Parametric Analysis of Wax Printing Technique for fabricating Microfluidic Paper Based Analytic Devices (µPADs)

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of MS Design and Manufacturing Engineering

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MASTER'S THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by **Muhammad Younas** (NUST201463082MSMME62014F) Titled: "**Parametric Analysis of Wax Printing** Technique for **Fabricating Microfluidic Paper Based Analytical Devices**" be accepted in partial fulfillment of the requirements for the award of **MS Design and Manufacturing** Engineering degree with (A grade).

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Date: 24 Aug 2016

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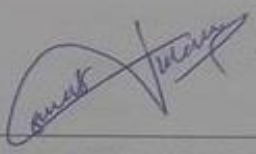
DECLARATION

I certify that this research work titled "*Parametric Analysis of Wax Printing Technique for fabricating Microfluidic Paper Based Analytic Devices (μPADs)*" is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources it has been properly acknowledged / referred.



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DEDICATIONS

To my loving parents and family…..

ABSTRACT

This study focuses on the detailed parametric analysis of wax printing technique for producing a paper-based microfluidic analytical device. The study of the important parameters for wax printing technique has been experimentally carried out. The simple process includes printing a pattern using wax based printer and melting the desired paper within the paper network producing a hydrophobic and hydrophilic network. The produced network confines the flow of fluid to the desired detection zones. A generalized model accounting for the spreading of molten wax within the porous paper material is derived and results obtained are compared experimentally and with the previously used Washburn's equation. The deviation of the Washburn's equation is due to the several assumptions made during the wax spreading in the paper. Most importantly, the derived analytical model is well validated by these experiments. The developed model shows improved results of the actual wax spreading and sufficiently gives the final width of the hydrophobic barrier accounting for varying printing widths, different working temperatures and time required for spreading of the molten wax within the paper network.

Table of contents

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

L	Average wax spreading	F_{HC}	Final Hydrophilic channel width
I	Width of printed line	I_{HC}	Initial Hydrophilic channel width
T	Temperature	D	Pore size
T	Time	γ	Surface tension
η	Viscosity	μm	Micrometer
µPAD	Microfluidic Paper based Analytical Device	SEM	Scanning Electron Microscopy

CHAPTER 1

INTRODUCTION

1.1.Motivation

Microfluidic devices have become a research area of interest for the application of detection and diagnostic purposes in low-resource areas. Microfluidic devices typically include a network of microchannel where fluids are transported and processed, and are often produced on the silicone-based polymer, PDMS [1]. The effectiveness of microfluidic devices in low-resource areas has provoked the World Health Organization to establish guidelines for developing microfluidic diagnostic devices. These guidelines are referred to as the ASSURED principles, indicating that microfluidic devices should aim to be Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end users.

Paper as a material is easy to stack, package, and store since it lays flat. Specificity and selectivity can be enabled with chemical reactions pre-programmed onto the paper, which involves patterning the paper and the placement of the reagents to control the reaction as the fluid wicks through the paper. Paper-based microfluidic devices are typically user-friendly, as they eliminate the need for sample treatment or fluid manipulation, and can provide a simple colorimetric signal readout. Rapidness and robustness will depend on the design details, such as reaction chemistry and the physical design of the device. The filter paper offers the additional benefit of being compatible with a variety of chemicals and biomaterials, as well as chemical treatments such as nitration. In this way, paper is an ideal platform for microfluidics and provides versatility in its physical and chemical flexibility.

In order to design an ASSURED paper-based microfluidic analytical device, a mathematical model is required to predict accurately the wax spreading in the paper such that fluid flow within the paper network can be guided accordingly. Previously models based on the well-established Washburn equation for capillary flow for flow in porous media have been developed to predict the

wax spreading process in paper. The structure of paper on the micro scale is complex, as it is classified as a porous medium as well as a fibrous medium, and the fluid transport mechanism has been identified as capillary-driven flow. Since the structure of the fibrous matrix in paper is highly complex, simplifying approximations are made at the expense of accuracy and precision. To apply the Washburn equation, the paper fiber structure is approximated as a bundle of capillaries created by an alignment of pores. However, this assumption is challenged by the ambiguity around selecting a representative pore size as the capillary radius.

Whereas, on microscale paper has a highly complex fibrous structure with interfiber pores and intra-fiber pores exhibiting a large pore size distribution within the paper surface [2] With this observation, it becomes counter-intuitive to assume that pores in paper would form continuous capillaries for fluid to flow through, compounded with the additional complication of identifying an appropriate representation for capillary size when such a large pore size distribution exists. Additionally, the influence of paper fiber effects, such as swelling and deformation, are not addressed in this model. Though the presence capillary forces are addressed in the Washburn model, it is challenging to determine an appropriate capillary size and resistances caused by the fibrous network are not addressed. While experimental studies for imbibition in paper have been unable to generate accurate predictions using the Washburn equation Here the concept of permeability attempts to encompass some of the complexities of flow through the fibrous paper network. Since permeability is an empirically determined parameter, it provides the versatility to incorporate a variety of parameters as an understanding of their impacts are developed. As such, a working model for fluid flow through paper-based microfluidic devices has yet to be established, but can be developed once a deeper understanding is acquired of the parameters that influence fluid flow in paper-based microfluidic devices.

In summary, the motivation for this thesis is the need for an understanding of wax spreading through the paper to assist in the design and application of an accurate paper-based analytical devices. The findings of this thesis can then be extended to paper-based microfluidic devices in a variety of applications.

1.2.Background

Whiteside's and coworkers [3-6] first proposed a diagnostics platform using paper microfluidics to perform multiplexed assays for on-site analysis. In their study, the paper was patterned with PDMS, photoresist or inkjet plotter to form hydrophobic barriers within the paper network. So far, various techniques used for patterning paper includes wax printing[7, 8] wax screen printing[9, 10], paper cutting[11], inkjet printing[12-14] photolithography[3], wax dipping[15], analogue plotting[4], etching[5], plasma treatment[16, 17] and laser treatment[18]. The hydrophilic-hydrophobic microchannel contrast of the paper network makes these devices suitable for handling small volumes of fluid for quantitative analysis of many potential applications including healthcare, medicine, quality control and environmental monitoring.

Carrilho *et al.* [8] introduced an inexpensive, fast and efficient process of wax printing for patterning the paper and producing a microfluidic device in less than 5 minutes. A simple equation based on well-developed Washburn's equation was presented to predict the wax spreading L and width of the hydrophobic barriers (W_B) for initially printed line (W_P).

$$W_B = W_P + 2L \qquad \textbf{(1)}$$

$$L = (\gamma D t / 4\eta)^{1/2} \qquad \textbf{(2)}$$

Washburn's equation[19] was used to predict the wax spreading (L) within the paper network. The formula conditions that the wax spreading is proportional to square root of time. Renault *et al*. [20] studied wax transport in Whatman Grade 1 and 3MM chromatography paper and the resulting final width of the barrier was predicted by using Washburn's equation. The heating temperature was set to 120C for 10 minutes for all the devices fabricated and compared the results experimentally. Zhong *et al.* [21] also investigated different paper material and wax types for fabricating optimal μPAD device and applied Washburn's equation to study the mechanics of fluid flow in the hydrophilic channel. Lu *et al.* [7] also used three different methods to pattern paper using wax and performed bioassays. The resulting width of the final

hydrophobic barrier was estimated from the difference between original printed line and the final spread line after heating step.

1.3.Microfluidic Paper-Based Analytical Devices

Paper-based microfluidic analytical devices also known as (μPADs) have significant potential for use in diverse application areas including point of care diagnostics, environmental regulations and food quality monitoring [22]. In recent years, μPADs have emerged as a research area of interest for detection and diagnosis in many applications because of their simple fabrication, low cost and flexibility and its functioning without adding an external source [23, 24]. Properties like good biological compatibility, strong capillarity, ease of fabrication and cheap availability of μPADs make them unique compared to conventional devices made of polymer and glass substrates[25].

Proper prevention and treatment of a disease require accurate diagnosis devices, but diagnostics devices used in developed countries are often difficult to use in underdeveloped countries due to high cost of the diagnostics, the sophistication of the technology and the lack of well-trained personnel. According to the World Health Organization, diagnostic devices for developing countries should be ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and deliverable to end-users.

Microfluidic paper-based devices often known as μPADs have serious potential to offer ASSURED diagnostics Here sensitivity is defined as avoiding false negative results, and specificity refers to avoiding false positive results [22]. According to these guidelines, paper is inherently advantageous as a platform for microfluidic devices. Not only is it an affordable and accessible material, but the wicking mechanism in paper also allows for the passive transport of fluids, eliminating the need for auxiliary equipment.

In summary, recent developments in different features of wax printed μPADs, and the applications for low-resource areas have demonstrated opportunities where a mathematical model that can effectively predict wax spreading behavior through paper devices would benefit the μPAD design process made by wax printing technique. A successful mathematical model will address the complexity of wax spreading and the impact of process parameters on the final achieved barriers.

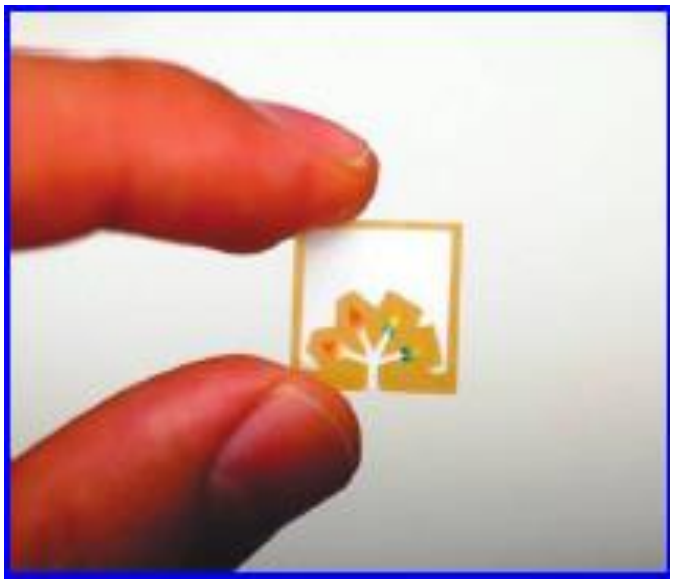


Figure 1. Feature summarizing Paper Microfluidic device [17]

1.4. Choice of paper

Paper is one of the good media for fluid transport, owing to the capillarity of the paper medium different types of paper have been used so far in microfluidic applications. The choice of paper also varies with the method used for fabrication and the particular application of the microfluidic device. Whatman no.1 filter paper having a pore size of 11 μM is most frequently used as a paper platform in many applications of choice due to the medium retention and flow rate. Thus it is also referred to as the standard grade filter paper[22]. A larger pore size 20 – 25 μM and hence the large surface area of Whatman Grade 4 filter paper has faster flow rate than Whatman Grade 1. When choosing a paper type, the flow rate is one of the important parameters to look after, with pore size and pore distribution which affect the retention time [26].

Also, the colour of the paper is very important for on-site colorimetric detection while performing image analysis the ideal colour of the paper must be white. However, the colour of the paper may change with age and humidity where paper may turn to yellow/brown [26].

Figure 2. SEM image of filter paper [26]

Grade 1 Filter Paper can be generally described as:

- A smooth surface, 0.18 mm thick
- with a linear flow rate (water) of 130 mm/30 min.
- Pore size of 11 microns.

1.5.Fabrication techniques

A number of fabrication techniques have been used in the recent years to fabricate paper based Microfluidic devices. Each method has its own limitations and advantages, but wax printing is observed to be the most effective way to fabricate paper-based devices. The table below summarizes the different methods used for patterning of the paper surface.

The natural ability of paper to wick fluids through capillary action provides a platform for microfluidic application of paper [22, 27, 28]. The fluid flow in all directions if no barrier is

provided in the surface. However, researchers have demonstrated the use of different kinds of barriers within the paper surface by selectively creating hydrophobic areas (restrictions/barriers) and hydrophilic areas (channels), thus directing the flow of fluid to the desired test zones in a chosen direction. There have been a lot of variations in the fabrication of these device since Whiteside's introduced the concept of paper microfluidics. However, the most common methods of patterning include wax printing, inkjet printing, screen printing, and photolithography. Liquid penetration pathways in these methods can be defined either physically or chemically [14].

The following fabrication methods have been published to date that pattern the paper such as:

1.5.1. Wax Printing

Paper is selectively hydrophobized by the deposition of wax on the paper surface and allowing it on a hot surface to melt completely. The fabricated devices have been used for simultaneous detection of protein and glucose in human urine [29].

1.5.2. Ink Jet Printing

This method uses Alkyl Ketene Dimers to chemically modify the paper surface and create hydrophobic-hydrophilic channel contrast. Microfluidic devices fabricated by this method has shown great promise in many potential applications and have been successful for the detection of nitrogen dioxide.

1.5.3. Photolithography

Paper surface is patterned using positive or negative photoresists to block the pores in the paper and create fluidic channels [5, 30]. Paper devices made by this technique have also shown for the application of detecting glucose and protein in a human urine sample.

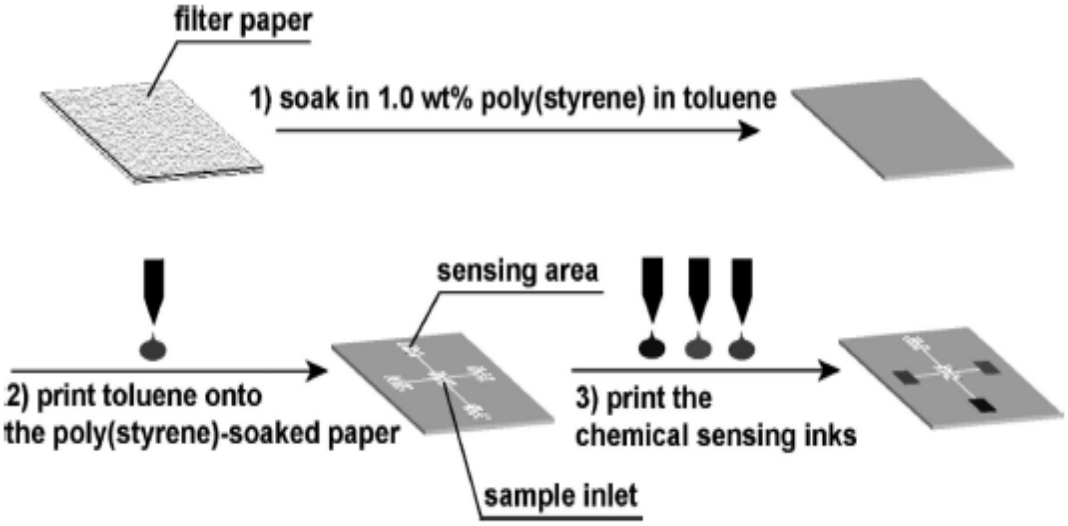


Figure 3. schematic representation of inkjet printed fabrication method [11]

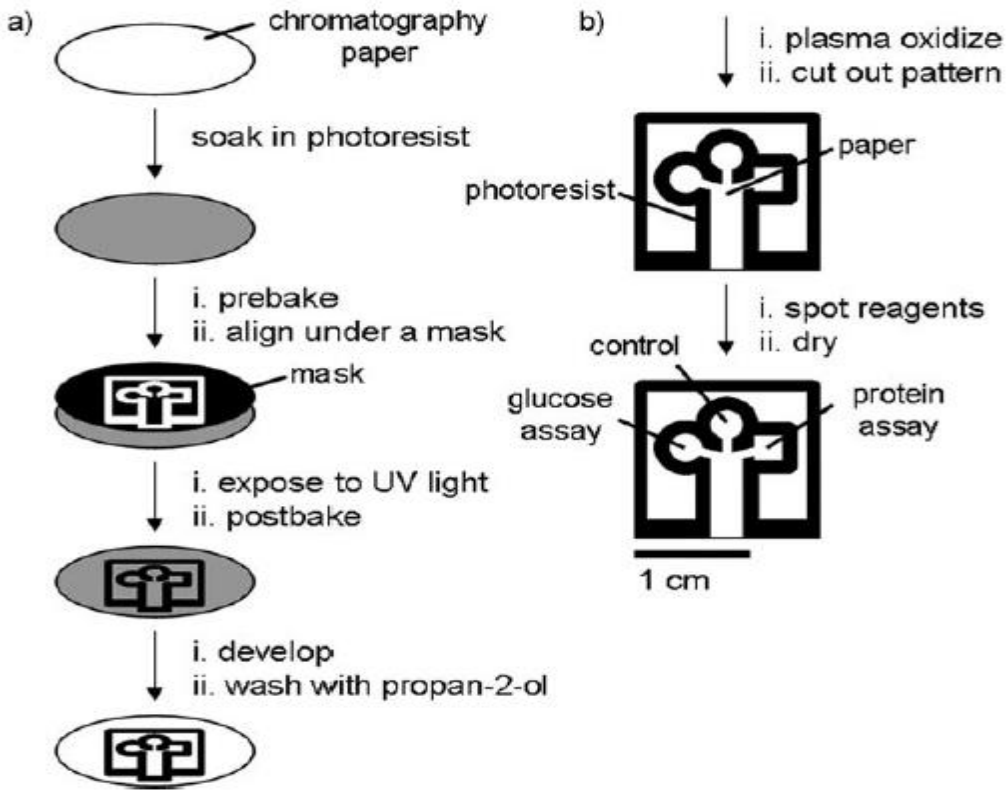


Figure 4. Photolithography method used for fabrication of paper-based device [17]

1.5.4. Analogue Plotting

Analogue plotter was used to make physical restrictions and produce fluid flow channels by using PDMS to block pores in paper. The device fabricated has been used for the detection of albumin in human serum samples [4, 31].

1.5.5. Ink Jet Etching

This is a physical deposition method of fabricating paper devices, this method uses polystyrene and the channels are made by hydrophobizing and dehydrophobizing of the paper surface. Detection of glucose in human urine samples have been demonstrated by this method [32].

1.5.6. Plasma Treatment

The paper sizing agent commonly used in paper making industry, alkyl ketene dimer (AKD), a cellulose reactive agent is used in fabrication of the devices. The devices fabricated in this way has also shown many potential applications in detection and analysis of many analytes [16, 17].

1.5.7. Laser Treatment

In this method the pores in the paper surface are blocked by hydrophobizing followed by selective dehydrophobization [33]. the devices fabricated have been successfully used for the detection of hemoglobin levels via a luminal reaction and chemiluminescence

1.5.8. Screen Printing

Screen printing method reported for the detection of bicinchoninic acid uses a local screen of a fine mesh size to physically deposit wax on the surface of paper and create zones for analytical applications[34, 35]

1.6. Wax printing method

Among all other techniques for patterning paper, we have used wax printing for patterning paper because wax printing is a simple and preferred technique for patterning the paper using solid ink printer and a hot plate as the process is accomplished without the use of clean room, UV lamps, organic solvent or other sophisticated instrumentation[36]. A desired pattern is printed on the paper surface and then placed on hot plate so as to spread the printed wax pattern in the paper network, thus resulting hydrophobic barriers and hydrophilic channels within the paper. Wax spreading in paper is faster in horizontal direction producing a wider line compared to the original printed line[8]. Therefore, the width of the initial printed line, the time needed for heating and the heating temperature has an important impact on the reproducibility and effectiveness of the device fabricated. The resolution of the device and the resulting channels and barriers dimensions have an important impact on the effectiveness of μPAD. So an accurate pattering of paper will ensure the best functioning of device. Paper porous medium is highly attractive for microfluidic applications and allows liquid to flow in the fiber matrix under capillary action[18]. The type of paper being used will affect the wax spreading as the pore size and porosity of the paper changes the flow of molten wax within the paper changes.[12]

1.6.1. Study of wax spreading in paper

The study of wax spreading is highly important for estimating the hydrophobic barriers and hydrophilic channel calculation. In order to achieve an accurate size of the channel the wax spreading must be analyzed and controlled so that we achieve the final dimension for the effectiveness and functionality of the device. Here a time series study of the wax spreading has been introduced, based on this analysis one can pre calculate the final required channel dimensions

1.6.2. key parameters of wax printing

The spreading of wax in paper may be impacted by several physical and chemical parameters related to the wax properties, paper properties, and surrounding conditions. The important process parameters of the wax spreading process in paper include the initial printed line, the heating temperature and the melting time. Experimental observations and analysis of the influence of these parameters will provide a foundation for the development of a mathematical model that can accurately predict wax spreading behavior in a paper-based microfluidic device. Parameters that demonstrate a significant impact on spreading behavior will be built into an expression for predicting the actual wax spreading within the paper network.

1.7.Applications

Currently, the majority of the devices made by wax printing and inkjet printing have shown great promise in many potential applications. However, other reported methods are also under development to be fully used for analytical purposes. Mostly the micro pad has been used for colorimetric detection of protein, glucose, and blood.

1.8.Properties of Solid Ink

Table 1. Properties of solid ink

Property	Density (kg/m^3)	Specific heat (kJ/ (kg. K))	Thermal conductivity (W/ (m.K))	Solidus temperature (^oC)	Liquidus temperature (^oC)
ColorStix 8200	820	2.25	0.18	60	90

1.9. Flow control in µPADs

Flow control in the microchannels is a very important aspect for the proper functioning of the microfluidic devices. Conventional microfluidic devices use pumps to control the flow of fluid within the channels and the theories and concepts regarding flow are well developed and can be best explained by fluid dynamics and hydraulics whereas, the flow control and the study of fluid flow in microchannels within the paper-based devices need innovation and best understanding the theories. Finally, the ability to control the flow of liquids and to control the flow direction and time is considered as the most important factor to produce powerful analytical device.

A typical method to control liquid flow speed is to change the hydrophilic channels length and width[37]. However, there is an upper limit to the size of a µPAD, especially when volumes of sample are limited. So some improvements are developed including cutting some paper of the channel to change its geometry and then adjusting the flow speed, building soluble flow barrier on the channel to change the time delay of liquid, and creating flow resistance on the channel to decelerate the speed.

Noh *et al.* [38] proposed an alternating method for controlling the flow rate by modulating the paraffin wax on the paper to regulate the wetting properties of the paper. It is capable of controlling the flow of fluids to detection zones with precise time delays of 6% of the total wicking time. In recent times with nearly the same idea, controlling the flow speed by varying paper permeability is reported by Jang *et al.* As the paper permeability can be adjusted by controlling the brightness of wax patterns in wax printing, this method is simpler to be implemented.

Parameters that affect the flow speed include the size of the hydrophilic channel, porosity of the paper, its permeability as well as the reagent properties. Up to now, theory is far less well-understood about the flow in microporous channels of µPAD. Some summaries of the recent theory studies of wicking in the porous matter can be found[39]. In the future, improving the controllability of liquid in the µPAD needs to be performed both in inventive design and theoretical study

1.10. Scope of the thesis

To design a Highly reliable, effective and accurate paper-based analytical device using wax printing technique, a mathematical model is needed to predict wax spreading behavior in paper. The paper is a complex medium due to its fiber network structure, and the parameters that influence the wax flow through this fibrous medium are not well understood. Considering these points, this thesis has two main objectives.

- To develop a comprehensive understanding of the parameters that influence wax spreading process in a paper porous medium, with particular emphasis on the impact of the change in temperature and initial printed line on final resulting width of the line through experimentation.

- To develop a mathematical model that accurately describes the wax spreading process in a porous medium and can predict the actual wax spreading by setting the parameters in the model.

CHAPTER 2

EXPERIMENTAL METHOD

This chapter introduces the complete experimental methodology used for fabrication of the paper-based microfluidic devices by a wax printing technique. The method of measuring and analyzing wax spreading has been introduced and the design of experiments has been presented systematically in this chapter.

2.1.Overview

The Experimental apparatus used in the study are shown in Figure 1. It consists of a wax printer, a hot plate, and the patterning tool. The patterns were designed in Microsoft Word and were printed using Xerox Solid Ink printer. The printer paper was then melted using an analog hot plate. The temperature of the hot plate was maintained using an Infrared thermometer. The change in the wax spreading before and after the melting was studied in an optical microscope with a built-in camera installed.

Figure 5. Wax printing method overview

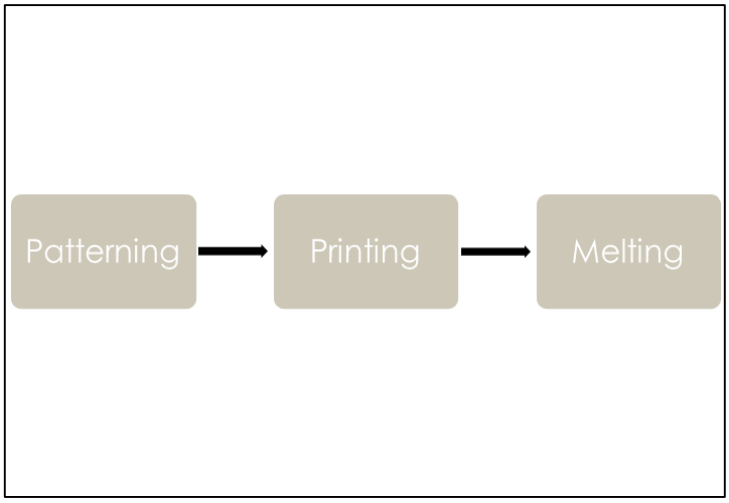


Figure 6. Sequence of the process.

2.2.Materials and Equipment

Whatman Grade 1. Filter paper available in sheets of 46cm × 570cm was chosen as a substrate material for fabricating μPAD. We subsequently cut the paper into four sheets of 215mm × 280mm, the A4 sized filter paper and fed directly to the wax printer. Different patterns made in Microsoft word were printed onto the paper surface using Xerox ColorQube 8580 printer. To provide a uniformly heated surface, we used an analog hot plate and a constant temperature of the plate was maintained using IR thermometer. The thickness of lines before and after spreading was recorded, measured and analyzed using an optical microscope installed with the camera. The measuring tool was calibrated for appropriate magnification of 5X and scaled to 500 μm.

2.3.Measurement Devices

2.3.1. Infrared Thermometer: To ensure the proper temperature requirement an IR thermometer was used.

2.3.2. Hot plate: An analog plate having a temperature range up to 350 ºC was used for the melting of printed patterns.

2.3.3. Microscope: An optical Microscope was used to study the wax spreading. It was calibrated to 5 X, and a scale of 500 µm was used for all the measurements.

2.4.Experimental Procedure

Experimental setup shown in the figure. 7 include a hot plate, an Infra-red thermometer, an optical microscope installed with the camera, measurement and analysis tool and other accessories used during the experimentation of this work.

Figure 7. An overview of the Experimental setup

2.4.1. Designing pattern

Different pattern shapes were designed in Microsoft Word for this study. The thickness of the lines was studied in a range of 100 to 900 micrometer for the complete spreading time.

2.4.2. Fabrication of paper device

The Designed Patterns were printed using solid ink printer which utilizes wax based ink to print. The printer deposits the wax on the surface of filter paper which is then placed on a hot plate at a certain temperature for the study of spreading phenomena.

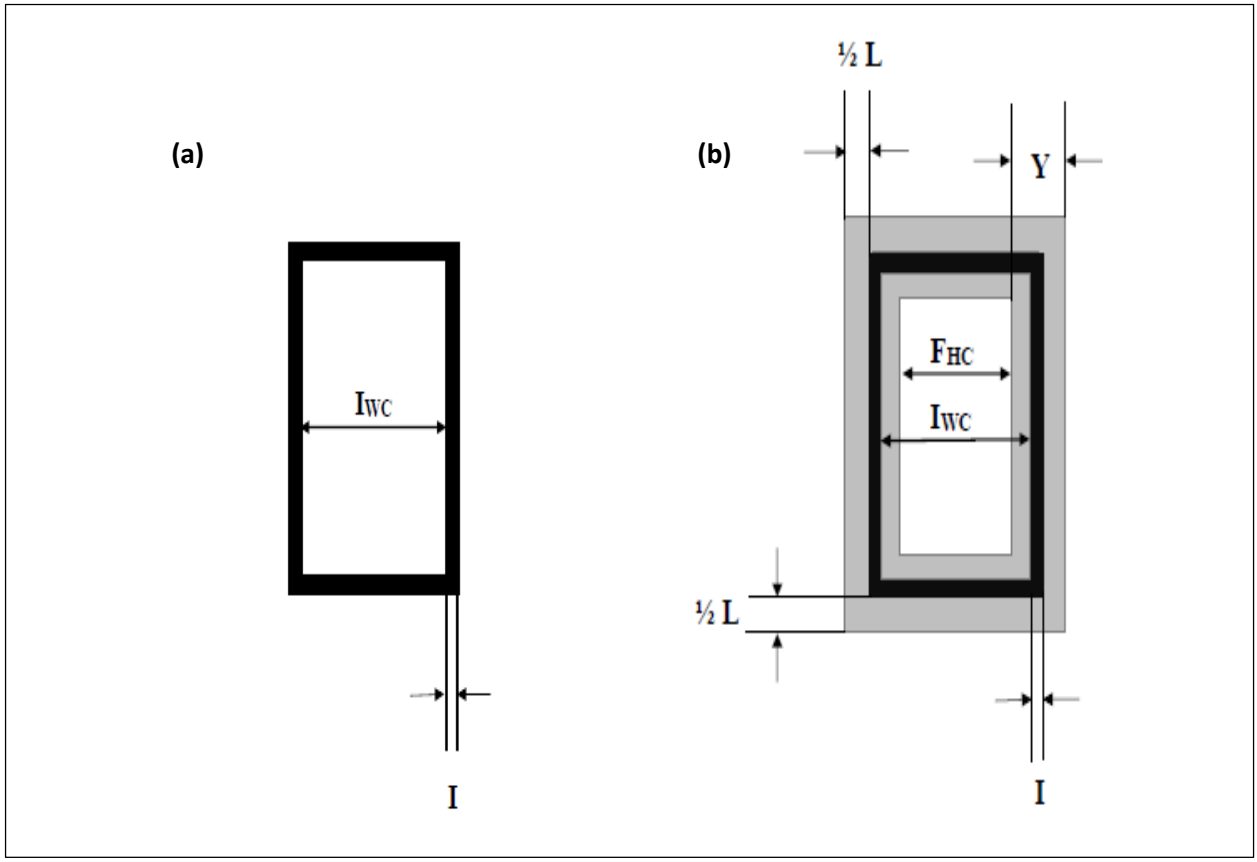


Figure 8. Simple pattern printed on paper. (a) Printed pattern before Spreading (b) pattern after spreading

The representation of the symbol used in figure 4. are as follow.

I initial width of printed line

I_{wc} Initial Channel width

L Average wax spreading in paper

Y Predicted wax spreading (hydrophobic barrier)

F_{Hc} Final width of the hydrophilic channel

Figure. 9 a wax pattern deposited on porous paper medium the three-dimensional spreading is shown. The black regions show the spreading through the depth of paper whereas, the grey portion show the spreading on the surface. The hydrophobic and hydrophilic channel are identified as well.

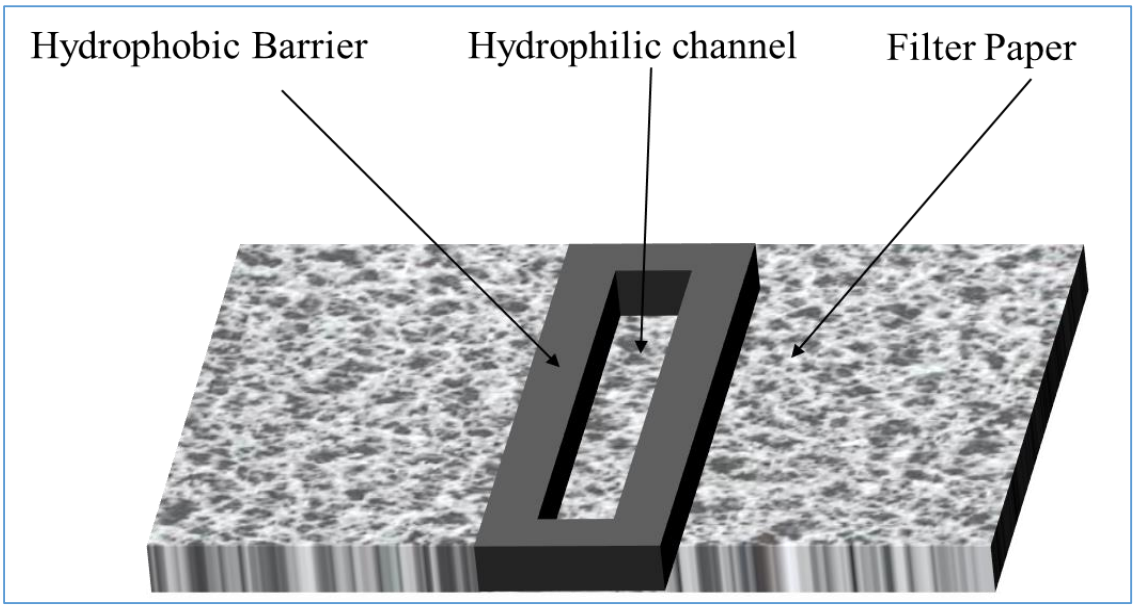


Figure 9. Three-dimensional Schematic representation of wax spreading in paper

2.4.3. Process flow chart

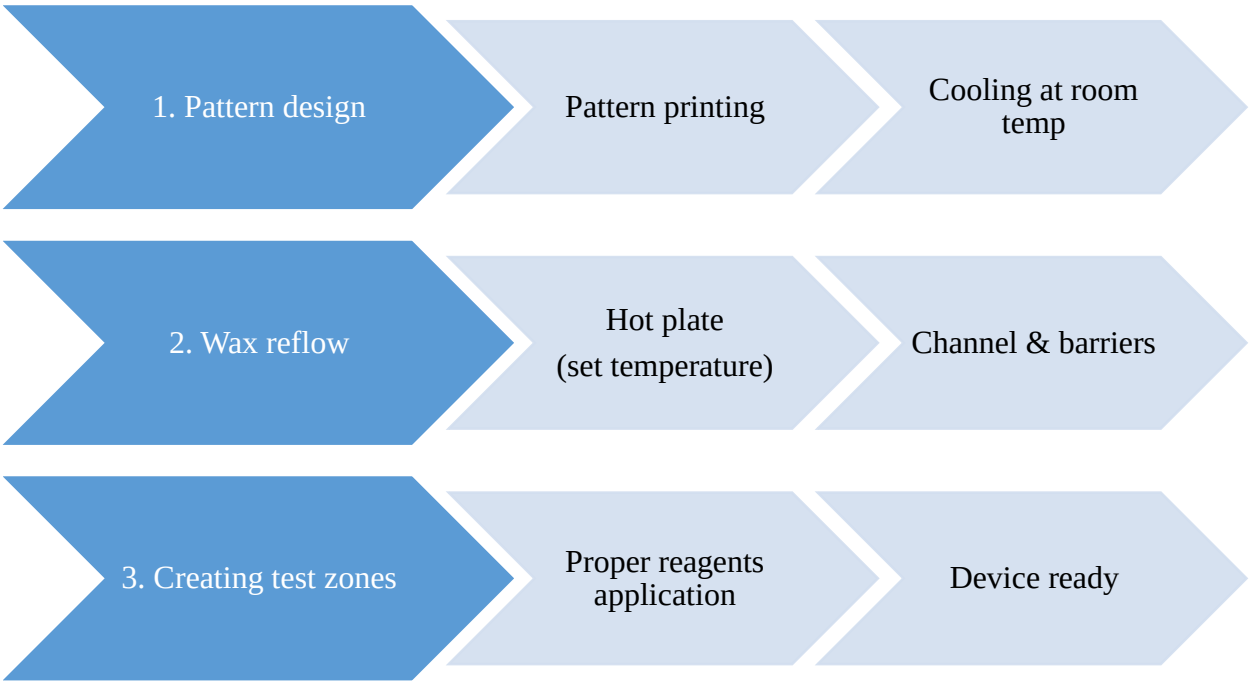


Figure 10. Flow chart of the wax printed fabrication method

2.4.4. Measurement of wax spreading

The printed lines after placed on hot plate were studied after each five seconds to observe the change in the width of the line till all the initially deposited wax spread completely in the paper. The wax spreading was observed in three different passions. First, the effect of the change in temperature on the final width of the hydrophobic barrier was observed for a complete range of printed lines. Then in a second attempt, these lines were also studied for the time required to complete the spreading at different temperatures. Finally, the effect of a change in the width of the printed lines on the final resulting barrier was studied for the given printed lines. The results were

recorded and a linear regression model was fitted for all the data points using data analysis software Minitab. Figure 1. show the actual process of measuring the line width before and after heating step.

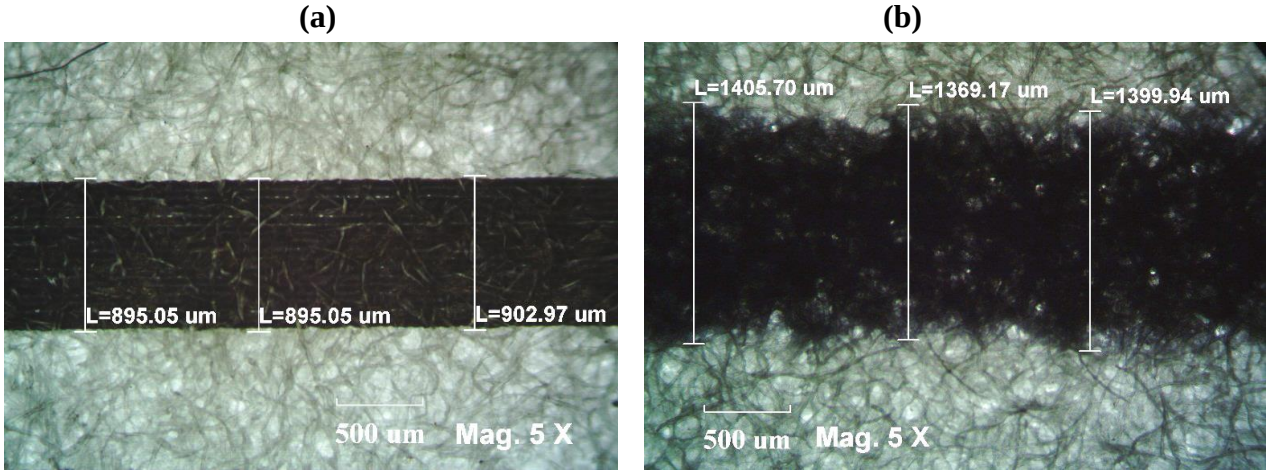


Figure 11. Images of Whatman grade 1 filter paper fabricated by wax printing (a) Wax printed line before spreading, (b) Spreading of wax after heating (captured under microscope at magnification 5 X).

2.5.Design of Experiments

A series of lines of different widths ranging from 100 μm to 900 μm were printed on filter paper, and the resulting final width of the lines was investigated at four different temperatures i.e. 90 ºC, 110 ºC, 130 ºC and 150 ºC. The patterns were designed using Microsoft Word 2016 and the width of the initial printed lines and final spread were observed in camera using an optical microscope. The wax spreading for all temperature sets were measured after each five seconds until complete spreading of the initial printed wax on paper. Table 1. Shows the range of values at which the experiments were performed.

Table 2. Range of selected parameters

Sr. #	Parameters	Range/Value
1	Initial printed width	100 μm, 200 μm, 300 μm, 400 μm, 500 μm, 600 μm, 700 μm, 800 μm, 900 μm.
2	Time	Reading after every 5 seconds till stability.
3	Temperature	90 °C, 110 °C, 130 °C, 150 °C.

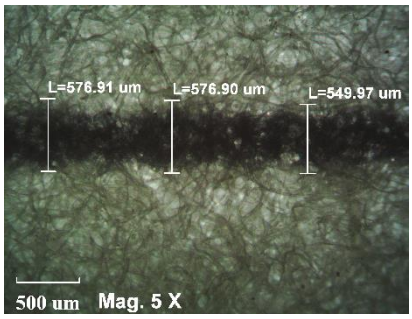
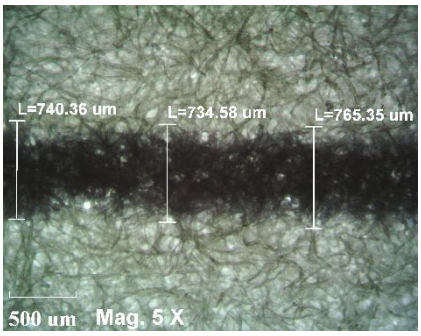
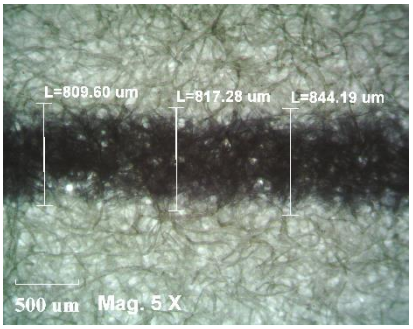
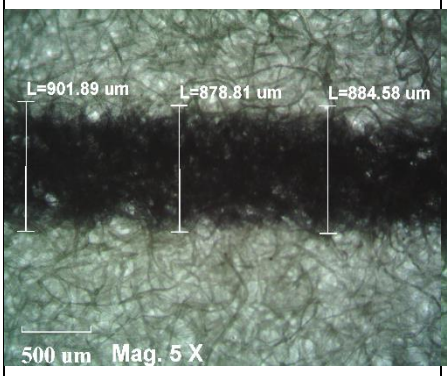
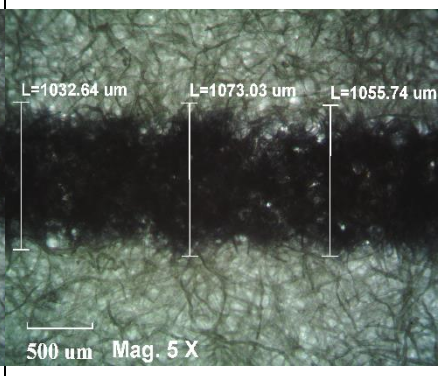
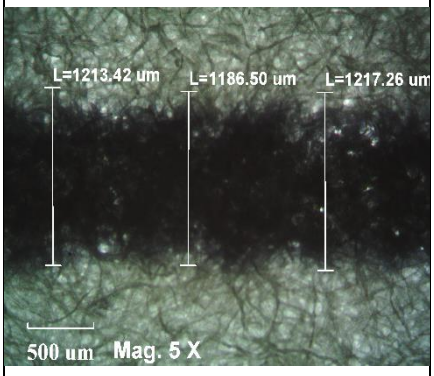
CHAPTER 3

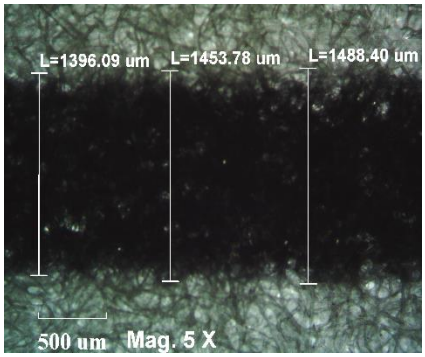
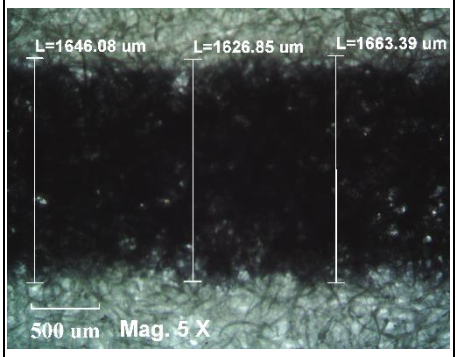
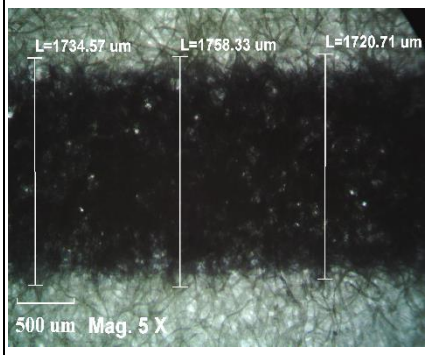
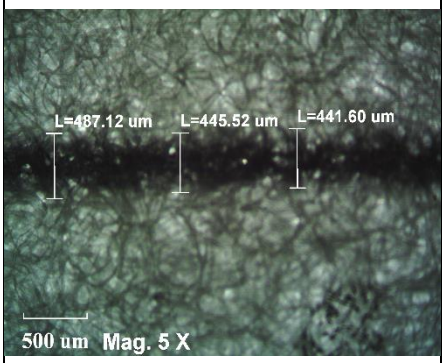
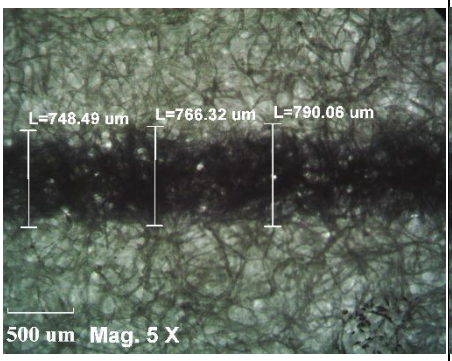
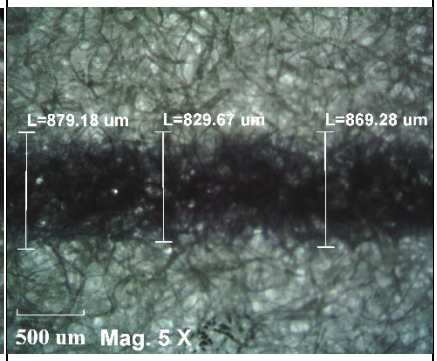
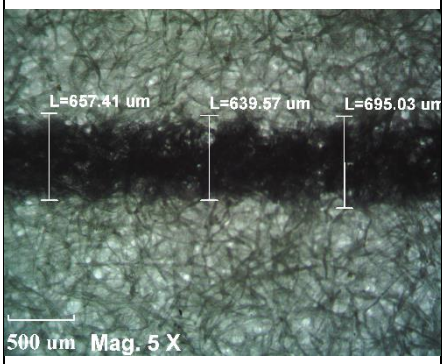
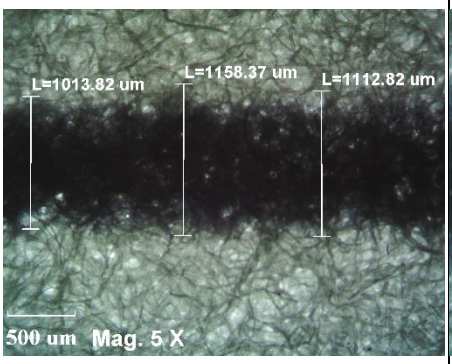
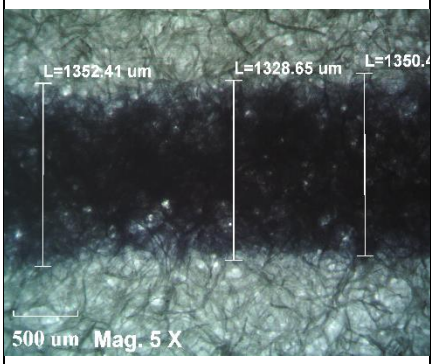
RESULTS AND DISCUSSION

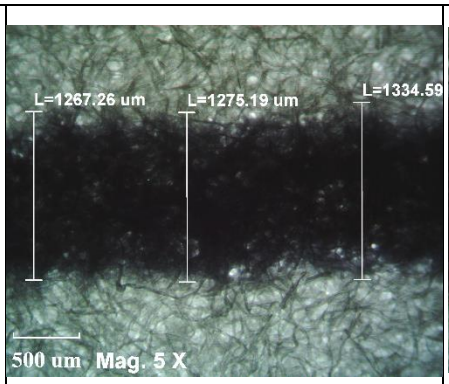
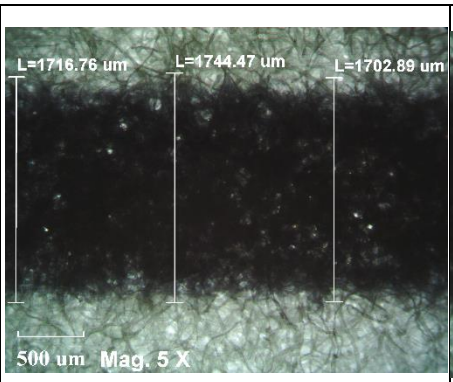
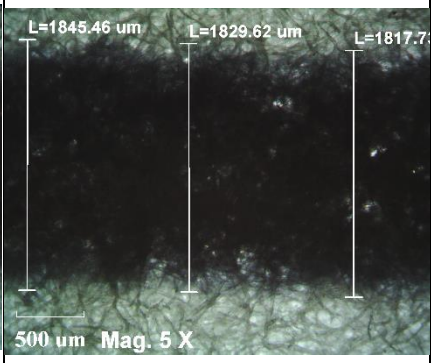
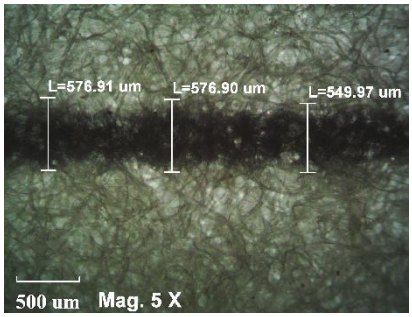
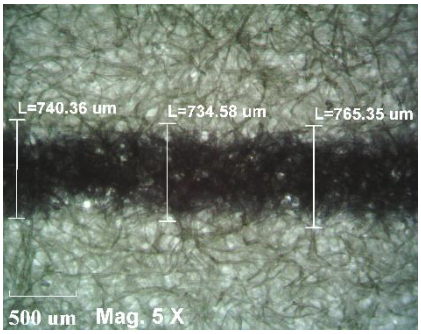
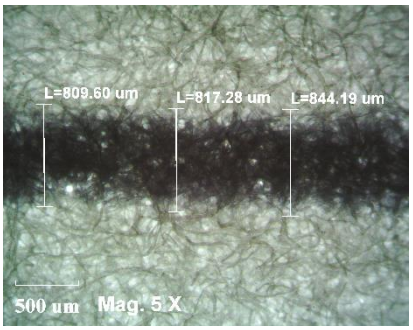
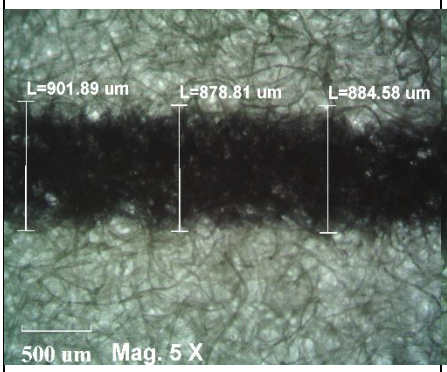
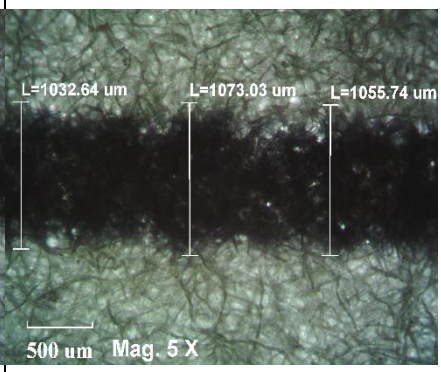
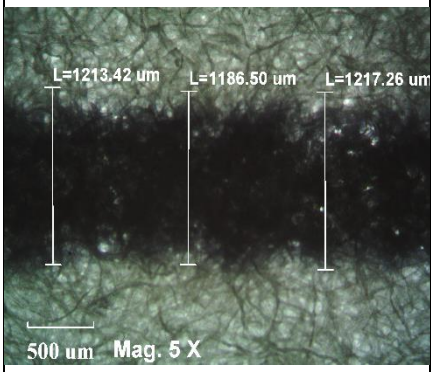
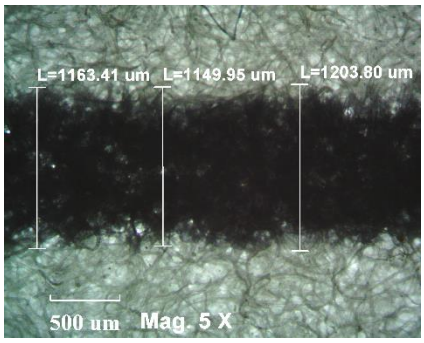
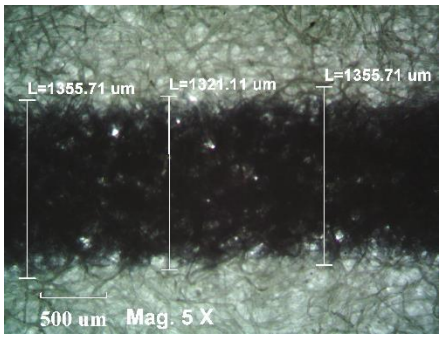
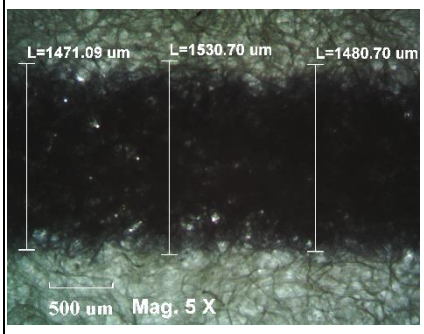
In this chapter, experimental results for parameters that influence wax spreading in paper network are presented and discussed. The parameters investigated include the initial printed line width, the heating temperature and the melting time required for complete spreading of wax.**Microscopic images of the wax spreading**

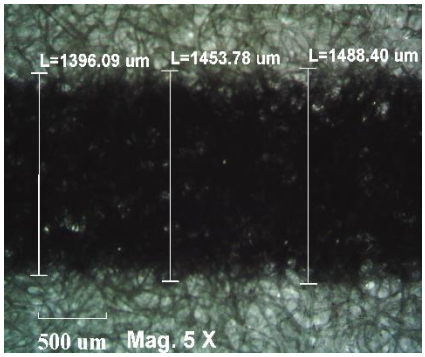
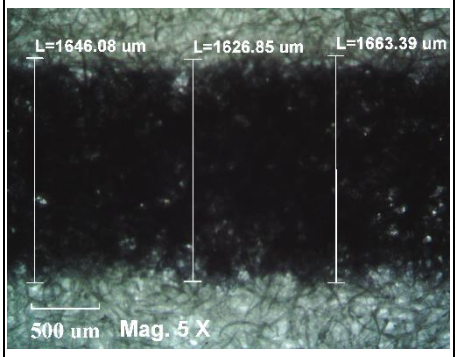
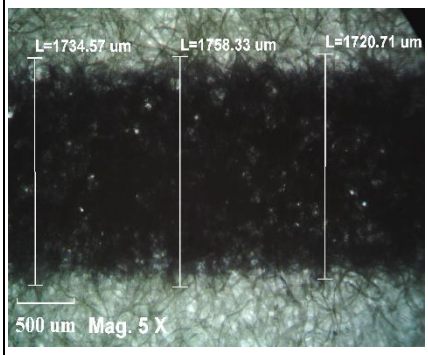
The paper porous medium was investigated on micro scale for the proper measurements. Table 3 show the micrographs of the change in the average width of the lines at different times. These images were recorded and analyzed at four different temperatures.

Table 3. Micrographs showing average change in the line width at different melting time

Line width	Change in width after 5sec	Change in width after 35sec	Change in width after 70 secs
100 at 150 C			
300			

900			
Line width	5sec	100 secs	200 secs
100 at 90C			
300			

900			
Line width	Change in width after 5sec	Change in width after 35sec	Change in width after 70 secs
100 µm at 130 °C			
300 µm			
600 µm			

900 µm			

3.2.SEM Images of paper:

SEM images of the Grade 1 filter paper were taken to observe the flow of molten wax within the capillaries. The micrographs suggested an uneven distribution of the pores within the paper and hence arise questions about the applicability of the Washburn's equation. To apply the Washburn equation, the paper fiber structure is approached as a bundle of capillaries formed by an alignment of pores. Though, this assumption is challenged by the ambiguity around selecting a representative pore size of the paper as the capillary radius. Figure 6 show the SEM micrograph of the filter paper presenting a large pore size distribution. A key observation from the SEM micrograph is that the micro-structure of paper cannot accurately be represented by the bundle of cylindrical capillaries approximation, especially considering the practical challenge of selecting an appropriate representation of capillary radius. With this observation, it becomes counter-intuitive to assume that pores in paper would form continuous capillaries for fluid to flow through. Secondly, the equation applies to capillary flow where the unlimited volume of the fluid reservoir is available whereas, in wax printing, a limited amount is deposited on the paper. Finally, the equation applies to constant pressure process unlike capillary driven process in the paper porous medium.

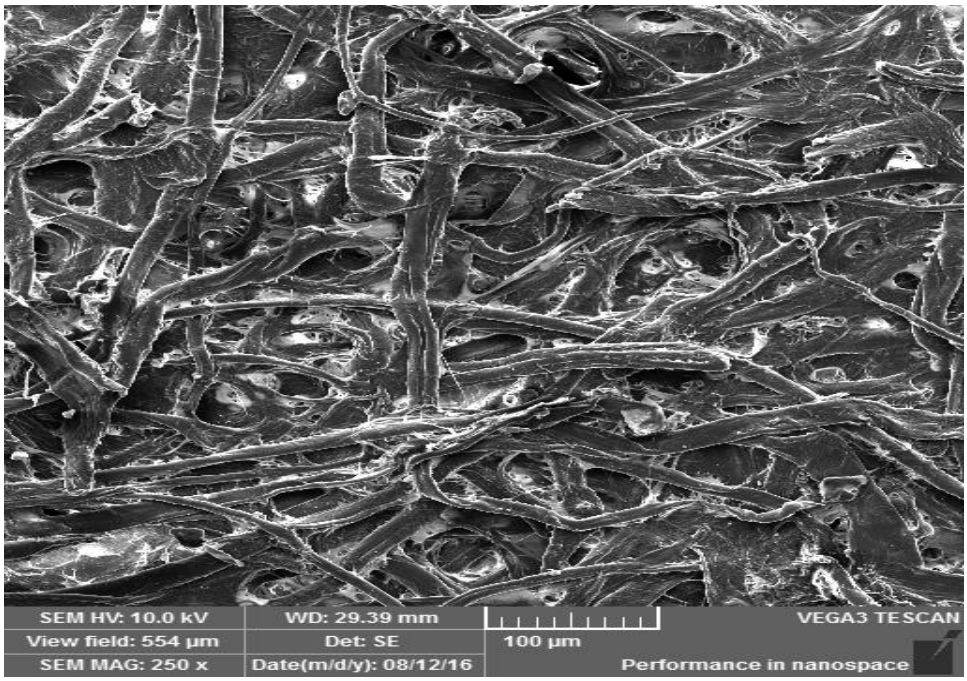


Figure 12. SEM Micrograph showing the pores and fibers arrangement in Grade 1 filter paper

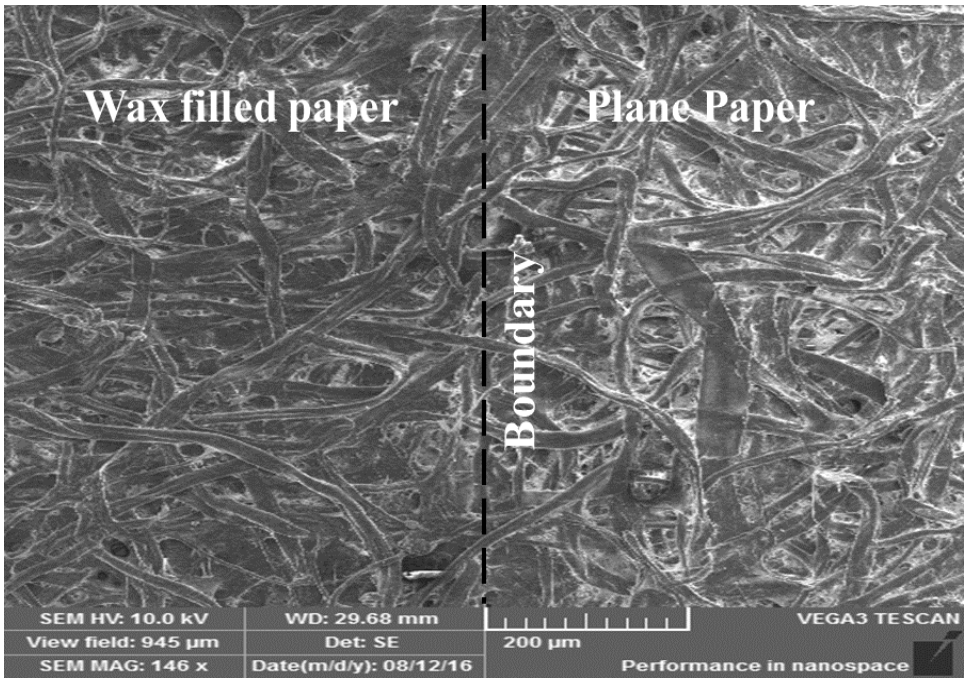
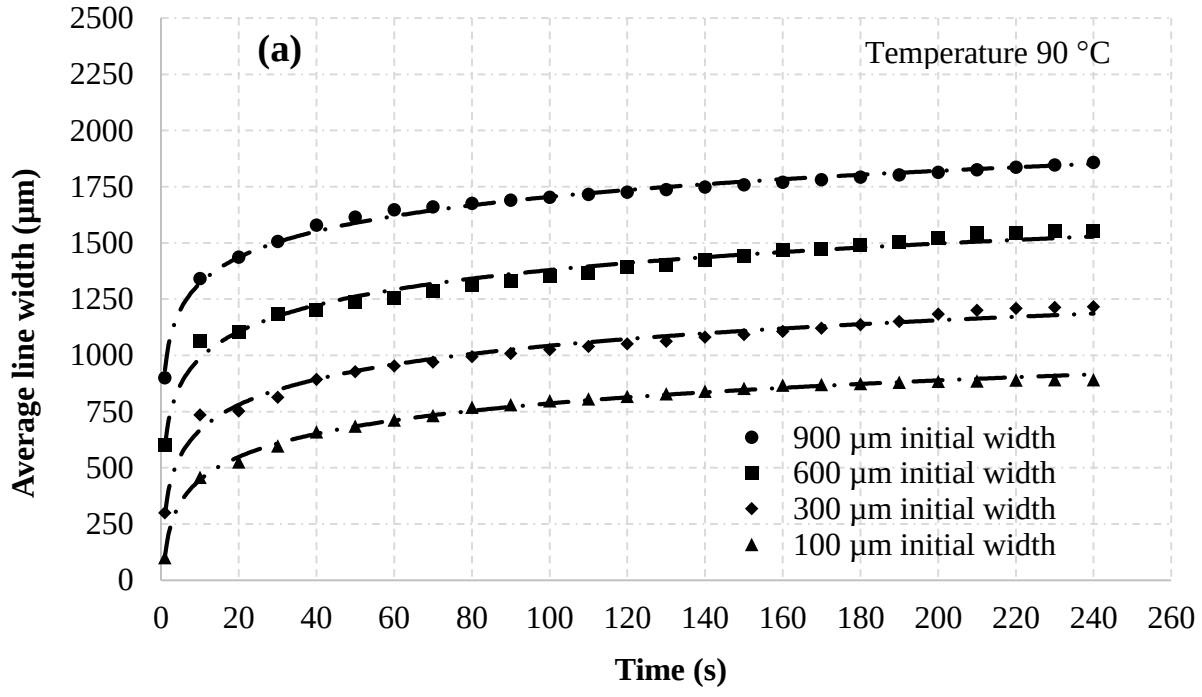
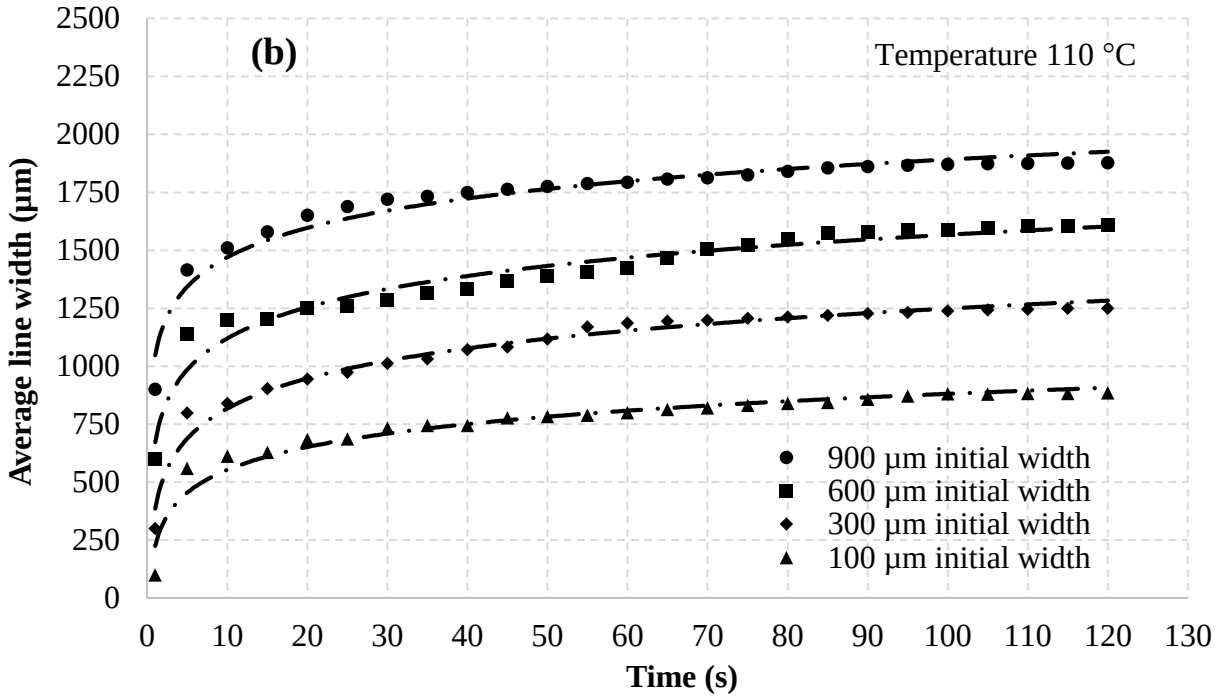


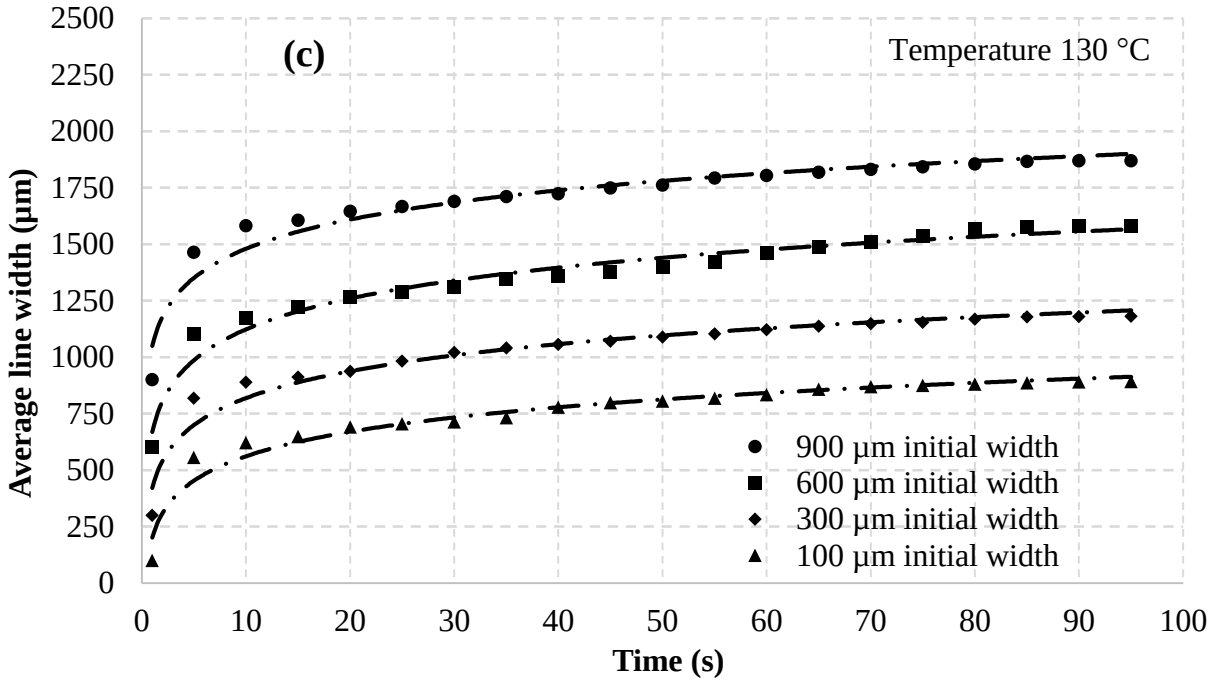
Figure 13. SEM image of the filter paper comparing the capillaries filled with wax with the plane paper

3.3.Melting Time

A series of lines printed on paper ranging from 100 µm to 900 µm were studied for the optimal time required to complete the spreading of wax in paper. The maximum time needed for spreading of wax in paper was recorded at four different temperatures for printed lines. Figure 1. (a) – (d) showing spreading time for lines of width 300 µm to 600 µm at four temperatures (90 °C, 110 °C, 130 °C and150 °C). It was observed that there is dominant effect of working temperature on the total time required for complete spreading. The spreading occurred at higher temperature were quick compared to low temperatures and melting above 110 °C resulted a fabrication time less than 100 seconds. Finally, the total fabrication time for devices at temperatures 90 °C, 110 °C, 130 °C and 150 °C resulted 240 secs, 120 secs, 95 secs and 70 seconds respectively. The optimal condition selected for better barrier integrity were 130 °C for 95 seconds.




(b)
Temperature 110 °C
Average line width (µm)
Time (s)
900 µm initial width
600 µm initial width
300 µm initial width
100 µm initial width
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
0
10
20
30
40
50
60
70
80
90
100
110
120
130


(c)
Temperature 130 °C
Average line width (µm)
Time (s)
900 µm initial width
600 µm initial width
300 µm initial width
100 µm initial width
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
0
10
20
30
40
50
60
70
80
90
100

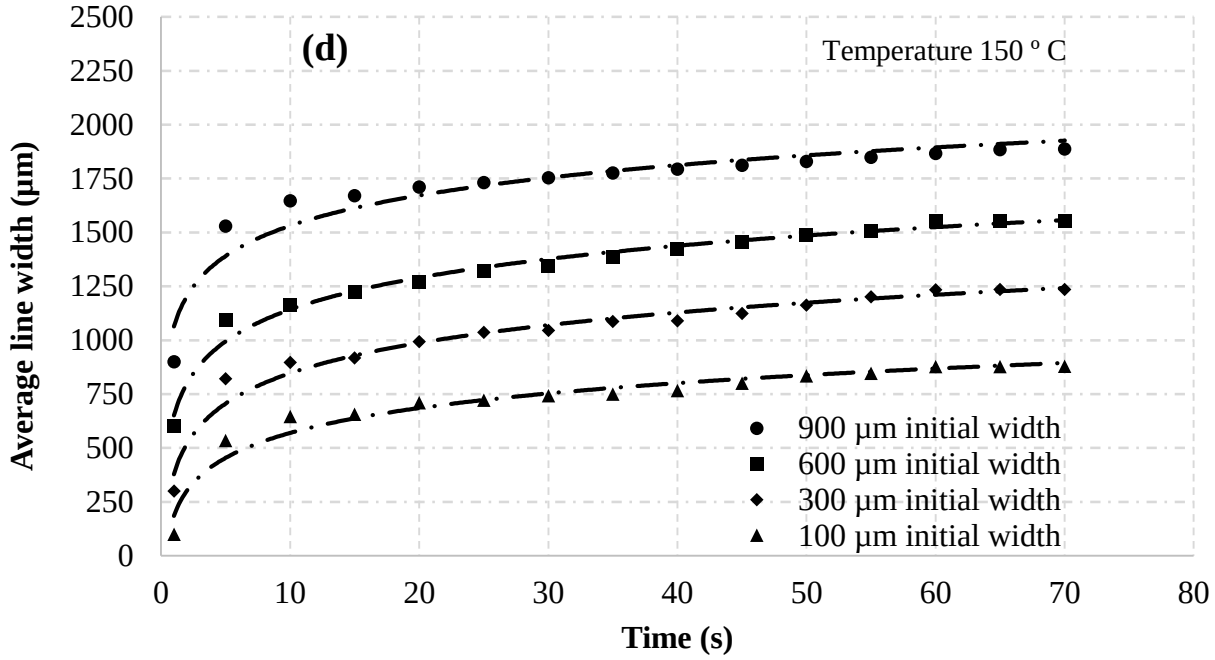


Figure 14. Graph of initial line thickness 100 µm, 300 µm, 600 µm and 900 µm Vs complete spreading time at; (a) a heating temperature 90 ºC, (b) a heating temperature 110 ºC, (c) a heating temperature 130 ºC and (d) a heating temperature 150 ºC.

3.4.Effect of temperature on the spreading of wax in paper

A series of 100 µm – 900 µm lines were printed on filter paper and each line was studied at four temperatures (90 °C, 110 °C, 130 °C and150 °C) to study the effect of temperature on the final achieved hydrophobic barrier. It was observed that the temperature effect is more pronounced when the line width is more than 300 µm. Figure 4. Shows that there is a significant change in the final width of the barrier with change in temperature (when observed at a similar time interval). The results show that the wax spreads very rapidly in the start of the process as the change from solid phase to liquid phase occurs when the paper is placed on a hot plate and then it keeps on spreading linearly until the complete deposited wax spreads to fill the capillaries.

The increase in the width of the barriers at higher temperature can be explained by considering the change in viscosity of wax as the temperature changes. At higher temperatures, the viscosity is less and makes the molten wax to flow easily within the capillaries as shown in Figure 9.

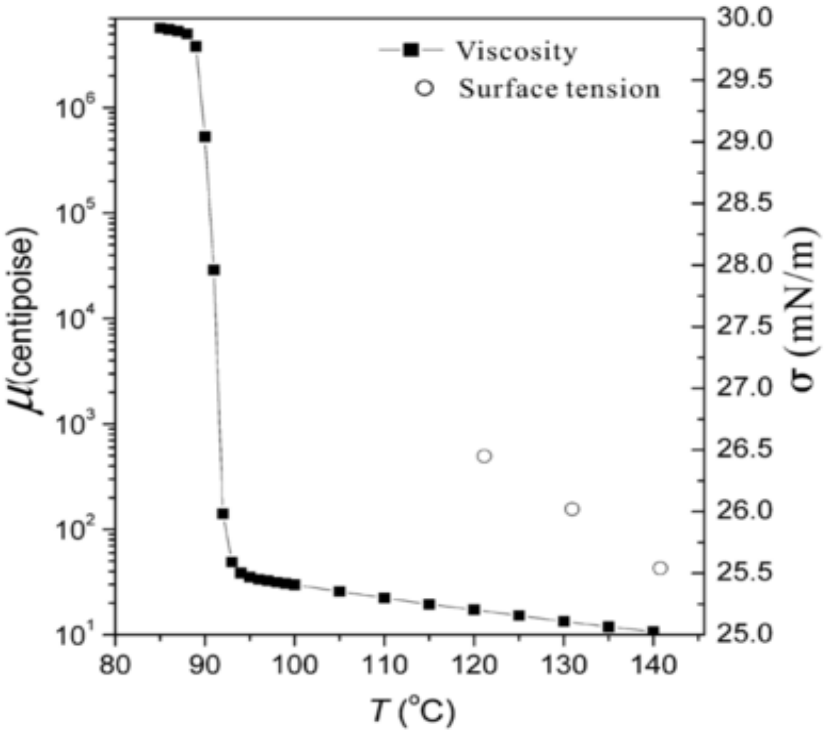
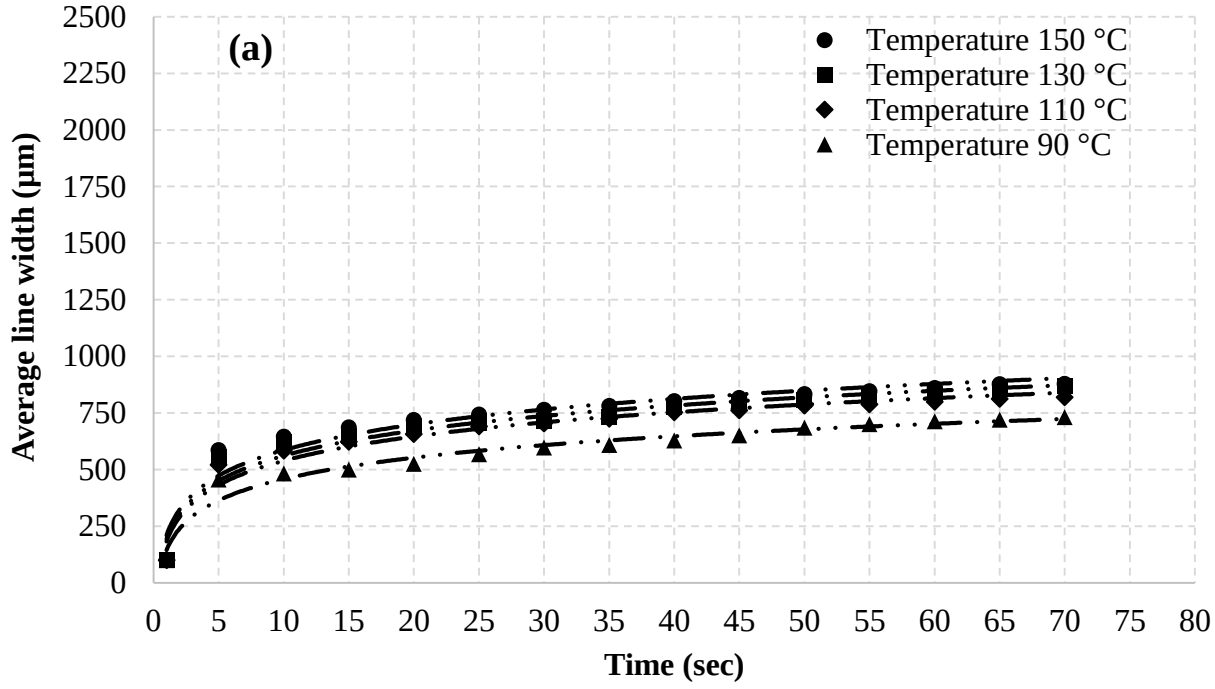
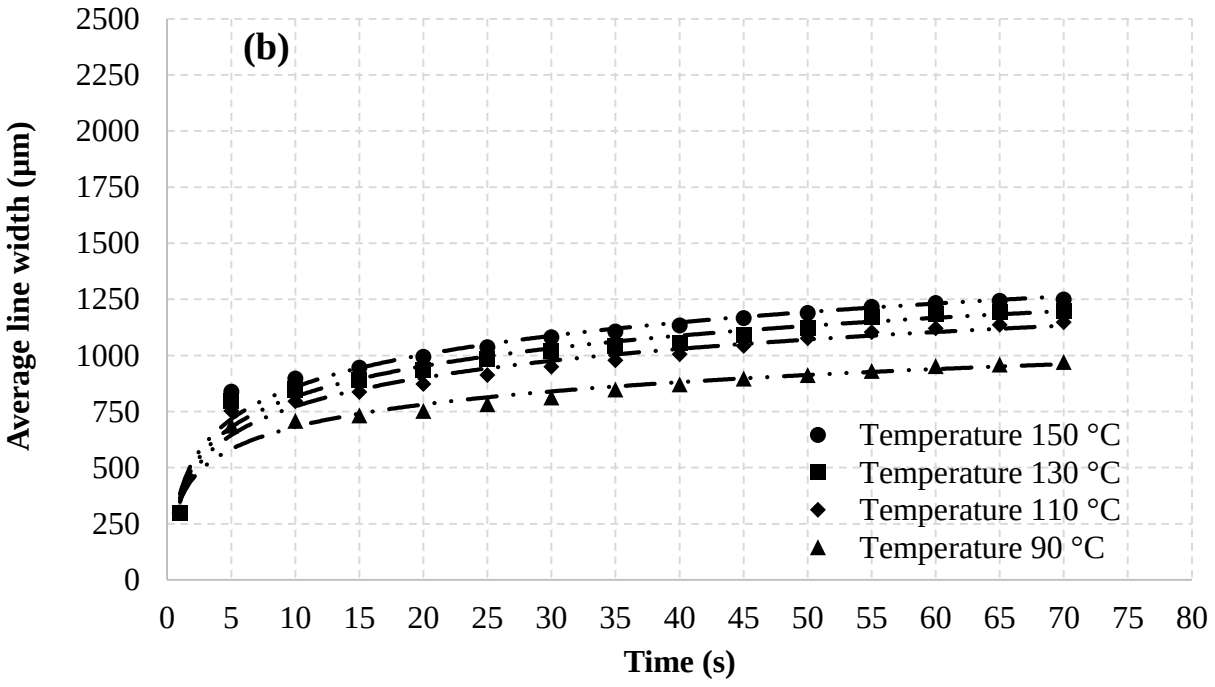


Figure 15. Dynamic viscosity and surface tension of solid ink with varying temperature [25]


(a)
Temperature 150 °C
Temperature 130 °C
Temperature 110 °C
Temperature 90 °C
Average line width (µm)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (sec)


(b)
Temperature 150 °C
Temperature 130 °C
Temperature 110 °C
Temperature 90 °C
Average line width (µm)
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Time (s)

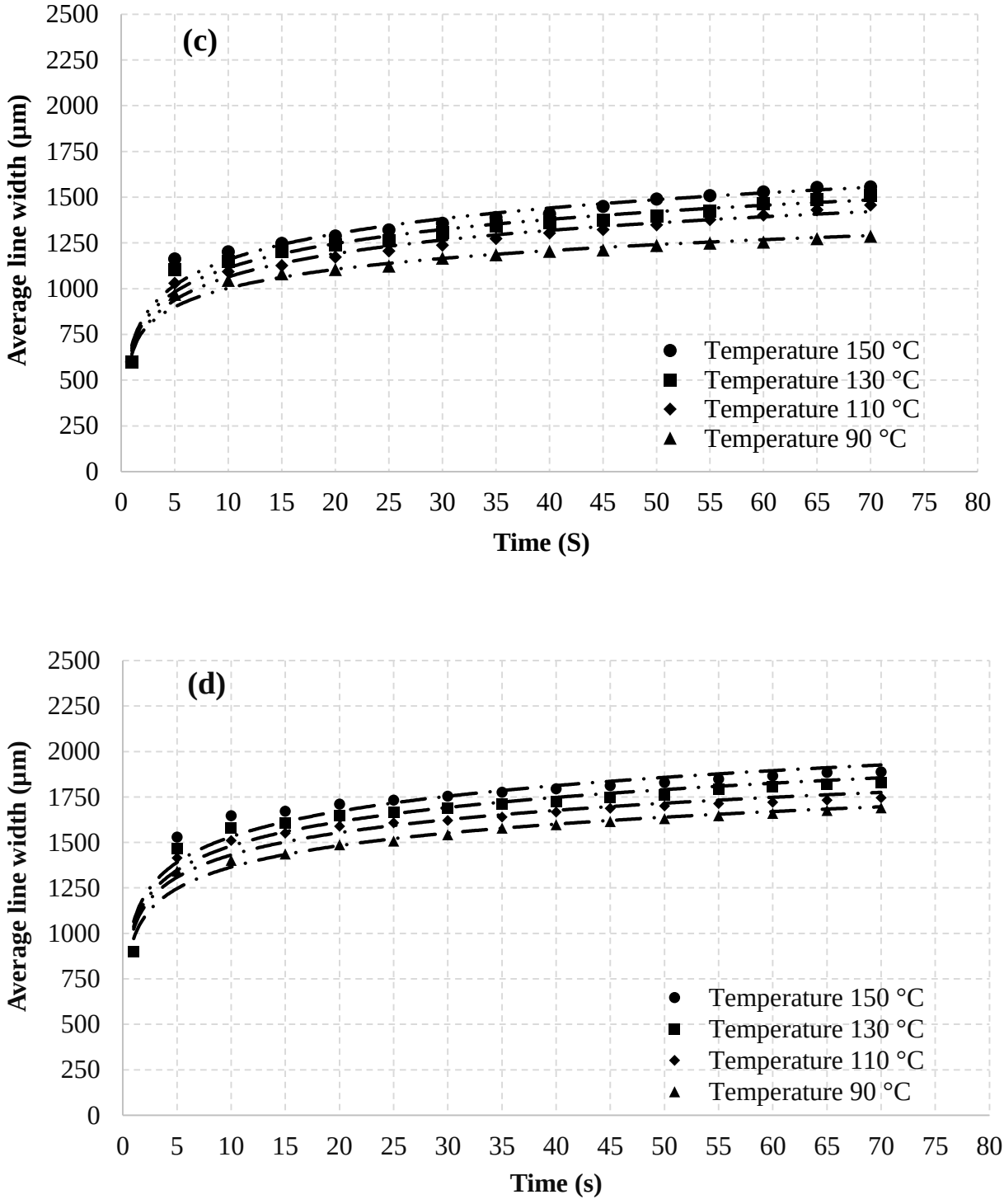


Figure 16. Spreading of wax at different temperatures (90 ºC, 110 ºC, 130 ºC and 150 ºC) for (a) 100 µm initial thickness, (b) 300 µm initial thickness, (c) 600 µm initial thickness and (d) 900 µm initial thickness.

3.5.Effect of printed width

Series of lines ranging from width (100 µm to 900 µm) were printed on paper and studied for the effect of initial printed line width on the final width of the hydrophobic barrier at different temperatures. It was observed that printed line width has higher impact on the width of final compared to temperature effect. Figure. 3 shows a linear fit resulted from the experimental data plotted.

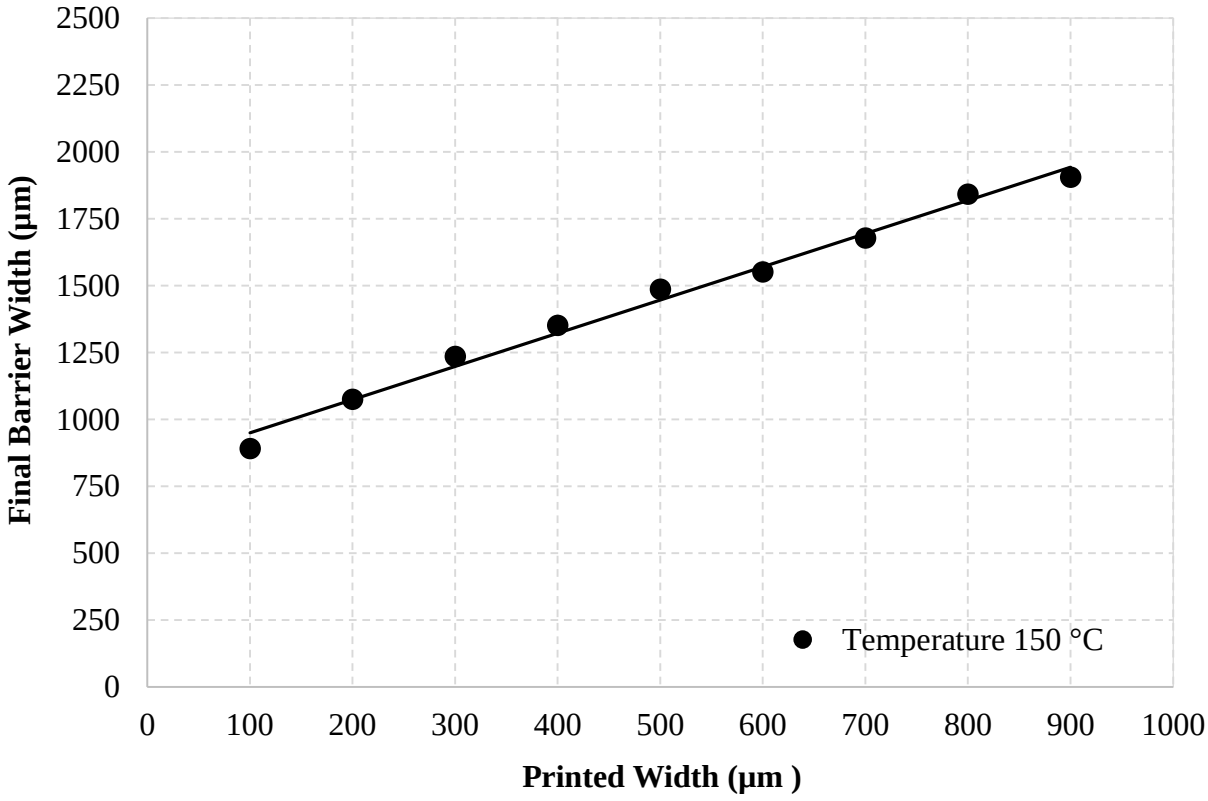


Figure 17. Assessment of change in printed line width on the final resulting hydrophobic barriers at temperatures (90 ºC, 110 ºC, 130 ºC and 150 ºC) respectively.

CHAPTER 4

WAX SPREADING MODELModel development

Three important process parameters that were focused during the experimentation were effect of initial printed width on the final barrier width, the temperature at which the process is carried out and the time for which paper placed on hot plate. The results obtained from the experiment were compiled and the data processed in Minitab by using a linear regression model. The resultant model is a function of time (t), temperature (T) and initial printed value (I). The model predicts an accurate response (Y) for the independent variables i.e. t, T and I with an accuracy of 96 %.

4.2. Washburn's Equation

The Washburn equation gives a very handy relationship for the flow of a fluid flow in a paper porous media under its own capillary action. A lot of work has been presented regarding the study of fluid flow in porous medium but still the complexity of paper fibrous structure and the pore size distribution complicates the applicability of Washburn equation to porous media. The Washburn equation describes that a volume of fluid (L) moves through a paper matrix according to the surface tension (γ) pulling the fluid into the paper determined by pore size (D), however opposed to this is a viscous resistance (η) which increases as the fluid travels over time (t) along a length eventually decreasing the flow of the fluid penetrating the paper, equation 3.

$$L = (\gamma D t/4\eta)^{1/2} \qquad (3)$$

4.2.1. Limitation of Washburn's Equation

The following assumptions are applied in the derivation of the Washburn equation.

- Flow in capillaries is continuous, fully developed, steady, and laminar throughout the length of the capillary.
- The capillaries are cylindrical and of constant radius.
- The liquid in the capillary is incompressible, with a constant viscosity and contact angle.
- There is no slip at the walls of the capillary, and the fluid has a finite velocity at the center of the circular pipe.
- The driving pressure gradient is linear in the x-direction, and constant with respect to time.
- The effects of gravity are negligible
- The effects of hydrostatic and atmospheric pressures negligible relative to capillary pressure

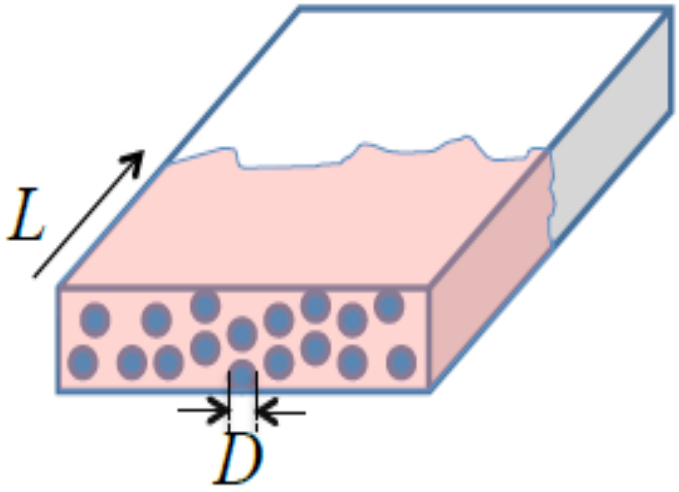


Figure 18. The washburn assumption of constant capillaries radius and the corresponding wax spreading represented by (L)

4.3.Proposed Regression Model

The results obtained from the experiment were compiled and the data processed in Minitab by using a regression method to develop an effective model.

The proposed model equation is;

$$\mathbf{Y\,(I, t, T) = Exp\ \{(A\ +\ B\ ln\ (\mathit{I}) +\ C\ ln\ (\mathit{t}) +\ D\ ln\ (\mathit{T})\}} \qquad \mathbf{(3)}$$

Where:

A = 3.8111, I = Initial printed line (μm)

B = 0.38644, t = Time (sec)

C = 0.09344 and T = Temperature (ºC)

D = 0.1317.

The above model results a final value in μm for the input values of I, t and T. Washburn's equation has also been used to predict the final values for the above three parameters independently and the results obtained are compared with the experimental results showing remarkable deviation. The Washburn's equation cannot be applied to the wax printing technique for the following reasons. First, the equation assumes the nominal pore size of the paper as the pore diameter. Secondly, the equation is applicable to capillary flow where unlimited volume of the fluid reservoir is available whereas, in wax printing a limited amount is deposited on paper. Finally, the equation is applicable to constant pressure process unlike capillary driven process in paper porous medium.

4.3.1. Model Summary

Table 4. Summary of the regression model

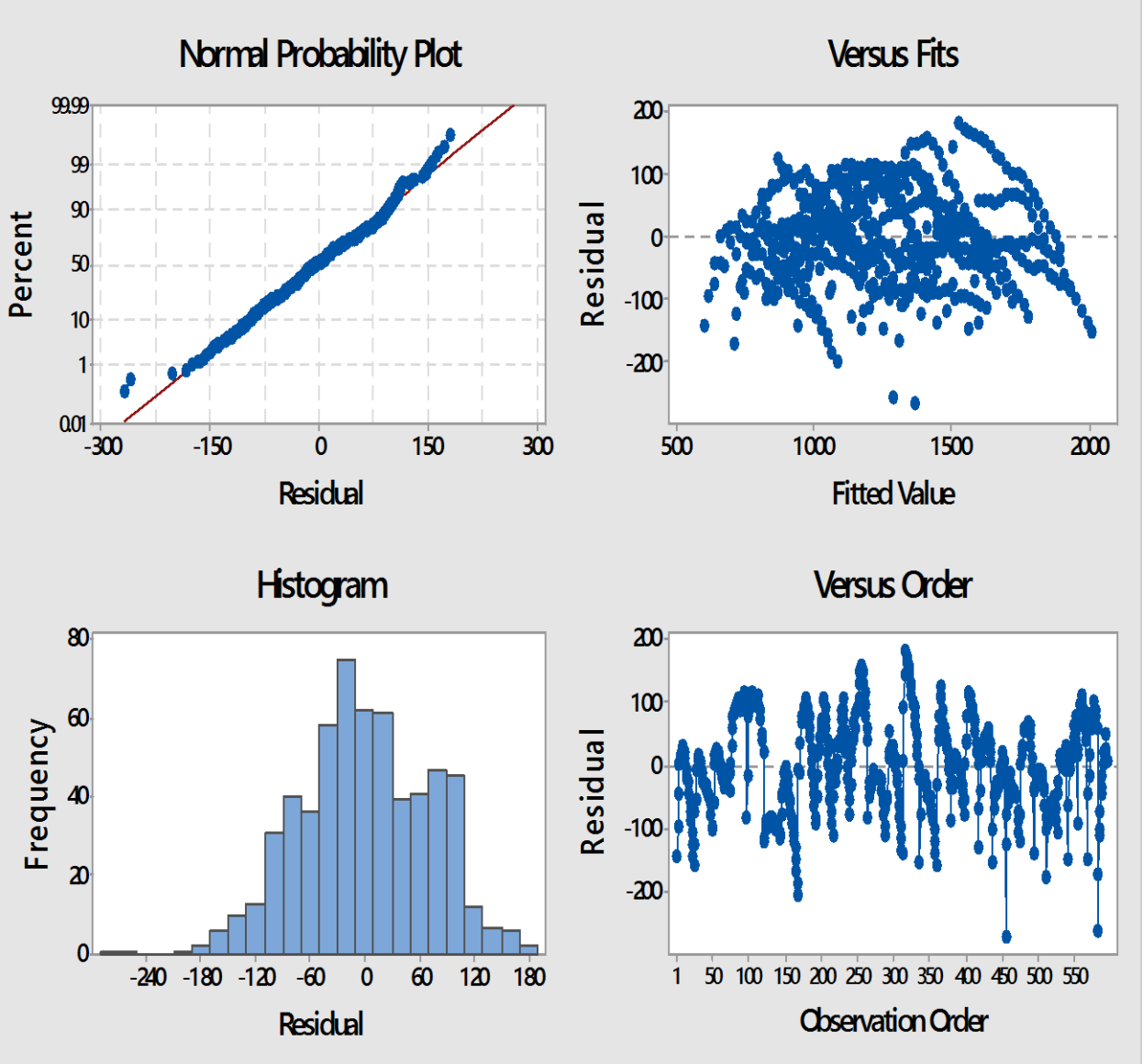
S	R-sq	R-sq(adj)	R-sq(pred)

69.3476	96.07%	95.76%	95.61%

Figure 19. Residual plots for all observation showing the model residual

4.4.Model validation

The suggested regression model was validated for additional printed lines of width 150 µm, 350 µm and 850 µm at temperatures 120 ºC and 140 ºC experimentally and were studied for the



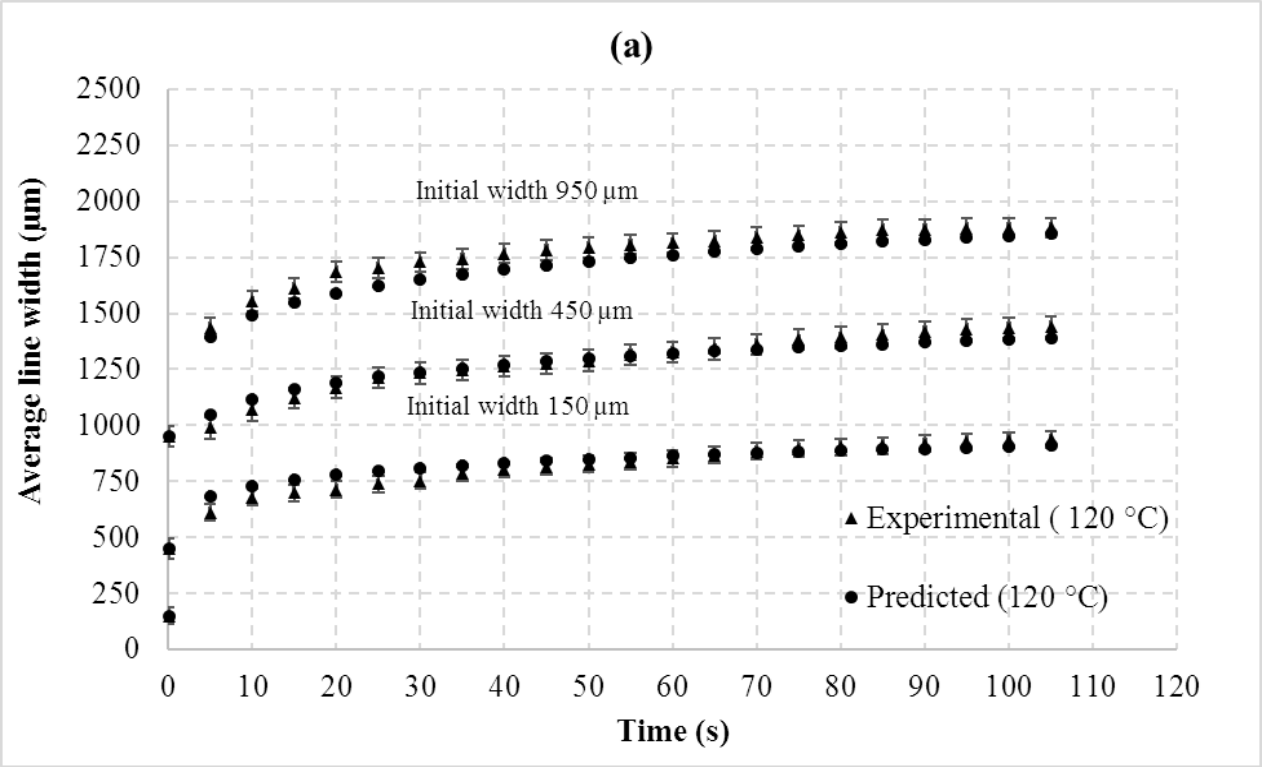
complete melting time. A maximum 6 % percentage error was observed in both experimental and

predicted data from the model. The experimental and predicted data plotted in Figure 7. Show that the model can best predict the final width of the hydrophobic barrier using equation. 3, by setting all the parameters in the equation. Furthermore, equation. 4 & 5 can be used to define the width of final achieved hydrophilic channel for producing an accurate microfluidic device in paper.

The additional parameters selected are shown in the Table. 5 below. These parameters were used to validate the regression model.

Table 5. Additional parameters and their range for model validation

Sr. #	Parameters	Range/Value
1	Initial width	150µm, 450µm, 950µm
2	Time	Reading after every 5 seconds
3	Temperature	120 °C, 140 °C.



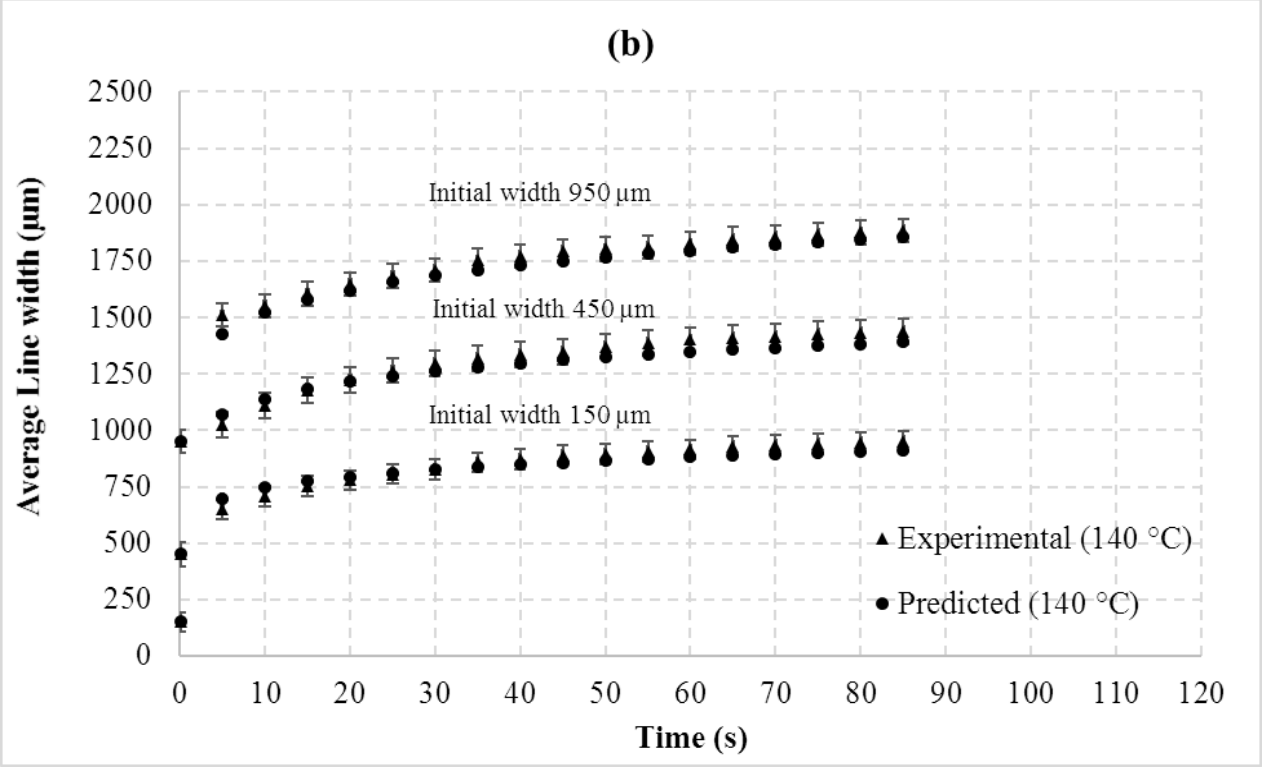


Figure 20. Graphs showing experimental and predicted trend lines (a) at temperature 120 ºC and (b) at temperature 140 °C.

4.5.Regression model vs Washburn equation

Regression model is compared with the experimental results and with that of the Washburn results. The Washburn's results showing a linear correlation and showing a large deviation from that of the experimental results. The experimental results show a strong correspondence with that of the regression model results. A maximum average error of 4 % was observed for all the results.

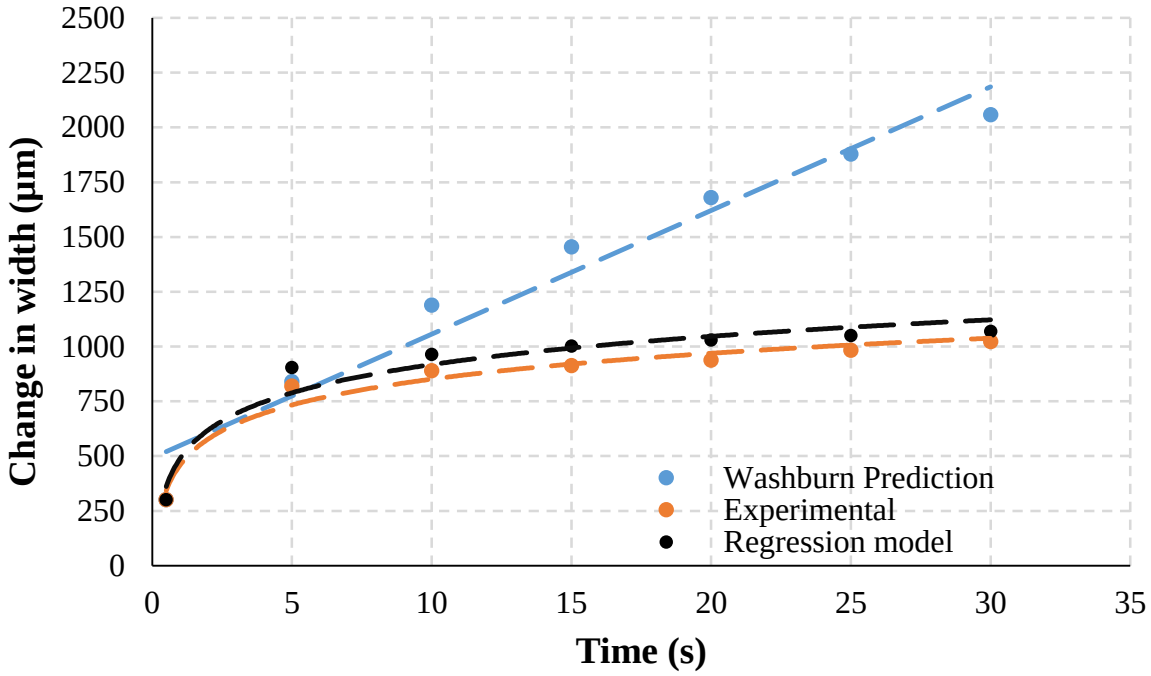
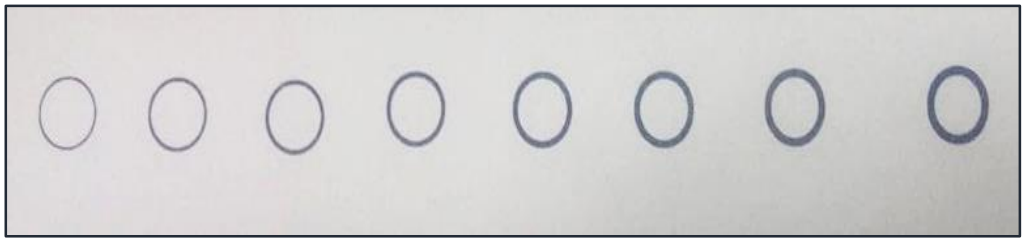


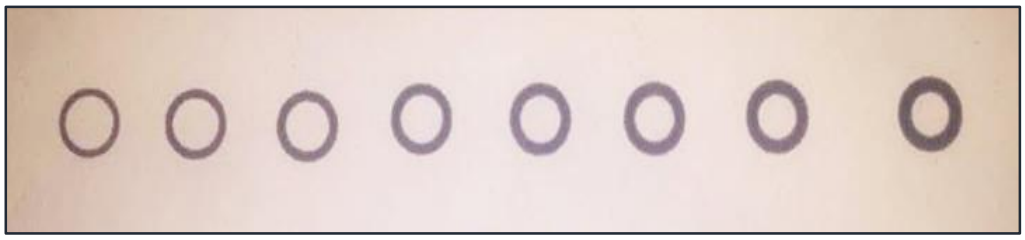
Figure 21. comparison between the experimental and regression model with that of the Washburn's equation

4.6.functional devices

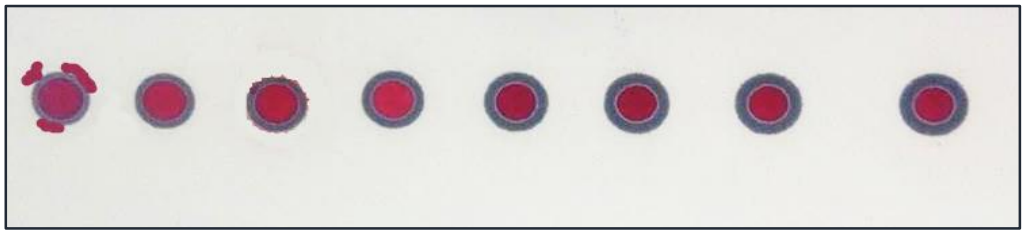
Series of circle printed with width ranging from 250µm to 900µm were printed in grade 1 filter paper to observe the functionality of these devices. It was deduced and observed that Line widths greater than 250µm resulted in a functional µPAD devices, thus volume of the sample can be calculated by setting the printed line width and the set channel width. Figure. 21 shows the circles tested for functionality.



(a)



(b)



(c)

Figure 22. Images showing the (a) initial printed circles (b) The final spreaded circle (c) leakage test performed for various width circles.

4.7.Device for application

Final shape of the pattern selected for the suitable application of these microfluidic devices is shown in figure.23. These devices were further used for the application of quantitative detection of contaminants in milk quality control. Thus the application of these devices has been demonstrated for the use of food quality control.

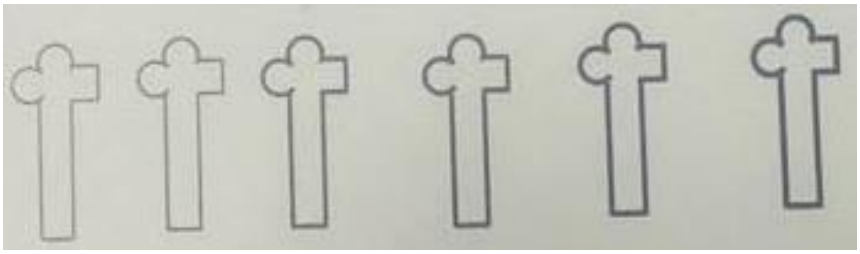


Figure 23. Final selected microfluidic devices for application in food quality control.

CHAPTER 5

CONCLUSIONS

The findings of this thesis will be summarized in this chapter, and recommendations for future work will be put forward. The objective of this thesis was to:

- Develop a comprehensive understanding of the parameters that influence wax spreading process in a paper porous medium, with particular emphasis on the impact of change in temperature and initial printed line on final resulting width of line through experimentation

- Develop a regression model that accurately describes the wax spreading process in porous medium and can predict the actual wax spreading by setting the parameters in the model

- To validate the proposed model for different values of these parameters

5.1. Conclusions

The wax spreading in the paper network is of a complex behavior and the model for wax spreading must be accounted for the viscosity changes that occurs with the changes in temperature, the width of the initially printed line and the heating time, which is well explained by our model presented here. In this research, an equation has been developed and presented that correlates (Y) as a function of (I, t and T). Experimental results show that this equation is in agreement with empirical observations within an accuracy of 96%. The important parameters studied here show a substantial impact of all these parameters on the final width of the hydrophobic barrier and the resulting channels. This model helps in future for fabricating paper-based analytical devices in mass production; wherein we can pre-calculate the desired dimension of the channels and barriers suited best to our applications desired.

RECOMMENDATIONS

The following are the next points and future recommendations that were beyond the scope of this thesis:

- The study of wax spreading for other paper types used for the analytical applications were not covered in this work. It is very useful to study the spreading in other paper types as well and model the wax spreading so that they can also be patterned accurately.

- The modeling of the fluid flow within the hydrophilic channel will aid in optimizing the complete designing of paper-based microfluidic devices

- The numerical simulation of the current work will further help reduce the rigorous experimentation. A successful investigation will lend significant prediction capacity not only for flow in paper-based microfluidic devices but also a large variety of porous media.

- The use of the current devices for numerous application including environmental monitoring, health science, and many other analytical venues.

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