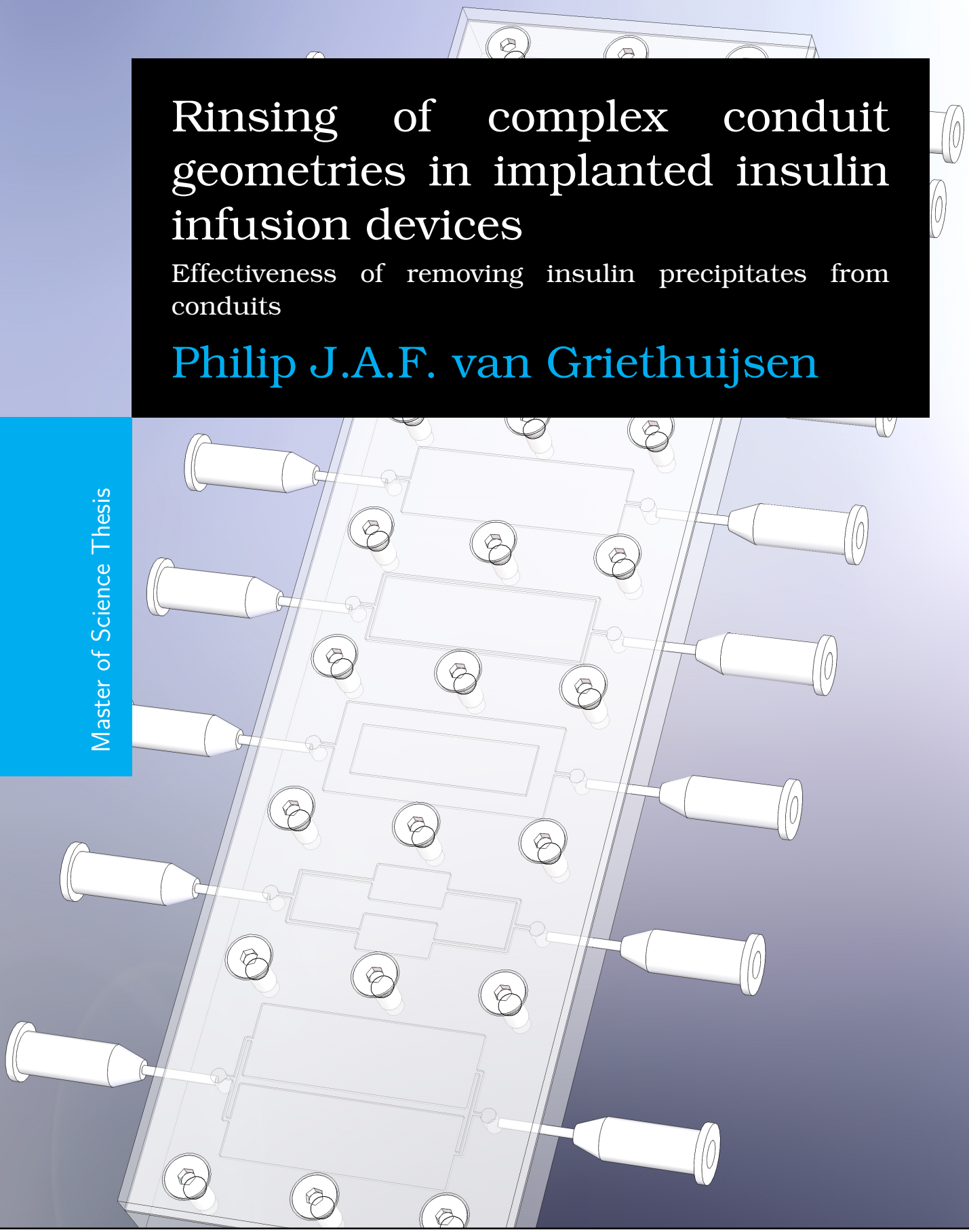
Rinsing of complex conduit geometries in implanted insulin infusion devices

Effectiveness of removing insulin precipitates from conduits

Philip J.A.F. van Griethuijsen

Master of Science Thesis



TU Delft Delft University of Technology

Dept. of BioMechanical Engineering

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Master of Science Thesis

For the degree of Master of Science in Biomedical Engineering at Delft University of Technology

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The undersigned hereby certify that they have read and recommend to the Faculty of Mechanical, Maritime and Materials Engineering (3mE) for acceptance a thesis entitled

Rinsing of complex conduit geometries in implanted insulin infusion devices

by

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in partial fulfillment of the requirements for the degree of

Master of Science Biomedical Engineering

Dated: October 4, 2017

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Abstract

Implanted intra-peritoneal insulin infusion ('i4') devices administer insulin into the peritoneum of diabetic patients with extreme blood glucose swings, that are highly difficult to control. The flushing procedures of i4 devices to clear obstructions from its conduits caused by insulin degradation are not always fully effective. The goal of this research was to investigate how the flushing effectiveness can be increased by adapting conduit geometries and adapting the flush flow rate. It was hypothesized that flushing of conduits with complex geometries (featuring a.o. widened sections, or parallel flow paths) would be less effective than simple geometries, and that a higher flush flow rate would increase flushing effectiveness. An ex vivo laboratory experimental setup was created by producing polymethyl methacrylate (PMMA) test conduits of differing geometries that model the in vivo implanted intra-peritoneal insulin infusion (i4) device's conduits, but are transparent to allow optical inspection. These test conduits were filled with acidified insulin, and precipitation thereof was artificially induced by heat incubation of the filled conduits, as a model for obstructed i4-device conduits. Experimental flushing procedures using sodium hydroxide (NaOH) as a flush liquid were performed on these test conduits at 2 different flush flow rates: 1.5mL/min, and at 20mL/min. After the flushing, the effectiveness of the procedure to clear the conduit of precipitates was evaluated by microscopically analysing the quantity of residual precipitates, and measuring Total Organic Carbon (TOC) of a liquid sample taken from the post-flushing conduit. All conduits with more complex geometries than straight tubes showed lower effectiveness of cleaning by flushing. The high flow rate showed an averaged 19% higher effectiveness of the flushing. In the sections of the conduits featuring widening, flushing often resulted in a narrow path being effectively cleaned, but significant regions of the conduit showed presence of residual precipitates. This research provides insight into the negative influence of complex geometries on effectiveness of flushing, and the positive influence of increased flush flow rate to improve effectiveness. Experimental repetition in this research is low, and the experimental methods were prone to much influence of material or measurement disturbances. This research merits elaboration and improvement of materials and methods to more precisely investigate the proposed relations. Outcomes suggest that future i4-devices should be designed to avoid complex conduit geometries as much as possible, and to be flushed with significantly higher flush flow rates than currently clinically applied.

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Delft, University of Technology — Philip J.A.F. van Griethuijsen
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Glossary

List of Acronyms

i4	implanted intra-peritoneal insulin infusion
IU	international Unit (unit of insulin dose)
PMMA	polymethyl methacrylate
PVC	polyvynil chloride
TOC	Total Organic Carbon
ppm	parts per million
NaOH	sodium hydroxide

Chapter 1

Introduction

1-1 Implanted intra-peritoneal insulin infusion

Implanted intra-peritoneal insulin infusion is defined in this thesis as the use of an implant to deliver insulin into a diabetic patient's peritoneal cavity. This infusion method is used by diabetics who cannot be treated effectively with subcutaneously infused insulin (using i.e. needles or needle-patches). The ineffectiveness of the subcutaneous method may have several causes, for example that blood glucose levels in so-called 'brittle diabetic' patients exhibit variations of such large amplitude and frequency that the absorption time of insulin administered subcutaneously, does not enable sufficient control of the blood glucose swings. Additionally, some patients exhibit insufficiently quick absorption subcutaneously infused insulin.Due to limited regulatory approval and low priority that industry provides this orphan disease, the pool of implanted intra-peritoneal insulin infusion (i4)-users is small However, those patients do benefit immensely from the method, because it provides effective ambulatory insulin therapy. Without this method they would likely be hospital-bound due to the severe impact of their kind of diabetes on their daily life [1, 2, 3, 4, 5].

1-2 Problem statement

Despite positive effect and success, i4-devices are also faced with challenges. This section clarifies one particularly challenge of obstructed conduits in i4-devices and introduces how the experimental research that was performed in this thesis contributes to tackling that challenge.

1-2-1 Insulin degradation

Insulin is a protein (or peptide) hormone, which is susceptible to degradation over time. Scientific literature describes the mechanisms of insulin degradation extensively, see a brief review of literature in Appendix A). Degradation of insulin consists of the following phases: denaturation, aggregation and precipitation. Denaturation of the protein structure is the so-called 'unfolding', and 'misfolding' resulting in the stacking of the insulin's β-sheets [6, 7]. During this process, the polar regions of the protein strands are exposed. The attraction between the polar regions of several insulin strands causes grouping of multiple proteins, which is called aggregation. Aggregation is a result of intermolecular forces, not of a covalent reaction. Continued aggregation then causes the formation of complex aggregate strands, called amyloid fibrils. If such fibrils have grown to increasingly large molecules, they can

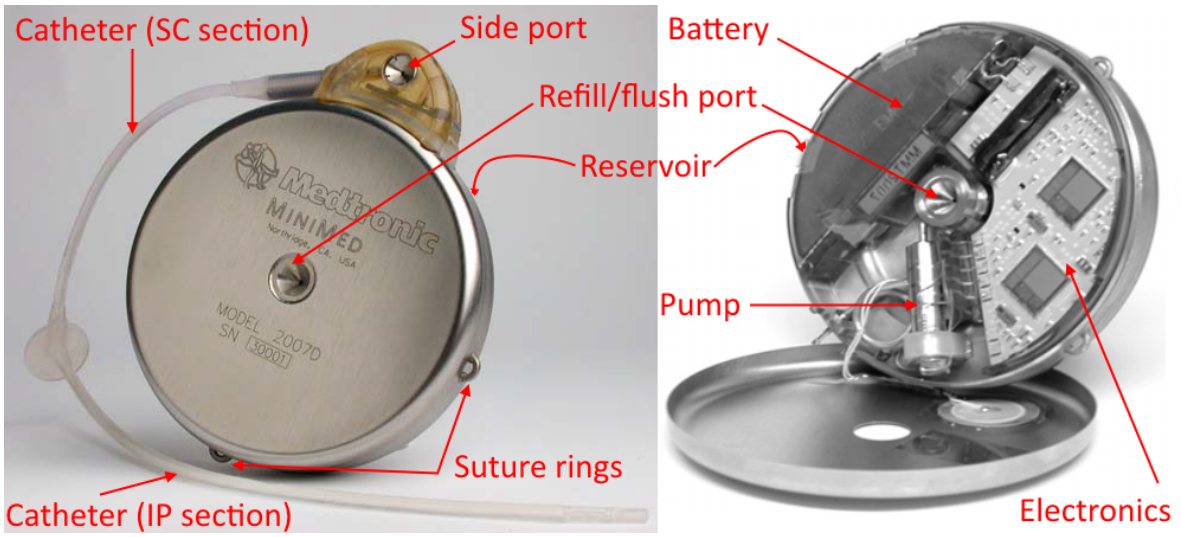

Figure 1-1: The MiniMed 2007D (Medtronic, Northridge CA) implanted intra-peritoneal insulin infusion (i4)-device.

no longer remain dissolved in liquid and they precipitate out of solution to become insulin precipitates (or crystals). Insulin in i4-devices is exposed to hydrophobic material surfaces, body temperature, agitation, shear forces due to insulin acting as a lubricant for the pumps' moving parts, and possibly even to trace amounts of contaminants of bodily origins. Each of these factors are known contributors to expedition of insulin degradation. Moreover, the literature also indicates that the prior presence of a threshold amount of insulin aggregates (often referred to as 'seeds'), may increase up to tenfold the rate of insulin aggregate formation [8]. This leads to the following assertion:

Insulin instability in i4-devices results in degradation in the form of denaturation, aggregation and precipitation

1-2-2 Obstructions in i4-devices

The literature review preceding this thesis research concluded that the only i4-device currently clinically used, the MiniMed 2007D (Northridge, CA, shown in Figure 1-1), is impeded in effectively administering the insulin due to insulin aggregation [5]. The literature only superficially describes that obstruction of moving components and insulin-carrying conduits render the device incapable of delivering the required insulin. Insulin degradation is said to be the underlying cause of these obstruction: precipitated insulin remains in the implant's conduits, and accumulates to form obstructions. A clinical intervention, a flushing procedure, can be performed by physicians in an attempt to dissolve and remove these obstructions. However, this often is not (sufficiently) effective. Ineffective flushing are suggested to lead to re-occurrence of obstructions, hence requiring yet more frequent flushing procedures. If, ultimately, an obstruction cannot be removed by flushing, the i4-device must be removed from the body and replaced with a new device (or an alternative method of insulin therapy must be chosen). Note that the literature does not specify in any significant detail which locations or components of the devices that become obstructed or to what degree. This is understandable given that the device's inner workings cannot be inspected while implanted, and, once

removed, the device is always returned to its manufacturer. No publications were encountered that offer post-removal analysis of obstructed devices. This leads to the following assertion:

Degraded insulin causes the conduits in i4-devices to become obstructed, resulting in failures in administering insulin

1-2-3 Flushing procedure

A preventive flushing procedure is performed on i4-devices every 9 months, and additionally whenever obstructions occur. This flushing procedure consists of percutaneously inserting two needles into the implanted device's two entry-ports. This creates one ingoing and one outgoing fluid flow route, enabling a flushing liquid to be pumped through the device's conduits in an attempt to dissolve and remove insulin precipitates. In the flushing procedure currently clinically applied to the MiniMed 2007D, the device is flushed with a sodium hydroxide (NaOH) solution (also named lye, or caustic soda) which causes dissolution of insulin precipitates. An NaOH solution is flushed through the device's insulin conduits only once , at a low flow rate. An (imperfect) vacuum is created in the syringe connected to the exit-port, initiating a flow through the device. It has been reported that this flushing procedure is effective in only ≈3 out of 4 cases of diagnosed obstructions [5]. The issue of an obstructed device is often seen to return before the scheduled next preventive flushing procedure, in some cases several times within a few weeks' interval. This leads to the formulation of the following **Problem Statement**:

The flushing procedure of i4-devices to clear obstructions from its conduits caused by insulin degradation are sub-optimal in effectiveness.

1-3 Hypothesis

Solving the problem of obstructions in the i4-devices could greatly contribute to the longevity of i4-device, and so significantly decrease patient burden and reduce expenses made for device maintenance and replacements. Learning how flushing effectiveness can be improved, can help to identify and evaluate possible solutions to this problem. Pharmaceutical research on increasing insulin stability is ongoing, which could contribute to lower incidence of obstructions. However, given the wide variety of contributors to insulin instability described above, and the complexity of influencing that pharmaceutical process, it is plausible that insulin degradation will for the near future continue to pose a significant risk of the formation of obstructions in i4-devices. Moreover, for new i4-system designs it is important to accumulate knowledge on the prevention of obstructions. Therefore, this research focuses on improving the effectiveness of the flushing procedure in removing obstructions from i4-devices. The chemical dissolving effect of NaOH and the fluid dynamics of flush flows together are deemed to be the prime influences on the flushing effectiveness. An analysis of the MiniMed 2007D's design and the flushing procedure has been performed and led to the formulation of hypotheses of the possible causes, which are described below.

1-3-1 Low flush flow rate

The flushing liquid is flushed through the device's conduits at a rate varying between ≈ 1μL/min up to ≈ 1.5mL/min. The laws that govern flow dynamics through miniaturized

vessels ('microfluidics') were consulted to analyse the fluid dynamics of this device [9, 10]. Stable insulin flowing through conduits can be treated as single-phase continuous microfluidic fluid flow. However, when insulin *precipitates* are carried along with the liquid, the flow must be treated as a two-phase fluid flow. Flow is driven by pressure differences resulting from pump or syringe-piston movement. It was assumed that there is no material in the gas phase in the implant's conduits, since all liquids are always de-gassed before being introduced into the implant, and no gas-producing reactions are known to occur. In microfluidics, inertial and gravitational effects become less important, whereas influences of capillarity, viscoelasticity and interface phenomena become more important. When viscous forces dominate inertia, a fluid flow is said to be laminar, i.e. the fluid flow lines are, at least locally, parallel and turbulences do not develop. Polymeric liquids, like liquids with dissolved proteins, and emulsions carrying solid particles, like precipitates, may exhibit increased viscosity. Increased viscosity results in lower liquid flow velocity directly at the interface between the liquid and the solid conduit walls (also called 'creep'). The design of the insulin holding, pumping, and carrying sections of the MiniMed 2007D features a broad variety of conduit geometries that are more complex than just straight tubes of constant diameter or cross-sectional surface (see also Figure 2-3). Particularly, cavities like those containing the plunger shaft or the plunger's magnetic pole as exist in the Maxon microfluidic pump in the MiniMed 2007D have an increase in the cross-sectional surface for the liquid flow, and so result in lower flow velocity (at constant flow rate). Such cavities are calculated to result in an average flow velocity of only approx. $8{\cdot}10^{-3}$m/s when the flush flow is at the observed procedural maximum of 1.5mL/-min. Additionally, flow velocity profiles often establish themselves such that flow velocity in subsections far away from the direct flow path is even lower. It is here hypothesized that such a low flow rate and low creep in the flushed conduits does not exert sufficient shear force to dissolve, dislodge, or break up and take away the insulin precipitates that constitute the obstructions. Complex conduit geometries as such feature spaces where insulin precipitates are more likely to accumulate, resist removal by the flush flow, and so bolster the formation of obstructions. An increased flush flow rate would result in larger overall flow velocities in all conduit subsections, with a corresponding increase in the shear forces exerted on the accumulations of insulin precipitates, and this might increase the effectiveness of the flushing. This analysis leads to the proposal of the following hypothesis:

Hypothesis 1: Using increased flush flow rates in i4-devices will be more effective in cleansing the insulin-carrying conduits with complex geometries.

1-3-2 Parallel flow paths

The conduits in the MiniMed 2007D feature several parallel pathways which the insulin can follow as it flows through the implant's components. If one of several pathways is (partially) fouled by degraded insulin, this causes both a higher viscosity and a reduced hydraulic diameter, and thus increased flow resistance in that pathway. The alternative pathway then has comparatively lower flow resistance and thus more flush flow will pass through that unobstructed pathway. The already obstructed flow paths thus may not be flushed as rigorously or even not at all, hence plausibly resulting in lessened effect in removing the accumulated precipitates - the very cause of the obstruction. This analysis leads to the proposition of the next hypothesis:

Hypothesis 2: Parallel flow paths in i4-device insulin conduits shield already

obstructed flow paths from the flush flow and thus disallow effective cleansing of conduits.

1-4 Research Question

The above problem statements and hypotheses lead to the formulation of the main research question of this thesis:

How can the effectiveness of the flushing procedure used in i4-devices be improved by adapting the design of conduit geometries and adapting the flushing procedure?

Keeping in mind the aim of verifying the two hypotheses formulated above, the following two research sub-questions are posed:

- **Do conduits with more complex geometries than straight tubes have a negative influence on flushing effectiveness?**
- **Can higher flush flow rates than currently clinically used, increase flushing effectiveness?**

1-5 Research Goals

To answer these research questions, the goal of this research was to design, build and use an experimental setup to investigate the influence both of differing conduit geometries on flushing effectiveness, and the influence of flush flow rates on the flushing effectiveness. This experimental setup must allow measurement of the effectiveness of the removal of insulin-based obstructions. The experimental setup must further allow the investigation of the influence of variations in conduit geometries, as well as differing flush flow rates. The ultimate aim in this research effort is to contribute to developing an improved i4-device, the 'DiaLin' produced by IPaDiC B.V.(Deventer, the Netherlands), that exhibits higher flushing effectiveness. To maintain a manageable scope in this research, the focus is exclusively on the goal of improving the effectiveness of the flushing procedure and on doing this by experimenting in an *ex vivo* laboratory environment which models the *in vivo* clinical problem described above. Naturally, many other efforts can be focused on reducing the impact induced by the occurrence of insulin-based obstructions in i4, but these are outside the scope of this research, as the Discussion will detail further. This report presents the methodology of the experiments which served to answer the research question. It presents the results of the experiments, then critically discusses these results, and presents the recommendations and conclusions emanating from them. Finally, this research is placed in the context to the broader developments foreseen in the field.

Chapter 2

Methodology

This chapter describes the experimental methodology used. It identifies the independent and dependent (or predictor and outcome) variables and confounding factors; it details the design choices and production of the materials and equipment; it presents the formulation of the experimental protocol, and it details the data analysis method.

2-1 General Experimental Methodology & Setup

To accomplish the research goal, an experiment was designed and performed to measure the influence of the independent variables - the conduit geometry and the flush flow rate - on the dependent variable - the flushing effectiveness. Because the physical characteristics of the MiniMed 2007D implanted intra-peritoneal insulin infusion (i4)-device do not allow comprehensive (optical) inspection of the conduit interiors and their state of cleanliness, transparent test conduits were produced to serve as a simplified model of the i4-device. The 9 different test conduits are milli-tubes or milli-channels that mimic the differing conduit geometries in the MiniMed 2007D. These test conduits were filled with insulin and exposed to environmental conditions chosen to expedite insulin degradation to such extent that insulin precipitated, thus resulting in the formation of precipitate particulates in the conduits. The precipitates are solids in the liquid and are visible in optical microscopic observation, and this allows to measure in which subsections of the conduits precipitates are present. Subsequently, the test conduits were flushed in an attempt to clear out the insulin (and its degradation products) from the conduits. At several moments during these steps, measurements are taken to register the (the location of) presence of precipitates in the conduits. The effectiveness of the flushing procedure is expressed in the degree of residual insulin precipitates remaining in the conduits after the flushing, and the flushing effectiveness is compared between the (9 different) conduit geometries, and the (2 different) flow rates. These experimental steps are presented visually in Figure 2-1, and a picture of the flushing setup is shown in Figure 2-2.

2-2 Test conduits

This chapter describes the design and production of the test conduits.

2-2-1 Test conduit design requirements

As is stated in the Introduction, it is not known precisely where obstructions in the MiniMed 2007D occur and what characteristics they have, so it is unclear which conduit geometries are

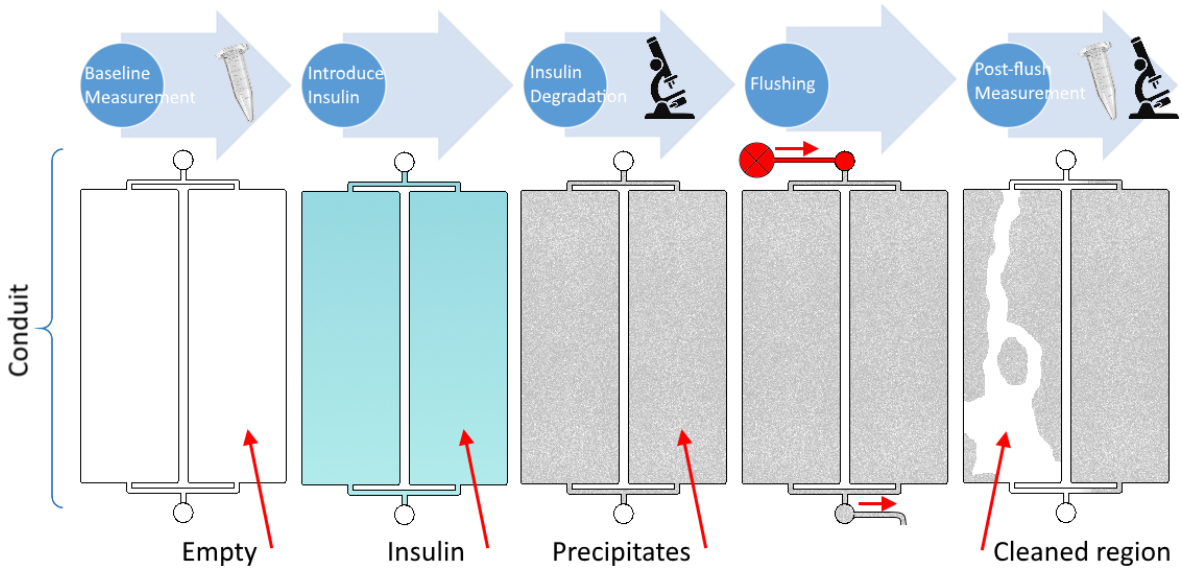

Figure 2-1: Graphical representation of the experimental steps. Insulin is inserted into a clean test conduit, and insulin degradation is then induced to cause precipitation in the conduits. The conduit is flushed, to clean the precipitates from the conduit. Flushing effectiveness is measured by the degree to which the precipitates are removed. Measurements were performed taking pre- and post-experiment liquid samples and measuring TOC content, and by ascertaining precipitate presence with an optical microscope.

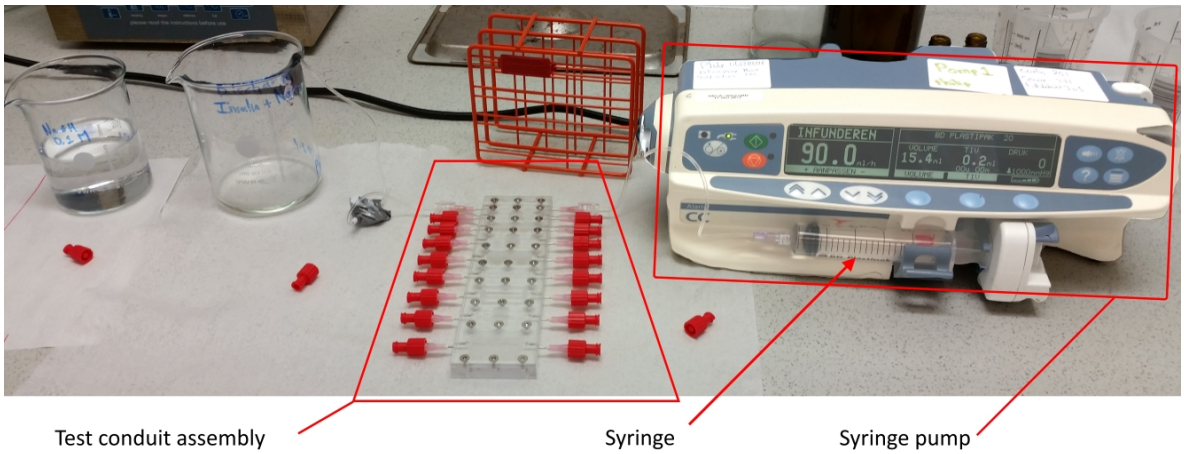

Figure 2-2: The experimental setup. One conduit on the assembly of test conduits is connected to the pump, which actuates a syringe with NaOH, to flush the conduit. Experiments are performed in a laboratory setting.

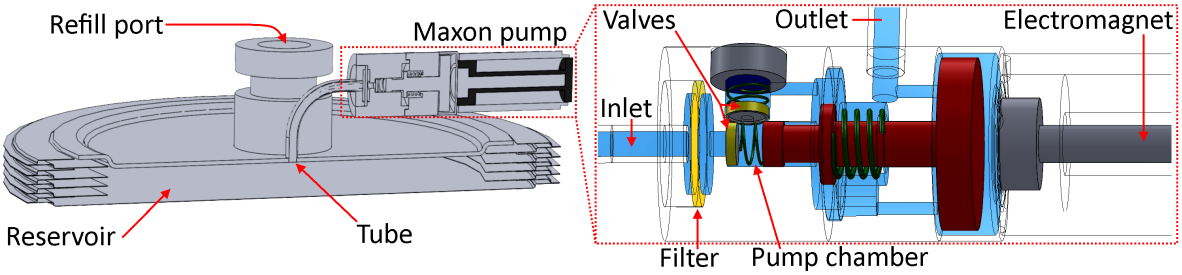

Figure 2-3: (*L*) Cross-sectional view of a MiniMed 2007D stripped of all but its entry port, reservoir, tubes and the Maxon Motors microfluidic pump. The bellows structure of the reservoir is clearly visible. (*R*) Cross-sectional view of the Maxon Motors microfluidic pump which is inside the MiniMed 2007D. When the electromagnet is engaged, the magnetic force pulls the plunger through its forward stroke. Once the electromagnet is disengaged, the plunger spring powers the return stroke. The valves around the chamber ensure flow directionality.

susceptible to obstructions [5]. Experimenting on an actual MiniMed 2007D was not deemed a practical option, because the conduits cannot (non-destructively) be optically observed. It also does not allow its variety of conduit geometries to be investigated *independently*, because the fluid flow patterns, fluid resistance and obstructions of consecutive conduit sections influence each other. Performing experimental flushings on **a series of differing test conduits geometries** is intended to have each test conduit represent one type of conduit geometry, and thus to enable the influence on the flushing effect to be measured independently. This approach required the test conduits to satisfy the following series of demands:

1. They mimic the conduits like those present in the Medtronic MiniMed 2007D.
2. They allow both optical microscopy identification and localization precipitate presence in the conduits, as well as chemical analysis to verify the visual observations.
3. Each conduit shows a structural variation of - ideally - only one design parameter compared to the next test conduit geometry in the series.
4. They allow a simple connectivity to insulin flows and flushing liquid flows, such that the materials can quickly be transferred between experiment steps.

2-2-2 Test conduit geometries

As is described in the Introduction, the differing conduit geometries comprise the first independent variable that is hypothesized to influence flushing effectiveness. The designs of the test conduit geometries were based on the conduits in the MiniMed 2007D device, and most specifically those in the Maxon microfluidic pump. Figure 2-3 shows the disctinctive conduit geometries of the MiniMed 2007D. An assembly of test conduits was built that features 9 differing geometries, that were chosen to represent three main categories. Each of the categories is detailed below. The numbering corresponds with Figure 2-4 which shows the test conduit assembly and its dimensions.

- **Straight (#1).** This simple straight conduit with a constant diameter of 0.5mm mimics the simplest tubes in the MiniMed 2007D, e.g. those transporting insulin from reservoir into the pump. It serves as the baseline conduit of minimal complexity. It is assumed that outcomes of this conduit are relevant to bent conduits with relatively large bend radii compared to conduit inner diameter, like in the MiniMed 2007D.

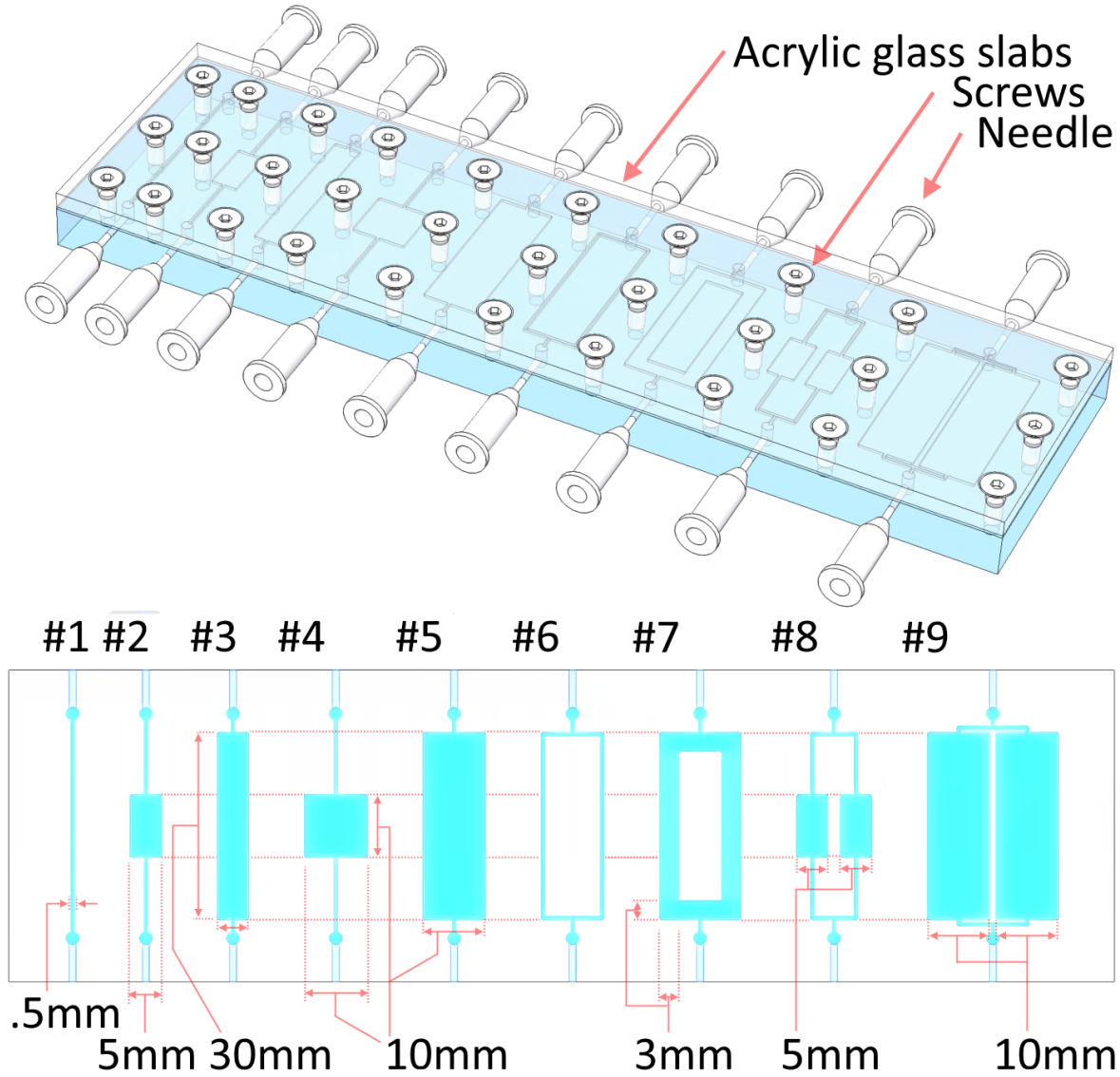

Figure 2-4: (*Top*) The assembly of 9 test conduits, produced from a block of transparent acrylic glass, with Luer-Lock needles to connect the conduits to the fluid flows. (*Bottom*) The different conduit geometries, in three categories: Straight conduits of constant diameter (#1); Featuring a section with a widened diameter (#2-5); And featuring a split into parallel branches, with respective widened sections (#6-9).

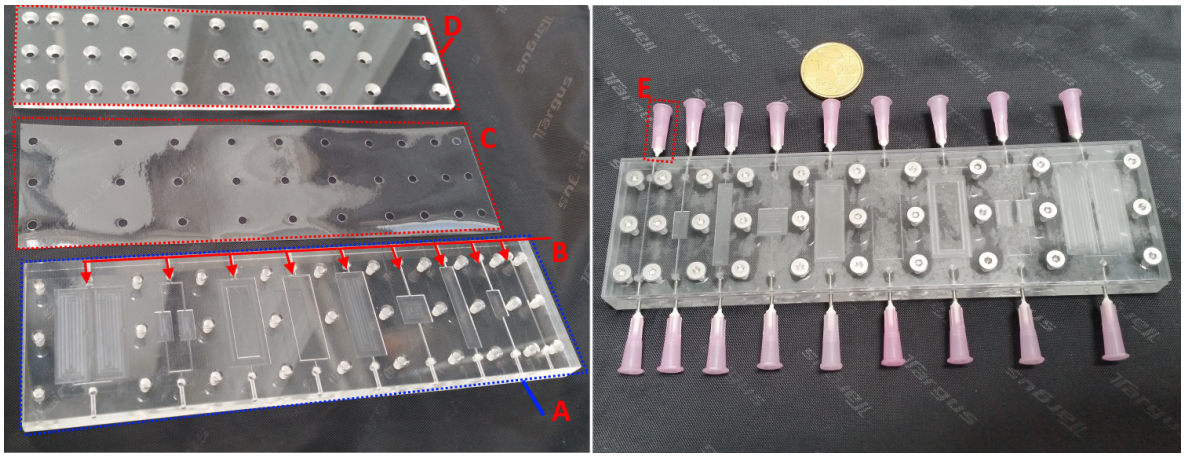

Figure 2-5: (*Left*) The assembly components: thick bottom PMMA slab (*A*) with milled conduits (*B*), the PVC film (*C*), and the top PMMA slab (*D*). (*Right*) The test conduits assembled, including the Luer-Lock needles (*E*); €0,50 coin for scale.

- **Cavities (#2-5).** These four comparable geometries mimic conduit cavities with larger hydraulic diameters and correspondingly larger volumes. Lengths and widths of the cavity are systematically varied, and these dimensions correspond to such cavities as the pump's chamber, or the pole's cavity. The explicit aim of this geometric category is to verify whether cavities contribute to precipitate remainder, and at which dimensions this effect has its onset.
- **Parallel branches (#6-9).** These four comparable geometries mimic parallel pathways. The different geometries of the parallel subsections mimic the geometries of the conduits featuring a cavity (#2-5), such that results of singular and parallel pathways can be compared against each other. The explicit aim of this geometric category is to verify whether parallel pathways result in the shielding effect of Hypothesis 2.
  - **#6** Both narrow parallel branches have constant diameters (0.5mm), allowing for direct comparison with test conduit #1.
  - **#7** This geometry has a wide diameter and parallel flow paths around a central 'obstacle'. This mimics the way insulin flows around the plunger shaft in the Maxon pump.
  - **#8** Both parallel conduits feature a cavity, which is similar to the plunger pole's cavity in the Maxon pump. To allow direct comparison, this cavities' dimensions are identical to those in conduit #2 (the *smallest* of that category).
  - **#9** Again, both parallel conduits feature a cavity. The cavity's dimensions are identical to those in conduit #5 (the *largest* cavity of that category).

The narrowest sections of *all* conduits have a diameter of 0.5mm, as is the case in the MiniMed 2007D. Of course the physical characteristics per test conduit could not be kept perfectly constant under systematic variation of single design parameters.

2-2-3 Production of the test conduit assembly

It was chosen to group all 9 conduit geometries on a single test conduit assembly to simplify handling the various miniature components, to facilitate all test conduits being exposed

Table 2-1: A comparison of carbon-atom contribution in different insulin concentrations.

	1 mol insulin	1 IU insulin	100 IU insulin / mL	400 IU insulin / mL
Insulin	5807.57 g	$3.47 \cdot 10^{-5}$ g	3.47 g/L	13.9 g/L
	1 mol	$5.98 \cdot 10^{-9}$ mol	$5.98 \cdot 10^{-4}$mol/L	$2.39 \cdot 10^{-3}$ mol/L
C	3050 g	$1.82 \cdot 10^{-5}$ g	1.82 g/L	7.29 g/L
	254 mol	$1520 \cdot 10^{-9}$mol	0.152 mol/L	0.607 mol/L

to exactly the same conditions, and to expedite interchange of conduits between successive flushing runs. Three such test conduit assemblies were produced by Meester Techniek B.V. (Leiden, Netherlands).
The conduits geometries were milled as 0.5mm deep grooves into a 8mm thick polymethyl methacrylate (PMMA) slate, and this groove was enclosed by a second PMMA slate of 3mm thickness to so create conduits. A thin sheet of flexible, transparent polyvynil chloride (PVC) foil was placed between the two PMMA slates as the sealant to make the conduit watertight. The top slate served as the medium for optical observation, while the transparent bottom slate allowed for trans-illumination for the purpose of optical observation. The slates of PMMA and the PVC foil are fixated together by a multitude of screws that were placed as close as possible to the conduits to reduce strain when pressure builds up inside the conduits. This facilitated disassembly to clean the assembly in between repetitions of the experiments. PMMA and PVC were chosen because of their transparency and resistance to the chemical solutions used in experimenting (see Sections 2-4 and 2-5). The test conduits' entry and exit ports were created using Luer-Lock needles pressed into the drilled holes. This satisfies the requirement of simple connectivity to Luer-Lock equipped medical materials (e.g. tubing, syringes) used for filling, emptying, flushing and closing the conduits. The production outcome is shown in Figure 2-5.
For a description of two *alternative* production methods that were initially attempted, but deemed unsuccessful, see Appendix B.

2-3 Insulin

2-3-1 Insulin types

The insulin molecule has chemical formula $C_{254}H_{377}N_{65}O_{75}S_6$, resulting in a molecular weight of 5807.57g/mol, of which 3050.72g/mol from carbon atoms. One international Unit (unit of insulin dose) (IU) of insulin represents 0.0347 mg insulin [11]. Two different concentrations of insulin were used in the experiments. The main flushing experiments were performed using same the highly concentrated insulin used clinically in the MiniMed 2007D: Insuman Implantable (Sanofi-Aventis, FR), with a concentration of 400IU/mL [12]. This insulin was outdated, but showed no precipitation. During the pilot experiments prior to the main experiment, Novo Nordisk Novorapid insulin was used with a concentration of 100 IU/mL (≈3.5mg of insulin/mL, see also Section 2-5-2) [13, 14]. This insulin is regularly used by diabetics administering insulin subcutaneously and is a readily available and affordable insulin type. This offered more freedom in the initial search for the adequate exposure conditions to induce insulin precipitation. Additionally, it was deemed to avoid the potential toxic hazard of accidentally mishandling highly concentrated insulin.

2-3-2 Processing insulin to induce precipitation

As was described in Section 2-1, the test conduits first needed to be successfully fouled with insulin precipitates, before flushing effect could be measured. As described in the Introduction, under implanted conditions insulin does show degradation, but *may* jst as well continue to be stable for months. Time management of this research required insulin precipitation to be induced in the conduits within a workable shorter time-span. Insulin was introduced into the test conduits and exposed to conditions known to contribute to insulin instability: heat, agitation and contamination. Precise values for these environmental conditions for precipitation were not available, so an extensive trajectory of trying different combinations of exposure methods was explored before insulin precipitates were successfully microscopically confirmed in the conduits. This trajectory is described and discussed in Appendix C. The protocol that eventually proved effective in inducing precipitation is based on the methodology of research into producing functional protein amyloid fibrils for micro electro-mechanical (MEMS) devices. There, insulin pH is lowered to strongly promote insulin denaturation. Subsequent incubation at high temperature of the denatured insulin molecules then expedites formation and growth of aggregates into precipitates, which finally creating an emulsion with increased viscosity [15, 16, 17, 18, 19, 6, 8]. Literature indicated a pH=2 and incubation 60 °Celsius for 22 hours temperature causes maximum accumulation between different fibrils and results in the largest size of precipitates, so that protocol was adopted [20]. A small liquid volume of insulin was placed in a test-tube and pH was lowered by addition of 1M hydrochloric acid (HCl) solution (Sigma-Aldrich). Correct pH=2 value was confirmed with pH color test strips (Fisher Scientific). The acidified insulin was then inserted into the test conduits, and the conduits were capped closed. This test conduit assembly of 9 filled conduits were then placed into an oven (Memmert UN 30 Oven) at 60 °Celsius for 22 hours.

2-4 Experimental flushing

With the different conduit geometries forming the first independent variable, the **flush flow rate** is the second independent variable in this experiment that is hypothesized to influence the flushing effectiveness.

2-4-1 Experimental flush flow rate

Observing a clinical flushing procedure. To establish suitable experimental flush flow rates, it was necessary to precisely understand the flushing characteristics of procedure performed clinically on the Minimed 2007D. The procedure is dictated in the device's Physicians Manual [21], but the literature review preceding this research identified that in attempts to increase it's success rate, adaptations were implemented in clinical practice over the years [5]. To obtain an up to date reference of the clinical practice, one such flushing procedure was observed. The most important conclusion was that a minimum volume of only 1 mL is prescribed to be flushed through the conduits, at a rate varying between $\approx 1\mu\mathbf{L/min}$ (the i4-device programmable flow rate for administrating a bolus dose) **up to** $\approx 1.5\mathbf{mL/min}$ (the average flow-rate in the case of one reservoir payload of 15mL being wholly emptied out over the time span of approx. 10 minutes).

Selecting experimental flow rates. Based on the above observation, the variation of the experimentally employed flushing characteristics was defined. To maintain a feasible scope

for this experiment, it was chosen to use **two** different flush flows. The *clinical maximum* flow rate was adopted as the *experimental minimum* flow rate, i.e. **1.5mL/min**. This approach was chosen because it allows a direct comparison with the clinical procedure. The other experimental flow rate was chosen at a significantly higher rate, namely **20mL/min**. This was intended to achieve the maximum influence on flushing effectiveness compared to the lower flush rate. A medical drug infusion pump (Alaris Asena CC [22]) was used to generate the flush flow. This pump generates flows up to 20mL/min, against pressures of up to 133kPa (1000mmHg). The pump is equipped with a pressure disk to to continuously measure pressure during in the infusion tubes from syringe into the conduit.

2-4-2 Flushing liquid

The flushing liquid used in the official Medtronic flushing procedure is a sterilized 0.1M sodium hydroxide (NaOH, or 'caustic lye') solution. In this experiment, sterilization was not a requirement, so industrial-grade 0.1M NaOH solution was used (Sigma-Aldrich). In order to maintain a feasible scope for the experiment, it was chosen not to introduce experimental variation in the form of different chemicals for dissolving.

A sterile 'rinse buffer' solution is used in the MiniMed procedure to flush away the NaOH, and it acts as a protective solution to the next insulin payload in the device. This buffer solution is a placebo solution comprising all the Insuman excipients, minus the insulin and zinc chloride, and is comparably costly to the Insuman insulin itself. Any chemical dissolving effect of the buffer excipients on insulin precipitates apart from the dissolving effect emanating form returning the solution to neutral pH are unknown, but are here assumed to be negligible. It was therefore assumed that the second rinsing flow only has effect as a fluid dynamic actor in dislodging and carrying insulin precipitates. In this experiment the sterility and the protective feature are not required, because the conduits are cleaned between successive runs. Therefore, it was chosen to simply use ultra-filtered water instead of the buffer solution for the second flushing instance.

2-5 Measurement

The flushing effectiveness, the dependent variable of this experiment, was measured qualitatively and quantitatively. This section describes the measurement equipment and methods.

2-5-1 Microscopic observation of precipitate presence in test conduits

Optical microscopy was used to observe obstructions in the test conduits. This was deemed preferential over alternative options (e.g. custom-built turbidity measurements or spectrophotometry), because this was a simple, non-invasive 'off the shelf' method, judged to provide sufficient resolution and ease of analysis without requiring an extensive calibration and validation trajectory. It was assumed that if insulin could reach such an evolved state of degradation that actual obstructions of fluid flow could occur, this must result in microscopically identifiable precipitate content in the liquid. Microscopically identifiable is here defined as: precipitate particulates can be individually resolved and distinguished from possible sources of noise (e.g. the surface roughness of the PMMA conduit material, or dust which was sometimes observed during pilot runs before an effective pre-experiment conduit cleansing protocol had been established). This measurement setup thus qualifies as a pretest - posttest design type, where measurements are taken before and after exposure to the experimental flush flow.

The Keyence VHX-5000 microscope provides magnification levels between 50-2500x at resolution down to 1μm. It is further capable of stitching multiple images to create a larger concatenated field of view. Using this option, images of the entire top view of all conduits were made, in which the macroscopic subsections with and without precipitates were identified. This microscope was used to image the pre-flushing and post-flushing conditions of all the test conduits.

The Motic microscope was also used to observe the presence of precipitate of the test conduits, before, during and after the various experimental steps [23]. This microscope served as a more versatile and most of all a quicker method of establishing mid-experiment conditions of precipitate presence in the test conduits. This microscope has no stitching capability and so is not used for post-flushing overview images, but was used for 'live' observations, e.g. of the dissolving effect of NaOH (see Section 2-5-2).

2-5-2 Quantitative measurement of effectiveness

To obtain a quantitative measurement of effectiveness of the flushing, the quantity of insulin remnants is not only observed microscopically, but also measured chemically. The precipitate remnants are dissolved from the conduits by inserting a solvent, and the concentration of insulin remnants in this solvent is measured using Total Organic Carbon (TOC) analyser. Such liquid samples are taken twice: one of the clean pre-experiment condition; and a second of the post-flushing condition.

Total Organic Carbon (TOC) analysis

The solution containing the dissolved residual insulin is removed from the conduit and captured, and tested for Total Organic Carbon. The measurement of the Total Organic Carbon content of the collected solution is performed using a Shimadzu TOC-V$_{CPH}$ oxidative combustion infrared TOC analyser, following the dedicated protocol [24]. Measurement results are given in mg/L (which corresponds $1:1$ to parts per million (ppm)), which qualifies this variable being measured on a ratio metric level. The TOC analyser can measure TOC content between 4μg/L - 50 mg/L, with measurement accuracy characterized by a coefficient variance of 1.5% between measurements.
A 400IU/mL insulin solution has $13.9 \cdot 10^3$mg/L of insulin, of which $8.48 \cdot 10^3$mg/L from carbon atoms - which thus sets the upper limit of measurable carbon content, see also Table 2-1. Given how the TOC analyser requires a minimum of 30ml sample volume, the ≈0.3mL NaOH sample with dissolved insulin that was taken from the conduits, had to be supplemented to 30ml, which means diluting the carbon concentration. If only 1/100th of the original insulin were to remain in the test conduit after flushing-condition, and this is fully dissolved by the NaOH, then this would theoretically result in a TOC measurement of ≈24μg/L. The dilution factor was measured by precisely registering the volume of each liquid sample taken for TOC measurement with a micro-pipette. TOC results were corrected for this dilution factor.

Dissolving insulin obstructions with NaOH

NaOH was also used as the solvent to dissolve the remaining precipitates in the test conduit for taking the TOC samples. With insulin being a denatured protein aggregate, the individual insulin molecules must be separated from the aggregation. In this case, insulin is in a far developed stage of aggregation and has precipitated out of solution, which is exactly the

reason why it needs to be broken down to become soluble again to be removed from the conduit. The individual insulin molecules de-fold again under influence of the now present OH^-− molecules, causing them to disassemble from the aggregate. As long as sufficient NaOH is present, insulin re-folding may continue until all polar regions from the OH^- are taken. This denaturation results in the breakdown of insulin precipitates into smaller fragments, and more of the insulin's own hydrophilic groups become exposed, thus rendering the fragments (more) soluble. The test conduits were agitated with ultrasonification (Shesto UT8031/EUK Ultrasonic Cleaner) to expedite the dissolution reaction. Unless proven otherwise (e.g. during post-dissolving microscopic analysis), it was assumed that the addition of NaOH resulted in **all** residual precipitates to be dissolved. The assumption is further made that the only source of carbon atoms in the solvent are be the insulin remainders that were not removed from the conduits in the flushing condition. The assumption is that the further employed materials (plastics, metals etc.) do not contribute to carbon-element in the liquid that were contained in the conduits. Out of consideration of practical scheduling of experiments, the TOC analyses were not performed immediately after taking the liquid samples from the conduits. Instead, all the liquid samples taken from the conduits were marked and stored.
It should be noted that some of the excipients in insulin (such as glycerol ($C_3H_8O_3$), metacresol (C_7H_8O), phenol (C_6H_5OH), could contribute to measured amount of TOC. The influence of these excipients however is assumed to be of no significant influence on the measurement outcome, as the concentration of excipients is constant in relation to the concentration of insulin in the Insuman insulin. A brief side-experiment was performed (see protocol in Appendix D) to investigate the potential of NaOH solely in (chemically) dissolving insulin precipitates, specifically in a non-flushing circumstance i.e. a situation where NaOH is simply in proximity to insulin, but not in an actuated fluid dynamical interaction. Insulin that was pre-exposed to the conditions as described in Section 2-3-2 was inserted into a conduit and then a small quantity of NaOH was also introduced into the same test conduit and immediately observed microscopically to investigate the speed of the dissolving reaction.

Selection of samples for TOC analysis

As is reported in detail in Section 3-1-2, great quantities of residual insulin precipitates were still observed in many of the test conduits even after the final introduction of NaOH to dissolve the remaining post-flushing precipitates. These post-flush liquid samples were unfortunately deemed to result in TOC measurement saturation and loss of specificity because solid materials needed to be filtered from the liquid sample. It was therefor chosen not to measure TOC content of *all* flushing experiments, but rather to measure TOC of a selection of liquid samples acquired throughout the different experiments performed (including other experiments than the main flushing experiments). A series of 10 liquid samples was so chosen, 2 measurements each in five categories:

1. Liquid samples taken from a cleaned test conduit. This serves as a baseline measurement.
2. Samples from a test conduit which had only briefly been flushed with insulin (but *not* exposed to induce degradation) before undergoing the NaOH flushing. This was chosen to investigate the possible effects of *not precipitated* insulin remaining in the conduit despite flushing (e.g. due to adsorption).
3. Post-flush samples from a conduit in which insulin *had* been exposed to degrading conditions but which had *not* resulted in microscopically identifiable precipitation. This

measurement was intended to provide data on whether not *visually* precipitated insulin might *also* remain in the conduits.

4. Post-flushing samples from test conduits the main flushing experiment series which had become optically wholly clean after flushing. This and the next samples fall within the initial intent of TOC measurement.
5. Post-flushing samples from test-conduits which had significant residual insulin precipitates after flushing.

2-6 Experimental protocols

The precise experimental protocols of this experiment are provided in Appendix D. It should be noted that essential precautions were taken to ensure a safe experimental environment, given how this experiment involved the use both of harmful chemicals as well as highly concentrated insulin, which could be poisonous if ingested or administered, e.g. accidentally injected. Hazardous chemicals were handled under supervision and in appropriately equipped laboratories. Insulin especially i.c.w. needles was handled conform directives for handling medicines, injectables and sharps.

2-7 Data analysis

The images taken with the microscope after performing the flushing were analysed to qualitatively and quantitatively determine the degree of residual precipitates. Each test conduit's overview image was manually analysed to precisely identify in what subsections precipitates had remained. Manual analysis was chosen instead of automatic image processing algorithms because the manageable quantity of images did not justify the overhead of developing a script, and because images were subject to much visual disturbances (e.g. presence of bubbles and opacity of the PVC-foil; for more details see Appendix B). All images of the conduit's surfaces were analysed on a scale of $\approx$0.005-0.01mm^2, and wherever a sufficient quantity of precipitates was observed to predominantly obscure the conduit's surface, this subsection was counted as 'containing residual precipitates', or else it was counted as having been 'cleaned effectively'. Thus, a macroscopic overview was obtained distinguishing between clean subsections, and subsections containing residual precipitates. These images firstly allowed *qualitative* identification which geometrical subsections of each conduit still showed residual insulin precipitates, whether specifically e.g. the corners of the wider sections or one of two parallel conduit branches were effectively flushed. Subsequently, using PDN image processing software [25], a simple pixel-counting technique was employed to the overview images of each conduit to *quantitatively* approximate the percentage of the conduit's surface area containing residual insulin precipitates: $(= n_{dirty}/n_{tot.surface} \cdot 100)$. A graphical representation of this manual imaging processing is provided in Figure 2-6.

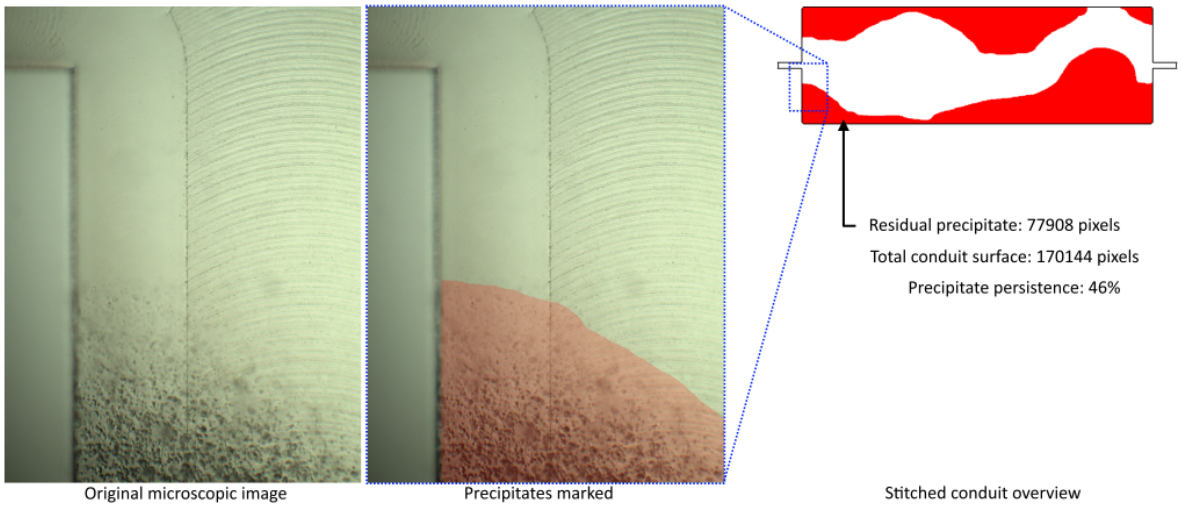

Figure 2-6: Graphic example of the manual image processing protocol. In the original microscope images, the regions containing residual insulin precipitates are marked. All microscopic images are stitched together to form a total test conduit overview. Counting pixels of the regions containing precipitates, and of the total conduit surface provides a percentage of surface containing residual precipitates.

Chapter 3

Results

3-1 Effectiveness of flushing

3-1-1 Microscopic observation of flushing effect

The results of the microscopic observation of effectiveness of the two flushing conditions are presented in Figure 3-1. The values represent the percentages of test conduit (top-view) surface that were observed to contain residual insulin precipitate fouling. The schematic figures show the overview of which regions precisely were observed not to have been flushed clean.

Influence of conduit geometry on effectiveness

For both flushing conditions, the baseline straight channels (#1) are observed to have become optically entirely clean under both flush flow rates. Under both flush flow rates in the series of straight test conduits featuring a cavity (#2-#5) were not entirely optically clean after flushing. Of those test conduits featuring parallel flow geometries (#6-#9), only channel #6 (without a cavity) is observed to be optically entirely clean after flushing and only after the 20mL/min flow rate flush. Also concerning the parallel channels, it is observed that in seven out of the total of eight flushing runs, the test conduit was observed not to have had *any* flush flow perfusion through one of the two parallel branches.

Apart from the baseline conduits (#1), only test conduit #6 (which *does* feature a parallel geometry, but *no* widened section in the parallel flow branches) exhibited one or both of the branches to have been flushed **wholly clean**; all test conduits with parallel flow branches geometries that *do* feature a cavity, showed at least some residual precipitates in both branches.

In the schematics in Figure 3-1, it can furthermore be observed quite markedly that in the case of conduit geometries featuring a (which *was* perfused by the flush flow), that shapes of the subsection that the flush flow had successfully cleaned were somewhat similar to that of a meandering riverbed. Only conduit #7 under the high flow rate condition, is an exception to this observation. In some cases this meandering path is quite narrow ($\approx 1mm$), where in other cases this path spans the majority of the conduit's width.

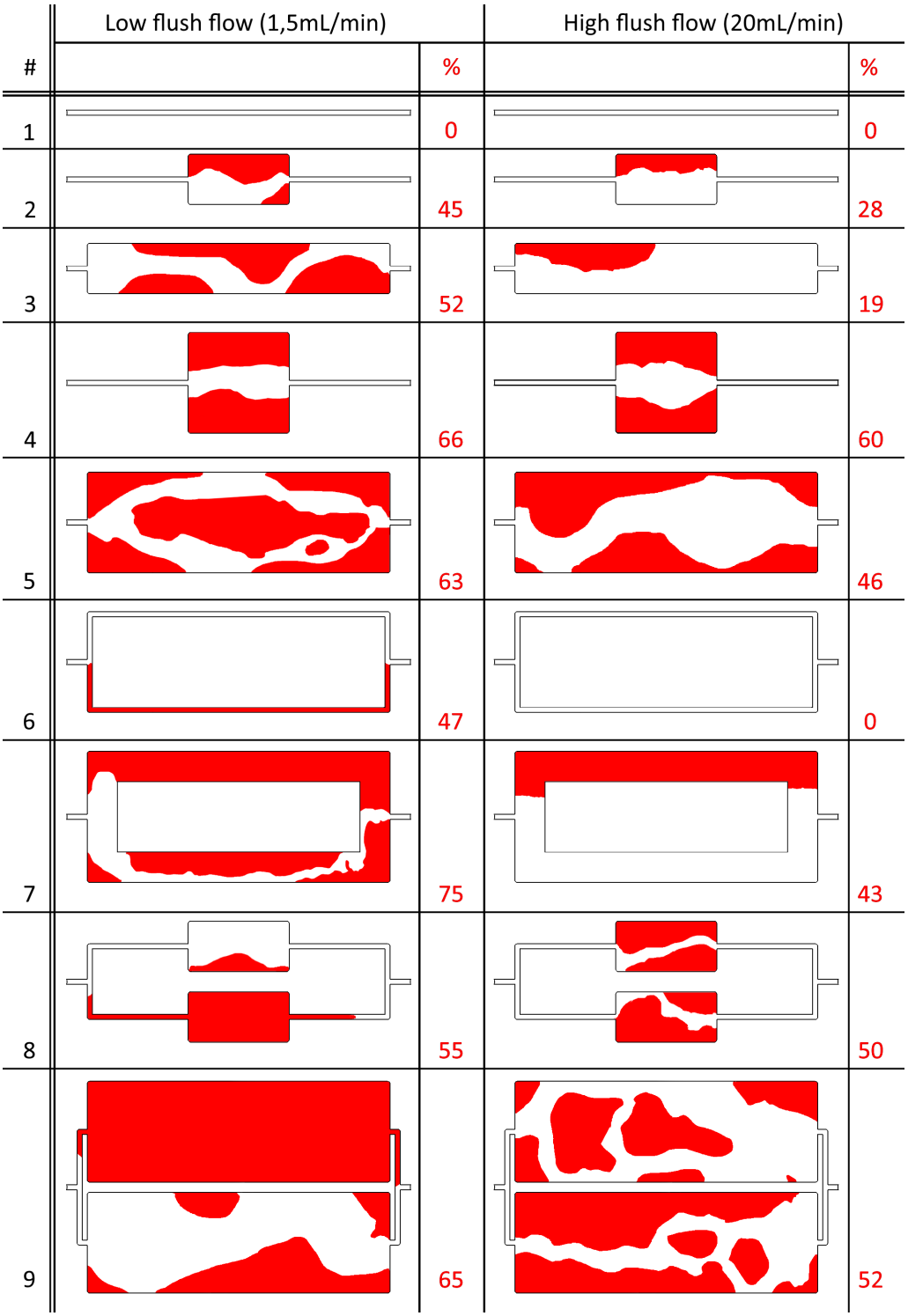

Figure 3-1: Qualitative and quantitative overview of the flushing effectiveness. The results of the two different flush flow rates are compared. In red shows the residual insulin precipitate after the flushing. Percentages of conduit top-view surface containing residual precipitates.

Table 3-1: Results of the TOC analysis. A total of 10 liquid samples were analysed for carbon content: Firstly 2 measurements of cleaned test conduits serving as baseline measurements, and then 8 samples of test conduits with various residual states of residual fouling after the flushing had been performed.

Liquid sample		Sample vol.	Dilution	Sample TOC
Condition	Conduit	[mL]	[-]	[mg/L]
Clean	#1	0.175	180	77.5
Clean	#1	0.250	130	247
Insulin rinse	#1	0.075	420	197
Insulin rinse	#9	0.375	85	90.0
No precipitates	#1	0.105	300	335
No precipitates	#9	0.300	105	121
Clean after flush	#1	0.175	180	841
Clean after flush	#1	0.030	1050	344
Dirty after flush	#9	0.400	80	3340
Dirty after flush	#9	0.350	90	2730

Influence of flush flow rate on effectiveness

The high flush flow rate resulted in an overall average increase of 19% of the test conduit surface area becoming cleaned after the flushing, compared to the low flush flow rate. Or if not taking the baseline straight conduit (#1) into account, high flow resulted in a 21% increase in cleaned surface area. The series of straight test conduits featuring a section with a cavity (#2-#5), showed an average 18% increase of conduit surface becoming clean. Qualitatively, in the case of the channels featuring parallel flow paths (#6-#9), in three out of four test conduits the high flush flow rate achieved **both** parallel flow paths to have been perfused with flushing liquid; where with the lower flush flow **none** of the conduits had shown proof of both parallel paths being perfused. Correspondingly quantitatively, test conduits #6-#9 flushed with the high flow rate on average showed a 24% increase in conduit surface being cleaned, compared to the low flow rate.

3-1-2 Total Organic Carbon (TOC) analysis

Table 3-1 shows the measured TOC contents of a selection of liquid samples taken from the test conduits, as described in Section 2-5-2. Despite the lengthy time allowed, and the ultrasonic excitation that the test conduits filled with sodium hydroxide (NaOH) were exposed to, and the deliberate agitation to facilitate proper mixing of insulin residues with NaOH, it was microscopically observed that a significant amount of insulin precipitates had not been dissolved by the NaOH at the time of capturing the post-flushing liquid samples. These remaining solid particles were filtered out before performing the TOC analysis on the samples. In some cases it proved difficult to capture all liquid volume from the test conduit, resulting in a high dilution factor when the liquid sample had to be supplemented to the TOC analyser's 30mL minimum sample volume.

3-1-3 Dissolving insulin precipitates with NaOH

The results of observing the effect of NaOH on the insulin precipitates in a non-flushing circumstance (following the protocol as described in Appendix D) is graphically represented in

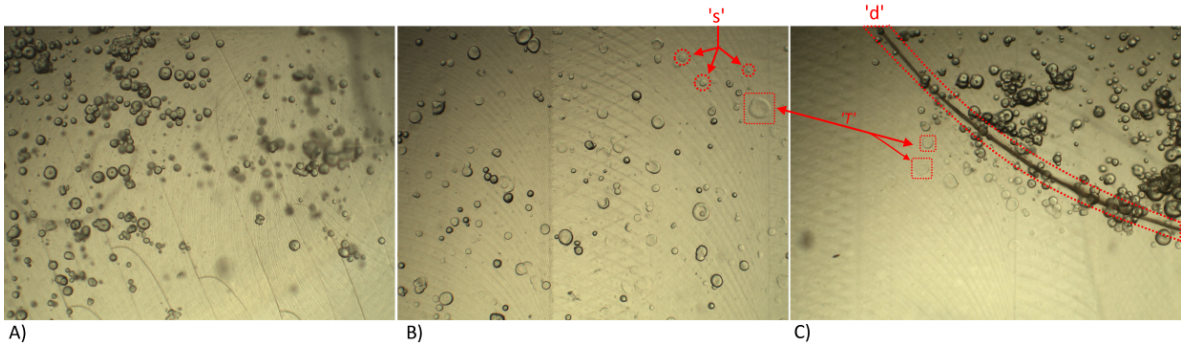

Figure 3-2: *A)* Microscopic image showing a section of a channel with low degree of insulin precipitate fouling, shortly after a small quantity of NaOH was introduced into the channel and (imperfectly) mixed. *B)* Image showing the various stages of dissolving of the insulin precipitates: some in their original state, some showing higher transparency (*'T'*), some having reduced in size significantly (*'s'*). *C)* Image showing a dark band that marks the approximate interface between the NaOH and the precipitates.

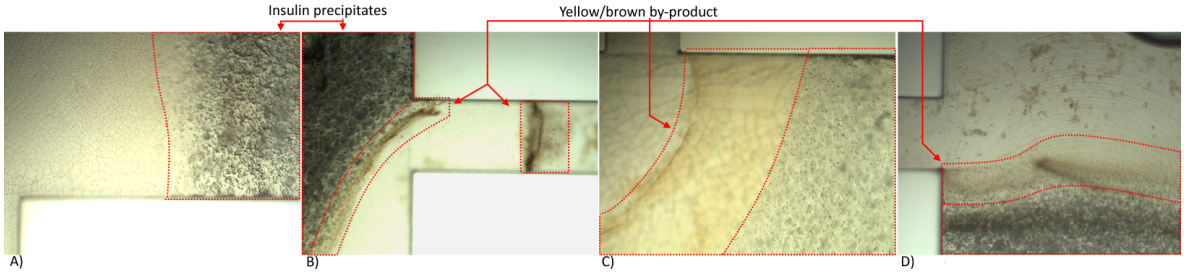

Figure 3-3: Collection of microscopic images of some of the observed constitutions of the unidentified yellow-brownish by-product which appeared upon flushing the insulin precipitates with NaOH and water. *A)* Interface between NaOH and insulin precipitates showing no brown material *B)* The material forming a thin layer *C)* The solid material having formed a thick band *D)* Chucks of the dislodged material flowing in the NaOH-flow.

Figure 3-2. The figure depicts insulin precipitate particulates sitting in a mixed solution of insulin and NaOH. The image of insulin mixed with NaOH shows insulin precipitate particulates with varying optical characteristics, that differ from their original pre-mixing state. Some precipitates particulates look alike the originals, but a substantial part shows decreasing size, and also a substantial part of the insulin particulates are observed to be significantly more transparent. Furthermore, at times a distinctly darkened band was observed. This darkened band in many cases coincided with the interface marking the transition from a region where NaOH was and insulin precipitates were visibly becoming smaller and more transparent, and regions where insulin precipitates could be observed to still remain unchanged. The dark band could be observed, within a time-span of several minutes, to slowly progress from the NaOH-side more toward the precipitate-side of the interface.

3-1-4 Byproduct of flushing insulin precipitates with NaOH

Throughout the flushing experiments the striking appearance was observed of a yellow/brown semi-transparent solid material, often appearing as a separating layer on the interface between NaOH and regions of insulin precipitate deposition. These layers were observed to often be

thin, and often to form a markedly sharp transition from clean region of a conduit to a region with significant amount of remaining insulin precipitate deposition. Figure 3-3 shows a collection of exemplary microscopic images of this material in various appearances. This brownish material was initially observed during the NaOH-flushing, but developed into a more profound presence during the subsequent water flushing. Also, chunks of this yellow-brown material were observed to sometimes be dislodged from the conduit and flow along with the flow of NaOH or rinsing water, and in this case stay in solid state. These brownish solid particulates also did not dissolve as quickly as the 'regular' insulin precipitate particulates (i.e. as generally observed prior to rinsing) were observed to be dissolved by NaOH like described in Section 3-1-3. The narrowest parts of the meandering cleaned paths as discussed in Section 3-1, often were lined with a distinct layer of this brown material.

This brownish material appeared in more regions of the conduits and in larger quantity in the case of the low flush flow rate, compared to the high flush flow rate. This was determined by comparing the bulk of the microscope images of the various channels, but was also apparent from the container in which all the flushing discharge liquid was caught. The discharge container for the low flow rate showed a significant amount of solids, see Figure 3-4, whereas the discharge container for the high flow rate flushing showed no such particles visible by the naked eye. Also, in comparing the small drill-holes that needle-end are fastened, it was observed that in 10 out of the 18 flushing runs, on the side where the flush flow entered the test conduit, more such material was observed than on the side where the flush flow exited the test conduit, see Figure 3-5.

3-2 Occurrence of conduit obstructions

This section describes the results of the experimental work in attempting to induce and prove the occurrence of insulin precipitation and establishment of the required insulin obstructions in the conduits.

3-2-1 Inducing insulin precipitation

Exposing the insulin to heat and sonication resulted in rather different outcomes than the insulin exposed to acid and overnight heating. The prior had a slurry-like optical appearance, with difficult to confirm possible flake-like crystalline formations. The acidified and incubated insulin showed spherical particulates of insulin precipitate. From a microscopic notion, the particulates were of a size varying between some 10 micron to 100 micron. In some cases there were precipitate constructs that looked like they might be several precipitate particulates coagulated in a clump with diameters of up to $\approx 500\mu m$. Clumps of such size are theoretically large enough to single-handedly clog the narrowest conduits (0.5mm) in whole. However, the actual structural integrity of these precipitate clumps may not be sufficient to stay intact under hydrostatic pressure and truly obstruct flow. From a slightly more macroscopic notion, it was observed that the vast majority of these precipitates were positioned on the bottom wall of the test conduit. It seems that when the test conduit assembly is left flat on its back most of the time, much of these precipitates settle on the bottom floor. Subsequently, higher concentrations of precipitate particulates were often identified on the side-walls of the conduits, and in yet lesser concentration on the top wall, i.e. the foil. Notably lower concentrations of precipitates were observed to be suspended 'floating' in mid-liquid. This suggests that the liquid in which the precipitates forms, is (or minimally *was* at some point

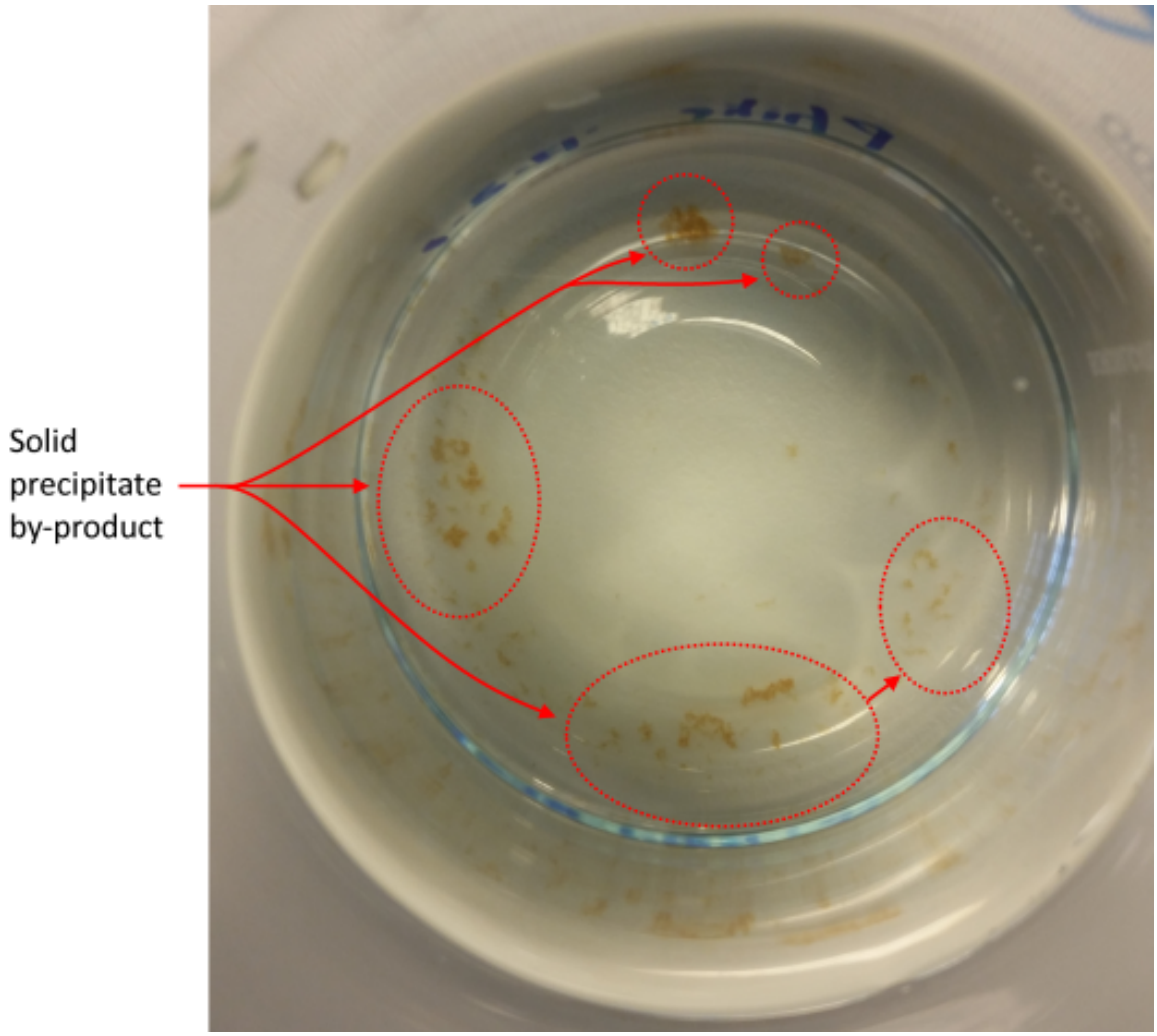

Figure 3-4: Top-view into the glass measuring cup in which the low flow rate flush liquid was collected (as shown in the left-hand side in Figure 2-2). Quite large accumulations of yellow-brownish material can be identified - marked in red.

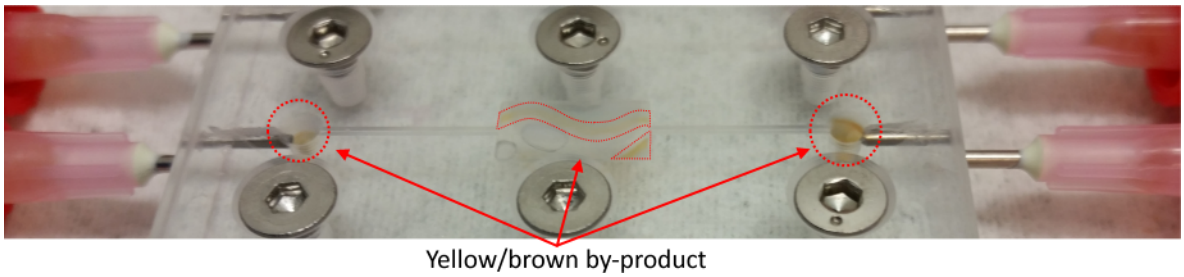

Figure 3-5: Microscopic images comparing of the bore between needle and start of the narrow sections of the conduits, which shows that the side where flow entered the conduit (right) showed a tendency for more remaining insulin and brownish material than the exit-side (left).

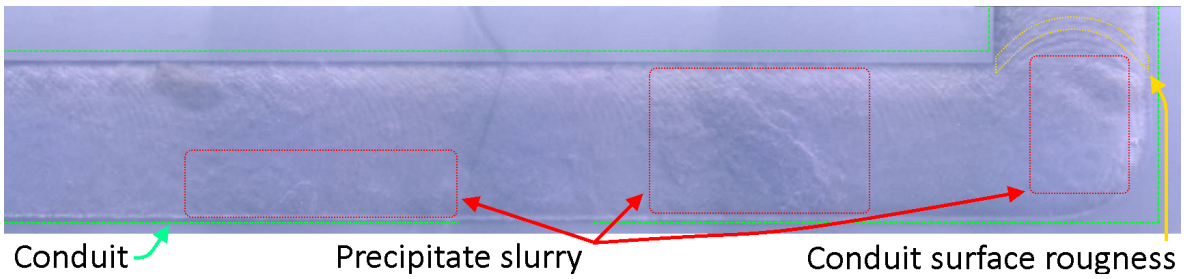

Figure 3-6: Microscopic image of the feeble insulin precipitate slurry observed in pilot experiments in which NovoRapid insulin was exposed to a temperature of 80 °Celsius and ultrasonic agitation for the duration of 1 hour.

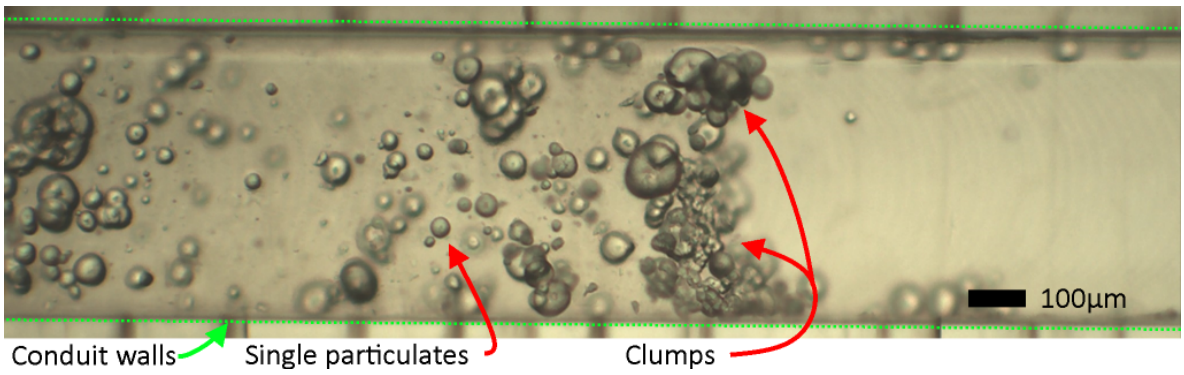

Figure 3-7: Microscopic image of the spherical precipitate particulates observed after exposing the insulin to acidification down to ≈ pH=1.5 and incubating at 60 degrees for 22 hours.

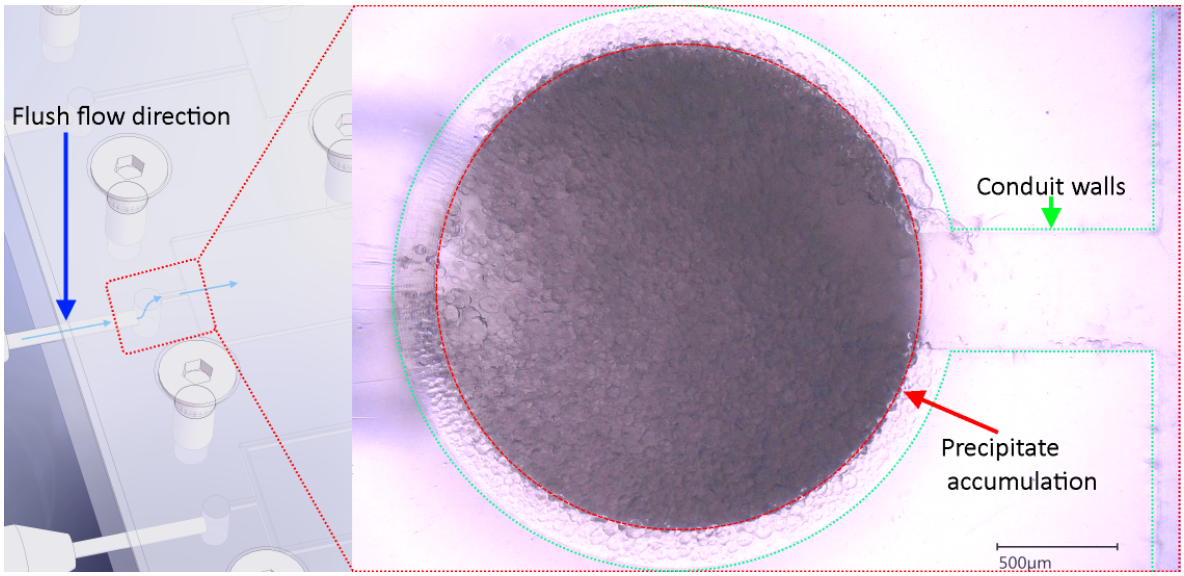

Figure 3-8: Keyence microscope image of a mass accumulation of insulin precipitates in the entry-section of conduit #9 after transferring pre-acidified and pre-incubated insulin into the channel. During infusing the insulin into this channel, a significant counter-pressure was manually felt, presumably resulting from an actual precipitate obstruction.

in time) in any case not so viscous that it prevented these precipitate solids from settling to the bottom.

3-2-2 Total conduit occlusion

None of the conduits in the main series of flushing experiments showed an obstruction in the interpretation of the word that fluid would no longer flow through the conduit. However, one single time when insulin which prior to introduction into the test conduit had been acidified and incubated in a cuvette and once precipitated subsequently been transferred into the conduits, it was very clearly noticed that a large accumulation of the precipitates occurred in the drilled-section directly between the needle & the narrow channel part. This space filled up entirely with tightly packed precipitate particulates and disallowed fluid flow to pass through. This resistance pressure unfortunately was not measured, only manually experienced. An image of the at that time observed bulk-accumulation of precipitates is depicted in Figure 3-8.

Chapter 4

Discussion and Recommendations

This chapter interprets experiment results, discusses the relevance and validity and draws the research conclusions. Recommendations for elaboration of the experiments are given, as well as for future research.

4-1 Discussion

4-1-1 Result Interpretation

Flushing effectiveness

The results of the flushing experiments under the conditions of differing conduit geometries and the two flush flow rates provide clear indications towards the answers of the Research Question, but also show much variability. A remarkable first observation is that all the narrowest (0.5mm width) conduits are entirely cleaned by flushing, as long as they are perfused. Flow velocity is maximum in these narrow conduits, and it is interpreted that the combined effect of the sodium hydroxide (NaOH)'s dissolution with the flow's shear force work effectively to clean the conduits, under both flow rates. It is also clear that all complex geometries indeed show more residual precipitates, than the baseline conduits do. Furthermore the increased flush flow rate results in an improvement of the flushing effectiveness of all conduits (other than the baseline conduits). There was much variability as to which subsections of the cavities in the conduits *are* cleaned successfully, and the shapes of these cleaned subsections. This variability is interpreted as being the result of the combined effect of NaOH's dissolving effect and of the flush flow. As was described in Section 3-2, the pre-flushing fouling by precipitates is locally inhomogeneous (in density of particulates and thus possibly also in viscosity). This in-homogeneity may have locally influenced the speed with which the NaOH flows through the viscous fluid, and also the speed with which NaOH dissolves those precipitates. Combined with the observed brown/yellowish byproduct, this is interpreted to have resulted in many different shapes of cleaned subsections of the widened sections of the conduits forming: sometimes narrow paths (e.g. conduit #5 at low flow, or conduit #8 at high flow), sometimes resulting in effective cleaning across the full width (e.g. conduit #9 at 20mL/min). For increasing 'complexity' of conduits, it is interesting to see that conduits #4 and #5 (widest and longest) show a remarkably higher percentage of residual precipitates than #2 and #3 do. Likewise, parallel conduits #7-#9 show higher residual precipitates than the baseline parallel conduit #6. On the other hand, conduits #8 and #9 show a much

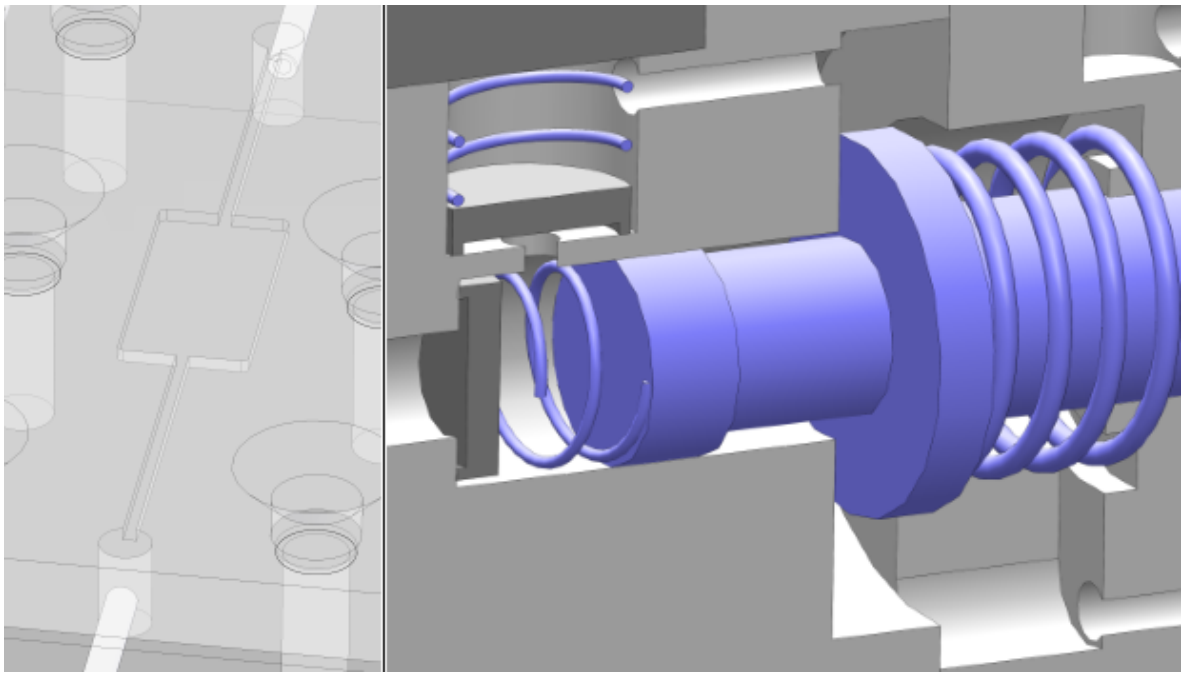

Figure 4-1: Example of the test conduit (L), which has geometric variations in only one direction, whereas the situation in the MiniMed 2007D (R) has three-dimensional variations.

wider cleaned subsection in the cavity at low flow rate, compared to the high flow rates. No conclusive correlation could be found between the width of a cavity and the degree to which such a cavity became clean. It *was* frequently observed that the outer corners of the widened sections did not become clean (e.g. #5 at low flow rate, or #9 at high flow rate). This suggests that abrupt widening and narrowing of conduits is not beneficial to flushing effectiveness, which corresponds to expectations based on microfluidics.
The fact that even the *higher* flush flow did not entirely clean the conduits, confirms the hypothesis that the *low* flush flow as employed clinically indeed may not be rigorous enough to ensure proper cleansing of the conduits of an implanted intra-peritoneal insulin infusion (i4)-device. In the MiniMed 2007D, however, some mechanical stirring may facilitate the flushing effect in ways that are not accounted for in this research. for example, the reservoir's deformation during filling and emptying reservoir, or the Maxon pump's piston movement may be effective in dislodging precipitates that in this 'static' test conduit remained undisturbed. Nevertheless, not all sections of the MiniMed's conduits have such a component of mechanical movement and thus may nonetheless be susceptible to the reduced flushing effect as observed. Also, this research made use of milled conduits of constant 0.5mm depth, and only feature a widening in one dimension. In the MiniMed 2007D, conduits exist that widen in two dimensions (e.g. see a graphical example in Figure 4-1), which significantly increases the total volume of presumably precipitate fouled regions, and correspondingly decreasing the likelihood of those regions becoming wholly clean.

TOC analysis

The results of the Total Organic Carbon (TOC) analysis are, unfortunately, deemed to only partially allow relevant interpretation. Firstly, with some samples only a limited sample

volume could be obtained, possibly resulting in the sample not being representative for the conduit's condition. The low sample volume also required high dilution factors, which may have influenced TOC accuracy. In any case TOC values show unexpectedly high variation. Especially the large discrepancy between the two measurement of what were supposed to be clean conduits are remarkable. It suggests that either the cleaning protocol was insufficiently ineffective, or that some source of contamination was introduced during handling of the liquid samples and performing TOC measurements. Results of the TOC measurement taken in the main flushing experiment, at least superficially confirm the specificity of TOC. The conduits that were microscopically observed to have become clean, show significantly lower TOC content than those conduits observed to have dirty post-flush. Unfortunately, TOC measurements are incomplete (not measured for all 9 geometries for both flow rates), which does not allow interpreting the flushing effectiveness based on TOC analysis. It is further most interesting to see that the TOC results of all samples from the 'clean', the 'insulin-rinsed', and from the 'no precipitates' are relatively close together, and lower than the TOC values for those conduits which *had* contained insulin precipitates. This, inconclusively, suggests that insulin that has *not yet* precipitated, is far less likely to remain in conduits, compared to insulin that *has* precipitated. It may have been of significant on TOC measurement results that precipitates had the opportunity to remain lodged not only in the conduit (the region of interest on the test-conduit assembly), but also in the drill holes and needles. This is deemed a contributor to TOC result disturbance.

4-1-2 Insulin

Insulin precipitates

Throughout the experiments, two different microscopically discernible alterations of the insulin compared to its 'maiden' condition were observed. The Novorapid (100IU/mL) insulin that was exposed to heating and ultrasonication formed microscopically barely discernible crystal flake-like solids, whereas the acidified and heat-treated insulin showed the origination of spherical solid coagulates. Given that these solid materials were not sampled and chemically analysed, it remains uncertain precisely what they were. One could even reasonably argue that the origin of these solids is not definitely confirmed. The various conditions to which the experimental material was repeatedly exposed to, might have resulted in some form of disintegration and creation of solid debris. Nonetheless, it is deemed highly likely that the solids observed in the insulin liquid after acidification and heat incubation are indeed insulin precipitates. This interpretation is based on several observations. Firstly, significantly more particulates were observed with use of the Insuman insulin (400IU/mL) than was observed using the Novorapid, under identical exposure conditions, which is in correspondence with observation in [6]. Secondly, the polymethyl methacrylate (PMMA), the polyvynil chloride (PVC)-foil, the needle tips, the pressure lines and the syringe material were all chosen to be inert with both NaOH and insulin. If, under the exposure conditions, some reaction did inadvertently occur between the hydrochlorid acid, and/or the NaOH and/or the employed materials, then it would be expected that one sees comparable degrees of fouling from that reaction product in both cases of using the same pH. However, as mentioned before, less particulates were observed in the case of pH$\approx$1 with lower insulin concentration than with higher insulin concentration. Continuing, the fact that the solid material readily dissolved upon contact of NaOH, is exactly the chemical reaction that was expected to occur between insulin and NaOH. Lastly and most significantly, the works of [16] show images of insulin

amyloid fybrils that are comparable to what was observed in the experiments performed for this research. The same work also reports that a pH>2 does not result in amyloid fibrils forming, as is confirmed in this experiment.

Inducing insulin precipitation by acidification and incubation is not what naturally occurs in the clinical situation - or at least is definitely not intended to. This elementary difference from the clinical situation is that in this choice of exposure, insulin structure is forcibly disrupted *in bulk* by the acid, whereas in the clinical situation this is hypothesized or described to occur (significantly slower) by means of inter-molecule forces or interaction with hydrophobic materials (titanium) of the implant. Precisely which 'final form' the precipitated insulin takes on can of course be hypothesized to have a significant influence on the likelihood of obstructions to form. Whether the precipitated condition of insulin as observed in the limited experiments in this research is even (remotely) similar to what might occur in an i4-device, is impossible to say based on this experiment only. Whether a few tenths of a mL of insulin in the conduits of an i4-device would also definitely cause as much precipitation under in vivo conditions, cannot be determined based on this research. It can at least minimally be stated that the two here observed insulin precipitate-conditions exist and they at least influence the liquid's viscosity. The fact that the here used Insuman insulin was already outdated before use, which may have had an certain altering effect on the precipitate characteristics. Given that in this experiment insulin was exposed to rigorously degenerative conditions, it is plausible that in the i4-device, much less precipitation would occur. However, as the MiniMed 2007D is scheduled to be flushed only once every nine months, equating to some 5 to 6 refills, this represents an estimated 75mL total of insulin passing through the device's conduits. If only a fraction of that volume undergoes enough degradation to form precipitates, over time as much insulin perfuses the device these solid particles can be hypothesized to flow along with the insulin and accumulate in regions where the conduits become narrower, or be settle in cavities where there is lower flow speed. These these precipitates might act as 'seeds' that expedite yet more insulin precipitation and grow larger, and they may not be cleaned effectively by the flushing as in correspondence with the observations described in Section 3-1.

The observed brown flushing by-product as described in Section 3-1-4 unfortunately has an unknown chemical composition. The material is deemed likely to be a reaction by-product of insulin and NaOH. This seems plausible, because the location of the brown by-product coincides with those darkened bands as observed in Figure 3-2 on the interface between the NaOH and the insulin precipitates. However, it should be kept in mind that the material might have originated from reactions between test conduit materials (like the needle's metal) and the hydrochloric acid; or from some (unidentified) pollutant introduced during experimenting. Such reaction products may have even been encapsulated by insulin precipitates, to form the observed brown material. Whether this brown by-product would form with any type of precipitated insulin, any concentration of insulin, and any concentration of NaOH, etc., has not been investigated here.
The yellow-brownish semi-solid layer appeared to separate the 'clean' NaOH flow from the regions where much precipitates were still present. This is identified as an aspect of potential strong influence on flushing effectiveness. The by-product layer seems to form a kind of barrier or sealant between NaOH and the yet-to-be-cleaned subsections and seems to prevent the NaOH from reaching the precipitates. If consecutive flushing procedures each leave only

a small amount of remaining insulin precipitates shielded by a thin film of this by-product, this might over time grow to a sort of layered, or even 'petrified' obstruction that is yet more difficult to clean.

Precipitate presence, or total occlusion. Presence of precipitates was readily observed. But the failure to measure an total occlusions (i.e. complete clogging, flow being entirely obstructed) in the test conduits may be due to a variety of factors. Of course it could be due to the fact that these occlusions are *not* the actual cause of the the MiniMed 2007D's reported failure to administer insulin. Perhaps those occurrences have an entirely different - as of yet unidentified - cause, but are wrongly accredited to insulin degradation in literature. It is however deemed more likely that the chosen methods of inducing insulin degradation simply did not cause enough influence on the insulin to actually form precipitates that accumulated enough to fully occlude fow paths. Also it may simply not be possible to speed up the degradation process that occurs in the Medtronic MiniMed 2007D in such an ex vivo environment and still achieve the same result.

The results of dissolving insulin precipitates using NaOH as described in Section 2-5-2 are interpreted as a confirmation of the dissolving power that NaOH has on the insulin precipitates. The optical characteristics of the insulin particulates after mixing with NaOH are interpreted to be effects of the precipitates in different stages of being dissolved by the NaOH. This result corresponds with expectations based on the prior-discussed literature describing the dissolution of insulin precipitates. However, the observations described in Section 3-1-2 also show the limitations of NaOH's dissolving power. It is hypothesized that the limited liquid volume of NaOH (e.g. 0.3 mL, the equivalent of the original volume of insulin in a test conduit) does not provide enough molecules to have all insulin precipitates wholly dissolve.

4-1-3 Functioning of the test conduits

Test conduit issues. This section briefly describes the most relevant points of discussion concerning the adequacy of the constructed test conduits in this experiment. A more detailed discussion is presented in Appendix B. Many of the type of conduit geometries encountered in the Medtronic MiniMed 2007D were not be recreated in the form of a test conduit for this experiment. Conduit geometries featuring moving components such as valves, plungers, or a bellow reservoir could not be produced within the limited time available for this thesis. The fact that the conduits' cross-sectional profile is square and not round as is mostly the case in the MiniMed 2007D, may - in accordance with Bertier et al. [9] - have resulted in yet lower flow velocity in the outer corners of the rectangular conduit. Also, despite the effort to ensure structured variation between the conduit geometries, the various designs are not all completely independent of each other in every aspect that might be influential. Examples include variation of the total volume held within each conduit and flow path length of each conduit. Test conduits unfortunately also exhibited several issues including leakage, damage to the PVC foil, unintended introduction of air bubbles into the conduits, disturbance to the pre-flushing condition resulting from capping and uncapping the conduits, and the the drilled holes on the conduit assembly forming a location for additional residual insulin to remain. The influence of these issues is more extensively discussed in Appendix B. A substantial part of the material that the insulin in this experiment came into contact with, is not actually part of the test conduit, but part of certain tubing, pumps, cuvettes etc. required to get the insulin

into and through the conduit. This introduces a variety of possible sources of disturbance to the absolute situation of interest in this research.

The wettability of titanium vs. PMMA was measured in a simple supporting experiment (see Appendix Section B-2), and insulin was found to have higher wettability on titanium than on PMMA. This can be interpreted to mean that in the case of small test conduits, insulin is more likely to wet the conduit-lining surfaces in titanium, than it would on PMMA. However, taking into account that in vivo all material surfaces are always intended to be fully wetted (as no gas is present in the conduits) this is deemed to be of limited relevance, and limited influence on the external validity of this research. Explicitly influential difference may lie in the fact that surface tension of insulin on PMMA is significantly different from titanium, which may result in different relative occurrences of the mechanisms of adsorption and insulin degradation. Titanium's higher wettability translates to a lower hydrophobicity than PMMA, which could contribute to an slower onset of denaturation in vivo than in this experimental setup. This may possibly expediting the occurrence of precipitation in the experimental setup using PMMA test conduits, relative to the in vivo case.

The surface roughness of PMMA may also be significantly different from that of the titanium used in the MiniMed 2007D reservoir, tubes or Maxon pump. Surface roughness provides a larger total surface available for insulin to adsorb to. Furthermore, roughness may contribute to the grip precipitates have on the surface, again possibly promoting precipitates to remain lodged in place. Also, surface roughness is cause for additional friction to flow, thus plausibly slowing down flow near the walls and shielding clumps of degraded insulin stuck to those walls from being removed.

4-2 Recommendations

Based on the observations and interpretations of the experiments described, a series of recommendations can be derived for further research and for the development of a novel i4-device.

4-2-1 Experiment improvements and elaboration

Test conduit production. The test conduits could better represent the clinical situation by using titanium, as in i4-devices, and avoiding other possibly influential materials such as PMMA. This could be realised by replacing the bottom of the conduits with a titanium slate rather than PMMA, while producing the covering slate in glass to allow microscopic observation. Instead of using a foil susceptible to damage and opacity, flexible (inert) rubber strips outlining the conduits could be used to waterproof the assembly. Instead of using screws to fixate the assembly, an external clamp-setup enclosing the assembly could be used which allows higher fixation forces to prevent conduit leakage. It might even be possible to fabricate the entire test conduit in glass, which would satisfy the demands for microscopic observation, inertness, and low surface roughness. Research into if the surface roughness of conduit materials in contact with insulin has an influence on the flushing effect is strongly recommended. In the future surface coatings (e.g. by anodization) could be investigated on influence flushing effectiveness.

Conduit geometries. More conduit geometries could be designed and tested. This research focused mostly on geometries mimicking the Maxon micropump. The body of knowledge

could be expanded by experiment on conduit geometries more closely mimicking the insulin reservoir. This reservoir has a bulky volume but has narrow grooves at the walls as a consequence of the metal bellows construction. Test conduits could be created that do not only have geometrical features in the flat (0.5mm depth) plane, but also in both radial directions. It is also recommended to look into effects of internal mechanical disturbances (deformations, piston movement, etc.) to induce more dislodging of settled precipitates and promote the mixing of precipitates with flushing liquids.

Flushing liquid volume. It is recommended that comparative research is performed into the influence of the total volume of flushing liquid that passes through the conduits. This experiment duplicated the use of a 15mL flushing volume, as is used in the clinical procedure. However, it is plausible that a flushing with a larger volume of NaOH may result in dissolution of more precipitates. An example is seen in Section 3-1-2, from which it may be deduced that the quantity of sodium hydroxide reaction ingredients simply is not sufficient to allow all insulin precipitates to be wholly dissolved.

Flow rates. This experiment applied only two different flow rates, but a broadened scope of flow rates may provide novel insights into ideal flush rates. A *lower* flow rate but with a corresponding longer time for NaOH to dissolve precipitates might even result in better dissolution effect and so unexpectedly result in better flushing effect. As described in Section 3-1-3, in these experiments it was observed that, in non-flushing circumstances, NaOH would only progress with dissolving through precipitates with less than a millimeter over a time-span of several minutes. Allowing for longer reaction times to wholly dissolve all insulin precipitates may result in more successful conduit cleansing. Also, methods could be devised to expedite the dissolving reaction in the clinical situation, e.g. increasing temperature or agitation, such as employed in this experimental setup. Furthermore, the effect of more complex flush flow characteristics could be investigated, e.g. intermittently activating and pausing flushing flow, or creating a reciprocating flow (intermittently flushing back and forth), and applying high enough flow velocity for turbulence to occur in the conduits (rates »20mL/min). It should be kept in mind that the application of higher flush flows in a novel i4-device may introduce certain design complications. In the MiniMed, flush flow is triggered by a pressure difference between the inlet port and the outlet port by creating a vacuum on the outlet port, i.e. *pulling* the flush liquid through. In this experiment, flushing liquid was *pushed* through the conduits by the syringe pump, which allows establishing higher pressure differences between inlet and outlet ports. However, in the in vivo situation, the vacuum at the outlet is required to prevent over-pressure at the inlet which could lead to leakage of fluid into the body.

Flushing liquids. The choice of flushing liquid could also be reconsidered. It is here hypothesized that it might be beneficial to the flushing effectiveness to make use of an *emulsion* containing (bioresorbible) microparticles that might act as an abrasive factor to dislodge insulin precipitates and better mix precipitates with NaOH to so promote dissolution. Naturally, higher concentrations than 0.1M NaOH or even chemical liquids other than NaOH could be investigated on effectiveness - naturally any medical risk these might pose to the i4-implant user must be considered.

Measurement techniques. In this experiment the microscopic observation provided most qualitative data, but unfortunately the results of the TOC measurements due to the practical complications and presumed contamination did not contribute to evaluating the flushing

effectiveness as planned. It is recommended that the protocol for TOC analysis is revisited to identify unintended sources of contamination and errors. The modes of data collection could also be expanded to include e.g. physico-chemical analysis methods to monitor the insulin condition throughout its various stages of degradation.

4-2-2 Follow-up research

Inducing insulin degradation and obstructions. More extensive research is recommended into which factors precisely cause insulin precipitates to occur, and how quickly this develops in an i4-like situation. Validity of experiment outcomes would increase if precipitates could be created in test conduits using less extreme conditions to induce degradation, e.g. lower temperatures, more neutral pH-values, or longer exposure times. Dedicated biochemical or pharmaceutical assessment techniques should be used to investigate the effect on insulin under structurally varied exposure to conditions of temperature, contaminants (e.g. acidification), agitation, and material interaction, and the duration of exposure to each of these factors are deemed important. Suggested techniques for precisely measuring insulin condition and precipitate presence includes: atomic force microscopy [19]; transmission electron microscopy; fluorescence microscopy [26], sub-visible particle identification of aggregate- and precipitate particulates; or the use of Thioflavin (ThT) assay to confirm presence of insulin amyloid fibrils [20]. If insulin simply shows a problematically high (e.g. seemingly spontaneous) tendency to occlude conduits, then improving insulin's chemical stability should be given priority. However, if further research confirms that small amounts of remaining degraded insulin cause expedited onset of insulin degradation in subsequent fillings, then increasing the success rate of flushing procedures should be given priority. he experimental method could also be adjusted to research how long-term (slow) perfusion of insulin through test conduits, as in the clinical case of a slowly pumping i4-implant contributes to formation of obstructions. The setup used in this research could be adjusted to facilitate the long-term perfusion of a test conduit. To limit the insulin consumption, a setup could be made which instigates a continuous recirculating perfusion in the conduit, instead of the reciprocating flow that was attempted in one pilot experiment in this research (see Appendix C).

Condition of insulin in clinical practice. An important diagnostic step that could clinically be performed to help identify the root cause of instances of failure to administer insulin from MiniMed 2007D devices, is to evaluate the condition of the residual insulin that is removed from the implant during refill or flush procedures. The relative presence of insulin in monomer, denatured, aggregated or precipitated state is a strong indication of which insulin degenerative occurrences are taking place. If this data is recorded over a long period of time and for a broad patient base, a correlation may be found between the (frequency of) occurrence of obstructions, thus providing pointers towards what mechanisms are most important in forming and removing obstructions.
The insulin solutions is exposed to rigorous manual shaking to de-gas it directly prior to insertion into the implant. It is recommendation to analyse the deteriorating influence this agitation may have on the insulin condition. A study could further be performed to compare degenerative influence of different de-gassing methods, for example centrifugal de-gassing or membrane de-gassing. Also, if this shaking to de-gas part of the procedure is maintained, it should be considered to mechanically perform this, instead of manually.

Brown by-products. Research into the origin and behavior of the insulin and NaOH reaction by-product is highly recommended. The chemical composition and environmental factors that influence its creation, and the degree to which this material shields precipitates from dissolution by NaOH should be researched. Using the test conduits from this experiment, simple follow-up experiments involving repeated steps of novel insulin insertion, inducing degradation, and flushing could be performed to verify if an explicitly problematic layered build-up of this material might occur, like proposed in Section 4-1-1. If the formation of the material could be recreated, and (a sufficient quantity of) the material captured, ThT-Flavin tests could be performed to identify presence of β-sheets to confirm insulin precipitates as a (partial) component [15]; or X-Ray Diffraction analysis, or a synchrotron could be used to identify the precise chemical bonds or structure.

NaOH residuals. Further research could also be focused on the effect of NaOH residuals in the implant's conduits after performing a flushing procedure. As this experiment shows, it cannot be assumed that all insulin precipitates are removed by the flushing flow. Likewise, in an ideal situation not even fouled by insulin precipitates, it should not be assumed that the flushing NaOH mixes perfectly with the insulin, and the buffer liquid, and therefore it is entirely possible that traces NaOH remain in the conduits upon filling it with a new insulin payload. This may expedite degradation of the new insulin payload.

Fluid dynamics. As the results describe, the viscosity of insulin was definitely increased after exposure to the acidification and incubation. This experiment lacks a precise rheologic quantification of the insulin solutions, which could be potentially very valuable in quantitatively modeling or computationally simulating the flow characteristics, and so the possible degree of mixing of the insulin-emulsion with NaOH and the resulting dissolution of the insulin precipitates.

Self-diagnostics As is also mentioned in [5], it is deemed very beneficial if novel i4-devices were outfitted with self-diagnostic methods to validate the correct functioning of critical components of the device. This may limit the time until detection of device malfunctioning, may limit the frequency and duration of implant maintenance interventions, and so limit both inconvenience and cost. Proposals for self-diagnostic methods are: flow meters to validate individual stroke volume, or average insulin flow; phisico-chemical sensors such as a built-in miniature turbidimeter to analyse the condition of the insulin solution held in the device (stable, denatured, in state of aggregation, or containing precipitates); sensors to determine exact positioning of moving components like the reservoir walls, the valves and plunger inside the Maxon pump to identify mechanical jamming.

i4 prototype evaluation Outcomes of this research provide general indications of how likely insulin is to cause obstructions in various conduit geometries and the tendency for insulin obstructions to be flushed away with rinsing currents. However, for the validation of a particular new i4 device, this research (or an adapted or improved version of it) would of course need to be performed anew using that actual device as a sample.

Relevance in other medical fields The leap toward applicability in comparable drug delivery implants (such as discussed in e.g. [5]) is logical. Results might even be transferred to the cleansing (decontamination, disinfection, sterilization) of operative instruments that feature cavities, conduits or lumens [27, 28].

Chapter 5

Conclusions

5-1 Occurrence of conduit obstructions

Following from the results and discussion concerning the artificial creation of obstructions in the test conduits, it can be concluded that total obstructions of conduits did not readily occur. The precipitation of insulin in reaction to exposure to insulin-degrading conditions was indeed measurable, but an obvious highly localized severe build-up of precipitates resulting in a total obstruction of the test conduits was not observed. From this it may be concluded that the situation of in vivo failures to administer insulin from implanted intra-peritoneal insulin infusion (i4)-devices purportedly resulting from insulin aggregation as reported in literature, remains yet to be recreated. The fact that insulin under the here-described (rather more extreme than in vivo) laboratory conditions, does not simply result in the expected total obstruction, is an indication for how reasonable it actually is to aim to achieve the creation of an i4 implant that is less susceptible to this problem. This outcome furthermore opens the road for further research into the precise occurrence of i4-device failures to administer insulin.

Insulin precipitation resulting from the acidification and heat incubation was readily observed in these experiments. Precipitates were observed as a large quantity of particulates suspended in liquid showing viscous properties. Here observed precipitates' visual characteristics correspond to those described in literature where a similar protocol was used to induce precipitation. The degree to which the here created precipitates corresponds to precipitates as may occur in the clinical situation of an implanted i4-device is unknown. The conditions to which insulin was exposed of expedite precipitation in any case are not representative of the in vivo circumstances. Given the large variability in which protein degradation may occur, it is in any case deemed plausible that the here created precipitates differ significantly from precipitates in the clinical situation. Regardless of correspondence of the precise constituency of the here resulting insulin precipitates, this research represents exploratory work in understanding the variables influencing flushing effectiveness. Further research in this direction is strongly recommended.

5-2 Effectiveness of flushing

The dissolving effect of sodium hydroxide (NaOH) on precipitates is confirmed as an effective measure to clean conduits that contain precipitate. However, this chemical effect is not by itself sufficient to dissolve all precipitates under these experimental conditions. Additionally, a

presumed by-product of the chemical reaction of NaOH with insulin precipitates, was observed to create a solid material layer that seemingly shields precipitates from the flushing flow and thus inhibits flushing effectiveness. Overall, precipitates indeed showed the tendency to resist being flushed from the device, so corresponding to the behavior of a viscous fluid showing when less shear force is induced closer to the conduit walls. All conduits with more complex geometries than straight tubes showed a lower effectiveness of the flushing. The high flow rate showed an averaged 19% decrease in test conduit surface area containing remaining precipitates. In those sections of the conduits featuring a cavity, the flushing experiment often resulted in a narrow path being effectively cleaned, whereas significant regions of the conduit still showed residual precipitates. Much variability is observed in the shapes of the cleaned regions, which could not be correlated specifically to geometrical characteristics. Remainder of this amount of precipitates is interpreted as a clear indication that the reported failures of i4-implants to deliver insulin may indeed be due to ineffective flushing procedures that result in accumulation of residual precipitates. Unfortunately, the limited and variable results of the Total Organic Carbon (TOC) analyses insufficiently allowed their use for interpretation of flushing effectiveness. Following from the results of the flushing experiment, this section comes to answer the two research questions:

- **Do conduits with more complex geometries than straight tubes negatively influence the effectiveness of flushing in i4 devices?**
- **Does the use of higher flush flow rates than currently clinically used, increase the effectiveness of flushing in i4 devices?**

Firstly, the considerably higher degree of residual fouling of complex conduit geometries after the experimental flushing provides a fundamental indication towards the answer to the first research question. Results strongly indicate that complex conduit geometries negatively influence the effectiveness of the flushing. This confirms the hypothesis as formulated based on the (micro)fluid dynamical laws governing flow characteristics in conduits. These results obtained in laboratory condition are expected to be transferrable to the in vivo, however this supposed validity must certainly be more extensively confirmed before extrapolation to clinical situations is justifiable.

Secondly, the fact that based on optical observation in eight out of nine conduits the higher flush flow rate resulted in a higher effectiveness of the flushing experiment, provides a fundamental indication toward the answer to the second research question: The use of higher flush flow rates increases the effectiveness of the flushing procedure. Again these results obtained in laboratory conditions are expected to be transferrable to the in vivo situation, however this supposed validity must certainly be confirmed before clinical application of i4-device flushing procedures with higher flow rates. Moreover, the difference between higher and lower flow rates was much higher than the difference in induced clearance. Hence, geometry seems to be a more dominant factor which can be manipulated in the clinical design.

5-3 Future i4-device development recommendations

Much continued and novel research are recommended to understand flushing effectiveness under influence of a.o. flow rate, total flushing liquid volume, flushing liquid chemical formulation, physical conditions inducing insulin degradation, and influence of yet more conduit geometries. The intention of the company IPaDiC is to develop a novel i4-device, which features an improved flushing effectiveness. Building directly from the preliminary answers to

the research questions formulated in this report, an answer to the main Research Question was formulated. The research question was:

How can the effectiveness of the flushing procedure of i4 devices be improved by adaptations to the design of conduit geometries and to the flushing procedure?

The answer to this research question is given in the form of the following two fundamental recommendations that are made for the development of future i4-devices. Firstly - and most pressingly given the observed strong influence caused by geometry - novel i4-devices are recommended to preferably make use of predominantly **simple, narrow, straight** conduits to facilitate the successful flushing of all conduits. Secondly, the device and flushing procedure are recommended to be designed to allow for a **significantly higher flush flow rate** than the currently clinically employed flow rate of 1.5mL/min. Although higher flow rates are more effective than lower ones we needed an increase of flow by a factor of 15 to obtain a increase in cleaning effectiveness of 19%. Hence, geometry is the most practical factor to optimize for optimizing cleaning effectiveness.

Appendix A

Insulin instability

A-1 Insulin in solution

The insulin monomer consists of two strings of amino acids, connected with one internal sulfide bridge and two cross-bridges. Insulin in solution exists in equilibrium its three 'native' forms: the monomer; or when in presence of zinc it can associate into its oligomers: the dimer, and the hexamer. The dynamical association equilibrium determines in which form insulin in solution predominantly exists. At high concentration, insulin hexamers predominate, at lower concentrations the amount of monomers and dimers increases.

The insulin monomer has some hydrophobic amino acid residues exposed outside and tends to associate into the dimer. Under certain conditions, three dimers associate to form a stable torus-shaped hexamer, in which both polar and non-polar residues are buried between the dimers.

During mixing of insulin at the apothecary, insulin crystals are solved to create the desired insulin concentration. Whether these crystals are the same as the crystals resulting from denaturation, aggregation and precipitation is yet unclear.

A-2 Instability

Three following main occurrences concerning instability exist:

- Physical instabilities:
  - Denaturation
  - Aggregation
  - Precipitation
- Chemical instabilities

These occurrences can cause drastic reduction of biological potency and obstruction of delivery routes, are suspected to cause elevated serum amyloid proteins, and could manifest diabetic amyloidosis, and trigger antigenic responses.

A-3 Denaturation

Denaturation refers to loss of conformational integrity through deformation of the protein's shape due to an external cause. Insulin has a protein string which can become unfolded, exposing the normally shielded insulin's inner components. Denaturation can be both reversible

and irreversible, depending on the (severity of the) cause. Causes for denaturation include (but may not be limited to):

1. Hydrodynamic shear forces, e.g. from flow through conduits, sonication, shaking, stirring, agitation or mechanical stress, stretching due to position in extensional liquid flow field.
2. Hydrophobic surface interactions (adsorption)
   - Hydrophobicity of materials is reported to be a major contributor to denaturation of insulin molecules. Adsorption occurs at:
     - Air-liquid interface
     - Solid-liquid interface
     - Oil-liquid interface
   - Especially monomers are susceptible to this, dimers and hexamers are hardly or not susceptible to adsorption denaturation. Dimers and hexamers actually have a shielding effect: by physically occupying the majority of hydrophobic material surface, monomers can no longer reach the surface and those undergo less denaturation. Increased insulin concentration is thought to contribute more dimers and hexamers, thus having shown higher stability. This is counter-intuitive for insulin, as normally higher concentration results in lower stability.
   - Surfactants (other solutes, like genapol) have higher affinity with the air or material surface and so can take on the same job of shielding monomers from the surface by entirely occupying it.
   - Adsorbed and denatured molecules may desorb from the surface back into the bulk. This occurs naturally, but is enhanced by external causes like solvent flow.
   - Surface roughness contributes to total available hydrophobic surface availability and so increases total occurrence of denaturation.
   - A composite parameter combining both surface wettability and roughness suggests that the ideal surface for slower nucleation should be hydrophilic and smooth.
3. Hydrophilic surfaces
   - Highly hydrophilic have been found to result in less denaturation at the interface.
   - Aggregation of insulin occurs on both hydrophilic and hydrophobic surfaces but with different nucleation rates
4. Electrostatic interactions.
   On certain surfaces adsorption of insulin occurs due to electrostatic interactions. Positively charged surface-molecules that attracts the insulin's negative charge (at specific pH).
5. Temperature
6. Pressure. Ambivalent. Increased pressure can either:
   - Protect insulin from aggregating (approx. 10kbar)
   - To some extent cause increase in aggregation (approx. 1kbar)
7. Presence of denaturizing agents (e.g. urea, acid, CO2, metal ions like Zn2+, Cu2+, Fe2+)

A-4 Aggregation (fibrillation)

An aggregate is when various molecules are held together by interacting forces between them. One cause for example is that hydrophilic regions of insulin molecules pull together. This

often is the result of denaturation of the insulin molecule having occurred. Aggregation occurs in two stages:

- First stage involves creation of the intermediate products ('amyloid skeletons')
  - Combination of small numbers of native (non-denatured, both monomer and oligomers) and denatured insulin molecules. Denatured insulin molecules interact (covalently, and non-covalently), to minimize total surface energy by shielding the exposed hydrophobic regions from the aqueous environment.
  - At least partially reversible, short-lived transients, often recombine.
  - The conditions in this first stage determine if the second stage will occur, and what the final product of the second stage will be.
- These intermediate aggregates may reach a critical size, causing them to become aggregation nuclei that now also interact with other insulin molecules. The time duration to form nuclei is called the lag time. This indicates a critical amount of protein has to accumulate on the surface before aggregation-promoting nuclei begin to form. The nuclei self-associate further and start elongating, laterally interacting with each other and forming irreversibly growing unbranched fibrils ('amyloid macrostructures'). From here on-forward amyloid formation takes flight. Hereafter the growth rate of insulin aggregates is largely independent of the nature of the surface. Some characteristic traits of the mature amyloid fibrils are the tendencies to bend, twist, and agglomerate. The rapid aggregate growth phase reaches a plateau when the soluble insulin pool is depleted. Upon further incubation, aggregates continue to detach from the surface. The driving mechanisms for this reaction are:
  - Further minimizing the exposure of the hydrophobic residues to aqueous environment
  - Saturating hydrogen bonding
  - Reaching an alternative non-native global free energy minimum
- There exists some alternative form of aggregates that is called 'amorphous aggregates'
- The presence of b-rich-form amyloid fibril leads to dense packing of fibril aggregates due to strong hydrophobic interactions between fibrils.

Contributors to speed of aggregation are:

- Insulin composition
  - Source (e.g. bovine, porcine, human). All undergo similar mechanism, but at different reaction speed
  - The relative existence of monomer/dimer/hexamer. More oligomers result in higher stability (!but! may delay onset-time of insulin action in the body) itemPresence of aggregation nuclei, or 'seeds'. The higher the concentration of seeds that is present, the quicker amyloid formation occurs.
  - Presence of other components (e.g. Zn, surfactants).
- Solvent flow (agitation)
  - Agitation is identified as a prime contributor to speed of aggregation, specifically in the presence of hydrophobic surfaces. Agitation only, with no hydrophobic surfaces present, is a far less drastic contributor to aggregation.
  - Mobility of air-liquid boundary causes much flow contribution, and formation of micro-bubbles contributes to huge increase in available hydrophobic surface. Similar occurrence with oil-liquid interface.
- Temperature

- Ionic strength
- pH

A-5 Precipitation (particulate formation)

Precipitation is the occurrence that an insulin molecule (or aggregate thereof) no longer can maintain being solved in the solvent, resulting in it precipitating from the solvent to become a suspended particle material, and the solution becoming a (turbid, cloudy) emulsion. The causes identified for precipitation are:

- Critically large size of aggregates no longer allow it's being soluble.
- Isoelectric precipitation
- Addition/removal of acid (H+, protons) cause a steep reduction in solubility over a very narrow pH-interval. This may occur under the influence of acid bodily environment or e.g. CO2 being solved, or presence of metal ions. This process can be overcome by stabilizing solution pH by using buffer, preventing CO2 diffusion into the insulin solution by an appropriate choice of catheter components (polyethylene lining in the MiniMed 2007D), and avoiding metal ion liberation from the pump materials.
- Being 'salted out': the addition of an excluded solute has caused the chemical potential of the protein to exceed that of the solid phase.

A-6 The case: in an implanted intra-peritoneal insulin infusion (i4) device

With implantable devices, all conditions are gathered to favour the aggregation of insulin molecules:

- Thermal exposure
- Long-term contact with (hydrophobic) metallic and synthetic surfaces
- Mechanical stress in the pump itself (high speed pump piston or valve motion (incurring estimated up to 10m/s flow velocity through a 0.1mm diameter tube))
- Stirring (agitation of reservoir or other bulk insulin content due to natural body movements)
- Possible interaction with CO2 which diffuses through catheter (suggested in articles), or bodily materials travelling from tip to device through the catheter (hypothesized)]
- Very remarkably also continuous presence (i.e. failure to rinse away) of aggregate nuclei (or 'seeds') that immediately cause renewed insulin amyloid fibril formation, entirely skipping the normally occurring lag-time

A-7 Sources

This overview was generated as result of a brief literature review, searching the SCOPUS scientific library using search-term (TITLE (insulin) AND TITLE (aggregat* OR precipit* OR denatur* OR instab*)) and following any relevant referenced literature [29, 30, 7, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45].

Appendix B

Test conduit evaluation

B-1 Test conduit production

Firstly it was attempted to produce the test conduits through the principle of lasercutting slates of transparent synthetic material, polymethyl methacrylate (PMMA) and glueing or welding several of these cuts slates together in a sandwich to have the cuts form an channel. The incentive was that this would allow for simple production and as such allow to update and improve conduit geometry throughout the experimenting steps based on preliminary results. The 4mm thick slates of PMMA were lasercut (BabyLion, Lion Lasers, Netherlands) to an outline handeleable size to serve as the 'bread' of the sandwhich , and a 0.5mm thin slate of PMMA was cut to the same outer dimension, but also with the designed conduit channel shapes cut out, to serve as the 'ham' on the sandwhich. The stack was attempted to be assembled using dedicated PMMA glue (EVONIK Acrifix 1R 0192), and through the use of dichloromethane (Sigma Aldrich Co. LLC), which serves as a solvent to PMMA and so welds the slabs together. This attempt was abandoned because the lasercutter was evaluated not to result in sufficiently flush cutting edges, and no matter the amount of precision care taken to perform this the glueing kept resulting in clogging of the channels and dichloromethane welding did not ensure complete bonding of the slates.

Secondly, it was attempted to 3D-print the conduits from a transparent resin. as 3D printing is exceptionally useful for printing hollow structures like these channels. Several attempts were made on a FormLabs Form 1+ stereolithography printer using a clear photoreactive methacrylate resin (FormLabs proprietary). Initial outcome seemed promising, but closer investigation showed flawed shape of the channel geometries, structural failure of the prints (parts breaking) and insufficient transparency of the material for qualitative optical observation, see also Figure B-1. Higher quality industrial printing services were outside of budget.

B-2 Wettability

A Kruss DSA100 Drop Shape Analyser was used to measure the shape of droplets of insulin placed on a slab of PMMA and on a slab of titanium (Ti_6Al_7Nb). Figure B-2 and Table B-1 show the result of the measurement.

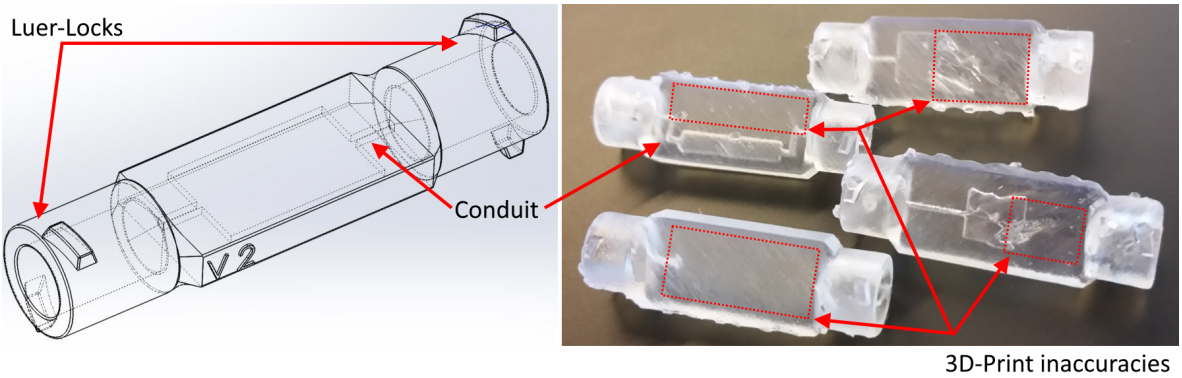

Figure B-1: (*L*) The design of a single stereolithography test conduit; and (*R*) the inadequate 3D-printed outcome.

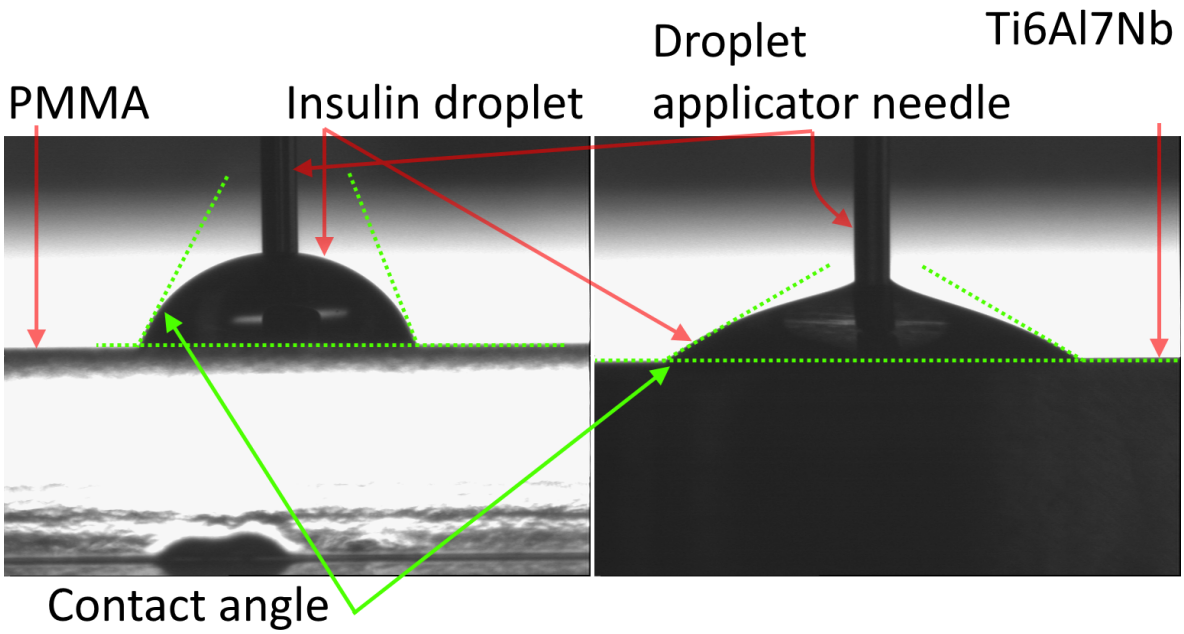

Figure B-2: Comparison of wettability of insulin on PMMA (*L*) and Titanium (*R*).

Table B-1: Measured contact angles of the insulin droplet on PMMA and Titanium.

	Ti_6Al_7Nb	PMMA
Contact angle	30.8 ±1.93	61.8 ±3.02

Table B-2: Leakage measurement

Channel	Flow rate	Pressure mmHg	kPa
1	0.1mL/hr	1000	
2	0.1mL/hr	1000	
3	0.1mL/hr	1000	
4	0.4mL/hr	1000	
5	1.2mL/hr	1000	
6	0.6mL/hr	1000	
7	0.25mL/hr	1000	
8	0.3mL/hr	1000	
9	20mL/hr	825	

B-3 Test conduit functional assessment

The outcome of the sample production satisfied the initial requirements up to a certain degree, but several problems or disadvantages were registered during the experimental work. These problems include leakage, difficulty of air bubbles in the channels, foil failures, and the disturbing influence of capping and uncapping the channels.

Leakage unfortunately occurred readily from the channels when increased pressure built up inside the channel. Apparently the multitude of screws fixating the PMMA slate assembly provided insufficient pressure to ensure a watertight seal. This was explicitly clear from the fact that more leakage occurred at the channels (#6-#9) where the screws are placed farther apart. At the highest pump output pressures or at high pressures caused by manually forcibly pressing a connected syringe, in some cases a trickling flow could be observed from the channel. The maximum pressure the conduit channels could withstand or the lowest measured liquid flows that resulted in no pressure-drop occurring are shown in Table B-2. It should be noted that 1000mmHg is deemed quite the significant pressure to expect to withstand. For example, in the MiniMed 2007D implant, such high pressures in any case would be unacceptable given the risk of insulin leakage occurring.

Also, damage to the polyvynil chloride (PVC) foil may have contributed to increased spreading of liquids in the capillary space between the PMMA plates. In any case, the damaged foil decreased the ability to completely rinse the test conduit assembly, especially the one which was damages by ultrasonification and could no longer be disassembled. This leakage was explicitly observable as insulin seems to light up milky/blueish after time, and with an oblique light source significant spreading of insulin could be seen, see Figures B-3 and B-4. Additionally, when red food-coloring was used, a neighboring channel showed appearance of coloration.

Air bubbles persisting inside the channels was a problem throughout the experimental work. The various actions required to perform on the conduits were all likely to introduce air into the conduit. The most difficult instance is the initial filling of the conduit. It required a steadfast hand, much care, and patience and practice to connect the syringes or fluid lines and initiate flow such that the liquid creep up, wetting all the walls of the conduits and take away the bubbles. Also when performing the connecting and disconnecting steps to initiate the various

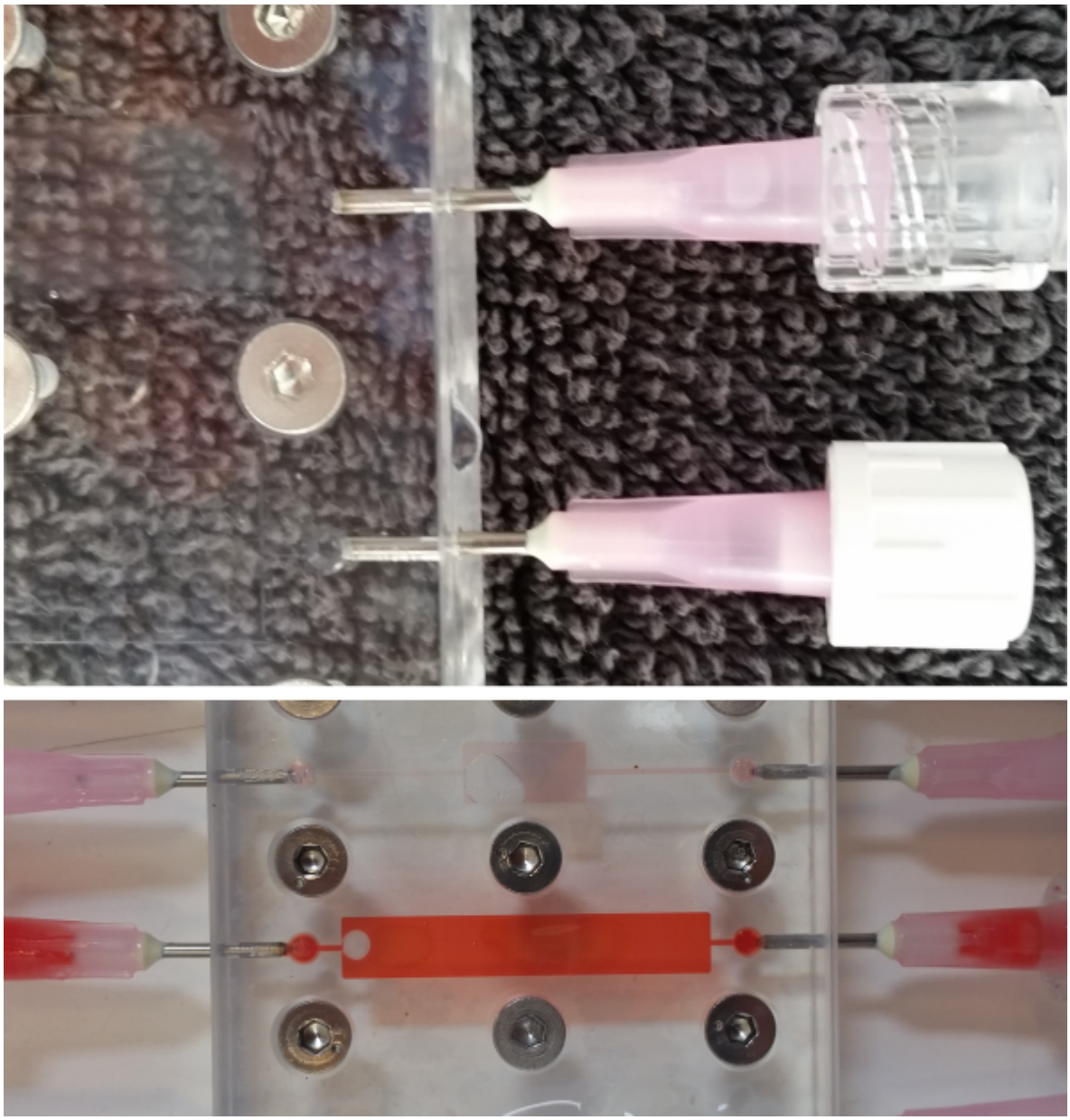

Figure B-3: Leakage from the channels into the capillary space or even other channels

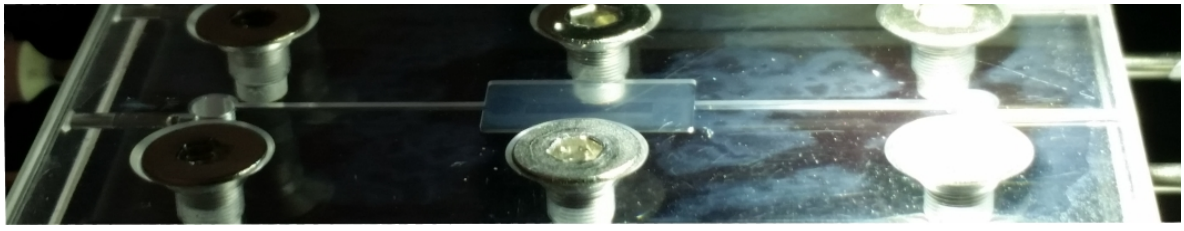

Figure B-4: Picture showing the milky/blueish haze of insulin that has leaked from the test-conduit into the capillary space between the two PMMA slabs and PVC foil.

fluid flows or take liquid samples, air bubbles easily were introduced into the channels. This could occur especially with the Alaris Pressure Lines and the volume in needle connecting opening. Once an air bubble is in the channel is it exceedingly tricky to get it out. In some cases soft rapping and tapping the conduit assembly would dislodge bubbles and cause them to willingly flow with the liquid. But in many cases it was clear that bubbles simply would not budge without rigorous handling. It often required manually instigated high flow speeds, sometimes back and forth, or much shaking. Precisely this rigorous handling was not always possible, as it would introduce influence on the pre-flushing precipitate-fouled condition of the insulin in the conduit. Because of the time-cost of preparing an experimental run anew when a bubble was identified in the channel, it was chosen to then continue experiments nonetheless. The presence of bubbles is a clear difference with the supposedly gas-less MiniMed conduits. It can be hypothesized that the presence of this air in the channels has resulted in unexpected exposure of insulin to yet more instigation of degradation (as described in Appendix A), and so limits the correspondence of insulin precipitates as made in experiment, compared to in vivo situations. Translating this to an actual implant, given imperfections of degassing the liquids introduced into the implant and the many dead-volume places in e.g. a Maxon pump, hypothetical gradual accumulation of air in the implant conduits might be significant and very difficult indeed to get rid of. Presence of air bubbles in the implant, as stated before, could contribute significantly to quicker onset of insulin aggregation. This may mean that methods to identify presence of bubbles in i4-implant conduits (e.g. low-energy ultrasonic activation and measurement and analysis of fluid response) and ability to perform high-speed bi-directional flushing flows could contribute to air-bubble removal.

Foil delamination and opacity both occurred seemingly specifically after exposing the filled channels to any form of heating. The PVC foil tended to delaminate from the top PMMA layer and 'hang' down into the channel's fluid space. Most predominantly specifically these sections of delaminated foil - but also other sections - tended to become less transparent over time and exposure. The precise cause hereof was not identified, but in any case it resulted in much difficulty in discerning structures in the channel below those opaque foil sections. Unfortunately because the 'floor' of the channels had a high surface roughenss from the production milling, observation from that side was not an alternative.

Capping disturbance occurred because as the Luer-caps are mounted on or dismounted from a channel's needle, the pin-section of the cap that is pressed into the needle causes quite a significant quantity of liquid displacement (column of ⌀4mm x 2mm height $\approx 3 \cdot 10^{-8} m^3$). Due to the small own volume of the channels ($1 \cdot 10^{-8} m^3$ up to $3 \cdot 10^{-7} m^3$), this capping-instigated fluid movement, causes a significant flow through the channel,as measured with the Alaris pump, pressure build-ups of up to 6kPa ($\approx$ 45mmHg). This flow unfortunately resulted in unwanted disturbance of the channel's condition for example directly after the exposure step: insulin was desired to have formed an obstruction, which susceptibility to disturbance by a experimentally varied flushing flow needed to be measured, but the capping caused a pre-flushing disturbance. It was in several cases observed that the capping-instigated fluid movement seemed to 'break open' a narrow path through the precipitated insulin slurry, and that later the flushing flow followed exactly *that* path. It can be postulated that whichever path through the precipitate (partial) occlusion was constructed by this uncapping disturbance would elsewise have been anyhow created upon engaging the flushing flow, as both flows will follow the path of least resistance anyhow, but this is deemed a relevant observation of a

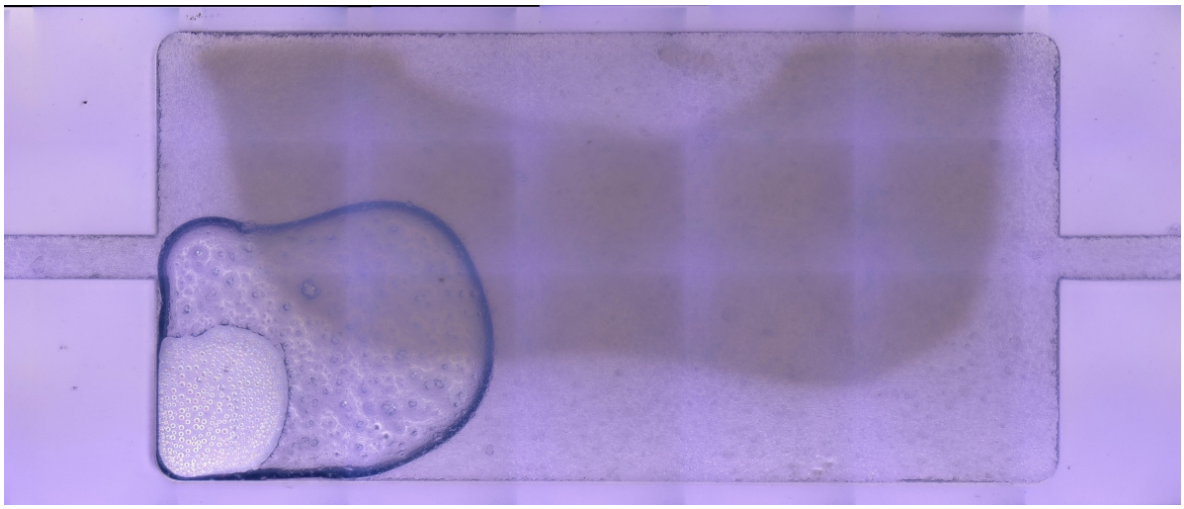

Figure B-5: Stitched microscopic image of a Chanenl #2 filled with insulin precipitates, showing a large section of PVC foil that has become less transparent.

possible noise contributor.

An additional location for precipitates to remain is formed by the entry and exit sections of the contuid assembly. The needles, and the drilled holes leading from the needles to the beginning of the 0.5mm conduit beginning all on various occasions observed to have residual insulin in them. This is expected most notably to have influenced outcome of the Total Organic Carbon (TOC) measurements. Because the TOC measurements however turned out not to be applicable in this experiment, the influence is deemed limited. This remains an issue that should be solved in possible future re-designs of test conduit.

Appendix C

Establishing the protocol to induce insulin precipitation

Various methods of inducing insulin precipitation were attempted, before the protocol as described in Section 2-3-2 was found successful, a variety of exposure conditions was attempted to induce precipitation. An overview of the different pilot exposure runs is provided in Figure C-1.

C-1 Exposure pilots

Substantial time was spent attempting to cause precipitation of insulin, before the final protocol using acidification was adopted. This Appendix describes the attempts which did not result in inducing precipitation that was readily microscopically observable and suitable for use in the experiment as intended.

C-1-1 Agitation: ultrasonic exposure

As follows from Appendix A-6, agitation contributes to the occurrence of aggregation in insulin. Because of the small cross-sectional channel diameter of the test conduits, it was expected that conventional sources of agitation like a shaking table or placing the conduit assemblies in a mixing machine, would not result in any actual turbulent-like flow patterns of the contained insulin. Therefor it was chosen to expose the insulin to ultrasonic pressure waves, by placing the entire conduit assembly into an ultrasonic bath (Shesto UT8031/EUK Ultrasonic Cleaner). From pilot experiments however it quickly turned out that extended ultrasonication of the conduit assembly caused overheating of the test conduits to occur, causing blackened burnt section to appear, and some plastic deformation to occur, hereby irreparably damaging one conduit assembly. This made it impossible to induce precipitation with ultrasonication while the insulin was *in* the test conduits.

As an alternative, insulin was placed in a reaction cuvette and then placed in the sonication bath for approx. 1 hour. This resulted in cloudiness and increased viscotity, visual with the naked eye. However, the thus precipitated insulin now needed to be inserted into the test conduit, but this unfortunately meant that the narrow channels of the needles and drilled holes leading to the test conduits caused the large precipitates to accumulate and get stuck, and this meant that insulin and the very precipitates that were sought for to cause an obstruction *in* the test conduits, were not actually arriving in the conduits.

Run	Insulin type	Conduit ('C')/ Reaction cuvette ('R')	Perfusion	Duration	Temperature	Ultrasound agitation	pH	Precipitates observed	Flushing
Pilot	U100	R	-	1u	45 – 50°C	Ja	-	No	-
	U100	C	-	15m	38 – 43 °C	Ja	-	No	-
	U100	C	-	8u 12u	65°C -15°C	Nee	-	No	1mL/min & 4mL/min
	U100	C	Yes	9 u	70 °C	Nee	-	No	1mL/min
	U100	R→C	-	1u	80 °C	Ja		Yes – feeble	1mL/min & 4mL/min
	U100 + *coloring*	R→C	Yes	1u	85 °C	Ja	-	Yes - feeble	-
	U100	R→C	Yes	12u	40 °C → 7°C	-	-	No	-
	U100	R→C	-	22u	60°C	-	≈2	Yes	≈ 1mL/min
Final	U100	C	-			-	≈3	No	1.5mL/min
	U400	C	-			-	≈2	Yes	1.5mL/min
	U400	C	-			-	≈2	Yes	20mL/min

Figure C-1: Overview of the attempted exposure conditions to induce insulin precipitation. The frames highlight which exposure combinations and intensities were differed per pilot run.

C-1-2 Temperature exposure

The test conduit containing insulin were also exposed to increased temperature. The test conduit filled with insulin was initially closed and placed in a heated bath, which was maintained at a constant temperature of 38^o Celsius. This temperature was chosen, to induce precipitation under conditions reasonably close to in vivo environment. Heating at this temperature did not result in formation of precipitates; and therefor higher temperatures were attempted: 45^o, 50^o, 65^o, 70^o, and 80^o, with exposure times up to 10 hours. In one pilot it was attempted to place the filled test conduits in high temperature for approx. an hour, and then into the fridge for approx. an hour and repeat this process many times. Finally, in one pilot experiment it was also attempted to freeze the insulin overnight. None of the above temperature exposure conditions (for completeness, *not* combined with prior acidification) resulted in microscopically visible precipitates being created. The temperature-exposure *along* can be presumed to have failed because denaturation probably had not yet occurred sufficiently, for the increased temperature to actually cause growth of denatured insulin molecules into aggregates or precipitates.

C-1-3 Conduit perfusion with insulin

In this experiment it was initially attempted to lock insulin inside the channels and cause such insulin degradation that it would cause obstructions to form. When this proved not to result in optically observable obstructions, it was attempted to cause a build-up of the expected insulin precipitates, by instigating an insulin perfusion through the test conduit with insulin, as is more close to the in vivo case of the MiniMed 2007D. To limit insulin consumption, it was chosen to instigate a reciprocating flow, which was achieved by connecting one infusion line to each of the conduit's outlets and connecting a syringe pump to each of the infusion lines. One pump was actuated to empty the syringe, and to perfuse the test conduit which was set up in the heated ultrasonicaiton bath, and to then fill the opposite syringe. When one syringe had been emptied, the opposite pump was engaged, reversing the flow. Due to

the fact that the exposure conditions (heating and mild sonication) at the time of perfusion were insufficient to induce precipitation, no precipitated formed and no accumulations thereof were formed either.

C-1-4 Coloring

In an attempt to facilitate visualization of the remaining insulin (which had not yet precipitated), it was attempted a few times to add a colorant (TRS Finest Food red Food coloring) to the insulin. This was attempted to enable identifying the distinction between the insulin and the flushing liquid. Unfortunately, the pilot exposure conditions performed with this colorant did not result in precipitates that were sufficiently distinguishable, and therefore no flushing was performed on the test conduits in this pilot experiment. the concept of Adding measurable markers (e.g. a colorant, but fluorescent markers should also be considered) may in future research effort contribute to identification of flushing effectiveness.

C-1-5 Acidification

The acificiation exposure condition was adopted from research in which insulin amyloid fibrils are used as functional components for MEMS-devices. The protocol was adopted. To approximate more closely the in vivo circumstances, it was attempted to apply lower acidification, i.e. not pH≈2, but pH≈3. That degree acidification with standard incubation of 22 hours at 60°Celsius however did *not* result in any precipitate formation. Therefore is was chosen to continue experiments with pH=2.

C-2 Choice of final exposure condition

Conditions were varied semi-structurally, attempting different combinations and intensity of exposure in anticipation of microscopically confirming success in inducing precipitates. Specific practical goals were considered to qualify an exposure method as applicable. Firstly it was aimed to induce precipitate formation while the insuline was *in* the conduit; not beforehand while in a reaction cuvette, because inserting the precipitated insulin into the conduit resulted in many precipitates being effectively filtered out by the narrow entrance into the conduit. Naturally, creation of insulin precipitates that were optically microscopically identifiable was key. The precipitates that were created using ultrasonic agitation, for example, were not deemed sufficiently identifiable. Additionally, the test conduits could not be exposed to ultrasonic agitation and therefor no precipitate formation could be induced while the insulin was in the conduit. Acidification and incubation resulted in precipitate formation that satisfied both above demands, and was therefore chosen as the protocol for the final experiments.

Appendix D

Experimental protocols

This Appendix contains, in that order, the experimental protocols for the main flushing experiment; and the protocols for the side-experiments in which the wettability of insulin on polymethyl methacrylate (PMMA) versus on Titanium was measured (see Section4-1-3; observing the dissolving effect of sodium hydroxide (NaOH) on insulin precipitates (see Section 3-1-3. The perfusion test involving a clean test conduit serving as a Total Organic Carbon (TOC)-baseline measuremet, as described in Section 2-5-2, follows the exact same steps as the **standard protocol**, except that steps in which insulin is *acidified* with HCl and the filled test conduits are *incubated* are **omitted**.

Protocol Step		Main Flushing Experiment Substep	Inventory of required materials / devices	#	Unit
1	Store insulin	Place vial in refrigerator, with identification label	Refrigerator to store insulin at 2°-8° Celsius	1	
2	Clean test conduit	Fill conduit with cleaning agent, check to ensure no air is present.	Ethyl alcohol	0.01	L
			3 ml Luer-Loc syringe	1	
		Place conduit sample (no caps) in box submerged in superfluous amount of cleaning liquid		1	
		Place the box in sonication bath; set and register time in sonication bath	Sonication bath	1	
		Remove conduit sample from sonication bath, empty out cleaning agent and rinse with ultrapurified - water			
		Cleanse optically needed sections with lens-cloth	Lens cloth	1	
3	Take liquid sample for **baseline TOC**	Choose quantity of solvent volume based on which conduit is being tested, register this volume on experiment form; fill the solvent-syringe and prime the syringe	NaOH	0.01	L
			3 ml Syringe with Luer-Loc	1	
		Connect syringe to conduit and hold syringe upright			
		Inject all the syringe's solvent into conduit. Take care not to cause overflow from opposite conduit opening. De-air by tapping conduit.			
		Close conduit's distal opening with a cap	Luer-caps to close conduit	2	
		Turn sample+syringe around so syringe is upside-down; remove syringe from conduit; and close conduit with cap			
		Place test conduit in sonication bath; set and register time in sonication bath.	Sonication bath	0	
			Timer	0	
		Remove test conduit from sonication bath, dry off	Tweezers / clippers / pincer to grab conduit	1	
			Towel or absorbant tissue	10	sheets
		Hold test conduit with exit-needle upright, remove cap and connect syringe	2 ml syringe with Luer Lock	1	
		Turn conduit sample upside down = syringe upright, remove opposite top cap, aspire syringe to pull solution from conduit. Allow for solvent to trickle down.			
		Transfer solution into cuvette, and close cuvette with cap.	Cuvette or reaction-tube with cap; 0.5 ml ?	3	
		Mark solvent sample for TOC with code, register code, place cuvette in cuvette holder	Permanent marker	0	
			TOC-sample cuvette holder	1	
		Safely discharge hazardous solute agent	Waste container 'Oxidizing liquid: NaOH'	1	
		Rinse cleaning solute from conduit two times; safely discharge hazardous solute	Rinsing liquid: ultra purified water	50	mL
		Fill conduit sample with UltraPurified-water, check that no bubbles are present, and close conduit Luer-connectors with caps.	Ultra purified water	10	mL
5	Optical observation of PRE-EXPOSURE cleanliness of test conduit; serves as **baseline <u>qualitative</u> measurement**	Transport conduit samples (filled with ultra-purified-water) to Biomaterials Lab	Ziploc	1	
			Conduit sample transport case	0	
		Place conduit sample under microscope			
		Optical observation; perform stitching algorithm.	Microscope	1	
		Code-name folders and files, register codes in Form, export images to USB / Ext. Hard Drive	USB / External Hard Drive	0	
	Precondition insulin to increase chance of occurrence of obstruction	Add 1M HCL solution to 1.5mL cuvette.	1M HCl solution, 2 ml syringe to aspire insulin from vial	0	
		Gently sway cuvette for 5 minutes to mix insulin and HCl		1	
		Discharge any remaining insulin in insulin-discharge container; discharge needle in sharps-container; discharge labware in CVE lab waste bin	**Waste** 'Cat III, halogeenarme org. vloeistof (insuline)'	1	
6		Aspire insulin from cuvette into insulin-syringe, return insulin vial to fridge; discharge needle into sharps-container	**Sharps** discharge container	0	

Protocol Step	Main Flushing Experiment Substep	Inventory of required materials / devices	#	Unit
	Remove cap from test conduit, hold upright, connect (new) empty syringe, turn sample+syringe so that syringe is upright, remove other cap from conduit and aspire syringe to remove Ultra-purified-water from conduit. Allow time for ultra-purified-water to trickle down.			
	Disconnect Ultra-Purified-water syringe and discharge fluid into sink			
Fill test conduit with insulin.	Connect insulin-syringe to conduit sample			
	Hold syringe upright together with the conduit sample, and inject insulin into the conduit sample until conduit is full.			
	Close off distal conduit with cap, and turn conduit + syringe upside down, then remove syringe and close off proximal conduit with cap	Luer-Loc caps	2	
	Discharge any remaining insulin in insulin-discharge container; discharge labware in CVE lab waste bin	**Waste** 'Cat III, halogeenarme org. vloeistof (insuline)'	0	
7 EXPOSE conduit samples containing insulin to conditions promoting	Place (insulin-filled) test conduit into oven.		0	
		Thermocouple + Datalogger	1	
	Set temperature at 60 Deg, and 22 hours, afterwards remove and clean.	Memmert Lab oven	0	
8 Optical observation of POST-EXPOSURE condition of conduit	Optical observation; perform stitching algorithm.	Microscope	0	
	Export images & code-name folders and files	External Hard Drive	0	
9	Prepare and prime flushing syringes: One 20 ml NaOH syringe and one 20 ml water-solution syringe;	20 ml BD Plastipak Luer-Loc syringes	2	
		Alaris Asena CC Pressure transducers	2	
		NaOH solution	40	mL
		Water	40	mL
	Place NaOH syringe in syringe-pump, connect the flushing Luer-tube, and engage syringe-pump until this tube is primed.			
	Set & register **flushing flow speed** and **flushing duration** based on the schedule for experimental condition	Alaris Asena infusiepomp	1	
	Choose and register **flushing flow total volume** based on which conduit is being tested, and fill the flushing syringe.			
	Hold entry-port of (insulin filled) conduit sample upright, and uncap.			
	Connect flushing Luer tube to conduit entry-port.			
	Turn conduit around so the conduit exit-port is upright, and uncap.			
Perform FLUSHING of conduit. Flushing is the experimental condition, and **flushing characteristics are the experimental variable**.	Connect the discharge Luer-tube to conduit-exit and lead discharge Luer-tube into **insulin** discharge container	Long Luer-Loc tubes	2	
	Engage syringe pump to start NaOH-flushing. Monitor flushing. Do not interrupt flow from discharge tube, as this will influence cause pressure variations and so influence flushing-condition.			
	Once syringe-pump has terminated the flusing procedure, hold conduit-sample with inlet upright; disconnect NaOH flushing Luer-tube; and close conduit inlet with cap.	Luer-Loc caps	1	
	Remove NaOH Syringe from pump, empty NaOH into chemical discharge container; dispose of NaOH-flushing tube	**NaOH** chemical discharge container	1	
	Use ample water to rinse NaOH from pressure-transducer equipped flushing tube	Ultra purified water	20	mL
	Place water-syringe in pump; place short Luer-Loc tube, engage pump to prime tube, and look-up, set & register water Total Volume and Flow speed			
	Hold entry-port of (NaOH filled) conduit sample upright, and uncap.			
	Connect flushing Luer tube to conduit entry-port.			
	Engage syringe pump to start water-flushing; monitor flushing progress and annotate remarkable observations and cancel in case of failure.			

	Protocol Step	Main Flushing Experiment Substep	Inventory of required materials / devices	#	Unit
		Once syringe-pump has terminated the flusing procedure, hold conduit-sample with inlet upright; disconnect water flushing Luer-tube; and close conduit inlet with cap.	Luer-Loc caps	1	
		Turn conduit sample around so that the conduit exit is upright, remove discharge Luer-tube and close conduit (filled with water) exit with cap.	Luer-Loc caps	1	
10	Optical observation of POST-FLUSHING condition of conduit sample (containing water); serves as qualitative		Conduit sample transport case	0	
		Optical observation of conduit	Microscope	0	
		Code-name folders and files, register codes in Form, export images to USB / Ext. Hard Drive			
		Backup Data	USB / Ext. Hard Drive & TU-PC	0	
11	DISSOLVE INSULIN REMNANTS in conduit with (**known volume**) solute	Hold water-discharge syringe upside-down, decap conduit sample exit, and connect syringe to conduit.	3 ml Luer-Loc syringe	1	
		Turn sample+syringe around so syringe is upright; and remove distal cap from conduit sample			
		Aspire syringe slowly (<1ml/min) to suck water from sample. Allow time for liquid to trickle down.			
		Disconnect syringe; lay conduit sample aside; and discharge water + insulin solution.			
		Choose quantity of solvent volume based on which conduit is being tested, register this volume on experiment form; fill the solvent-syringe and prime the syringe	NaOH	0.01	L
			3 ml Luer-Loc syringe	1	
		Connect syringe to sample and hold syringe upright			
		Inject all the syringe's solvent into conduit. Take care not to cause overflow from opposite conduit opening. De-air by tapping conduit.			
		Close conduit's distal opening with a cap	Luer-Loc caps to close sample	2	
		Turn sample+syringe around so syringe is upside-down; remove syringe from conduit; and close conduit with cap; check to verify there are no bubbles			
		Place conduit sample in sonication bath; set and register time in sonication bath.	Sonication bath	0	
		Remove conduit sample from sonication bath.			
12	CAPTURE this solution (=NaOH + dissolved insulin remnants).	Hold sample with exit-connector upright, and remove cap from conduit sample exit-connector.			
		Hold syringe upside down and connect syringe to sample exit connector; then turn conduit+syringe around so syringe is upright.	3 ml Luer-Loc syringe	1	
		Remove opposite conduit cap; and aspire syringe to pull solution from conduit. Allow time for solvent to trickle down.			
		Once empty, disconnect syringe from conduit sample.			
		Transfer solvent into cuvette for TOC-analysis; close glass container with cap.	Cuvette or reaction-tube with cap; 0.5 ml ?	3	
		Mark cuvette for TOC-analysis with code, and register code in Form	Permanent marker	0	

	Protocol Step	NaOH Dissolution Protocol Substep	Inventory of required materials / devices	#	Unit
1	Store insulin	Place vial in refrigerator, with identification label	Refrigerator to store insulin at 2°-8° Celsius	1	
2	Clean test conduit	Fill conduit with cleaning agent, check to ensure no air is present.	Ethyl alcohol, 3 ml Luer-Loc syringe	0.01	L
		Place conduit sample (no caps) in box submerged in superfluous amount of cleaning liquid		1	
		Place the box in sonication bath; set and register time in sonication bath	Sonication bath	1	
		Remove conduit sample from sonication bath, empty out cleaning agent and rinse with purified -water			
		Cleanse optically needed sections with lens-cloth	Lens cloth	1	
3	Optical observation of PRE-EXPOSURE cleanliness of test conduit; serves as **baseline qualitative measurement**	Transport conduit samples (filled with ultra-purified-water) to Biomaterials Lab	Ziploc	1	
		Place conduit sample under microscope			
		Optical observation; perform stitching algorithm.	Microscope	1	
		Code-name folders and files, register codes in Form, export images to USB / Ext. Hard Drive	USB / External Hard Drive	0	
	Precondition insulin to increase chance of occurrence of obstruction	Add 1M HCL solution to 1.5mL cuvette.	1M HCl solution, 2 ml syringe to aspire insulin from vial	0	
		Gently sway cuvette for 5 minutes to mix insulin and HCl		1	
		Discharge materials	**Waste** 'Cat III, halogeenarme org. vloeistof (insuline)'	1	
4	Fill test conduit with insulin.	Aspire insulin from cuvette into insulin-syringe, return insulin vial to fridge; discharge materials	**Sharps** discharge container	0	
		Remove cap from test conduit, hold upright, connect (new) empty syringe, turn sample+syringe so that syringe is upright, remove other cap from conduit and aspire syringe to remove Ultra-purified-water from conduit. Allow time for ultra-purified-water to trickle down.			
		Disconnect Ultra-Purified-water syringe and discharge fluid into sink			
		Connect insulin-syringe to conduit sample			
		Hold syringe upright together with the conduit sample, and inject insulin into the conduit conduit filled			
		Close off distal conduit with cap, and close off proximal conduit with cap	Luer-Loc caps	2	
		Discharge any remaining insulin in insulin-discharge container; discharge labware in CVE lab waste bin	**Waste** 'Cat III, halogeenarme org. vloeistof (insuline)'	0	
5	EXPOSE conduit samples containing insulin to conditions	Place (insulin-filled) test conduit into oven.	Thermocouple + Datalogger	0	
		Set temperature at 60 Deg, and 22 hours, afterwards remove and clean.	Memmert Lab oven	0	
6	Optical observation of POST-EXPOSURE condition of conduit	Optical observation; perform stitching algorithm.	Microscope	0	
		Export images & code-name folders and files	External Hard Drive	0	
7	Perform FLUSHING of conduit. Flushing is the experimental condition, and **flushing characteristics are the experimental variable**.	Prepare NaOH syringe	1mL ml BD Plastipak Luer-Loc syringes	2	
			NaOH solution	40	mL
		Hold entry-port of (insulin filled) conduit sample upright, and uncap.			
		Connect NaOH syringe to conduit entry-port.			
		Turn conduit around so the conduit exit-port is upright, and uncap.			
		Connect the discharge Luer-tube to conduit-exit and lead discharge Luer-tube into **insulin** discharge container	Long Luer-Loc tubes	2	
		Manually insert <0.1 mL into test conduit			
		Hold conduit-sample with inlet upright; disconnect NaOH flushing Luer-tube; and close conduit inlet with cap.	Luer-Loc caps	1	
7	Optical observation of POST-Dissolution condition of conduit		Conduit sample transport case	0	
		Optical observation of conduit	Microscope	0	
		Code-name folders and files, register codes in Form, export images to USB / Ext. Hard Drive			
		Backup Data	USB / Ext. Hard Drive & TU-PC	0	

Appendix E

Article

This Appendix additionally presents this thesis in the form of an article.

Rinsing of complex conduit geometries in implanted insulin infusion devices

Philip (J.A.F.) van Griethuijsen - *Thesis for the degree of MSc. in Biomedical Engineering*

Abstract
Obstructed implanted intra-peritoneal insulin infusion ('i4') require a flushing procedure to clean its conduits, which are unreliable in effectiveness. The goal of this research was to investigate how the flushing effectiveness can be increased by adapting conduit geometries and adapting the flushing flow rate. Transparent PMMA test conduits of differing geometries were produced to model the i4 device's conduits. These test conduits were filled with insulin, which was made to precipitate. Experimental flushing procedures were performed on these test conduits at 2 different flushing flow rates: 1.5mL/min, and at 20mL/min. The flushing effectiveness was evaluated microscopically to measure the quantity and location of residual precipitates. Conduits with complex geometries often showed that only a narrow path was cleaned, whereas significant regions of the conduit showed residual precipitates. The high flow rate showed an averaged 19% higher effectiveness. Results suggest that future i4-devices should be designed to avoid complex conduit geometries, and to be flushed with significantly higher flushing flow rates than currently clinically applied.

Supervisors: Dr. ir. Arjo (A.J.) Loeve; Prof. dr. Jenny (J.) Dankelman; dr. ir. Luigi (L.) Sasso; Prof. dr. Jos (J.A.E.) Spaan

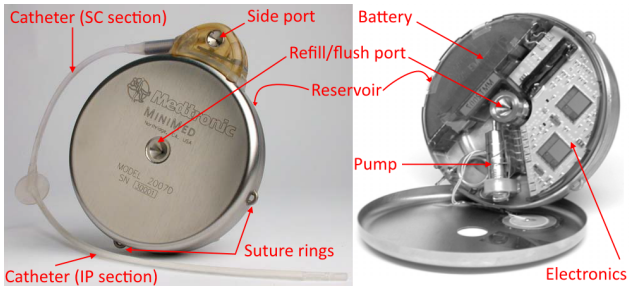

Figure 1. The MiniMed 2007D (Medtronic, USA) i4-device.

1. Introduction

Implanted intra-peritoneal insulin infusion ('i4') is a method of administering insulin into a diabetic patient's peritoneal cavity from an implanted device. This method of ambulatory insulin therapy is generally used by diabetics who cannot be treated effectively with subcutaneously infused insulin. Device users benefit immensely from the method, because without this method they would likely be hospital-bound due to the severe impact of their type of diabetes [1, 2, 3].

I4-devices are however faced with challenges. Insulin is a protein (or peptide) hormone, which is susceptible to degradation over time, in the form of denaturation, aggregation and precipitation. Insulin in i4-devices is exposed to hydrophobic material surfaces, body temperature, agitation, and shear forces due the pumps' moving parts. All of these factors are known contributors to insulin degradation. Moreover, literature indicates that the prior presence of a threshold amount of insulin aggregates ('seeds'), may increase up to tenfold the rate of insulin degradation [4]. Insulin precipitates are reported to remain in the implant's conduits, and accumulate to form obstructions that impede administration from the MiniMed 2007D (Medtronic,USA; see Figure 1), the only i4-device currently clinically used [3]. A clinical intervention, a flushing procedure, can be performed by physicians in an attempt to dissolve and remove the degraded insulin. It is performed preventively every 9 months, and additionally whenever obstructions occur. The device is first flushed with a NaOH solution ('lye') which dissolves insulin precipitates, and then with a insulin buffer-solution. However, this flushing procedure is (sufficiently) effective in only approx. 75% of cases of diagnosed obstructions [3], which leads to (costly and burdening) frequent re-occurrence of obstructions or even the need for the implant to be replaced.

The research question of this thesis is: **How can the effectiveness of the flushing procedure used in i4 devices be improved by adapting the design of conduit geometries and adapting the flushing procedure?** The design of the conduits of the MiniMed 2007D features differing geometries that are more complex than just straight tubes of constant diameter or cross-sectional surface (see also Figure 2), which are hypothesized to present spaces where precipitates are more likely to accumulate, resist removal by the flushing flow, and so bolster the formation of obstructions. The research sub-question is posed: **Do conduits with more complex geometries than straight tubes have a negative influence on flushing effectiveness?** During flushing procedures, NaOH is flushed through the device's conduits at a low flow rate of $\approx$ 1.5mL/min, which is hypothesized not to exert sufficient shear forces to dissolve, dislodge, and take away the obstructions. The second research sub-question is posed: **Can increased flush flow rates than currently clinically used with a corresponding increase in the shear forces exerted on the ac-**

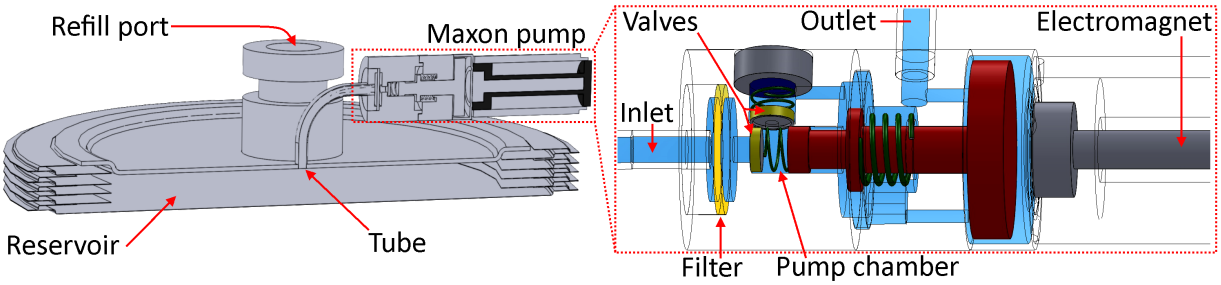

Figure 2. (L) Cross-sectional view of a MiniMed 2007D stripped of all but its entry port, reservoir, tubes and the Maxon Motors microfluidic pump. The bellows structure of the reservoir is clearly visible. (R) Cross-sectional view of the Maxon Motors microfluidic pump which is inside the MiniMed 2007D.

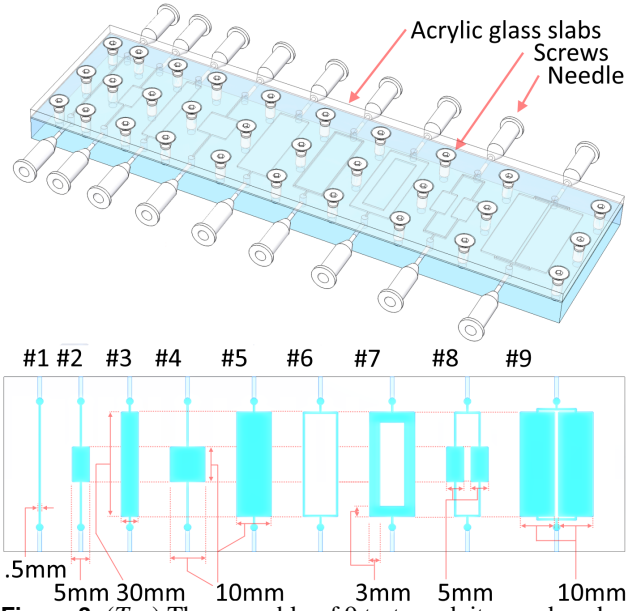

Figure 3. (*Top*) The assembly of 9 test conduits, produced from a block of transparent acrylic glass. (*Bottom*) The different conduit geometries.

cumulations of insulin precipitates, increase flushing effectiveness? The ultimate aim in this research effort is to contribute to developing an improved i4-device, the 'DiaLin' produced by IPaDiC B.V. (Deventer, the Netherlands), that exhibits higher flushing effectiveness.

2. Methods

2.1 Experimental setup

Test conduits Transparent test conduits were produced to serve as a simplified experimental ex vivo model of the i4-device to investigate the influence on flushing effectiveness (the dependent variable), both by differing conduit geometries and by different flush flow rates (the independent variables). Performing experimental flushings on **a series of differing test conduits geometries** is intended to independently measure their influence on the flushing effectiveness. The designs of the test conduit geometries mimic the conduits in the MiniMed 2007D device; Figure 2 shows the distinctive conduit geometries of the MiniMed 2007D. An assembly of test conduits was built that features 9 differing geometries, that were chosen to represent three main categories:

- **Straight (#1)** This simple straight conduit with a constant diameter of 0.5mm mimics the tubes in the MiniMed 2007D, e.g. those transporting insulin from reservoir into the pump. It serves as the baseline conduit of minimal complexity.
- **Cavities (#2-5)** These four comparable geometries mimic conduit cavities with larger hydraulic diameters and correspondingly larger volumes. Lengths and widths of the cavity are systematically varied.
- **Parallel branches (#6-9)** These four comparable geometries mimic parallel pathways. The different geometries of the parallel subsections mimic the geometries of the conduits featuring a cavity (#2-5).

The test conduit assemblies were produced by Meester Techniek B.V. (Leiden, Netherlands). The conduits geometries were milled as 0.5mm deep grooves into a 8mm thick polymethylmethacrylate (PMMA) slate, on which a thin sheet of flexible, transparent PVC foil was placed as the sealant, and on this a second PMMA slate of 3mm thickness to so create enclosed conduits. PMMA and PVC were chosen because of their resistance to the chemical solutions used in experimenting. The test conduits' entry and exit ports were outfitted with Luer-Locks to connect Luer-Lock equipped medical materials (e.g. tubing, syringes) for filling, emptying, and flushing the conduits.

Inducing insulin precipitation Under implanted conditions insulin does show degradation, but *may* stay stable for months, thus insulin precipitation in the conduits had to be induced within a workable shorter time-span. A protocol was adopted and adapted where insulin (Insuman Implantable 400IU/mL, Sanofi-Aventis, FR; the same highly concentrated insulin as used in the MiniMed 2007D) is first acidified to pH=2 using HCL to strongly promote insulin denaturation, and then inserted into the test conduits, which is incubated at 60 °Celsius for 22 hours (Memmert UN 30 oven) to expedite formation and growth of amyloid fibril aggregates into precipitates inside the conduits [5, 6, 7, 4, 8].

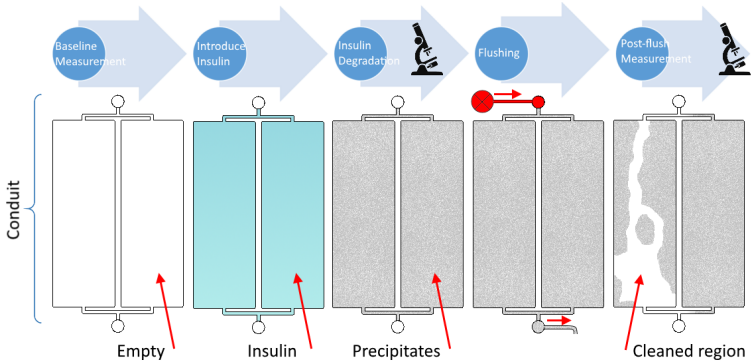

Figure 4. The experimental steps: Insulin is inserted into a clean test conduit, and precipitation is induced. The conduit is then flushed. Flushing effectiveness is measured by optical microscopy.

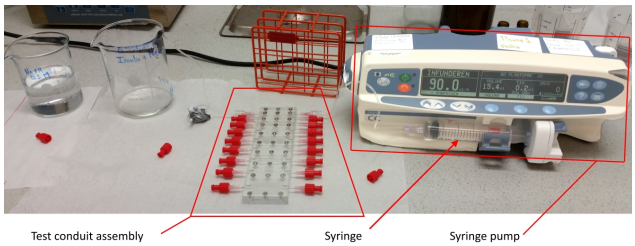

Figure 5. The experimental setup.

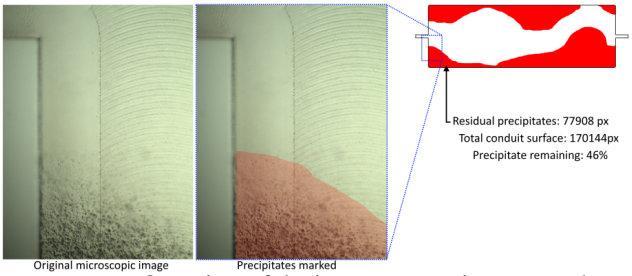

Figure 6. Overview of the image processing protocol.

Flushing condition Subsequently, the test conduits were flushed in an attempt to clear out the precipitates. In clinical practice, i4-devices are flushed with 15mL of flush liquid at a maximum rate of ≈ **1.5mL/min**. This *clinical maximum* flow rate was adopted as the *experimental minimum* flow rate. The second experimental flow rate was chosen at the significantly higher rate of **20mL/min**. This approach was intended to allow a direct comparison with the clinical procedure, and to achieve the maximum difference between flushing effectiveness compared to the lower flush rate. A medical drug infusion pump (Alaris Asena CC) [9] was used. Industrial-grade 0.1M NaOH solution (Sigma-Aldrich), and ultra-filtered water instead of the Insuman Implantable insulin buffer-solution for the second flushing instance, were used as flushing liquids.

2.2 Measurement and Analysis

Optical Microscopy Optical microscopic (Keyence VHX-5000 microscope) measurements were taken to identify and locate the presence of precipitates in the conduits. Images were taken of the pre-flushing and post-flushing conditions. The microscope was capable of stitching multiple images to create a concatenated field of view of the entire top view of all conduits.

Data analysis Each test conduit's overview image was manually analyzed to *qualitatively* identify in what regions precipitates had persisted. Images were analyzed on a scale of ≈0.005-0.01mm^2, and wherever a sufficient quantity of precipitates was observed to predominantly obscure the conduit's surface, this region was marked as 'containing residual precipitates' (see Figure 6). Subsequently, using PDN image processing software [10], a simple pixel-counting technique was employed to *quantitatively* approximate the percentage of the conduit's surface area containing residual insulin precipitates: $(= n_{dirty}/n_{tot.surface} \cdot 100)$. The experimental steps are presented in Figure 4, and the flushing setup in Figure 5.

3. Results

3.1 Flushing effectiveness

Conduit geometry The flushing results are presented in Figure 7. For both flushing conditions, the baseline straight channels (#1) *are* observed to have become optically entirely clean under both flushing flow rates, but the straight test conduits featuring a cavity (#2-5) were *not* entirely clean after flushing. Of those test conduits featuring parallel flow geometries (#6-9), *only* channel #6 (without a cavity) at high flow rate is observed to be entirely clean after flushing. Also concerning the parallel channels, in seven out of the eight flushing runs, *no* flushing flow perfusion was observed through *one* of the two parallel branches.

Apart from the baseline conduits (#1), only test conduit #6 (which *does* feature a parallel geometry, but *no* cavities in the parallel flow branches) exhibited one or both of the branches to have been flushed wholly clean.

In Figure 7, it can be observed quite markedly that in the case of conduit geometries featuring a cavity (which *was* perfused by the flushing flow), that the cleaned regions were shaped somewhat similar to a meandering riverbed. In some

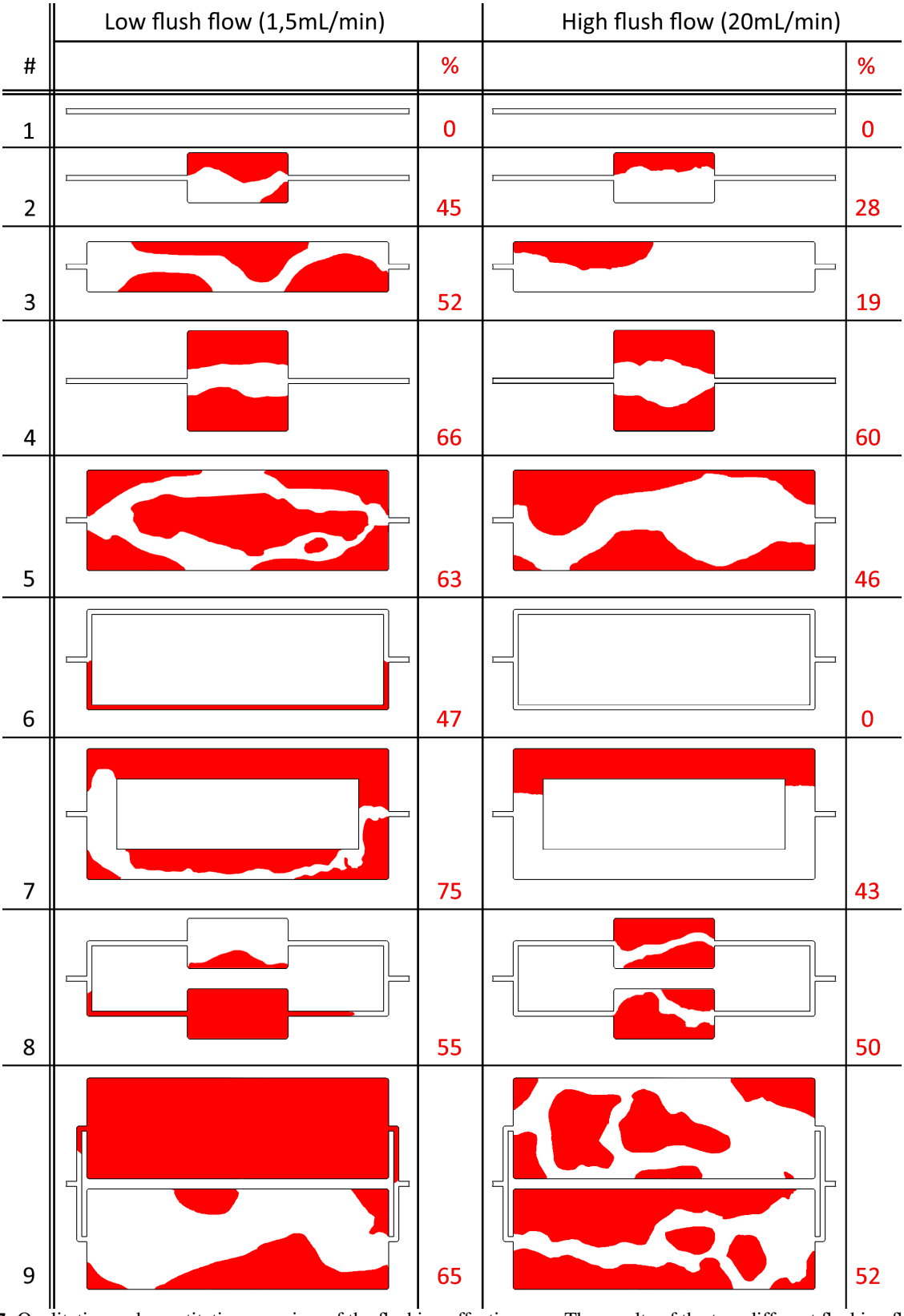

Figure 7. Qualitative and quantitative overview of the flushing effectiveness. The results of the two different flushing flow rates are compared. In red shows the residual insulin precipitate after the flushing. Percentages of conduit top-view surface containing residual precipitates.

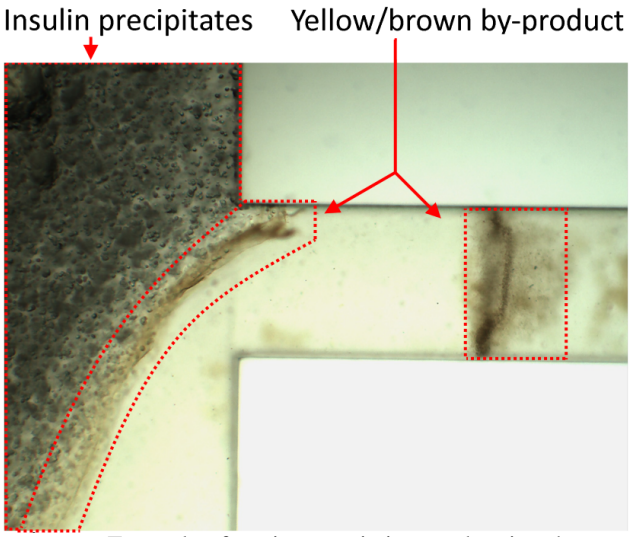

Figure 8. Example of a microscopic image showing the yellow-brownish by-product

cases this meandering path is quite narrow ($\approx 1mm$), where in other cases this path spans the majority of the cavity's width.

Flow rate The high flushing flow rate resulted in an overall average increase of 19% of the test conduit surface area becoming cleaned after the flushing, compared to the low flush flow rate. The series of straight test conduits featuring a cavity (#2-5), showed an average 18% increase of conduit surface becoming clean. Qualitatively, in the case of the channels featuring parallel flow paths (#6-9), in three out of four test conduits the high flushing flow rate achieved *both* parallel flow paths to have been perfused with flushing liquid; whereas with the lower flushing flow *none* of the conduits had shown proof of both parallel paths being perfused. The parallel conduits geometries (#6-9) flushed with the high flow rate showed on average a 24% increase in conduit surface being cleaned, compared to the low flow rate.

3.2 Insulin degradation

Microscopic observation showed the acidified and incubated insulin as spherical precipitate particulates, of a size varying between some 10 micron to 100 micron. In some cases there were precipitate constructs that looked like they might be several precipitate particulates agglomerated in a clump with diameters of up to $\approx 500\mu m$. Clumps of such size are *theoretically* large enough to single-handedly clog the narrowest conduits (0.5mm) in whole. However, these clumps may not have sufficient structural integrity to stay intact under hydrostatic pressure to truly obstruct flow. None of the conduits in the main series of flushing experiments showed an obstruction in the interpretation of the word that fluid would no longer flow through the conduit. A striking side-observation was that of a yellow/brown semi-transparent solid material, often appearing as a separating layer on the interface between NaOH, and regions of insulin precipitate deposition, see Figure 8.

4. Discussion

Flushing effectiveness The flushing results show much variability, but do provide clear indications towards the answers of the Research Questions. A remarkable first observation is that all the narrowest (0.5mm width) conduits are entirely cleaned by flushing, as long as they are perfused. Flow velocity is maximum in these narrow conduits, and it is interpreted that the combined effect of the NaOH's dissolution with the flow's shear force work effectively to clean the conduits. It is also clear that all complex geometries indeed show more residual precipitates, than the baseline conduits do. Furthermore the increased flush flow rate results in an improvement of the flushing effectiveness of all conduits (other than the baseline conduits). There was much variability as to which regions of the cavities in the conduits *are* cleaned successfully, and the shapes of these cleaned regions. The pre-flushing inhomogeneity precipitate presence (in density of particulates and thus possibly also in viscosity) may have locally influenced the speed with which the NaOH flows through the viscous fluid, and the speed with which NaOH dissolves those precipitates. This is interpreted to have resulted in the many different shapes of cleaned regions in the cavities.

For 'increasing complexity' of conduits, conduits #4 and #5 (widest and longest) show a remarkably higher percentage of residual precipitates than #2&3 do. Likewise, parallel conduits #7-9 show higher residual precipitates than the baseline parallel conduit #6. On the other hand, conduits #8&9 show a much *wider* cleaned subsection in the cavity at low flow rate, compared to the high flow rates. No conclusive correlation could be found between the width of a cavity and the degree to which such a cavity became clean. It *was* frequently observed that the outer corners of the widened sections did not become clean, suggests that abrupt widening and narrowing of conduits is not beneficial to flushing effectiveness, which corresponds to expectations based on microfluidics.
The fact that even the *higher* flush flow did not entirely clean the conduits, confirms the hypothesis that the *low* flush flow as employed clinically may not be rigorous enough to ensure proper cleansing of the conduits of an i4-device. In the MiniMed 2007D, however, some of the mechanically moving components may facilitate the flushing effectiveness by dislodging precipitates.

Insulin precipitates The 'final form' precipitated insulin takes on in clinical practice can be hypothesized to have a significant influence on the likelihood of obstructions to form, and be flushed away. Whether the precipitated condition of insulin as observed in these experiments is similar to what might occur in an i4-device, is impossible to say based on this experiment only. Given that insulin here was rigorously acidified and incubated, which is not what naturally occurs in in vivo at all, it is plausible that in the i4-device, much less precipitation would occur. However, in the 9 months period in between scheduled MiniMed 2007D flushing, ≈75mL insulin passes through the device's conduits. If only a *fraction* of

that volume degrades to form precipitates, this may over time settle and accumulate, acting as 'seeds' to expedite yet more insulin precipitation and cause obstructions.

The yellow-brownish semi-solid layer appeared to form a barrier separating the 'clean' NaOH flow from the regions where much precipitates were still present, which seems to prevent the NaOH reaching the precipitates. This is identified as an aspect of potential strong influence on flushing effectiveness. Consecutive ineffective may thus result in the formation of, possibly yet more difficult to clean, layered residue.

5. Recommendations

Experiments could be performed on yet more conduit geometries, e.g. with geometric variations in 3 dimensions, instead of only the flat plane. This would yet better represent the situation in i4-devices. It is also recommended to look into effects of internal mechanical disturbances (deformations, piston movement, etc.) to induce more dislodging of settled precipitates and promote the mixing of precipitates with flushing liquids. This experiment applied only two different flow rates, but a broadened scope of flow rates may provide novel insights into ideal flush rates. Furthermore, the effect of more complex flush flow characteristics could be investigated, e.g. reciprocating flow. More extensive research is recommended into which factors precisely cause insulin precipitates in an i4-like situation. Validity of experiment outcomes would increase if precipitates could be created in test conduits using less extreme conditions to induce degradation, e.g. lower temperatures, or pH-neutral.

6. Conclusions

Precipitates were observed as a large quantity of particulates suspended in liquid showing viscous properties. The conditions to which insulin was exposed to expedite precipitation in any case are not representative of the in vivo circumstances. Given the large variability in which protein degradation may occur, it is in any case deemed plausible that the here created precipitates differ significantly from precipitates in the clinical situation.

The dissolving effect of NaOH on precipitates is confirmed as a functional means to clean conduits that contain precipitate, this effect is not by itself sufficient to dissolve all precipitates under these experimental conditions. The observed large quantity persistent precipitates is interpreted as a clear indication that the reported failures of i4-implants to deliver insulin may indeed be due to ineffective flushing procedures that result in accumulation of residual precipitates. In answer to the research sub-questions: Firstly, the considerably higher degree of residual fouling of complex conduit geometries after the experimental flushing strongly indicates that **complex conduit geometries do indeed negatively influence the effectiveness of the flushing**. Secondly, the fact in eight out of nine conduits the higher flushing flow rate resulted in a higher effectiveness of the flushing experiment, strongly indicates that **the use of higher flushing flow rates does indeed increase the effectiveness of the flushing procedure.**

In answer to the main Research Question: **The effectiveness of the flushing procedure of i4 devices may be improved by adapting conduits design to feature predominantly *simple, narrow, straight* geometries, and by employing a *significantly higher flushing flow rate* than the currently employed flow rate of 1.5mL/min.** These results obtained in laboratory conditions are expected to be transferrable to the in vivo situation, however this supposed validity must be more extensively confirmed before clinical application in i4-device is advised.

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