomy* was chosen to be the most distinctive for this technology. Finding an optimum between the costs and benefits could be achieved by technical and control policy provisions as well as through discussion and adjustment of the direction of technological development.

A successful introduction of novel technology greatly depends on how the technology is communicated. The most appropriate communication method is a committed, open, and timely discussion between all stakeholders. A new legal institute where the public has power of intervention is not needed. Through political influence and pressure from critical organisations, the public has sufficient influence to co-determine the direction of technology. Until now, this power has mostly been used reactively (downstream). With multi-stakeholder discussions in a novel technology's early phases, the public's influence could become a more contributive power. The discussions could make use of scripts as a tool to investigate perceived views of the good life and the associated benefits and costs. As the technology's proponents stand to benefit most from decreased technological and economic damage caused by a market backlash, it is in their best interest to initiate such discussions. The ultimate practical applications of microreactor technology and their success could depend on the outcome of these discussions.

References

Akrich, Madeleine. 1992. The de-scription of technological objects. In *Shaping technology/Building society.*, eds. W.E. Bijker and J. Law, 205–224. Cambridge, MA: MIT Press.

Apple. 2008. A greener apple. URL http://www.apple.com/hotnews/ agreener-apple/, visited May 22 2008.

Auroux, Pierre-Alain, Dimitri Iossifidis, Darwin R. Reyes, and Andreas Manz.

2002. Micro total analysis systems. 2. Analytical standard operation and applications. *Analytical Chemistry* 74(12):2637–2652.

Barker, Todd, Michael Lesnick, Tim Mealey, Rex Raimond, Shawn Walker, Dave Rejeski, and Lloyd Timberlake. 2005. Nanotechnology and the poor: opportunities and risks, closing the gaps within and between sectors of society. Tech. rep., Meridian Institute.

Belder, Detlev, Martin Ludwig, Li-Wen Wang, and Manfred T. Reetz. 2006. Enantioselective catalysis and analysis on a chip. *Angewandte Chemie International Edition* 45:2463–2466.

Belt, Henk van den. 2003. Debating the precautionary principle: "guilty until proven innocent" or "innocent until proven guilty"? *Plant Physiology* 132:1122–1126.

Bentham, Jeffrey. 1948. *The principles of morals and legislation.* New York.

Better Regulation Task Force. 2003. Scientific research: innovation with controls. Tech. rep., Better Regulation Task Force.

Blow, Nathan. 2007. Microfluidics: in search of a killer application. *Nature Methods* 4(8):665.

Borchelt, Rick E. 2001. Communicating the future: report of the research roadmap panel for public communication of science and technology in the twenty-first century. *Science Communication* 23(2):194–211.

Broughton, Edward. 2005. The bhopal disaster and its aftermath: a review. *Environmental Health* 4(1):6.

Burns, Mark A. 2002. Analytic chemistry: Everyone's a (future) chemist. *Science* 296(5574):1818–1819.

Chess, Caron. 2001. Organizational theory and the stages of risk communication. *Risk Analysis* 21(1):179–188.

deMello, Andrew J. 2006. Control and detection of chemical reactions in microfluidic systems. *Nature* 442:394–402.

Dyer, C.K. 2002. Fuel cells for portable applications. *Journal of Power Sources* 106:31–34.

Elkington, John. 1994. Towards the sustainable corporation: Win-win-win business strategies for sustainable development. *California Management Review* 36(2):90–100.

ETC group. 2003. The big down: From genomes to atoms. Tech. rep., ETC group.

Fischoff, Baruch. 1995. Risk perception and communication unplugged: Twenty years of process. *Risk Analysis* 15(2):137–145.

Folch, A., A. Ayon, O. Hurtado, M.A. Schmidt, and M. Toner. 1999. Molding of deep polydimethylsiloxane microstructures for microfluidics and biological applications. *Journal of Biomechanical Engineering* 121:28–34.

Funtowicz, Silvio O., and Jerome R. Ravetz. 1993. Science for the post-normal age. *Futures* 9:739–755.

Gordijn, Bert. 2005. Nanoethics: from utopian dreams and apocalyptic nightmares towards a more balance view. *Science and Engineering Ethics* 11(4):521–533.

Greenpeace International. 2008. How the companies line up. URL http://www.greenpeace.org/international/campaigns/toxics/electronics/how-the-companies-line-up , visited 22 May 2008.

Groth III, Edward. 2001. The debate over food biotechnology in the united states: is a societal consensus achievable? *Science and Engineering Ethics* 7(3):327–346.

Grunwald, Armin. 2004. Ethische Aspekte der Nanotechnologie. Eine Felderkundung. *TechnikfolgenabschÃdtzung - Theorie und Praxis* 13(2):71–78.

———. 2005. Nanotechnology - a new field of ethical inquiry? *Science and Engineering Ethics* 11(2):187–201.

Gurabardhi, Zamira, Jan M. Gutteling, and Margôt Kuttschreuter. 2004. The development of risk communication: an empirical analysis of the literature in the field. *Science Communication* 25(4):323–349.

Hornig, Suzanna. 1995. Information equity, public understanding of science, and the biotechnology debate. *Journal of Communication* 45(1):39–54.

Huikko, Katri, Risto Kostiainen, and Tapio Kotiaho. 2003. Introduction to micro-analytical systems: bioanalytical and pharmaceutical applications. *European Journal of Pharmaceutical Sciences* 20(2):149.

Janasek, Dirk, Joachim Franzke, and Andreas Manz. 2006. Scaling and the design of miniaturized chemical-analysis systems. *Nature* 442:374–380.

Jensen, Klavs F. 2001. Microreaction engineering - is small better? *Chemical Engineering Science* 56:293–303.

Jönsson, Christina, Stina Lundgren, Stephen J. Haswell, and Christina Moberg. 2004. Asymmetric catalysis in a micro reactor - Ce, Yb and Lu catalysed enantioselective addition of trimethylsilyl cyanide to benzaldehyde. *Tetrahedron* 60:10,515–10,520.

Kikutani, Yoshikuni, and Takehiko Kitamori. 2004. Micro-flow reaction systems for combinatorial synthesis. *Macromolecular Rapid Communications* 25:158–168.

Kling, Jim. 2006. Moving diagnostics from the bench to the bedside. *Nature Biotechnology* 24(8):891–893.

Köhler, J. Michael, and Thomas Henkel. 2005. Chip devices for miniaturized biotechnology. *Applied Microbiology and Biotechnology* 69:113–125.

Komduur, Rixt H., Michiel Korthals, and Hedwig te Molder. 2008. The good life: living for health and a life without risks? On a prominent script of nutrigenomics. *British Journal of Nutrition* 101(3):307–316.

Korthals, M. 2007. Ethics of personalized nutrition. In *Personalized nutrition: Principles and applications.*, eds. F. Kok, L. Bouwman, and F. Desiere, 221–234. London: Taylor and Frances.

Korthals, Michiel. 2004. Ethics of differences in risk perception and views on food safety. *Food Protection Trends* 24(7):498–503.

Lee, S.J., and S.Y. Lee. 2004. Micro total analysis system (μ-TAS) in biotechnology. *Applied Microbiology and Biotechnology* 64:289–299.

Lewenstein, Bruce V. 2005. What counts as a 'social and ethical issue' in nanotechnology? *Hyle – International Journal for Philosophy of Chemistry* 11(1):5–18.

Löwe, Holger, Volker Hessel, and Andreas Mueller. 2002. Microreactors. prospects already achieved and possible misuse. *Pure and Applied Chemistry* 74(12):2271–2276.

Macoubrie, Jane. 2005. Informed public perception of nanotechnology and trust in government. Tech. rep., Woodrow Wilson International Center for Scholars.

McInerney, Claire, Nora Bird, and Mary Nucci. 2004. The flow of scientific knowledge from lab to the lay public: the case of genetically modified food. *Science Communication* 26(1):44–74.

Pollan, Michael. 2006. *The omnivore's dilemma: a natural history of four meals*. New York (NY): Penguin Press.

Raffensperger, Carolyn. 1998. Wingspread statement. In *Wingspread Conference on the Precautionary Principle*.

———. 2007. The precautionary principle and biotechnology: Guiding a public interest research agenda. In *Genetically Engineered Crops, Interim policies, uncertain legislation*, ed. Iain E. P. Taylor, 153–171. New York: Haworth Food & Agriculture Products Press.

Räikkä, Juha. 1998. Freedom and a right (not) to know. *Bioethics* 12(1):49–63.

Renegar, Gaile, Christopher J. Webster, Steffen Stuerzebecher, Lea Harty, Susan E. Ide, Beth Balkite, Taryn A. Rogalski-Salter, Nadine Cohen, Brian B. Spear, Diane M. Barnes, and Celia Brazell. 2006. Returning genetic research results to individuals: points-to-consider. *Bioethics* 20(1):24–36.

Reyes, Darwin R., Dimitri Iossifidis, Pierre-Alain Auroux, and Andreas Manz. 2002. Micro total analysis systems. 1. Introduction, theory, and technology. *Analytical Chemistry* 74(12):2623–2636.

Sandler, Ronald, and W.D. Kay. 2006. The gmo-nanotech (dis)analogy? *Bulletin of Science, Technology & Society* 26(1):57–62.

Schummer, Joachim. 2001. Ethics of chemical synthesis. *Hyle – International Journal for Philosophy of Chemistry* 7(2):103–124.

———. 2005. "Societal and ethical implications of nanotechnology": meanings, interest groups, and social dynamics. *Techné: Research in Phylosophy and Technology* 8(2):56–87.

Service, R.F. 2002. Shrinking fuel cells promise power in your pocket. *Science* 296:1222–1224.

Service, Robert F. 1998a. Labs on a chip:coming soon: the pocket DNA sequencer. *Science* 282(5388):399–401.

———. 1998b. Labs on a chip:miniaturization puts chemical plants where you want them. *Science* 282(5388):400–.

Shell. 1998. The shell report 1998 - profits and principles - does there have to be a choice? Tech. rep.

———. 1999. The shell report 1999 - people, planet & profits - an act of commitment. Tech. rep.

Slovic, Paul. 1987. Perception of risk. *Science* 236(4799):280–285.

Swierstra, Tsjalling, and Arie Rip. 2007. Nano-ethics as NEST-ethics: Patterns of moral argumentation about new and emerging science and technology. *Nanoethics* 1(1):3–20.

Talary, M.S., J.P.H. Burt, and R. Pethig. 1998. Future trends in diagnosis using laboratory-on-a-chip technologies. *Parasitology* 117:S191–S203.

Terpstra, Paul. 2008. Consument-en-technologie in het perspectief van duurzame ontwikkeling.

The Royal Academy of Engineering. 2003. The societal aspects of risk. Tech. rep., The Royal Academy of Engineering.

———. 2004. The risk debate. Tech. rep., The Royal Academy of Engineering.

U.S. Department of Transportation. 2004. Summary of fuel economy performance. Tech. rep., U.S. Department of Transportation.

Walt, David R. 2005. Chemistry: Miniature analytical methods for medical diagnostics. *Science* 308(5719):217–219.

Wynne, Brian. 2006. Public engagement as a means of restoring public trust - hitting the notes, but missing the music? *Community Genetics* 9:211–220.

Yager, Paul, Thayne Edwards, Elain Fu, Kristen Helton, Kjell Nelson, Milton R. Tam, and Bernhard H. Weigl. 2006. Microfluidic diagnostic technologies for global public health. *Nature* 442:412–418.

Appendix 6-I
Structure of interviews

This appendix lists the items and questions that were used as a guideline for the interviews. Not all items and questions were covered to an equal degree by all interviewees; this depended largely on the background knowledge of the interviewees.

1. Introduction of interviewer and definition of microreactors
2. Introduction of interviewee's work
3. Introduction of interviewee's relation to microreactor technology
4. In what direction will microreactor technology develop?
5. What are typical products/applications from microreactor technology?
6. Who will use these applications?
7. What are the benefits and risks for those whose use the applications?
8. What are the benefits and risks for others, e.g., people close to the users or society at large?
9. What should be the role of limitation by policy and control?
10. How will these applications change the way people live?
11. Are these changes desirable?

Appendix 6-II
List of interviewees

Due to privacy agreements, the list of interviewees is anonymized. The list links the shorthand code for the interviewees used in the main text to their background, the date and place of interview.

Abbreviation for interviewee	Background	Date and place of interview
University1	Technical university, experience with microreactors	November 1st, 2007 (NL)
University2	Technical university, a lot of experience with microreactors	November 30th, 2007 (NL)
Policy and control1	Governmental organisation, no experience with microreactors	January 7th, 2008 (NL)
Policy and control2	Governmental organisation, no experience with microreactors	January 7th, 2008 (NL)
Industry1	Production/research company, some experience with microreactors	January 23rd, 2008 (NL)
Industry2	Production/research company, experience with microreactors	January 25th, 2008 (NL)
NGO1	Critical think-tank, no experience with microreactors	August 11th, 2008 (UK)
NGO2	Consumer organisation, no experience with microreactors	August 13th, 2008 (NL)

Table 6.5: List of interviewees with background, date and place of interview.

Chapter 7

General discussion

7.1 Introduction

The overall goal of this thesis is to study the potential of using microreactors for enzymatic conversions. The question this thesis tries to answer is similar to Klavs Jensen's paper 'Microreactor engineering: is smaller better?', but focussed at enzymatic microreactors (Jensen, 2001). In a nutshell; when should you use enzyme microreactors and how should they be used? First, we investigated thermal aspects of the microreactor, more specifically the thermal performance in practice in chapter 2 and the thermal design in chapter 3. Next we investigated two enzymatically catalyzed reactions in these microreactors.

We found that a slow reaction such as a lipase catalyzed esterification behaves similarly on micro scale and bench scale (chapter 4). The kinetic results can be used interchangeably between the two scales. Therefore, microreactors can be used very well to accurately determine the kinetics of enzymatically catalyzed reaction systems. A faster enzyme such as β-galactosidase has the same kinetic behavior on both scales. However, diffusion effects on the enzyme activity became noticeable for residence times below 10 seconds (chapter 5).

Finally, experts from various fields were interviewed to obtain different perspectives on the social and ethical dimensions of the application of microreactor technology in

Part of this chapter has been accepted for publication in *Lab on a Chip* as Tetala, Kishore K.R.; Swarts, Jan W.; Chen, Bo; Janssen, Anja E.M.; van Beek, Teris A. A three-phase microfluidic chip for rapid sample clean-up of alkaloids from plant extracts.

practice (chapter 6). Central issues here were the typical applications that might evolve from microreactor technology, their benefits and costs, and their communication to the public.

In this chapter, we will first discuss some aspects of the subject that were not yet covered. The previous chapters dealt with micro reactions. Reactions usually involve downstream processes. Any microreactor design should involve downstream processing. On-chip extraction has much potential in this respect. An alternative to immiscible liquid phases is the use of a membrane, leading to a micro pertraction process.

We will discuss the work on two and three phase extractions in microchips and the development of CNC-milled polymer microreactors with membranes. These two examples illustrate the possibility of tuning the design to the desired (downstream) process conditions. These conditions could include the separation of different fluid phases, or the highest possible extraction rates of a certain component. The separation of phases could be done by coating the microchannel walls or using membranes in the CNC-milled chips. A high extraction rate could be obtained by selecting the right solvent.

Next, we will discuss the application of enzyme microreactors and their practical use. This discussion is divided into the choice of process scale, the design, the use, and the eventual routine application of enzyme microreactor systems. These aspects will be discussed using the results from this thesis research.

7.2 Microfluidic chips for two and three phase extraction

Due to the short diffusion paths and the large interfacial areas between phases, several researchers have considered microsystems for extraction purposes (Hibara et al., 2001; Tokeshi et al., 2002; Žnidaršič Plazl and Plazl, 2007). The work on three phase extraction chips with stable interfaces is limited compared to two phase extraction chips because of the obvious increase in complexity. We have investigated both two and three phase extraction chips. A microsystem was designed to suit the extraction of strychnine for sample preparation (Žnidaršič Plazl and Plazl, 2007). The design included the design of channels, the coating of channels, the choice of solvents, and the required residence time. The chip was made of borofloat glass by Micronit (Enschede, The Netherlands) with a 40 μm deep and 300 μm wide channel, 3.5 or 9.3 cm long

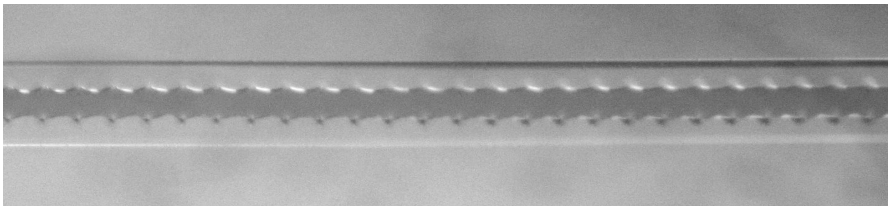


Figure 7.1: Streams of water, chloroform (with Sudan dye) and water in a three phase chip.

channel. To promote parallel (stratified) flow of the three fluids, two rows of pillars were positioning along the length of the channel (dots in fig. 7.1). These pillars were shaped as sloped cones, 10 μm at the top and 90 μm at the bottom.

The goal of the process was to keep the solvents separated and to maximize the extraction. The first step was to make the chip facilitate stable two or three phase parallel flows. These flows were typically water-chloroform or water-chloroform-water. Because of the hydrophilicity of the glass chip, a hydrophobic coating was needed to make the non-polar chloroform 'wet' the middle part of the channel (figure 7.1). Conversely, if a hydrophobic material has to be used, hydrophilic coating could be applied on the side channels.

With this system it was possible to attain a stable three phase system. Strychnine could be extracted from the original alkaline sample stream, through the chloroform phase, into the acidic acceptor stream. The practical results from experiments at various residence times were compared to numerical calculation results. Two dimensional computer models were constructed in COMSOL Multiphysics from COMSOL (Burlington, MA). The results from these models and experiments are shown in figure 7.2.

Figure 7.2 shows that for increasing residence times, the strychnine is extracted from the alkanline phase (initial concentration 0.2 mM) through the chloroform (initial rise, then drop in concentration) to the acidic phase. The numerical results (lines) were obtained with *ab initio* estimated physical parameters and corresponded well to the experimental findings (symbols). These models were then used with other parameter values to investigate the effect of such variations.

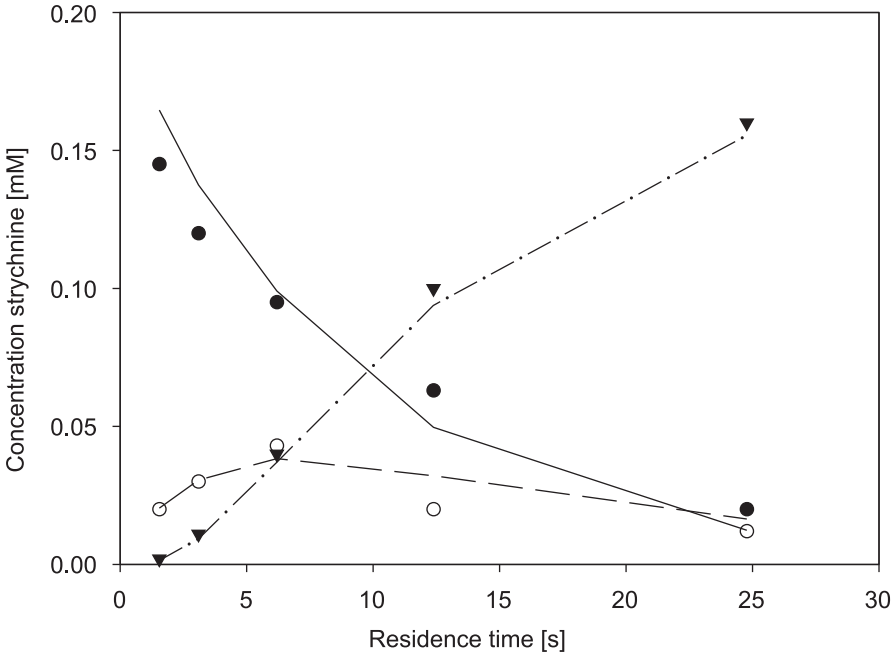


Figure 7.2: Strychnine concentration at end of a three phase microchip (3.56 cm length) from the basic phase through chloroform to the acidic phase at different residence times. Experimental data (● - alkaline aqueous phase; ○ - chloroform phase; ▼ - acidic aqueous phase) and model data (– is alkaline aqueous phase, - - is chloroform phase, -.- is acidic aqueous phase).

Table 7.2 shows numerical simulation results where specific parameters are varied. With increasing residence times the strychnine concentration goes up at the exit of the acidic (acceptor) channel. At low residence times, reducing the width of the middle channel will boost the extraction. Increasing the extaction rate could be done by decreasing the flow rate ratio between the middle and the side channels or by redesigning the chip, i.e. making the middle channel physically narrower. At long residence times, such effects seem to become smaller as the extraction approaches equilibrium.

The highest extraction could be reached by changing the solvents in the middle channel. Such a new solvent could have a higher partitioning coefficient of strychnine (favoring presence in the solvent over the donor stream), or the solvent could have a viscosity allowing the donor phase flow at a lower flow rate. A twofold higher partitioning coefficient and a twofold lower flow rate for the middle channel resulted

Table 7.1: Effect of different channel and solvent properties on the strychnine concentration obtained at the exit of the acidic channel.

	Residence time		
	12.5 s	25 s	150 s
Standard case	0.0961 mM	0.1592 mM	0.1846 mM
Middle channel width reduced from 112 to 56 μm	0.1228 mM	0.1624 mM	0.1714 mM
Viscosity of middle channel same as side channels, flow rate ratio 1:1:1	0.0976 mM	0.1576 mM	0.1914 mM
Partitioning coefficients changed to 1:66.7 (base:$CHCl_3$) and 1:20.6 ($CHCl_3$:acid)	0.1096 mM	0.1699 mM	0.1914 mM

Standard case: flow rate ratio 1:2:1, channel widths 95.5-112-95.5 μm, viscosity $1{\cdot}10^{-3}$ Pa.s (water) $0.54{\cdot}10^{-3}$ ($CHCl_3$), partitioning coefficients 1:133 (base:$CHCl_3$) and 1:40.6 ($CHCl_3$:acid)

in a 0.0086 mM loss of the strychnine to either the base or the chloroform phase, compared to the 0.0154 mM strychnine loss in the standard case. The results show that in such a microsystem, diffusion times cannot be neglected. Thus, we can say that the design of micro-extraction systems should be based on good estimations of diffusion times. This is similar to the conclusion of chapter 2, in which the temperature control was found to be much more complex than usually thought for microsystems.

This example demonstrates that by tuning the design of the microfluidic chip, the coating of the microchannel, or the solvents used, a good process can be designed. In this case, a good design could have included separate mono-phasic streams exiting the system, high extraction rates, or relatively high extraction rates at moderate residence times. The use of computer models can reduce the need for many experiments, or designing and fabricating many microfluidic chips. The computer models in this paper can be used to find an optimal chip and process design relatively simply, cheaply, and quickly.

7.3 Membrane microchips

Instead of having a hydrophobic immiscible solvent as an extraction phase, one can also use a membrane as a solid barrier between the reaction mixture and the extract. The possibility of constructing such membrane chips in-house was investigated. Microchips were computer designed and constructed with Computer Numerical Control

milling of polymer plates. The chips were designed in such a way, that they could be used in an existing chipholder. The research focused on producing chips comprised of two plates with channels and a membrane clamped in between.

Based on the requirements for the desired process, design parameters were established. The main requirements were chemical stability, processability, and transparency of the material. Different materials were scored against these properties. PVC proved to be most suitable, due to its relatively good resistance against solvents, transparency and the availability of very flat plates. Using polymers instead of glass chips is fairly common and a wide range of production methods can be used (Martynova et al., 1997; McCormick et al., 1997; Xia and Whitesides, 1998; Xu et al., 2000). As opposed to glass chips, the bonding of thermoplastic polymer chips is relatively easy (Tsao and DeVoe, 2009).

To separate the fluids a regenerated cellulose membrane (PLCC15005 from Millipore, Billerica MA) was placed in between two PVC plates with milled channels (200 μm wide, 50-200 μm deep) as shown in figure 7.3. The sides of the chip were polished and then pressed against a 130°C hot plate for 5 to 10 seconds, three times each side. By heating the PVC to above its glass temperature, the sides of the chip softened and upon cooling hardened again, sealing the sides of the chip. While thermal fusion bonding is a common method for bonding microfluidic chip, bonding of PVC has not been reported before.

The chip was used in the existing chipholder (top right in fig. 7.3. Two syringes with water were connected to chip, resulting in a co-current flow with the membrane in the middle. The chip could be operated at flow rates up to 40 μl min^{-1} without leaking. Due to PVC's relatively good resistance against solvents, these chips could be used for extraction in a two phase system, keeping the streams separated. Furthermore, these chips can be redesigned and produced with relative ease, based on new process requirements.

7.4 Choice of process scale

Before any process is run, one has to choose the right system dimensions. This choice of system is dependent on the required throughput, required level of process control, reaction intrinsic requirements such as process temperatures and many other aspects. Microreactors and microseparators are a relatively new addition to the range of tools

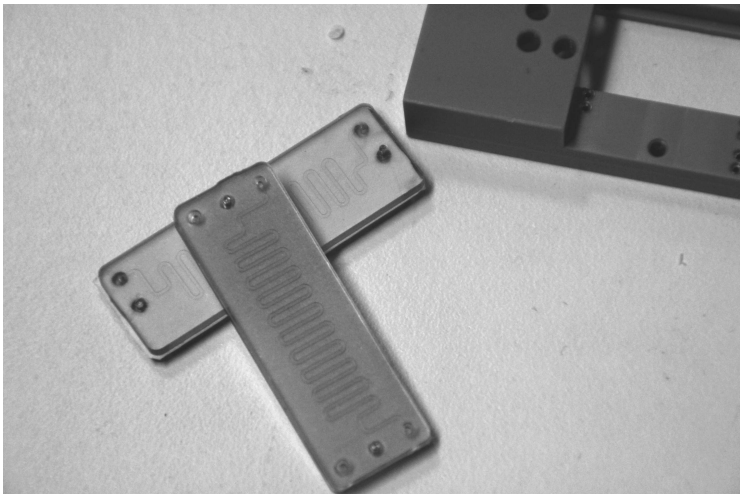


Figure 7.3: PVC chip with membrane and chipholder.

available to the process engineer. The choice of a microreactor should be based on the merits of the microsystem and the requirements of the process.

The benefits of enzyme microsystems are most likely to be found in the analysis of enzyme reactions or very small scale production. Micro scale processing is suitable for reagents that are very costly or difficult to obtain, or for complex reaction-extraction systems. As we showed in chapter 3, 4, and 5 for many enzyme reactions, heat and mass transfer is not a rate limiting step. The reaction will not be faster inside a microreactor than in a stirred vessel. This implies that one can use microreactors to measure the reaction kinetics which may be required for designing a larger scale process. In this way, the kinetic data can be obtained at a much lower consumption of enzyme and chemicals. For instance, the Michaelis-Menten parameters in chapter 5 were obtained with 500 times less enzyme on micro scale compared to bench scale.

When only a very small amount needs to be produced (micrograms to milligrams, sometimes even more), production on micro scale could be most appropriate. Especially when the optimization was carried out on micro scale, production can easily be done in the same (type of) chip. Production in a microreactor is often continuous so the exact volume can be produced very accurately. This is very convenient when the chemicals used or produced are highly toxic, explosive, or expensive.

When a process requires the specific extraction of an intermediate or end product, microreactors may be a good choice. Due to the highly tunable design of microchannels, extraction of certain components can be programmed at very specific positions in the reaction channel. Furthermore, three-phase extraction with two miscible and one immiscible liquid phase can be carried out continuously with complete separation of all phases. In addition, other systems such as membrane systems can be realized.

7.5 Design

When the choice has been made to use microreactors, an appropriate microsystem needs to be designed. This design should focus on critical parameters. Some examples are:

- The required residence time
- How fast should the components be mixed
- What temperature profile is required
- Which extraction is needed
- Is numbering up of the microfluidic chip required

These process parameters determine what the system should look like. In this thesis we have demonstrated the effect of system design on temperature (in chapter 2) and reaction rates (chapters 4 and 5)

In many cases the fluid temperature is important for the conversion rate of the enzyme. In some cases a stable temperature for continuous processing is desired, in other cases a temperature gradient is better. The fluid temperature is mainly dictated by the design of the chipholder. The position and size of the heater/cooler determine where the major heat flux will be. The design of the chipholder determines how this energy will flow to the chip and the surroundings, and how uniform the temperature in the chip will be.

As the chipholder represents a far bigger reservoir for heat, heating and cooling of a chip is not as fast as one could expect based on the assessment of the chip itself. Similarly, the chipholder will strongly influence the temperature distribution on the chip, i.e. will determine whether the fluids in the chip will 'feel' a stable isothermal or

a temperature gradient profile. Thus, in the design one should never only consider the chip alone, but consider it as an integrated system with heater/cooler and chipholder.

7.6 Using microsystems

The final goal of using a microsystem is to obtain a product (an extract or new component) of stable quality for a longer period of time. The system has to be robust and should have sufficient control possibilities. The reaction or extraction should take place in an environment which is (fluid-) mechanically, thermally, and chemically stable. The first stability criterion involves stable fluid flow profiles and rates. The system should have microchannels which do not clog or deteriorate. Furthermore, the pumping (EOF or mechanical) of fluids should be constant and well controlled. Within the project of this thesis pressure sensors were developed which could be fitted between the syringes and the syringe pumps. These sensors could detect and warn for pressure build-ups or drops which could indicate clogging or leaks. When the microfluidic chips are to be parallelized (scaled out), an equal distribution of residence time or pressure over the chips is crucial. Also, the system should be robust that the break-down or blockage of one chip will not influence surrounding chips too much.

Second, the temperature of the fluids inside the system should be stable. As shown before, there is strong interaction between chip and chipholder in the heating process. Temperature can influence reaction rates as well as physical properties such as viscosity. A microsystem with a proper design should include sensors and actuators and a temperature control mechanism. This system should be robust under different processing circumstances. The microsystem from this thesis was tested and described in chapter 3.

In chapter 3, the fluid temperature inside the chip was measured using a fluorescent dye. The fluid temperature proved to be independent from the fluid flow rates ($<$ 8 μl min^{-1}) inside the chip and movement of air over the chipholder ($<$ 1 m s^{-1}). These are typical conditions under which the microsystem was operated. As such, the microsystem was thermally robust during practical use.

The final criterion is the chemical stability of the system. There should be no unwanted adsorption or desorption to the channel walls. Proteins such as enzymes may adsorb to the channel wall thus increasing the enzyme load per volume of microchannel, or blocking immobilized enzymes by covering them with an adsorbed

(possibly denatured) layer. This proved not to be the case with the lipase and β-galactosidase tested in this research (chapter 4 and 5). Hydrophilic or hydrophobic coatings may also hydrolyze or otherwise change their properties. The hydrophobic coating as discussed earlier for three phase extraction systems was stable for weeks, ensuring relatively long term use of the microsystem. However, cleaning procedures using high or low pH, chemically aggressive solvents, in combination with elevated temperatures may well induce irreversible changes in any wall modification.

7.7 Application

Microfluidic technology is still a rather new technology. At the moment microsystems are mainly applied in research environments, with a few early adopters in industry. When microsystems' advantages are maximized and practical issues are dealt with, applications might enter a wider market. With a growing audience for microreactor technology applications, the impact of this technology on the public will also grow. The discussions around the introduction of GMO-food have demonstrated that technological advances can not be pushed into the market at will. Social and ethical issues can be critical for the success or failure of a novel technology.

In general, the success of a new product or technology application depends on its benefits and costs for the stakeholders. However, even with great benefits (much cheaper production) and low costs (a very small number of additional injuries during use of the product) implementation of a new technology is not straightforward. The distribution of benefits and costs, for instance over producers and consumers or over present and future generations should be considered, since all the different stakeholders have to accept the technology to make it a success. Many of the benefits and costs are related to the perceived goals and views of the good life.

In chapter 6 of this thesis we discussed a simple framework for surveying possible social and ethical aspects of a new technology's application. Possible microreactor technology applications in food, pharmaceuticals, and energy were given and their benefits and risks assessed. These examples were based on the current state and direction of microreactor technology and the current perception of what we might need or want in 30 to 50 years. The most unique features of these examples could be centered around the aspect of autonomy.

It is unsure which exact applications will come from microreactor technology. However, when they appear, the methods employed in this thesis may be helpfull in the assesment of social and ethical issues of any new application. Whatever direction the technology might take, communication with society shall be instrumental in its possible success. A timely discussion with a wide range of stakeholders including industry, consumers, government, and NGOs will increase the chances of a new technology benefiting a large group of consumers, while minimizing the costs. This multi-stakeholder communication is a first cautious step which will demand all participants to rethink their traditional role in the process.

7.8 Conclusions

In this thesis we have shown that microreactors can be a useful tool for reaction engineering. We have shown that enzyme kinetics of two enzymatically catalyzed reactions are similar on both micro and bench scale. Thus, one can use the kinetic data obtained on micro scale and apply it to the design of large scale reactors. Since measurements on micro scale can be obtained with much less chemicals and enzyme, enzyme microsystems make a suitable platform for enzyme exploration. We further conducted short time, high temperature experiments on this micro scale, which are difficult to achieve on a larger scale. One does have to keep in mind that even at the micro scale diffusion can limit the enzyme reaction. We showed under which conditions such diffusion limitation is most likely to occur.

The activity of an enzyme in any system is dependent on the temperature it experiences throughout its presence in the reactor. We showed that the temperature an enzyme might encounter during its flow through a microreactor is dependent on the design of the chipholder. The geometry and material of the chipholder and the position and size of the heater dictates the temperature profile in the whole micro reactor. One therefore has to consider the microreactor chip and the chipholder as one integrated system, and design the heating and cooling system for this ensemble. By adapting the design of the chipholder different temperature profiles can be achieved at the fluid level in the microreactor. The operation of such a system was shown not to influence the temperature of the fluid inside the chip. The microsystem therefore presents a robust platform for enzymatic experimenting.

When microreactor development succeeds and more applications enter the public market, social and ethical issues surrounding these applications should be addressed.

We have proposed a framework for assessing such issues in the case of three exemplary applications. The eventual success of microreactor technology may well depend on the manner these issues are dealt with and communicated.

References

Hibara, Akihide, Manabu Tokeshi, Kenji Uchiyama, Hideaki Hisamoto, and Takehiko Kitamori. 2001. Integrated multilayer flow system on a microchip. *Analytical Sciences* 17:89–93.

Jensen, Klavs F. 2001. Microreaction engineering - is small better? *Chemical Engineering Science* 56:293–303.

Martynova, Larisa, Laurie E. Locascio, Michael Gaitan, Gary W. Kramer, Richard G. Christensen, and William A. MacCrehan. 1997. Fabrication of plastic microfluidic channels by imprinting methods. *Analytical Chemistry* 69(23):4783–4789.

McCormick, Randy M., Robert J. Nelson, M. Goretty Alonso-Amigo, Dominic J. Benvegnu, and Herbert H. Hooper. 1997. Microchannel electrophoretic separations of DNA in injection-molded plastic substrates. *Analytical Chemistry* 69(14):2626–2630.

Tokeshi, Manabu, Tomoko Minagawa, Kenji Uchiyama, Akihide Hibara, Kiichi Sato, Hideaki Hisamoto, and Takehiko Kitamori. 2002. Continuous-flow chemical processing on a microchip by combining microunit operations and a multiphase flow network. *Analytical Chemistry* 74(7):1565–1571.

Tsao, Chia-Wen, and Don L. DeVoe. 2009. Bonding of thermoplastic polymer microfluidics. *Microfluidics and Nanofluidics* 6(1):1–16.

Žnidaršič Plazl, Polona, and Igor Plazl. 2007. Steroid extraction in a microchannel system-mathematical modelling and experiments. *Lab on a Chip* 7:883–889.

Xia, Younan, and George M. Whitesides. 1998. Soft lithography. *Angewandte Chemie International Edition* 37:550–575.

Xu, Jindong, Laurie Locascio, Michael Gaitan, and Cheng S. Lee. 2000. Room-temperature imprinting method for plastic microchannel fabrication. *Analytical Chemistry* 72(8):1930–1933.

Summary

Microreactor technology is reported to have many benefits over regular chemical methods. Due to the small dimensions over which temperature and concentration gradients can exist, mass and heat transfer can be very quick. This could minimize the time needed for heating and mixing, due to a reduction in diffusion limitation. Furthermore, a very low fluid to chip volume ratio could facilitate a very stable fluid temperature.

The goal of this thesis research was to investigate the effect of the use of microreactors on enzyme kinetics and the thermal behaviour of fluids inside the chip. First, the effect of the design and use of a microsystem on the fluid temperature inside the microfluidic chip was investigated experimentally and with computer models. A stable and predictable temperature is of great importance for running (enzymatic) processes in a microchip. Next, we used model enzyme reactions to investigate whether the enzyme kinetics were different on micro and bench scale, and when diffusion would play a role. Furthermore, some social and ethical aspects of microreactor technology applications were studied.

To ensure a stable and predictable temperature of the fluids inside the microreactor, the microsystem should be properly designed and used. To test these two aspects, we investigated the effect of practical use (chapter 2) and design parameters (chapter 3) on this fluid temperature. The micro system used in this research consisted of a PEEK chipholder, a relatively small heater, a glass microchip, and surrounding air. We conducted experiments and used computational fluid dynamics models to understand the effect of all varied parameters. In the design of the system, the chipholder shape and material (with its density, specific heat, and thermal conductivity) dominated the temperature of the fluid inside the chip. A temperature gradient as large as 40 °C was observed over the length of the chip. This temperature profile at fluid level

can be changed by adapting the geometry and material of the chipholder. The results show that a uniform temperature is highly dependent on the correct design of the integrated system of chip, chipholder, and heater. The practical use of the chip with moderate air flow over the chip and moderate fluid flow rates through the channel had no effect on the fluid temperature. A well designed micro system can therefore be considered thermally robust under moderate processing conditions.

The microsystem from chapters 2 and 3 was used for enzyme reactions on micro scale. The kinetic parameters of a lipase catalyzed esterification reaction (chapter 4) and a β-galactosidase catalyzed hydrolysis reaction (chapter 5) on this micro scale were the same as those found on bench scale. Kinetic and thermal (in-)activation results obtained on micro scale can be used for large scale processing. This can bring down optimization costs by reducing the required amount of enzyme and chemicals.

Next, we found that at residence times below a few seconds, diffusion effects limited the reaction rate and therefore reduced the conversion per volume of enzyme microreactor. This effect of diffusion on the conversion increased quadratically with channel width, increased with enzyme concentration, and decreased with substrate concentration. When an enzyme microreactor system should be run efficiently, these factors should be explored to avoid diffusion limitation and subsequent reduced volumetric productivity.

With microreactor technology reaching maturity, a wider application of the technology could be imagined. With increasing application the impact it will have on society will also increase. In chapter 6, three examples of microreactor technology applications in nutrition, in medicine, and in energy carrier supply were investigated. The benefits and costs, and their distribution were discussed for these examples. Furthermore, possible strategies of communication surrounding a public introduction of such a novel technology were considered. The applications proposed in this chapter were only three out of an infinite number of possibilities. However, the discussion of these examples can be used as a framework for discussing future applications as they might be developed in the future. A societal backlash as with the GMO-scare in 1990s, can be avoided when the relevant issues are communicated appropriately and timely. This could improve the chances of success of this technology in the market.

In this thesis we have shown that microreactors can be a useful tool for reaction engineering. Their use could reduce the required amount of enzyme and chemicals

for optimization. Furthermore, they can be used to study processes with a very short residence time. To use microreactor technology effectively, one does have to consider whether the scale is appropriate, and whether that the system, including chipholder, interfaces to the outer world and thermal actuators, is properly designed and used.

Samenvatting

Microreactor technologie zou in vergelijking met reguliere chemische reactie methoden veel voordelen hebben. Door de kleine afstanden waarover temperatuurs- en concentratieverschillen kunnen bestaan, kan warmte en massa transport erg snel plaats vinden. Door de reductie in diffusie limitatie kan de benodigde tijd voor verwarmen en mengen verkort worden. Ook kan door de verhouding van een groot chipvolume tot een klein vloeistofvolume, de vloeistoftemperatuur heel stabiel gehouden worden.

Het doel van dit proefschrift was het onderzoeken van het effect van microreactoren op enzym kinetiek en van de temperatuursgedrag van vloeistoffen in deze microchips. Eerst werd het effect van het ontwerp en het gebruik van een microsysteem op de temperatuur binnenin de microchip onderzocht met experimenten en computer modellen. Een stabiele en voorspelbare temperatuur is van groot belang bij het draaien van (enzymatische) processen in een microchip. Hierna gebruikten we enzymatische modelreacties om te zien of de enzym kinetiek op micro of bench schaal anders zou zijn en wanneer diffusie een rol zou gaan spelen. Ook hebben we gekeken naar de sociaal-ethische aspecten van toepassingen van microreactor technologie.

Om een stabiele en voorspelbare vloeistoftemperatuur in de microchip te bewerkstelligen moet het hele microsysteem op een juiste wijze ontworpen en gebruikt worden. Deze twee aspecten zijn getest door het effect van praktisch gebruik (hoofdstuk 2) en ontwerp-parameters (hoofdstuk 3) op de vloeistoftemperatuur te onderzoeken. Het microsysteem wat gebruikt werd in dit onderzoek bestond uit een PEEK chiphouder, een relatief klein verhittingselement, een glazen chip en de omgevingslucht. We deden experimenten en gebruikten computational fluid dynamics modellen om het effect van alle gevariëerde parameters te onderzoeken. In het ontwerp van het systeem bleek de vorm en materiaal van de chiphouder (dichtheid, soortelijke warmte en warmtegeleidingscoëfficiënt) de vloeistoftemperatuur in de chip te dicteren. Er werd

een temperatuursgradiënt van wel 40°C over de lengte van de chip waargenomen. Het temperatuursprofiel op vloeistofniveau in de chip kan veranderd worden door de vorm en materiaal van de chiphouder aan te passen. De resultaten laten zien, dat een uniforme temperatuur sterk afhankelijk is van het juiste ontwerp van het gehele systeem met chip, chiphouder en verwarmingselement. Het praktisch gebruik van de chip met gematigde langsstromende lucht en gematigde vloeistofdoorstroming bleek geen effect te hebben op vloeistoftemperatuur. Een goed ontworpen microsysteem kan daarmee als thermisch robuust verondersteld worden onder gematigde procescondities.

Het microsysteem uit hoofdstuk 2 en 3 werd hierna gebruikt voor enzym reacties op micro schaal. De kinetische parameters van een lipase-gekatalyseerde veresteringsreactie (hoofdstuk 4) en een β-galactosidase-gekatalyseerde hydrolyse reactie (hoofdstuk 5) op micro schaal bleken gelijk aan die op bench schaal. Kinetische en thermische (in-)activatie resultaten, welke verkregen zijn op micro schaal, kunnen gebruikt worden voor processen op grote schaal. Dit kan de kosten voor optimalisatie verlagen door de benodigde hoeveelheid enzym en chemicaliën te beperken.

Hiernaast vonden we dat bij verblijftijden van minder dan een paar seconden, diffusie effecten de reactiesnelheid limiteerden en daarmee de volumetrische productiviteit van de microreactor verlaagden. Dit diffusie-effect op conversie schaalde kwadratisch met de kanaalbreedte, steeg met toenemende enzymconcentratie en daalde met toenemende substraatconcentratie. Wanneer een enzymatische microreactor efficiënt gebruikt zou moeten worden, dan verdienen deze aspecten de aandacht. Diffusie limitatie en een vervolgens verlaagde volumetrische productiviteit kunnen zo vermeden worden.

Met het volwassen worden van microreactor technologie kan men zich een bredere toepassing van deze technologie voorstellen. Met een groeiend aantal toepassingen zal de impact van deze technologie op mensen ook groter worden. In hoofdstuk 6 werden drie voorbeeld-toepassingen van microreactor technologie gegeven op het gebied van voeding, medicijnen en energievoorziening. De voordelen, kosten, en hun verdeling bij deze voorbeelden werd besproken. Tevens werden er mogelijke communicatiestrategieën voor de marktintroductie van deze nieuwe technologie besproken. De voorbeelden uit dit hoofdstuk zijn er slechts drie uit een oneindig aantal mogelijkheden. De bespreking van deze voorbeelden kan echter gebruikt worden als uitgangspunt voor de bespreking van andere toepassingen wanneer deze in de toekomst ontwikkeld worden. Een maatschappelijk verzet, zoals gezien bij de GMO-discussie in de jaren

90, kan worden voorkomen, wanneer de relevante kwesties op gepaste en tijdige manier gecommuniceerd worden. Dit kan de kansen op marktsucces van de technologie vergroten.

In dit proefschrift hebben we aangetoond, dat microreactoren een nuttig hulpmiddel kunnen zijn in de reactie technologie. Ze zouden de hoeveelheid enzym en chemicaliën, benodigd voor optimalisatie, kunnen verminderen. Daarnaast zouden ze gebruikt kunnen worden bij het bestuderen van reacties met een zeer korte reactietijd. Om microreactor technologie effectief te gebruiken, zal men moeten overwegen of de (micro) schaal in het specifieke geval wel de meest effectieve is. Ook zal men het gehele systeem, inclusief de chiphouder, de verbindingen met de buitenwereld en de temperatuursactuatoren, op een juiste manier moeten ontwerpen en gebruiken.

Dankwoord

Zoals zoveel AiO's merken, vliegen de aanvankelijk oneindig lang lijkende vier (of vijf) jaar voorbij voordat je het weet. De jaren worden vaak gevuld met periodes van stagnatie en dan plotselinge reuzenstappen. Eenvoudig lijkende metingen blijken onmogelijk, problemen blijken oplossingen. Het opsporen van deze problemen en oplossingen kun je nooit alleen doen en ik wil dan ook graag van deze mogelijkheid gebruik maken om een aantal mensen te bedanken.

Allereerst wil ik natuurlijk Anja bedanken, die me al die tijd ondersteund en vooruitgeholpen heeft. Zonder jou had ik nog geen fractie van wat er nu ligt, kunnen bereiken. Ik heb door de geringe afstand tussen onze kantoren wellicht wat erg vaak bij je op de stoep gestaan met (futiele) vragen. Dat had ik echter nooit gedaan als ik niet dacht dat je daar voor open stond. Remko, bedankt voor je inbreng tijdens onze bijeenkomsten. Ik blijf me er over verbazen hoe iemand binnen 5 minuten overleg meer ideeën kan generen dan de AiO die zich er al maanden over buigt. Michiel, erg bedankt dat je zo'n 'ongeletterde' beta wilde begeleiden in een voor hem nieuw vakgebied.

Daarnaast zijn er binnen de vakgroep een aantal mensen onmisbaar geweest tijdens het onderzoek. Hierbij wil in het bijzonder, maar niet uitsluitend, Maurice en Pieter noemen. Jullie hebben mijn werk op het gebied van respectievelijk de experimenten/analyses en de cluster/berekeningen mogelijk gemaakt. De themagroep (Daniël, Tim, Marieke, Ruben, Tamara en Nirmal) wil ik bedanken voor al hun bijdragen tijdens de AiO-overleggen en het ontelbaar aantal keren dat ik daarbuiten een beroep op ze deed. Ook moet ik Koen nog noemen, die ik zo vaak lastig gevallen heb met STAR-CD vraagstukken en wat al niet meer.

Buiten deze met naam genoemden zijn er zo veel mensen van de vakgroep, de werkplaats, of de WUR, die me vak-inhoudelijk, praktisch of sociaal op de rails gehouden hebben. Namen durf ik hier niet meer te noemen, het waren er zo veel, dat ik er vast een paar vergeet. Maar gedurende koffiepauzes, lunches, borrels, PhD-trips (+vakanties), en veel meer hebben jullie allemaal indirect bijgedragen aan dit boekje. Applaus voor jezelf. Also for all of you who I have shared an office with over the years, thank you for the pleasant atmosphere and the assistance when I needed it.

Mijn onderzoek maakte deel uit van een groter project met projectpartners in Nederland en Duitsland. Kishore, Teris, Geb, Pieter, Kaspar, Floris, Jan, Robert, Martin, Burkhard, Khiem, Peter, and Michael thank you for the great cooperation over all these years. I think we can be proud of what we have achieved with all these people from different backgrounds.

Een zeer groot deel van de metingen uit mijn onderzoek heb ik niet zelf gedaan. Tijdens mijn promotie heb ik kunnen steunen op het werk, inzichten en ideeën van mijn afstudeerstudenten. Ruben, Constant, Yu-Ming, Marieke, Petra, en Mieke; jullie hebben een groot deel van het werk gedaan, heel veel dank daarvoor. Het is leuk te zien dat ik jullie niet al te zeer afgeschrikt heb, aangezien al de helft van jullie ook AiO is geworden. Zelf ben ik ook tweemaal afstudeerder geweest en ik wil mijn begeleiders van destijds, Dane en Janneke, bedanken voor hun inspiratie en hun voorbeeld in het doen van onderzoek.

Ook buiten de universiteit zijn veel mensen die mij in enig moment in mijn leven gevormd, opgevoed, en gesteund hebben. Natuurlijk wil ik allereerst mijn ouders en broer en zus bedanken. Jullie hebben me gemaakt tot wie ik nu ben. Als laatste in het dankwoord, maar als eerste in mijn leven, wil ik Marike noemen. Jij zorgt ervoor dat alles wat ik op het werk gedaan heb, betekenis heeft. Bij het verschijnen van dit boekje ben je inmiddels mijn vrouw en ik weet zeker dat we nog heel veel jaren samen gelukkig zullen zijn.

Publications

Bicanic, Dane; Swarts, Jan; Luterotti, Svjetlana; Pietraperzia, Giangaetano; Dóka, Otto; Rooij, Hans de. 2004. Direct quantification of lycopene in products derived from thermally processed tomatoes: optothermal window as a selective, sensitive, and accurate analytical method without the need for preparatory steps. *Analytical Chemistry* 76(17):5203-5207.

Bicanic, Dane; Swarts, Jan; Luterotti, Svjetlana; Helander, Per; Fogliano, Vincenzo; Anese, Monica. 2005. Optothermistor as a breakthrough in the quantification of lycopene content of thermally processed tomato based foods: Verification versus absorption spectrophotometry and High-Perfomance Liquid Chromatography. *Journal of Agricultural and Food Chemistry* 53(9):3295-3299.

Bicanic, Dane; Fogliano, Vincenzo; Luterotti, Svjetlana; Swarts, Jan; Piani, Giovanni; Graziani, Giulia. 2005. Quantification of lycopene in tomato products: comparing the performances of a newly proposed direct photothermal method and high-performance liquid chomatography. *Journal of the Science of Food and Agriculture* 85:1149-1153.

Kromkamp, Janneke; Bastiaanse, Annet; Swarts, Jan; Brans, Gerben; Sman, Ruud G.M. van der; Boom, Remko M. 2005. A suspension flow model for hydrodynamics and concentration polarisation in crossflow microfiltration. *Journal of Membrane Science* 253:67-79.

Swarts, Jan W.; Vossenberg, Petra; Meerman, Marieke H.; Janssen, Anja E.M.; Boom, Remko M. 2008. Comparision of two-phase lipase catalyzed esterification on micro and bench scale. *Biotechnology and Bioengineering* 99(4):855-861.

Swarts, Jan W.; Janssen, Anja E.M.; Boom, Remko M. 2008. Temperature effects during practical operation of microfluidic chips. *Chemical Engineering Science* 63(21):5252-5257.

Tetala, Kishore K.R.; Swarts, Jan W.; Chen, Bo; Janssen, Anja E.M.; van Beek, Teris A. 2009. A three-phase microfluidic chip for rapid sample clean-up of alkaloids from plant extracts. *Lab on a Chip* DOI: 10.1039/b822106e

Swarts, Jan W.; Janssen, Anja E.M.; Boom, Remko M. Modelling thermal interactions in a heated microsystem. To be submitted for publication.

Swarts, Jan W.; Kolfschoten, Ruben C.; Jansen, Mieke C.A.A.; Janssen, Anja E.M.; Boom, Remko M. Diffusion limitation on enzyme activity in a microreactor. Submitted for publication.

Curriculum Vitae

Johannes Wilhelmus (Jan) Swarts was born in Veghel on June 17^{th} of 1978. After primary school in his home town Vorstenbosch, he continued with his secondary education at the Zwijsen College in Veghel, where he graduated in 1996. In this year he started studying Food Technology at HAS Den Bosch, specializing in product development. During the course of the study he did internships at Royal de Kuyper in Middelharnis and dairy farm Baraka Farm in Eldoret (Kenya). After his graduation in 2000, Jan continued with an MSc in Food Technology at Wageningen University and Research Centre, specializing in Process Technology. He did two thesis research projects on 'the quantification of carotenoids in tomato products' at the Biophysics group and on 'the construction of a Lattice-Boltzmann model for crossflow microfiltration' at the Food Process Engineering group, both at Wageningen University. He graduated with honors in 2004, after which he started his doctoral research at the latter group. This research was focused on the application of enzymes in microfluidic systems, studying enzyme kinetics, thermal microsystem behavior, and social and ethical aspects of microtechnology applications. On April 1st 2009, Jan started working as researcher process technology at FrieslandCampina.

Johannes Wilhelmus (Jan) Swarts werd geboren in Veghel op 17 juni 1978. Na de basisschool in zijn woonplaats Vorstenbosch, deed hij het VWO op het Zwijsen College in Veghel, waar hij afstudeerde in 1996. In dat jaar begon hij de studie Levensmiddelentechnologie aan de HAS Den Bosch, waar hij specialiseerde in productontwikkeling. Tijdens zijn studie liep hij stage bij Koninklijke de Kuyper te Middelharnis en bij zuivelboerderij Baraka Farm in Eldoret (Kenia). Na zijn afstuderen in 2000, ging hij aan de Wageningen Universiteit en Researchcentrum de MSc studie Levensmiddelentechnologie volgen, met Levensmiddelenproceskunde als specialisatie. Hij deed afstudeeronderzoeken naar 'de bepaling van carotenoïde-concentraties in tomatenproducten' bij de vakgroep Biofysica en 'het bouwen van een Lattice-Boltzmann model

voor crossflow microfiltratie' bij de Levensmiddelenproceskunde vakgroep, beide aan de Wageningen Universiteit. Hij slaagde cum laude in 2004, waarna hij bij de laatste vakgroep zijn AiO onderzoek begon. Dit onderzoek was gericht op de toepassing van enzymen in microfluïdische systemen, waarbij gekeken werd naar enzymkinetiek en warmtehuishouding in microsystemen en naar sociale en ethische aspecten van microtechnologische toepassingen. Op 1 april 2009 begon Jan als onderzoeker procestechnologie bij FrieslandCampina.

Overview of completed training activities

Discipline specific activities

Courses

Microchemical systems - Principles and applications (TuD, 2004)
A unified approach to mass transfer (OSPT, 2004)
Numerical methods in chemical engineering (OSPT, 2005)
Introduction to STAR-CD (CD-Adapco, 2005)
Short course on Modelling and Computation of Multiphase Flows (ETH, 2007)
Advanced Biocatalysis (BSDL, 2008)

Congresses and symposia

MicroTAS International Conference on Miniaturized Systems for Chemistry and Life Sciences (2004, 2005)
Netherlands Process Technology Symposium (2004, 2006, 2007, 2008)
Process on a Chip kick-off symposium (2004)
Soft matter: Food for Computers (2006)
International Conference on Microreaction Technology (2006)
MinacNed Symposium (2006)
Symbiosis (2007)
American Institute of Chemical Engineers Annual Meeting (2007)

General courses

VLAG PhD week (VLAG, 2004)
Supervising and guiding a BSc/MSc thesis (OWU, 2005)
Scientific writing and presenting in English (CENTA, 2006)
Effectief omgaan met bestuur (2006) Philosophy and ethics in Food Science and Technology (VLAG, 2007)
Carreer Perspectives (VLAG, 2007)

Optionals

PhD trip Process Engineering (WUR, 2004, 2006)
Analysis methods course (WUR, 2005)

Typesetting: by the author using LaTeX 2_ε.

Cover: schematic representation of seven streams with different colors converging, flowing in a parallel laminar manner, and separating again, draining into the thesis' title and author's name. Inspired by a picture of Felice Frankel, printed as the cover of the July 2nd 1999 edition of *Science* magazine.

The research described in this PhD thesis was co-financed by the EU-Program INTERREG IIIA of the Euregion Rijn-Waal and by the ministries of Economic Affairs of the Netherlands and Nordrhein-Westfalen.