

Copyright 2010 Michael Richard Thorson

MICROFLUIDIC PLATFORMS FOR SCREENING OF CRYSTALLIZATION  
CONDITIONS OF ACTIVE PHARMACEUTICAL INGREDIENTS

BY

MICHAEL RICHARD THORSON

THESIS

Submitted in partial fulfillment of the requirements  
for the degree of Master of Science in Chemical Engineering  
in the Graduate College of the  
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

Advisers:

Professor Charles F. Zuksocki  
Associate Professor Paul J. A. Kenis

## Abstract

The loss of candidate drugs (CD) due to poor physiological properties or incompatibilities with a patients (more commonly referred to as attrition) in the pharmaceutical research and development (R&D) process limits throughput, hinders productivity and results in large overhead costs. Much of the attrition in the pharmaceutical industry is due to poor physiological properties. As a result, these properties need to be identified as early as possible in the R&D process. The initial step in screening for these properties is identifying (1) all the stable forms of a CD and (2) the conditions resulting in nucleation of these solid forms. Highly complex robotic systems have been developed to increase screening efficiency. These robotic systems enable researchers to work on a scale infeasible by hand; however, the number of conditions screened is still limited by the volumes required (~100  $\mu$ l per well), thus the amount of material required, and the limited number of wells per device. Using a microfluidic approach allows for earlier screening due to the reduced volumes required. The development of a microfluidic platform to screen for crystallization conditions of CDs is presented. The chip using as little as 50 nl per well on a 96 well chip. The reduced volume and improved control over fluid handling in our microfluidic platforms allow for more extensive screening before production has been scaled up. The microfluidic platform studied in this thesis utilizes Free Interface Diffusion, Temperature control and Evaporation to control the supersaturation of CDs and therefore induce nucleation. Operation and potential has been demonstrated with Acetaminophen screens on-chip. Successful chip operation is demonstrated in all three modes using a common Active Pharmaceutical Ingredient (API), acetaminophen. Additionally, the chips have been

modified to accommodate analysis by Raman spectroscopy for crystal and polymorph identification.

Incompatibility of PDMS with a wide range of organic solvents has limited the analysis on-chip. To overcome this challenge, more resistant microfluidic platforms are needed for example using glass instead of PDMS as the main material for chip fabrication. We are in the process of developing a glass-based microfluidic platform to reduce the amount of absorbent material.

*To my Wife  
For Her Love and Support*

## **Acknowledgements**

This work is possible because people supported me, helped me and provided me with direction when I needed it throughout my masters. First, I would like to thank my wife, Candi Thorson, for being supportive of me and making my time in Illinois enjoyable.

I am grateful for such a supportive adviser and friend, Paul Kenis, who has provided direction and instruction. He has made working as a graduate student productive in a friendly atmosphere. I appreciate the insight, direction and support provided by my adviser, Charles F. Zukoski. His positive attitude towards research has made this work enjoyable. This research project has been made possible through funding from Abbott laboratories. The continuous support and input by Geoff GZ Zhang and Yuchuan Gong from Abbott laboratories has greatly helped me to stay focused and make rapid progress. My coworkers Benjamin Schudel and Sachit Goyal as well as the rest of the Kenis group have been instrumental in my research.

I thank my father for providing support, motivation and advice throughout my time in Illinois. He continually inspires me to work harder through praise. I thank my mother for the love and support she provides. I thank my grandfather for the support he provides. Most importantly, I want to thank God for providing me the opportunity to complete this degree

# Table of Contents

Chapter 1: Introduction .....	1
1.1 Challenges in the Pharmaceutical Industry in Drug Development .....	1
1.2 Present Challenges in Screening for Crystalline Forms.....	4
1.3 Microfluidic Approaches for Crystallization Screening .....	6
1.4 Overall Objectives.....	7
1.5 Figures .....	9
Chapter 2: Design and Fabrication of a Microfluidic Platform for Crystallization Screening.....	12
2.1 Introduction.....	12
2.2 Results and Discussion .....	13
2.3 Concluding Remarks.....	21
2.4 Figures .....	22
Chapter 3: Application of Crystallization Screens for Identification of Conditions Favorable for Crystallization of Active Pharmaceutical Ingredients (API) and Analysis 26	
3.1 Introduction .....	26
3.2 Results and Discussion.....	27
3.3 Challenges Associated with Solvent Absorption .....	30
3.4 Concluding Remarks .....	32
3.5 Figures .....	33
Chapter 4: Overcoming On-chip Solvent Absorption .....	36
4.1 Introduction .....	36
4.2 Literature Survey.....	37
4.3 Coatings to Eliminate Solvent Absorption.....	39
4.4 Alternative Materials and Approaches to Eliminate Solvent Absorption .....	40
4.5 Concluding Remarks .....	41
4.6 Figures .....	43
Chapter 5: Conclusion.....	45
5.1 Concluding Remarks .....	45
5.2 Future Work .....	46
Chapter 6: References.....	47

# Chapter 1: Introduction

## 1.1 Challenges in the Pharmaceutical Industry in Drug Development

Historically, the top priority regarding drug development has been reducing the time to market of candidate drugs (CD) and potential active pharmaceutical ingredients (API)[1]. “Time-to-market” is measured as the time from the identification of a CD to federal registration and commercial production of an API. This priority is motivated by the long times and associate costs required for CDs to reach production. In 2003, the median time was found to be 7.23 years for a compound to reach market (with a large range of 3-21 years) [2]. Increasingly strict standards and regulations have and will continue to require more stringent research and development (R&D), thus further extending the time for production of APIs. The long time to market of APIs negatively affects the bottom line of pharmaceutical companies due to high R&D costs and the deferred time for a return on investment (ROI).

The pharmaceutical industry also suffers from high attrition regarding CDs [1-3]. Attrition, defined as the failure of CDs to reach the market, is seen throughout the entire drug development process (see Figure 1.1). Once a lead target is identified in the R&D process, it is sent to lead optimization where information is built-up regarding crystalline forms, efficacy, and safety of a potential API. At this point, 93.3% of CDs are eliminated based on potential problems, as shown in Figure 1.2 [1, 4]. The CDs that pass lead optimization go through preclinical development for feasibility investigations regarding scalability, IP, and costs. Ninety percent of the CDs in preclinical development fail, leaving 0.67% of the original CDs for scale-up and clinical studies (phases I, II and III)

[1, 4]. Only 0.04% make it past the clinical studies, 0.027% are federally approved and only 0.003% of the CDs initially identified based on their pharmaceutical properties have a satisfactory ROI [1, 4]. The high rate of attrition amongst CDs makes production of APIs very expensive. The cost to bring a drug to production, a significant portion of which is due to attrition, was estimated to be 800 million dollars in 2003 [5]. The high rate of attrition amongst CDs and attrition's effect on the overall cost of bringing APIs to market has resulted in drug development priority focusing on early screening of CDs. Early screening of CDs assists in identifying physiological properties of CDs. Knowledge of these properties lead to the prioritization of CDs based on their likelihood for success, therefore, minimizing long R&D processes and attrition. Early screening also allows researchers to prioritize the selection of CDs based on (1) their probability for success throughout the R&D process and (2) their potential for a positive return on investment, because it eliminates high R&D costs associated with CDs that will otherwise fail at a later stage.

A significant amount of the attrition up through phase III of clinical testing can be attributed to poor physiochemical and preformulation properties [6, 7]. These problematic properties include solubility, the ionization constant, surface activity, stability, solution vehicles and toxicity. Knowledge of these properties can be used to prioritize CD development based on probabilities for success. Figure 1.3 illustrates the various contributing factors for attrition. Below the major problematic properties are highlighted in order to better understand how they bring about attrition and why they are important to identify early in the R&D process.

*Solubility* affects dissolution rates, solvent compatibility, toxicity and ultimately the performance of APIs [7]. Thus, solubility is a critical factor affecting the length of the R&D process as well as a CD's potential for success or failure. Solubility data can be used to weed out the lowest solubility compounds because of the problems they cause in development, e.g. due to low dosages. Therefore, solubility is one of the key properties affecting CD effectiveness.

The *ionization constant* determines how pH affects solubility, dissolution rate and other properties. The ionization constant also gives insight regarding how APIs behave when co-administered with drugs that change stomach pH [6]. Additionally, ionizable compounds can form salts, which increases the number of available solid forms; which, in turn, can overcome problematic properties such as hygroscopicity and solid-state stability [6]. Identification of ionizable compounds can assist in the identification of CDs with high probabilities of success or failure.

*Surface activity* is another important property as it can be either harmful or helpful, depending on the properties of the CD. The surface activity typically increases solubility in aqueous media, improving compatibility, while it disrupts membranes, which can make CDs toxic [6, 8]. Knowledge of this activity is important depending on the CD's function in the body. Stability of a compound drastically affects its potential for reaching the market due to the need for drugs to remain stable on store shelves for consumers. Early knowledge of these properties gives insight regarding the propensity of compounds for challenges and attrition in the R&D process.

To lower attrition, physiochemical and preformulation properties need to be identified as early as possible. Because the first step in identifying these properties is identifying all the solid forms [1], screening needs to be explored as early as possible to identify all the available solid forms. Once all the available solid forms and the conditions favorable for crystallization are identified, larger crystals can be nucleated using the conditions identified. These crystals can be tested for their physiochemical and preformulation properties in order to improve the selection criteria. Employing earlier detection and establishing a prioritizing system can drastically reduce the cost of bringing APIs to market. Screening needs to (1) begin sooner in the R&D process, (2) encompass more extensive sets of conditions to guarantee identification of all solid forms, (3) consume less material so testing can commence well before production is scaled up, and (4) improve the solid form analysis.

## **1.2 Present Challenges in Screening for Crystalline Forms**

Early screening allows for identification of both problematic compounds and compounds with the highest probability for success. While compounds are initially selected for their pharmacological and toxicological properties, it is the suboptimal physiochemical parameters (e.g. stability, lipophilicity and stability) as well as preformulation challenges (e.g. solution vehicles, toxicity) which bring about long R&D processes and attrition [1]. Early screening yields insight regarding these parameters and understanding regarding CDs' propensity for failure, thus allowing researchers to focus efforts on CDs with higher probabilities for success. Improved selection reduces development times, costs, and complications while providing a faster and safer return on investment.

The first step in identifying the potentially problematic properties is identification of all the available solid forms and the conditions favorable for crystallization. This level of identification requires extensive screens due to the large number of variable parameters (e.g. solvents, pH, precipitants, counterions, evaporation rates, temperatures, modes of mixing) [9-12]. Additionally, the amount of analyte available for testing is typically limited to a few milligrams due to the expenses associated with the production of the solid form of CDs in the early stages of drug development. Because these screens require many conditions to be tested with limited material, tests of each parameter must consume less material while maintaining consistency and accuracy in testing.

The ever increasing need for improved early screening has caused pharmaceutical screening technology for the identification of solid forms of CDs to grow by leaps-and-bounds over the last several decades. The industry transitioned from technicians manually screening for crystallization conditions with pipettes to complex, combinatorial screening robotic systems for solid form screening. The combinatorial chemistry techniques used today can be traced to the 1960s for solid phase peptide synthesis; however, combinatorial screening became widely applied to small pharmaceutical compounds in the 1990s [13]. The shift is partially due to the usage of microwell plates for crystal screens before the 1980s, which was tedious, time consuming and limited in applicability due to the small number of conditions tested [13]. Active ingredients were mixed with precipitants, salt solutions, and other solvents by technicians in micro well plates who then monitor these plates for the occurrence of crystallization. This technique required large sample volumes (microliters per condition tested), had limited capability regarding the brute number of conditions that could be tested, and was quite time

consuming. In the 1980s, robotic systems were developed to take the place of technicians [13]. An example robotic screening tool can be seen in Figure 1.4 [14]. These robotic systems enable researchers to work on a scale infeasible by hand. Robotic systems accurately pipette API solutions with various compounds in micro wells. While this technology is faster, more accurate, and less wasteful of sample than manual methods, the number of conditions screened is still limited by the volumes required (~100  $\mu$ l per well) and the number of wells per device [14]. The development of very large-scale integrated (VLSI) microfluidic techniques for screening has opened the door to more efficient pharmaceutical screening with promise to improve the early stage screening of APIs for favorable solid forms [15].

### **1.3 Microfluidic Approaches for Crystallization Screening**

The development of a microfluidic platform for crystallization screening has the potential to identify a much larger number of available solid forms of CDs early in the R&D process which, in turn, can provide much needed data regarding the solubility, bioavailability, dissolution rate, impurities and stability of APIs. Advantages of a microfluidic approach include reduced sample volume per test condition, increased number of conditions tested, reduced peripheral equipment, lower cost and improved fluid control.

Microfluidics as a field has grown by leaps and bounds since the 1990s to the point that large microfluidic chips with precise control and on-chip metering can be integrated into stand-alone unit operations [4-6]. Additionally, microfluidics for the application of crystallization screening have seen tremendous growth as is seen by the

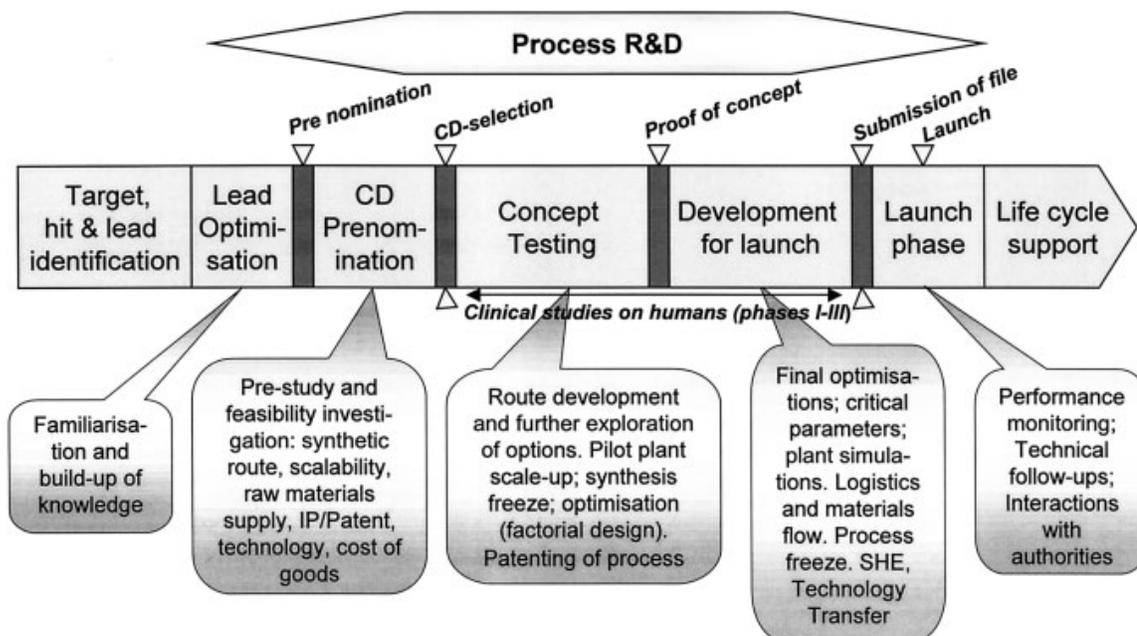
development of microfluidic platforms that screen for crystallization conditions, solubility, and nucleation kinetics of crystalline materials [7-11]. Highly integrated microfluidic platforms have been developed to screen for crystallization conditions of proteins using FID [9]. However, these platforms do not incorporate all of the modes of crystallization seen in macroscale crystallization, including evaporation and temperature control. Additionally, the valves used in these microfluidic devices require positive pressure to maintain isolation between adjacent chambers and lines [6, 9, 12-15]. The use of valves which fail closed would allow chips to be disconnected from peripheral connections, e.g., for X-ray or raman analysis. Other groups have developed microfluidic devices to screen for crystallization conditions using oil submersion methods utilizing two phase flow on PDMS chips [7, 8, 16-18]. These devices are well adapted to carry out one method for crystallization (FID, Temperature or Evaporation) and employ simple fabrication techniques. Microfluidics has demonstrated itself as an excellent platform to screen for crystalline conditions due to its inherent advantages of small volumes, high level of fluid control, and ability for scaling-out.

#### **1.4 Overall Objectives**

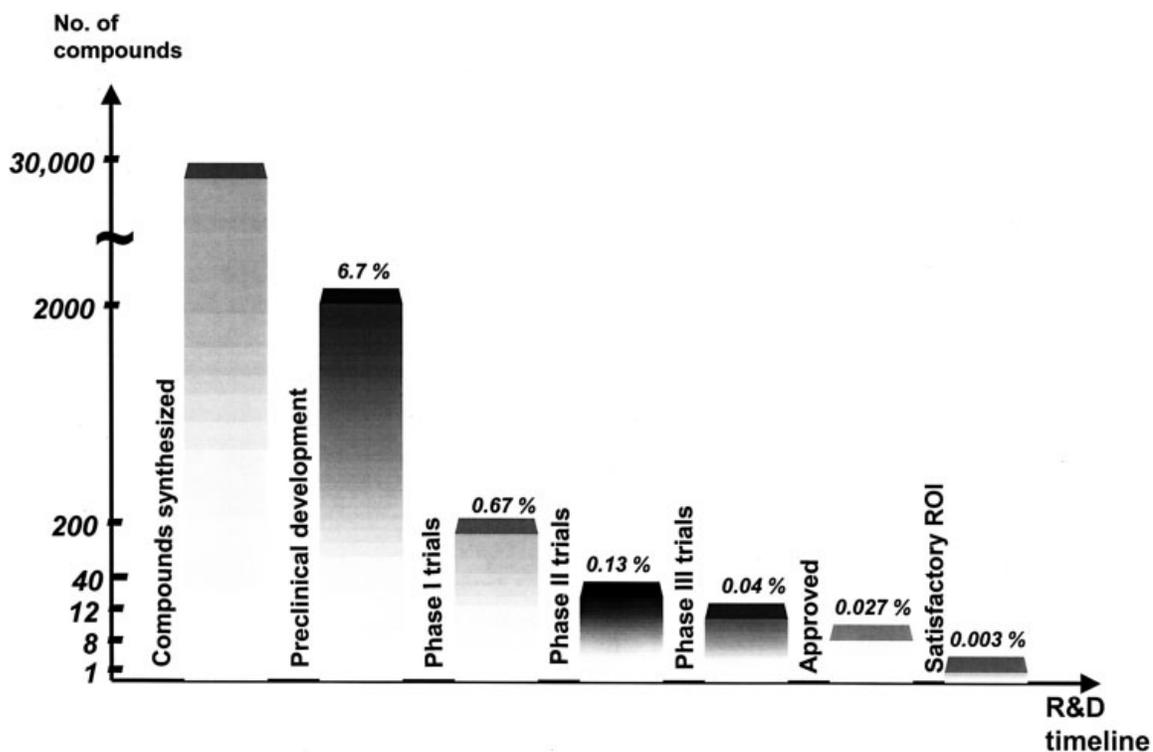
The overall objective of our research is to develop microfluidic chips to improve screening efficiency for solid forms of CDs. This will enable earlier screening in the drug development process thereby increasing the information available to researchers regarding crystalline properties. This information will allow researchers to focus on the materials with the highest probability of short R&D timetables and profitable returns on their investments.

This thesis presents the development of a microfluidic platform for the screening of a wide range of solid forms of CDs and for the conditions favorable for crystallization. The PDMS chip mixes a CD in a combinatorial fashion with precipitating agents, antisolvents, or counterions. Nucleation is induced through free interface diffusion, temperature control, or evaporation. Chapter 2 discusses the microfluidic platform's design, fabrication and operation. Chapter 3 discusses the application of the microfluidic platform for the screening of crystallization conditions of acetaminophen, a common API. Chapter 4 discusses challenges regarding solvent absorption of non-aqueous solvents into PDMS. A variety of materials from literature and materials used in our lab are discussed as potential alternatives for a PDMS platform. Current progress towards the development of a glass based microfluidic device is discussed in detail. Finally, Chapter 5 summarizes the key conclusions from this work and describes the future directions of this project.

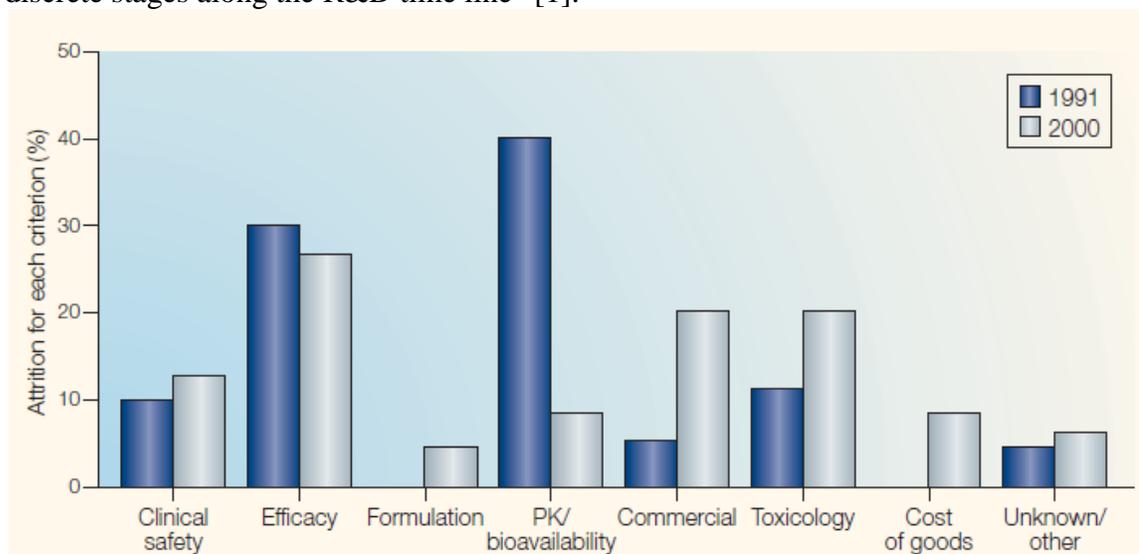
## 1.5 Figures



**Figure 1.1** The R&D process from lead discovery (left) through CD development to launch of an API [1].



**Figure 1.2** Attrition throughout the R&D process. The “likelihood of project survival at discrete stages along the R&D time line” [1].



**Figure 1.3** Contributing factors to attrition in the pharmaceutical industry. Of note are the bioavailability, formulation, efficacy, clinical safety and toxicology which can be reduced with improved screening capabilities [16].



**Figure 1.4** Example of a SYMYX system used for crystallization screening. This highly complex robotic system used robotic pipettes to meter fluids into wells and perform crystallization studies [17].

## **Chapter 2: Design and Fabrication of a Microfluidic Platform for Crystallization Screening**

### **2.1 Introduction**

The cost of bringing an Active Pharmaceutical Ingredient (API), better known as a pharmaceutical drug, to market was estimated to be 800 million dollars in 2003 [5]. Much of the expense associated with bringing APIs to market comes from the extensive R&D of candidate drugs (CD) that fail in testing [13]. As a result, it is critical to speed up the testing process and provide the information needed to remove CDs from development early on to reduce R&D on CDs that fail testing and ultimately lower the cost of bringing a compound to market. In order to provide information regarding bioavailability, stability, etc. of CDs earlier in the R&D process, more efficient screening technology needs to be developed for the identification of all available solid forms of CDs.

The development of a microfluidic platform has the potential for providing much needed data regarding solubility, bioavailability, dissolution rate, impurities and stability of CDs early in the R&D process. This information will provide researchers insight regarding whether CDs will ever reach the market, thus allowing researchers to focus on compounds more likely to reach the market. We propose to develop a microfluidic platform to screen for all the available solid forms of CDs while using less material, screening for more conditions and providing better control than state-of-the-art robotic macroscale approaches. Additionally, our microfluidic plan will utilize the microfluidic technology already demonstrated to screen for proteins and small molecules [18]. This

platform employs evaporation, temperature control and free interface diffusion as the method to drive supersaturation up and therefore nucleation. These methods have the highest probability of identifying crystallization conditions, thus maximizing the number of solid forms identified. Building on the designs of other microfluidic combinatorial mixers, we developed a device that mixes API solutions with precipitant and salt solutions in a combinatorial fashion [19]. The combinatorial mixing maximizes the number of unique conditions screened. We utilize Actuate to Open (ATO) valves, which close when disconnected from peripheral connections, thus allowing the device to be transported between analytical tools [19, 20]. Using acetaminophen, chip operation has been demonstrated using evaporation, temperature control and free interface diffusion. For analysis, we use Raman spectroscopy and birefringence on-chip. The inclusion of Raman is critical as it allows for polymorph detection [21]. The device has a total of 96 wells and uses 50 nl per well. The platform has the scalability, volume, and fluid control advantages associated with microfluidics while maintaining the versatility of macro-scale tools.

## **2.2 Results and Discussion**

First, the design of the microfluidic platform is detailed. Then, the fabrication is explained and finally, device operation is demonstrated with dyed solutions.

### **2.2.1 Design of Microfluidic Crystallization Screening Platform**

This microfluidic device was designed to mix API solutions with many precipitating solutions, salt solutions, and solvents in a combinatorial fashion to maximize the number of conditions analyzed for solid formation. The device uses a

repeating structure that enables the metering of fluids in a combinatorial fashion, simple diffusion mixing, well isolation, minimal sample waste, simple controls and a robust design. Figure 2.1 lays out the repeated structure critical to the device operation, which consists of three components: (1) the chamber for the pharmaceutical component, (2) the chamber for the precipitating agent, salt solution or other solvent and (3) the chamber for evaporation. These chambers are filled independently to create unique combinations of CD solutions with their respecting precipitating agents, salt solutions or solvents. Once filled, mixing is employed within these chambers. When using the evaporation mode, the fluid is transferred to the evaporation chamber which allows the solvent to evaporate at a rate set by the evaporation lines (Figure 2.1).

The power of using a microfluidic approach comes with the ability to scale out and thus screen a large array of conditions, with the microscale advantage of reducing the sample volume per condition screened. Figure 2.2 demonstrates how the repeated structure shown earlier can be replicated on a 7x12 chip to create a large number of integrated wells.

To accomplish the filling, mixing, transfer of fluids and evaporation rates discussed earlier, several layers are incorporated into the design. The fluid layer houses all the fluids and, therefore contains the chamber for the API solution, the chamber for the precipitant solutions, salt solutions, or other solvents as well as the evaporation chamber and fluid lines, as shown in Figure 2.3. Fluid transfer and mixing is controlled in this layer. PDMS (polydimethyl siloxane) is used for this layer due to its propensity for bonding to other surfaces and its elastomeric properties.

A control layer with valves embedded is used to control the movement and mixing of fluids in the fluid layer. The control layer sits on top of the fluid layer, as shown in Figure 2.3, and controls the fluid movement through valve actuation. The control layer is made out of PDMS to improve bonding with the fluid layer.

Traditionally, positive pressure valves are used for on-chip metering of fluids [18, 22]. The typical structure of a positive pressure valve is shown in Figure 2.4. When positive pressure is applied to the valve control line, the valve compartment is pushed up into the fluid layer, closing the channel or well. While this technique works for closing channels, these valves are prone to leaks along the edges and the valves require external pressure to hold the valves closed. These challenges force all testing to be done in one location and limit the time of experiment. In order to overcome these challenges, actuate-to-open (ATO) valves have been used. The structure is shown in Figure 2.5. ATO valves are closed in rest, thus keeping fluidic compartments isolated, and minimize leaks, thus allowing us to disconnect the device from peripherals for testing. However, the incorporation of ATO valves into a high density chip is challenging due to pressure losses on-chip. Large chips require complex valve structures; which result in long valve lines. Longer valve lines are problematic because long valve lines lose pressure over long distances due to the permeability of gas in PDMS. These pressure drop losses required changes in the valve design. The pressure loss was found to decrease when the ratio of the channel cross sectional area to the channel surface area increased. The control lines were made thicker which increased the cross sectional area relative to the surface area and thus allowed for valve actuation on longer valve lines.

Additionally, several layers which are not typically used in microfluidics have been incorporated to enhance operation. An impermeable layer, a standard self adhesive laminating sheet (polyvinyl chloride), is used above the control layer, as shown in Figure 2.3, to eliminate solvent evaporation out of the PDMS into the ambient air. Additionally, a gold coating, shown in Figure 2.3, has been applied to the glass slide backing of the device. The gold coating eliminates noise from the glass backing when using Raman microscopy on-chip.

### **2.2.2 Fabrication of Microfluidic Platform**

The microfluidic device consists of several layers. Each layer is made of a different material, thus requiring a complex fabrication procedure. The fluid and control layers are patterned using standard soft lithographic procedures [23, 24]. A silicon wafer is patterned with SU8-50 via spin coating at 2000 RPM to make a 50  $\mu\text{m}$  thick pattern. The coated wafer is prebaked, patterned in a UV exposure tool using a computer-generated negative mask, postbaked and developed. The fluid and control layers are patterned by coating the silicon wafer with PDMS (Slygard 184). The control layer is 5:1 (slygard 184: crosslinking agent) and the fluid layer is 15:1. PDMS is spun onto the silicon wafers at 1250 RPM to make each layer 75  $\mu\text{m}$  thick. After each layer is partially baked for 20 minutes at 70°C, the control layer is transferred from its corresponding silicon master to the fluid layer where it is aligned and fully baked. Because the control layer is only 75  $\mu\text{m}$  thick and the features are 50  $\mu\text{m}$  thick, the device cannot be removed from its silicon master using standard procedures, as the device will tear. As a result, a stamp, shown in Figure 2.5, is used to transfer the control layer onto the fluid layer. A PMDS stamp in the form of a window (5:1) is bonded to the control layer beyond the

features of the device to make the device rigid enough to be removed. However, the control layer still cannot be removed from the silicon wafer without tearing the device. As a result, hexane, a highly absorbent solvent in PDMS, is used to de-seal the device from the silicon wafer. Hexane swells PDMS enough that the control layer can be easily removed as shown in Figure 2.6. After the control layer is removed, it is washed with water to restore the device to normal size, dried with nitrogen gas and aligned to the fluid layer where it is permanently sealed with an additional bake at 70°C for 30 minutes.

Once holes are punched in the PDMS device for inlets, an impermeable layer is applied to the top of the control layer via a self adhesive to eliminate absorption. The fluid and control lines sit on top of evaporation lines which are made out of NOA 71 (Norland Optical), a Thiol-Ene material. The fluid lines are fabricated out of a PDMS master. The same technique discussed earlier for the fabrication of silicon masters for PDMS molding is used to create positive silicon masters. The silicon master is used in turn to fabricate PDMS masters. The PDMS (5:1) is poured on the ~2 mm silicon wafer and baked for 45 minutes at 70°C. The PMDS mold is then used to make the evaporation channels. NOA 71 is spun onto the PDMS mold at 500 RPM and crosslinked using UV exposure for 10 minutes at 10 mW/cm<sup>2</sup>. Once crosslinked, the combined fluid layer, control layer, and impermeable layer is adhered to the Thiol-Ene layer with pressure. The whole device is pressure-bonded to a glass slide with a Cr/ Au coating. The glass slide is coated with the Cr / Au combination in a sputter coater (AJA International). The chrome is used to improve the adhesion of gold, and gold is used to enhance Raman spectroscopy on-chip.

### **2.2.3 Operation of Microfluidic Platform for Filling, Mixing, Free Interface Diffusion, Temperature Control and Evaporation**

For proper device operation, various solutions containing a CD must be introduced into the chip to fill different compartments. Once on-chip, the solution must be mixed, transferred, evaporated, etc. This chip gives an operator the control and the screening potential necessary to analyze many conditions with one sample. Below, chip operation to maximize the screening potential of CDs is discussed.

CDs for testing are produced off-chip. The compounds are dissolved in solvents of interest and loaded onto the chip with Teflon tubing or by pipetting fluid directly over the access holes on-chip. Once loaded, the fluid is introduced into the chip through the actuation of the ATO valves and the application of vacuum pull fluid in. By selectively opening a series of valves, fluid can be directed and moved through a chip to fill the appropriate chambers. The valves have been designed to fill the chip in a combinatorial fashion. Figure 2.2 highlights the combinatorial sample formulation capabilities using food dye for demonstration purposes. On-chip, we create a large array of conditions with unique API: precipitant mixtures to maximize the number of conditions screened in a single test.

More specifically, the device is filled in a series of steps illustrated in Figure 2.6. The API and precipitating agent are filled independently via valve actuation in a combinatorial fashion. Once filled, one of the following methods is employed depending on the mode of crystallization desired:

*Free Interface Diffusion* – The valves separating the chamber housing the precipitating agent and the chamber housing the pharmaceutical component is opened as shown in Figure 2.7, allowing for slow diffusional mixing between the two chambers. The time for the API solution to fully mix with the precipitating solution can be estimated with the following equation:

$$t = \frac{x^2}{2D} \quad (2.1)$$

where  $t$  is the time for mixing,  $x$  is the distance for mixing and  $D$  is the diffusivity of the compound [25, 26]. Using the diffusivity of acetaminophen,  $4.25 \times 10^{-6} \frac{\text{cm}^2}{\text{s}}$  [26], and estimating the distance for mixing to be 1mm, we calculate the time required for mixing to be between 19 and 20 minutes. Dyed solutions with diffusivities similar to acetaminophen have been observed to mix in roughly 20 minutes, which agrees with the above calculation.

In some cases, it may be desirable to speed up mixing to better simulate microbatch macro-scale techniques. To accomplish this, a valve has been added which can push the fluid from the two wells back and forth. Through visual observation with food dyes, the mixing time has been determined to be less than 3 minutes.

*Temperature* – After mixing using the technique described above, the temperature is controlled externally via a peltier heater/cooler (20/20 Technologies) to induce crystallization. The temperature can be controlled from 0°C to 60°C. The peltier device can be placed on the microscope stage with the chip placed within the temperature

control device for in situ and live analysis. The wide temperature range increases the likelihood for nucleation.

*Evaporation* – After mixing using one of the techniques of microbatch or FID, the fluid is transferred into the evaporation chamber by opening a valve connecting the evaporation chamber to the chamber for the pharmaceutical component. The evaporation mode is shown in Figure 2.7. Once in the evaporation chamber, the evaporation rate is set by the dimensions of the evaporation line. The evaporation rate depends on the cross-sectional ( $A_c$ ) area of the evaporation channel and the length ( $L$ ) of the evaporation channel according to the following equation [25]:

$$J = \frac{A_c}{L} \cdot D \left( \frac{P^*}{P_T} \right) \rho \cdot MW \quad (2.2)$$

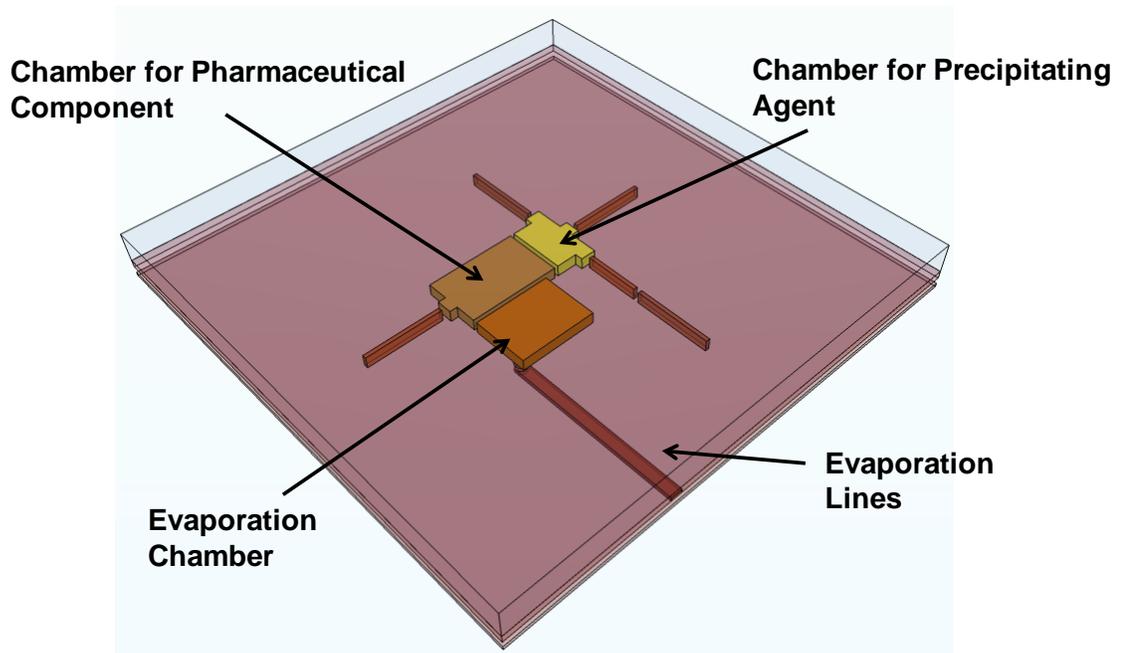
where  $A_c$  is the cross sectional area,  $L$  is the length,  $D$  is the diffusivity,  $P^*$  is the vapor pressure,  $P_T$  is the total pressure,  $\rho$  is the density and  $MW$  is the molecular weight.

Using this design equation, the chip has been designed such that four different sets of wells evaporate between 10 and 100 hours. Experimentally, we have found that the wells evaporate in 7, 24, 36, and 41 hours when using aqueous media. The difference can be attributed to the absorption of solvent into the PDMS. Although the solvent loss is minimized through the reduction of the PDMS thickness and the incorporation of an impermeable layer, some solvent still is absorbed into the surrounding PDMS. Nonetheless, the absorption rates can be accounted for via modifications of the evaporation channel dimensions to give the desired evaporation rates.

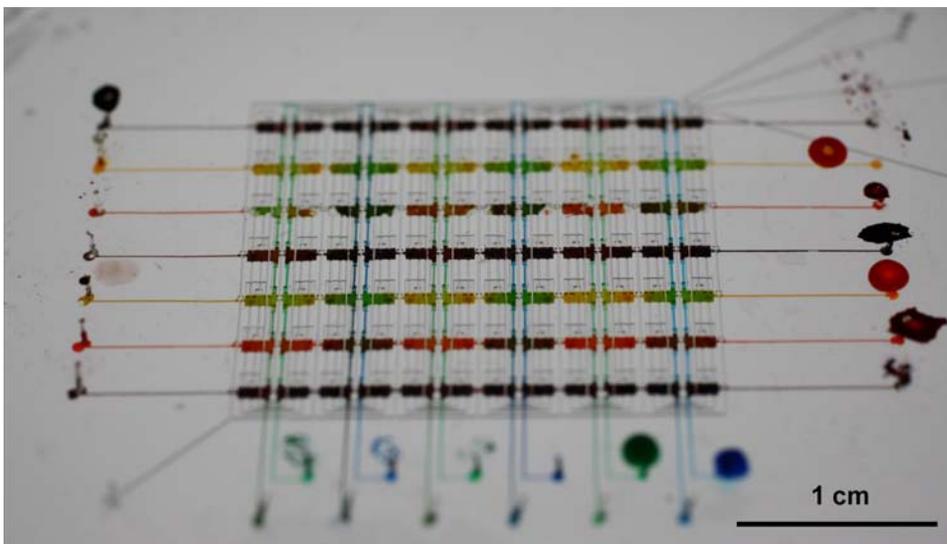
### **2.3 Concluding Remarks**

This chapter described the design and fabrication of a microfluidic device for crystallization of CDs. The microfluidic chip described integrates the technology and robustness seen on the macro-scale in a microfluidic device without sacrificing the variety of testing methods and versatility seen on the macro-scale. Using a microfluidic approach allows for the reduction of the volume required per condition screened (~50 nl), provides large arrays, improves fluid control in mixing, minimizes peripheral equipment, and provides portability. Additionally, this platform has several advantages over other microfluidic crystallization screening approaches reported in literature. We utilize a fail closed valve, thus eliminating leaks between adjacent compartments and providing portability by allowing the device to be disconnected from peripheral equipment. We integrate several modes of driving supersaturation to induce nucleation and growth. This device has the potential to generate many conditions favorable for crystallization and identify many more solid forms of CDs in the initial stages of drug development. Thus, the platform has the potential to reduce time and cost related to bringing new drugs to the market.

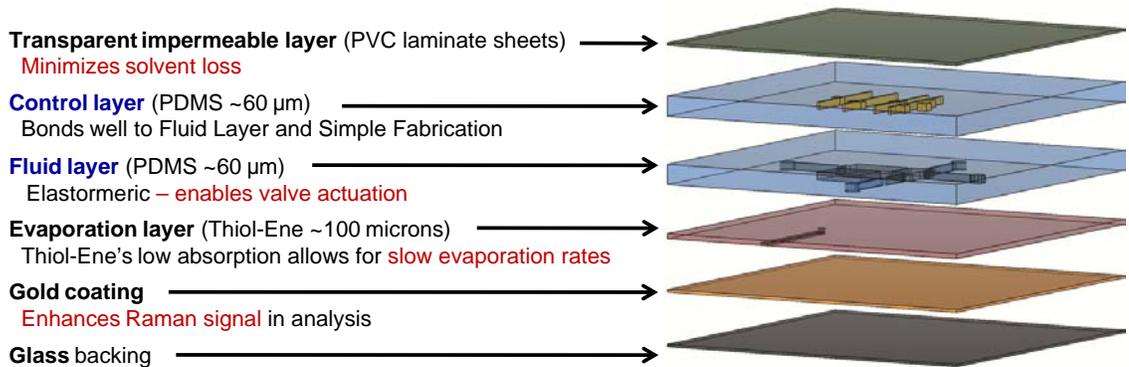
## 2.4 Figures



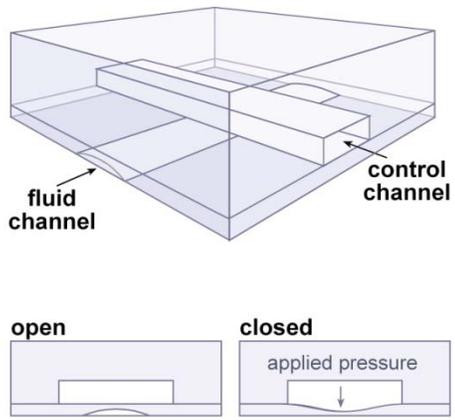
**Figure 2.1** The repeated structure of the microfluidic device. Each set of wells contains chambers for (1) the pharmaceutical component, (2) the precipitating agent, and for (3) evaporation. The precipitant and pharmaceutical chambers are filled independently. These chambers are mixed following filling and transferred to the evaporation chamber.



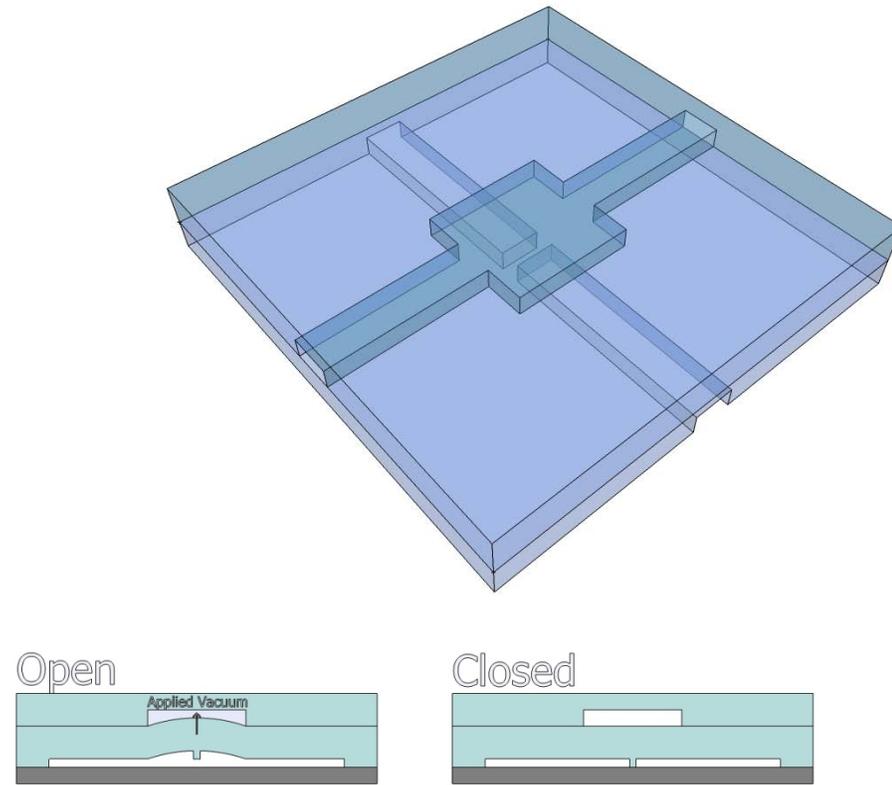
**Figure 2.2** Demonstration of the screening on our 7x12 microfluidic chip. Red, yellow and orange lines are filled horizontally and mixed with green and blue vertical lines.



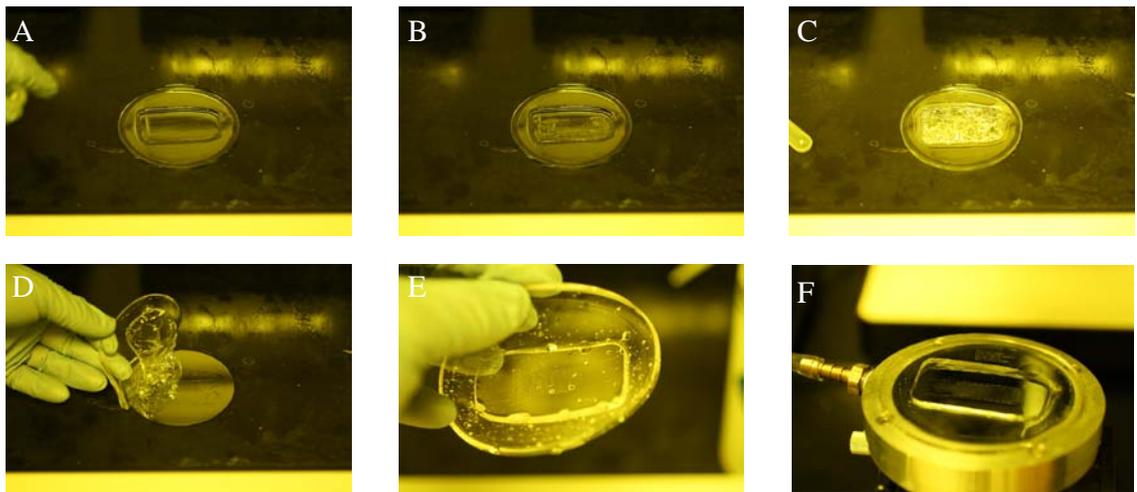
**Figure 2.3** Schematic overview of the layered assembly of the microfluidic crystallization chip. The impermeable layer eliminates absorption and evaporation beyond the control layer. The control layer is made of PDMS because the fabrication is well understood and it bonds well to the fluid layer. The fluid layer is made of PDMS because its elastomeric properties allow for valve actuation. The evaporation channels are made of a Thiol-Ene material for its low absorption. This device sits on a gold coated (to eliminate raman signal from glass) glass slide (to provide a rigid backing).



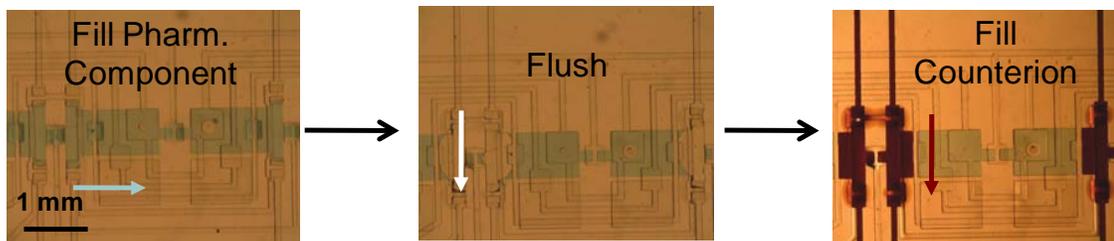
**Figure 2.4** Actuate-to-Close valves made with soft-lithographic techniques [27]. Shown schematically is the actuation of the valve with pneumatic pressure. Figure from Josh Tice [28].



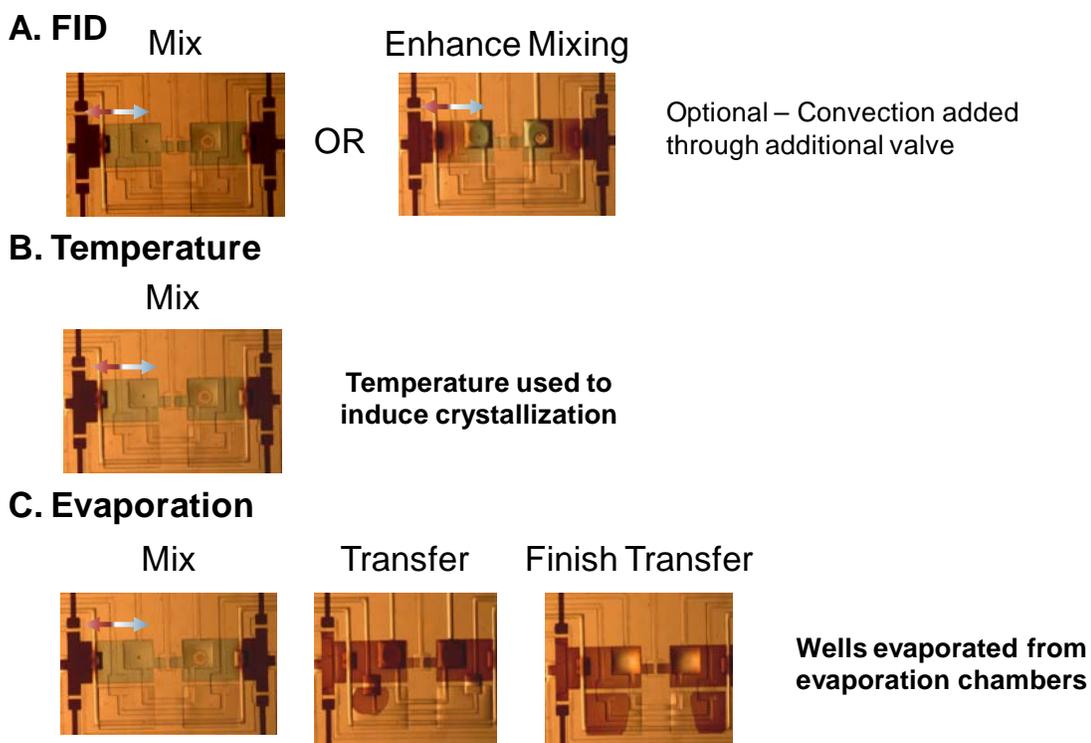
**Figure 2.5** Actuate-to-Open (ATO valves made with soft-lithographic techniques. Shown schematically is the actuation of the valve.



**Figure 2.6** Use of highly absorbent solvent to remove control layer from silicon wafer. The (A) 75 $\mu$ m control layer is de-sealed with the (B&C) addition of hexane. This allows the control layer to be (D) removed without any tears. Once removed, the PDMS film can be (E) cleaned with water to remove hexane, (F) bonded and aligned to the fluid layer.



**Figure 2.7** Filling procedure for a set of wells. Initially, a compartment is filled horizontally with the CD by selectively actuating valves which enable the fluid to transfer horizontally. Then the precipitant chamber is flushed by actuating the valves allowing fluid to flow horizontally and pulling the fluid out with a vacuum. Once flushed, the precipitating chamber is filled with a precipitant.



**Figure 2.8** Operation of microfluidic platform for (A) FID, (B) temperature control and (C) evaporation.

# **Chapter 3: Application of Crystallization Screens for Identification of Conditions Favorable for Crystallization of Active Pharmaceutical Ingredients (API) and Analysis**

## **3.1 Introduction**

The previous chapters demonstrated the operation and development of a microfluidic chip with the proposed application of screening for crystalline conditions of candidate drugs (CDs). The chip can screens many conditions using minimal material, and employs exceptional fluid control with regard to combinatorial filling, precise metering, controlled diffusional mixing, and controlled evaporation. Chip operation for the following three modes of nucleation will be demonstrated: (1) FID mixing with the ability to add convective mixing to simulate microbatch, (2) temperature control with the ability to control the temperature on-chip between 0°C and 60°C, and (3) evaporation with the ability to control the time for evaporation between 7 and 50 hours.

The ability of this chip to screen for conditions favorable for the crystal nucleation and growth of an active pharmaceutical ingredient (API) is demonstrated using the known API, Acetaminophen, as an example. Acetaminophen (more commonly known as Tylenol) was chosen due to its wide use as an over the counter drug. Acetaminophen is used as a target compound and is tested on-chip using FID, temperature control and evaporation to identify conditions favorable for crystal nucleation and growth.

Additionally, two methods have been developed for on-chip analysis of CDs. Birefringence is used to determine if crystalline material have formed and identify the solid form of the crystalline material. However, birefringence doesn't provide insight regarding the exact nature of the crystal formed. It cannot distinguish between different polymorphs. Researchers have shown that Raman spectroscopy can identify different polymorphs of compounds [29]. Due to the improved identification possible with the incorporation of Raman spectroscopy, we have adapted our microfluidic chips to enable analysis of crystals on-chip using Raman spectroscopy.

### **3.2 Results and Discussion**

Acetaminophen is supersaturated and nucleated using FID in an antisolvent setup, temperature control and evaporation. All experiments can be carried out on an upright microscope. Chips are filled manually with a vacuum pump. Once filled and mixed, the microscope stage is programmed to monitor activity in the cells over specified intervals at specified coordinates. Additionally, the temperature profile is programmed. Once set up, the stage is allowed to run as programmed. Finally, the data is analyzed after the experiment has completed.

#### **3.2.1 On-chip Crystal Screening of Potential APIs**

The first on-chip experiment discussed is crystallization of acetaminophen with FID. An acetaminophen (Sigma Aldrich) in methanol stock solution at the temperature solubility of 50°C was used in the subsequent experiments to identify nucleation conditions. The stock solution was screened against several antisolvents including water, methanol, ethanol, isopropanol, and butanol. Using pipettes, solutions were loaded onto

the microfluidic device at the inlet ports and pulled into the device via valve actuation with negative pressure. After filling and mixing, each well was monitored for crystallization conditions. We observed that 10 of the 15 tested conditions resulted in nucleation.

For comparative purposes, identical concentrations were mixed using an oil submersion method (microbatch). Using the oil submersion method on the macro-scale, we observed nucleation in 7 of the 15 conditions tested. The increased number of “hits” seen in the microfluidic experiment compared to the macro-scale microbatch experiment can be attributed to the increased levels of supersaturation seen in a FID approach. The higher levels are supersaturation and observed as the compounds slowly diffuse into the corresponding precipitant solutions.

The next on-chip crystallization experiment involved nucleation of Acetaminophen with temperature control. Acetaminophen solutions with concentrations ranging from 0.0015 g/ml to 0.0045 g/ml in water were created on-chip through our combinatorial mixing approach. Once loaded and mixed, the temperature of the device was lowered from 50°C to -5°C to induce crystal nucleation and growth. We observed nucleation in wells with acetaminophen concentrations greater than 0.002 g/g. This result demonstrates the ability to generate crystalline material using a temperature controlled microfluidic approach. Beyond identifying crystalline forms of Acetaminophen, the solubility of Acetaminophen was identified. The device temperature was raised, which caused the crystals in the wells to dissolve. The temperature at which the wells dissolved correlated to the solubility temperature of the crystals.

The third on-chip crystallization experiment used evaporation to induce nucleation. The evaporation rates are controlled through a combination of absorption and evaporation. The absorption rate is governed by the ratio of PDMS to solvent and the dimensions and design of the channels. The evaporation rates are governed by the ratio of the cross sectional area to length of the channel as discussed in chapter 2. In Figure 3.2, we demonstrate the nucleation of crystals in wells via evaporation for demonstration purposes. The device has been designed to control evaporation rates such that the solvent within the wells evaporate in 10 to 50 hours. These slow nucleation rates improve the probability of crystalline growth rather than amorphous material. Acetaminophen at the temperature solubility of 30°C was introduced into the microfluidic chip through inlet ports and transferred to the evaporation chambers where the solvent in the wells was evaporated. The API within the wells was consistently nucleated.

### **3.2.2 Characterization of Crystals Grown On-chip**

The identification of the solid forms of CDs on-chip both in terms of solid formation (crystalline or amorphous) and polymorphism is critical to proper analysis. Birefringence can be used for solid form identification. Two polarized filters are fitted to the light source and the detection microscope so that crystalline material can be visualized with the microscope. Figure 3.3 demonstrates the incorporation of birefringence in the FID experiment discussed earlier, revealing easily identifiable crystalline forms.

While microscope analysis using birefringence is simple to implement and provides identification of solid formations of the crystals, the method does not typically

provide insight regarding polymorphism. Thus, we chose to use Raman spectroscopy for polymorph, crystal structure and compound identification. Enabling Raman spectroscopy of crystals on-chip required several modifications to the chip design. Early designs which used a chip with 2 mm of PDMS and a 1mm glass backing resulted in poor spectroscopy (we tested Acetaminophen, Adipic Acid and Lysozyme). Reducing the PDMS thickness to <150  $\mu\text{m}$  and applying a gold coating to the glass slide allowed us to eliminate noise from both PDMS and glass. Applying a gold coating to the glass slide reflected the Raman laser back through the device, rather than through the glass slide, thus completely eliminating the noise from the glass. Both of these changes allowed us to obtain quality Raman spectra of APIs on chip. Figure 3.4 demonstrates quality Raman spectra obtained on-chip for acetaminophen. Of note is that the peaks observed from acetaminophen off-chip correlate strongly with the peaks of acetaminophen on-chip.

### **3.3 Challenges Associated with Solvent Absorption**

The success of this chip for crystallization of APIs in aqueous solvents has been demonstrated with relatively long time scales (order of days) and organic solvents with short time scales (order of hours). However, PDMS tends to absorb organic solvents, limiting us to short term experiments when using organic solvents. This absorption presents a challenge due to the higher solubilities of most APIs in organic solvents. We would like to carry out longer experiments with organic solvents (e.g. methanol, ethanol, isopropanol) to identify more solid forms and conditions favorable for crystallization. The original chip was designed with aqueous media in mind; thus, a reduction of the PDMS layer to 150  $\mu\text{m}$  was sufficient to minimize aqueous absorption (see equations below). In order to quantify the fraction of solvent available to absorb into the

surrounding PMDS, the solvent on-chip, the amount of PDMS in contact with the solvent, and the absorbance of the PDMS for the solvent must be known.

The solvent on chip can be calculated based on the number of wells on-chip ( $N_{wells}$ ) and the volume of these wells ( $V_{well}$ ). Using the equation below, the amount of solvent on-chip was calculated to be 6.5  $\mu$ l.

$$Solvent_{on-chip} = N_{wells} \cdot V_{well} \quad (3.1)$$

The amount of PDMS in contact with the fluid is the volume under the impermeable layer ( $V_{PDMS}$ ) minus the volume of the solvent ( $V_{solv}$ ). The Amount of PDMS on-chip was calculated to be 68  $\mu$ l.

$$PDMS_{on-chip} = V_{PDMS} - V_{solv} \quad (3.2)$$

The amount of solvent absorbed is the volume of PDMS available for absorption ( $V_{PDMS}$ ) times the absorption of PDMS ( $Abs_{PDMS}$ ) for the appropriate solvent. Amount of water available to absorb in PDMS was calculated to be 66 nl.

$$Solvent_{Absorb} = V_{PDMS} \cdot Abs_{PDMS} \quad (3.3)$$

The fraction absorbed is the volume of solvent absorbed into PDMS ( $V_{abs}$ ) divided by the volume of the solvent on-chip ( $V_{solv}$ ). The fraction of water absorbed was calculated to be 0.75%

$$Fraction_{Absorbed} = \frac{V_{abs}}{V_{solv}} \quad (3.4)$$

Because PDMS will absorb 20 times more material from organic solvents than water, shown in Figure 3.5, experiments with organic solvents cannot be carried out over

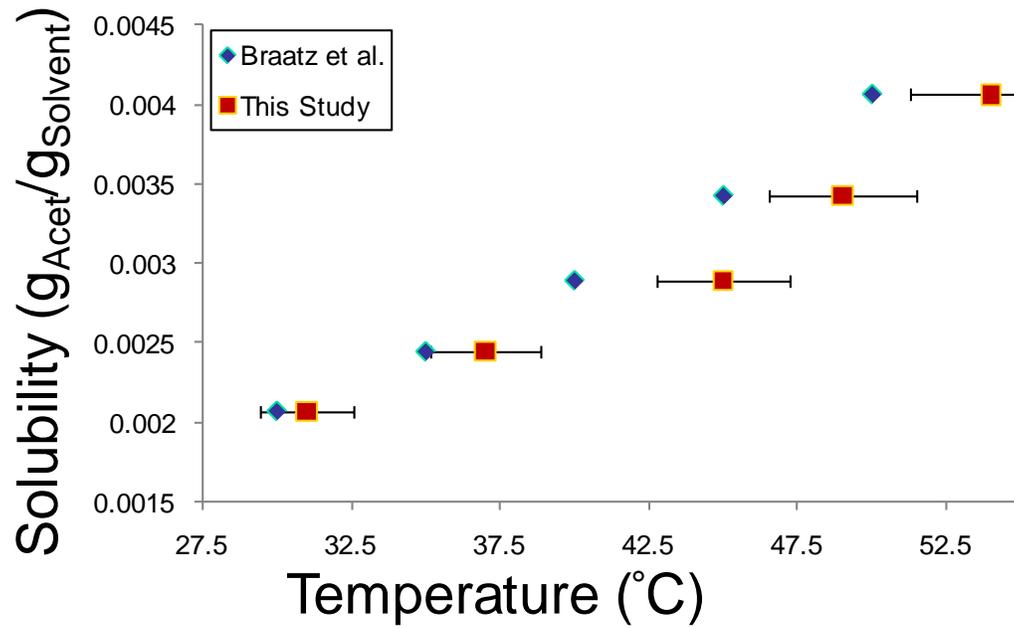
a several day period. Using equations 3.1 to 3.4, the maximum amount of solvent absorption was calculated to be greater than the amount of solvent on-chip (with organic solvents).

Due to PDMS' propensity for absorbing organic solvents into PDMS, long term experiments using organic solvents can't be carried out on-chip (e.g. temperature control and evaporation). As a result, we are pursuing alternative approaches to crystallizing these materials on-chip.

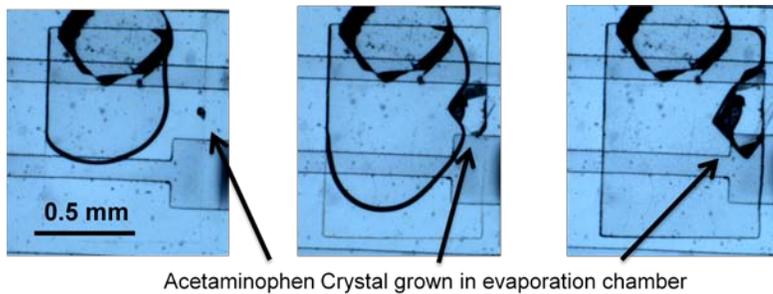
### **3.4 Concluding Remarks**

This chapter demonstrates a successful chip for the crystallization of APIs on-chip using FID, temperature control and evaporation. Additionally, methods to analyze the crystals on-chip using birefringence and Raman spectroscopy was developed and tested. The current challenge with our chip is its absorption of organic solvents. As a result, the current chip needs to be modified such that organic media can be used on-chip.

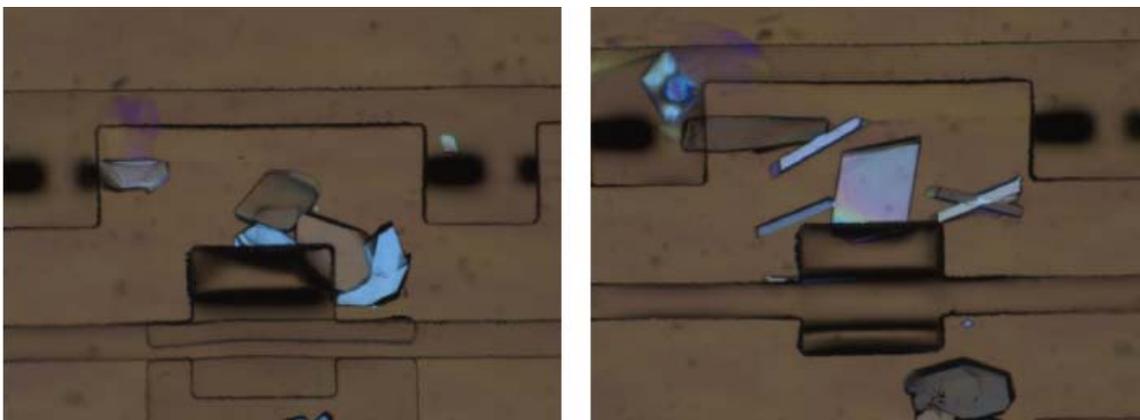
### 3.5 Figures



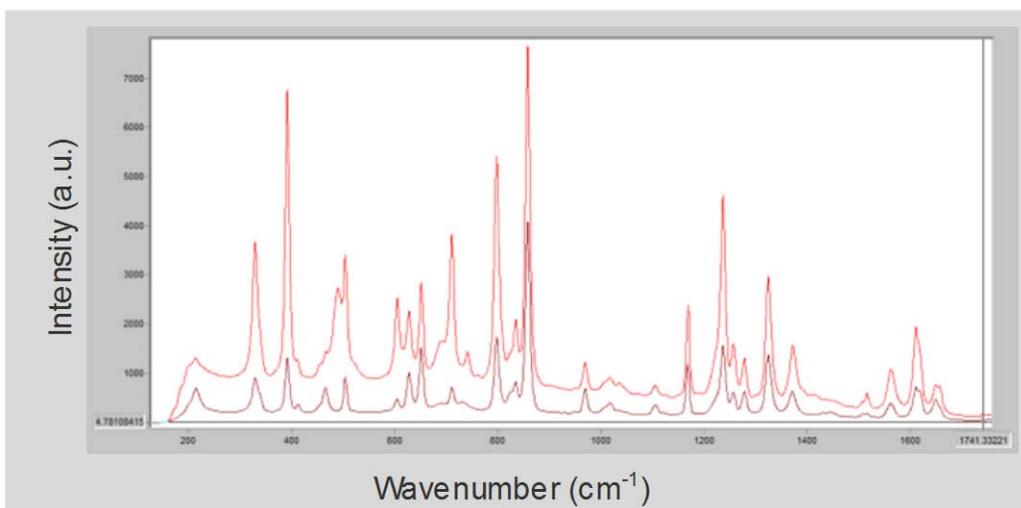
**Figure 3.1** Solubility of Acetaminophen in water. Comparison of data obtained in this study and data obtained Braatz *et al.* [30].



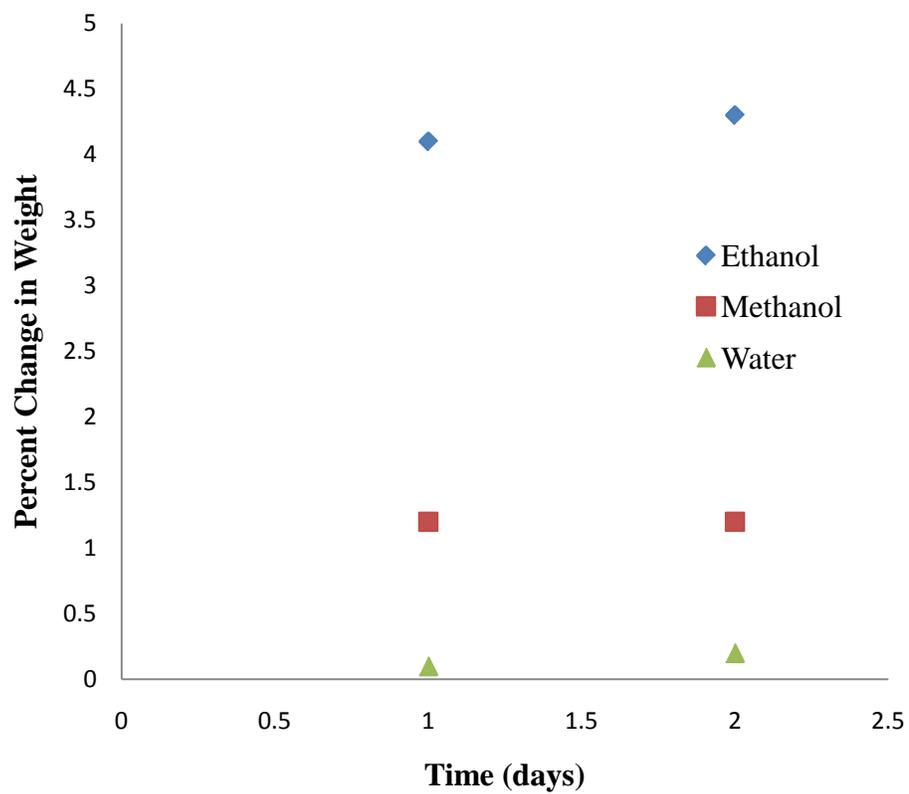
**Figure 3.2** Nucleation of Acetaminophen on-chip via evaporation.



**Figure 3.3** Birefringence of Acetaminophen on-chip. The above image demonstrates birefringence's ability to identify crystalline material on-chip.



**Figure 3.4** Raman spectrum of Acetaminophen crystals on-chip. Light red: the spectrum of Acetaminophen crystals on-chip and dark red: acetaminophen crystals off-chip.



**Figure 3.5** Absorbance of common solvents used for crystallization experiments of CDs.

## Chapter 4: Overcoming On-chip Solvent Absorption

### 4.1 Introduction

Microfluidic technology has grown by leaps and bounds over the last decade. Much of the success of microfluidics can be accredited to the material polydimethylsiloxane (PDMS). PDMS' well understood fabrication procedures and elastomeric properties have made it the primary material used in microfluidic devices [31-34]. As a result, PDMS microfluidic devices can be seen in biological applications, concentration studies, crystallization screening, nucleation studies, PCR analysis, and DNA separation to name a few [35-38].

While PMDS enables researchers to develop many needed devices and applications, use of PDMS is mostly limited to aqueous media [39] due to its inherent tendency to absorb certain solvents [40]. Hard materials such as glass and silicon have been explored as an alternative for PDMS and other solvent-absorbing polymers [39]. These hard materials have been used in microelectromechanical systems and microfluidic applications; however, these materials lack the properties and valve control needed for high levels of fluid manipulation and control [34, 41].

Solvent-resistant materials are particularly important for crystal screening of CDs in microfluidic platforms due to the wide variety of solvents typically used in these screens. Typical solvents for crystallization include isopropyl acetate, ethanol, methanol, isopropanol, heptane, acetonitrile, 1-octanol, toluene and 2-butanone, all of get absorbed to different extents in PDMS [9, 40]. As a result, a platform for crystallization that is compatible with these solvents needs to be developed.

## 4.2 Literature Survey

Many approaches have been explored in an attempt to overcome solvent absorption. The attempts can be classified in three categories (1) material coatings on PDMS devices to create a physical barrier to absorption, (2) alternative materials which are both elastomeric and solvent-resistant to replace PDMS, and (3) material reduction strategies. Of these strategies, the most successful are the coating and material reduction strategies.

In regards to material coatings on PDMS, Cytop, a fluoropolymer from Asahi glass, has shown the most promise. Kanai *et al.* [42] reported the passivation of PDMS microfluidic channels with a 0.2-5 $\mu$ m thick coating of Cytop. Cytop channel coatings create a barrier to absorption due to their hydrophobicity. Cytop can be applied on PDMS using several techniques including spin coating, dipping, flowing, and hot pressing. These coatings increase the overall young's modulus of the material, therefore, increasing the resistance to valve actuation. However, valve actuation has been demonstrated with increased pressure in Cytop-coated devices. Cytop was found to reduce but not eliminate both the swelling and the rate of absorption from organic solvents.

Other coatings, including Viton, Teflon AF, and metals, have shown promise limiting solvent absorption. Viton, a liquid-castable FKM fluorelastomer, is a coating with good chemical resistance which has shown moderate success eliminating solvent absorption [39]. Viton (coated via spin coating on PDMS) reduced absorption into PDMS where uniform coatings were applied; however, Dam *et al.* struggled creating a

uniform barrier and thus, an effective barrier [39]. Teflon AF, Teflon in a perfluorinated solvent, was applied via spin-coating [39]. Teflon AF was found to have extremely high solvent resistance; however, Dam *et al.* found that the high temperatures required for annealing cracked the material preventing a uniform coating [39]. Other more common and hard materials, such as metals, have been used in an attempt to eliminate absorption; however, valve actuation tends to crack the material causing it to fail [39].

Beyond material coatings, researchers have evaluated alternative materials for PDMS. The challenge becomes finding a material with the elastomeric properties needed for valve actuation, solvent resistance and well understood fabrication techniques. Natali *et al.* has developed a multilayer thiolene (NOA61) microfluidic chip fabrication technique enabling the manipulation of solvents on-chip. However, valve actuation wasn't incorporated in the thiolene chip [43]. Another material of note is isobornyl acrylate. This material can be easily crosslinked with UV light in the presence of crosslinker and photoinitiator. Khoury *et al.* have developed a simple fabrication technique; however, the cured polymer is too hard for valve actuation [44]. The major advantage of PDMS over alternative materials is its elastomeric property. While there are many options, the challenge in developing a replacement for PDMS becomes finding an elastomeric material with well understood and high density fabrication techniques.

Researchers have looked into alternative approaches to minimize absorbent material. Mathies *et al.* has developed a material reduction strategy to minimize absorption to the point that organic solvents can be used, as shown in Figure 4.1 [20]. This approach employs glass fluid and control layers with a thin flexible film between the two layers. The flexible film between the glass layers is made of PDMS, which enables

valve actuation. Using this approach, the thickness of the PDMS membrane can be reduced from more than 2 mm to less than 100 microns. This approach has been demonstrated to be compatible with organic solvents due to the reduced amount of PDMS required. Manalis *et al.* have taken Mathies' approach and replaced the PDMS membrane with a solvent resistant Teflon FEP (a fluorinated ethylene propylene from DuPont) film [45]. Incorporating this Teflon film eliminates all unwanted absorption. Teflon FEP films with glass chips have been demonstrated to work with organic solvents in a leak-free fashion.

### **4.3 Coatings to Eliminate Solvent Absorption**

Coatings such as Cytop have been employed in an attempt to reduce solvent absorption. Cytop was spun onto the PDMS device, dried, annealed and tested for resistance to solvent absorption. Cytop 809-A was mixed 1:10 with Fluorinert FC-75 and spun onto PDMS at 500 RPM to produce a roughly 5 $\mu$ m coating. Following spin coating, the chip was baked at 75°C for 30 minutes and annealed at 115°C for 30 minutes. Cytop appeared to form a uniform coating. The coating reduced the elasticity of the PDMS mold; however, the valves could still be actuated with increased pressure. Unlike previous studies, our studies avoided crack formation within the Cytop material as the result of valve actuation. This is believed to be due to the incorporation of actuate-to-open valves rather than actuate-to-close valves. The actuate-to-open valves flex the material less, which prevented the material from cracking. While the material coated the device well and formed a uniform coating without cracks, we experienced solvent loss with the coated chip to a similar extent on an uncoated chip with water solutions. These results show that Cytop does not form a sufficient physical barrier for solvents.

#### 4.4 Alternative Materials and Approaches to Eliminate Solvent Absorption

A variety of thiolene materials (NOA 60, 61, 63, 65, 68, 70, 75, 76, 81) were also coated on our PDMS chip to reduce absorption. These materials have a wide range of properties regarding adhesion and elasticity. Each thiolene material was spin coated (1) directly onto the PDMS mold and (2) directly onto the silicon master. When spinning onto a silicon master, the materials (a) failed to adhere to the PDMS master poured on top of it, (b) permanently adhered to the silicon master, or (c) prevented valve actuation due to increased stiffness of the film. When spinning thiolene directly onto PDMS, the materials would either (a) not uniformly coat the device or (b) prevent valve actuation due to increased stiffness of the film. Thiolene was found to make a poor coating material due to adhesion incompatibilities and insufficient elasticity.

We also attempted to replace the PDMS material altogether with thiolene (NOA 76). NOA 76 was chosen due to its low Young's modulus (6600 kPa), which is 7.5 times that of PMDS (870 kPa). Silicon masters were made from masks which selectively blocked UV light to develop the desired structure on a silicon wafer. The pattern on the silicon wafer was used to mold a PDMS master, which, in turn, was used to mold the fluid and control layers out of thiolene. The thiolene material was spun onto the PMDS master and exposed to UV light to crosslink the material. Once crosslinked, the layers were combined and further crosslinked under UV light for permanent bonding. While the fabrication procedure was simple, the higher Young's modulus of thiolene prevented the material from flexing under a vacuum pump with a pressure as high as 0.2 bar. Higher pressure gradients than are available in an atmospheric environment are necessary to actuate these valves.

Due to the challenges associated with incorporating alternative materials and coatings to PDMS devices, glass-based microfluidic devices based on technology Mathies et al. developed were explored. The design used in the PDMS approach discussed in chapter 2 was transferred to glass slides. Our control and fluid design patterns have been transferred to glass slides. These glass slides are sandwiched together with a thin PDMS or Teflon FEP film separating the glass slides. See Figure 4.1 for explanation regarding the alignment of the layers.

The procedure for glass fabrication is as follows: Glass slides are coated with 30 microns of chrome with 150 microns of Gold. The second chrome and gold coatings improve adhesion and eliminate pin-hole defects observed with only one coating. The coated slide is selectively patterned with SPR220 using computer-generated masks. The exposed Cr/ Au is etched, exposing the glass. The channels and wells are subsequently etched with HF where the glass is exposed. After the glass slide is etched, the remaining photoresist, chrome and gold are removed, exposing the glass slide. The etched glass slides are sandwiched together with a 20 micron PDMS film between.

The combined devices worked moderately well. Valve actuation was successful. However, the chip leaks around the valves and chambers, as shown in Figure 4.2. The chamber and valve spacing need optimization to improve fluid containment.

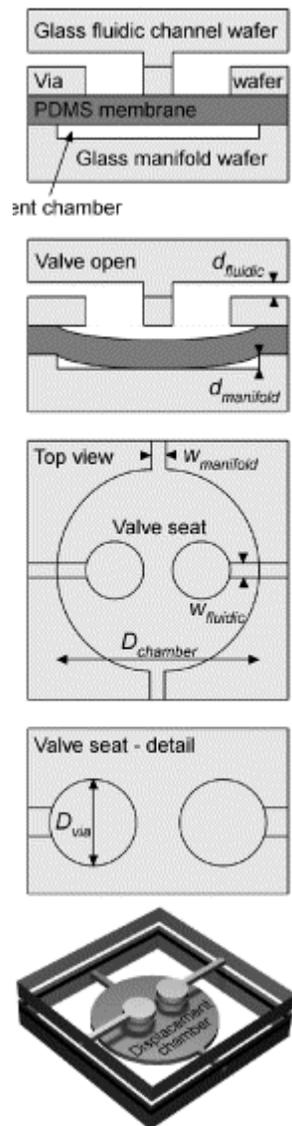
#### **4.5 Concluding Remarks**

A major problem with PDMS microfluidic chips is its high rate of solvent absorption. Many materials, coatings, and alternative approaches have been explored in an attempt to discover a chip that can meter, isolate and control fluid flow on-chip while

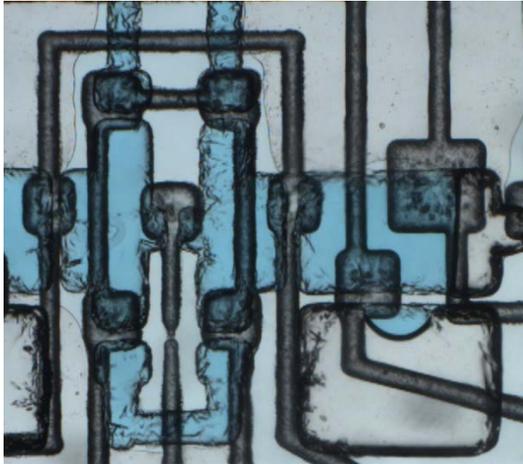
maintaining solvent compatibility. The alternative approach based on a glass chip developed by Mathies shows promise to eliminate solvent absorption.

With the proper modifications, this approach holds promise for development of a chip that will isolate and control fluid movement on-chip while overcoming our challenges of solvent absorption.

## 4.6 Figures



**Figure 4.1** Microfluidic valve fabrication in glass as reported by Mathies *et al.* [20].



**Figure 4.2** Depicted is a glass based microfluidic device built for crystallization screening. The filled chambers are in the fluid layer and the unfilled chambers are in the control layer. This figure demonstrates successful etching, and fluid filling. Leaking can be seen around the fluid lines.

## Chapter 5: Conclusion

### 5.1 Concluding Remarks

This thesis reported the development, fabrication and application of a microfluidic chip for the screening of candidate drugs (CD) in regards to conditions favorable for crystallization. The platform screens for available solid forms of a CD, which is the first step in identifying the physiological properties of CDs. In turn, this provides insight regarding the propensity of the CD for attrition or long R&D processes. Three main methods are used to increase supersaturation, and thereby to induce crystal nucleation and growth: (1) free interface diffusion which increases the likelihood of nucleation compared to microbatch the most common macroscale technique, (2) temperature control by an external source and (3) evaporation, incorporating an additional layer with evaporation channels, with evaporation times between 10 and 50 hours. The microfluidic platform is comprised of 96 wells that each consume 50 nl of CD solution, so typically less than 5 micrograms of a CD per well.

The functionality of this platform is demonstrated with acetaminophen using the three methods discussed above. Using free interface diffusion (with antisolvents), temperature control and evaporation, conditions favorable for nucleation were identified. The free interface diffusion technique was compared to a conventional microbatch oil submersion method. In this comparison, more hits were observed on-chip than off-chip. Additionally, the chip was modified for Raman spectroscopy to be obtained from crystals on-chip. This allows for the identification of crystalline solids and polymorphs.

## 5.2 Future Work

While the platform's capabilities have demonstrated screening potential with aqueous media, the microfluidic platform is incompatible with most organic solvents for long term experiments (>8 hrs) due to solvent absorption. As a result, alternative materials and coatings have been explored. While using alternative materials and coatings, challenges with sealing between layers, swelling, cracking and limited valve actuation prevented their successful incorporation. Similar challenges are reported in literature. Mathies *et al.* has developed a valve design that limited the PDMS material via incorporating channels in glass slides with a thin PDMS or Teflon membrane. Incorporating the current PDMS design discussed in Chapter 2 with Mathies' technique shows significant promise for limiting solvent absorption. Thus so far, a significant amount of leaks on-chip due to poor sealing and improper spacing of valves, lines, chambers, etc have limited immediate incorporation. To overcome this challenge, additional experiments are needed to better understand the design rules such as proper spacing between valves and valve widths. By first doing experiments to understand the design rules associated with this approach, we will gain a better understanding of the optimal dimensions that will allow us to develop a glass based microfluidic platform capable of screening for crystallization conditions of pharmaceuticals. This device will use the same approach seen in the PMDS based microfluidic chip with the advantage of improved compatibility with organic solvents.

## Chapter 6: References

1. Federsel, H.-J., *Facing Chirality in the 21st Century: Approaching the Challenges in the Pharmaceutical Industry*. Chirality, 2003, **15**(S1): S124-S142.
2. *Centre for Medicines Research*.
3. Kola, I. and J. Landis, *Can the pharmaceutical Industry Reduce Attrition Rates?* Nature Reviews Drug Discovery, 2004, **3**.
4. Kuhlmann, J., *Drug Research: from the idea to the product*. Institute of Clinical Pharmacology and Therapeutics, 1997, **35**: 541-552.
5. Dimasi, Hansen, and Grabouski, *The Price of Innovation: new estimates of drug development costs*. Journal of Health Economics, 2003, **22**: 151-185.
6. Chen, X.-Q., M.D. Antman, C. Gesenberg, and O.S. Gudmundsson, *Discovery Pharmaceuticals - Challenges and Opportunities*. The AAPS Journal, 2006, **8**: E402-E408.
7. Florence, A.A., D, *Physiochemical Principles of Pharmacy*. 1998, London, UK: Macmillan Press.
8. Kodavanti, U.M., HM, *Cationic amphiphilic drugs and phospholipid storage disorder*. Pharmacological Reviews, 1990, **42**: 327-354.
9. Carlson, E., P. Cong, W.H. Chandler, Jr. , H. Chau, E. Danielson, P.J. Desrosiers, R. Doolan, and L. Wu, *Apparatuses and Methods for Creating and Testing Pre-Formulations and Systems for Same*. 2003: United States.
10. Gardner, C.R., O. Almarsson, H. Chen, S. Morissette, and e. al., *Application of high throughput technologies to drug substances and drug product development*. Computers and Chemical Engineering, 2004, **28**: 943-953.
11. Ware, E.C. and R.D. Lu, *An Automated Approach to Salt Selection for New Unique Trazodone Salts*. Pharmacological Research, 2004, **21**(1): 177-184.
12. Fogg, M.J. and A.J. Wilkinson, *Higher-throughput approaches to crystallization and crystal structure determination*. Biochemical Society Transactions, 2008, **036**(4): 771-775.
13. *Handbook of Modern Pharmaceutical Analysis*, ed. S. Ahuja and S. Scypinski. 2001, Orlando, FL: Academic Press.
14. Desrosiers, P., *The Potential of Preform*. Modern Drug Discovery, 2004: 40.
15. Su, F., S. Ozey, and K. Chakrabarty, *Concurrent Testing of a Droplet-Based Microfluidic Systems for Multiplexed Biomedical Assays*. International Test Conference, 2004.
16. Kola, I.L., John, *Can the Pharmaceutical Industry Reduce Attrition Rates?* Nature Reviews Drug Discovery, 2004, **3**.
17. *Symyx system for salt and polymorph screening*. [cited 2010 March 8].
18. Hansen, C.L., S.R. Quake, and J.M. Berger, *High throughput screening of crystallization materials*. 2007: US.
19. Schudel, B.R., C.J. Choi, B.T. Cunningham, and P.J.A. Kenis, *Microfluidic chip for combinatorial mixing and screening of assays*. Lab on a Chip, 2009, **9**(12): 1676-1680.
20. Grover, W.H., A.M. Skelley, C. Liu, E.T. Lagally, and R.A. Mathies, *Monolithic membrane valves and diaphragm pumps for practical large-scale integration into glass microfluidic devices*. Sensors and Actuators B, 2003, **89**(3): 315-323.

21. Alfred Y. Lee, I.S.L., Severine S. Dette, Jana Boerner, Allan S. Myerson, *Crystallization on Confined Engineered Surfaces: A Method to Control Crystal Size and Generate Different Polymorphs*. Journal of the American Chemical Society, 2005, **127**: 14982-14983.
22. Hansen, C.L., E. Skordalakes, J.M. Berger, and S.R. Quake, *A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion*. Proceedings of the National Academy of Sciences of the United States of America, 2002, **99**(26): 16531-16536.
23. Duffy, D.C., J.C. McDonald, O.J.A. Schueller, and G.M. Whitesides, *Rapid prototyping of microfluidic systems in poly(dimethylsiloxane)*. Analytical Chemistry, 1998, **70**(23): 4974-4984.
24. Thorsen, T., S.J. Maerkl, and S.R. Quake, *Microfluidic Large-Scale Integration*. Science, 2002, **298**: 580-584.
25. Bird, R.B., W.E. Stewart, and E. Lightfoot, N., *Transport Phenomena*. 1960, New York: John Wiley & Sons.
26. Atkins, P.W., *Physical Chemistry*. 5th ed. 1994, New York: W. H. Freeman.
27. Unger, M.A., H.P. Chou, T. Thorsen, A. Scherer, and S.R. Quake, *Monolithic microfabricated valves and pumps by multilayer soft lithography*. Science, 2000, **288**(5463): 113-116.
28. Tice, J.D., *Electrostatically Actuated Microvalves Fabricated with Soft-lithographic Techniques for Integrated Microfluidics*, in *Chemical and Biomolecular Engineering*. 2009, University of Illinois: Urbana, IL.
29. Lee, A.Y., I.S. Lee, S.S. Dettet, J. Boerner, and A.S. Myerson, *Crystallization on confined engineered surfaces: A method to control crystal size and generate different polymorphs*. Journal of the American Chemical Society, 2005, **127**(43): 14982-14983.
30. Fujiwara, M., P.S. Chow, D.L. Ma, and R.D. Braatz, *Paracetamol Crystallization Using Laser Backscattering and ATR-FTIR Spectroscopy: Metastability, Agglomeration, and Control*. Crystal Growth & Design, 2002, **2**(5): 363-370.
31. Boone, T.D., Z.H. Fan, H.H. Hooper, A.J. Ricco, H. Tan, and S.J. Williams, *Plastic advances microfluidic devices*. Anal. Chem., 2002, **74**: 78A-86A.
32. Chou, H.P., M.A. Unger, and S.R. Quake, *A microfabricated rotary pump*. Biomedical Microdevices, 2001, **3**(4): 323-330.
33. Liu, J., C. Hansen, and S.R. Quake, *Solving the "World-to-Chip" Interface Problem with a Microfluidic Matrix*. Anal. Chem., 2003, **75**: 4718-4723.
34. Soper, S., S. Ford, S. Qi, R. McCarley, K. Kelly, and M. MC, *Polymeric microelectromechanical systems*. Anal. Chem., 2000, **72**(19): 642A-651A.
35. Pal, R., M. Yang, R. Lin, B.N. Johnson, N. Srivastava, S.Z. Razzacki, K.J. Chomistek, D.C. Heldsinger, R.M. Haque, V.M. Ugaz, P.K. Thwar, Z. Chen, K. Alfano, M.B. Yim, M. Krishnan, A.O. Fuller, R.G. Larson, D.T. Burke, and M.A. Burns, *An integrated microfluidic device for influenza and other genetic analyses*. Lab on a Chip, 2005, **5**(10): 1024-1032.
36. Gunawan, R.C., E.R. Choban, J.E. Conour, J. Silvestre, L.B. Schook, H.R. Gaskins, D.E. Leckband, and P.J.A. Kenis, *Regiospecific control of protein expression in cells cultured on two-component counter gradients of extracellular matrix proteins*. Langmuir, 2005, **21**(7): 3061-3068.

37. Namasivayam, V., R. Lin, B. Johnson, S. Brahmasandra, Z. Razzacki, D.T. Burke, and M.A. Burns, *Advances in on-chip photodetection for applications in miniaturized genetic analysis systems*. Journal of Micromechanics and Microengineering, 2004, **14**(1): 81-90.
38. Lucchetta, E.M., J.H. Lee, L.A. FU, N.H. Patel, and R.F. Ismagilov, *Dynamics of Drosophila Embryonic Patterning Network Perturbed In Space and Time Using Microfluidics*. Nature, 2005, **434**: 1134-1138.
39. Dam, R.M., *Solvent-resistant elastomeric microfluidic devices and applications*, in *Applied Physics*. 2006, California Insitute of Technology.
40. Lee, J.N., C. Park, and G.M. Whitesides, *Solvent Compatibility of Poly(dimethylsiloxane)-Based Microfluidic Devices*. Anal. Chem., 2003, **75**: 6544-6554.
41. Jensen, K.F., *Microreaction engineering - is small better?* Chemical Engineering Science, 2001, **56**(2): 293-303.
42. Kanai, M., D. Uchida, S. Sugiura, Y. Shiraski, J.S. Go, H. Nakanishi, T. Funatsu, and S. Shoji. *PDMS microfluidic devices with PTFE passivated channels*. in *Miniaturized Chemical and Biochemical Analysis Systems*. 2003.
43. Natali, M., S. Begolo, T. Carofiglio, and G. Mistura, *Rapid prototyping of multilayer thiolene microfluidic chips by photopolymerization and transfer lamination*. Lab on a Chip, 2008, **8**(3): 492-494.
44. Lopez-Garcia, M.D.C., D.J. Beebe, and W.C. Crone, *Characterization of poly(isobornyl acrylate) as a construction material for microfluidic applications*. Journal of Applied Polymer Science, 2007, **105**(4): 1894-1902.
45. Grover, W.H., M.G. von Muhlen, and S.R. Manalis, *Teflon films for chemically-inert microfluidic valves and pumps*. Lab on a Chip, 2008, **8**(6): 913-918.