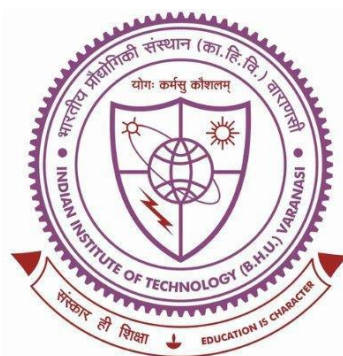


Development of Affordable Paper-based Sensors for Blood-plasma Related Microfluidic Analysis and Pathological Diagnostics



**Thesis submitted in partial fulfilment for the
Award of Degree
DOCTOR OF PHILOSOPHY**

By

NEHA GAUTAM

**Department of Mechanical Engineering
Indian Institute of Technology
(Banaras Hindu University)
Varanasi-221005, India**

20131512

2024

Dedication

“To my beloved parents”

Certificate

It is certified that the work contained in the thesis titled "*Development of Affordable Paper-based Sensors for Blood-plasma Related Microfluidic Analysis and Pathological Diagnostics*" by **Neha Gautam** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has satisfactorily fulfilled all the requirements of Comprehensive Examination, Candidacy, SOTA, Pre-submission seminar for the award of the Ph.D. degree.



Supervisor

Prof. Arnab Sarkar

Professor

Department of Mechanical Engineering,

Indian Institute of Technology

(Banaras Hindu University)

Varanasi-221005, India

Declaration by the Candidate

I, **NEHA GAUTAM**, certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of **Prof. ARNAB SARKAR** from **DECEMBER 2020** to **FEBRUARY 2024**, at the **DEPARTMENT OF MECHANICAL ENGINEERING, Indian Institute of Technology (BHU), Varanasi**. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not wilfully copied any other's work, paragraphs, text, data, results, etc., reported in journals, books, magazines, reports dissertations, theses, etc., or available at websites and have not included them in this thesis and have not cited as my own work.

Neha Gautam

Date: 14.8.24

(Neha Gautam)

Place: Varanasi

Candidate

CERTIFICATE BY THE SUPERVISOR

This is to certify that the above statement made by the candidate is correct to the best of my knowledge

Sarkar
14.8.24

Prof. Arnab Sarkar

प्राध्यापक / Professor

Supervisor

एन.आई.टी. (आई.आई.टी.) / Dept. of Mechanical Engg.

भारतीय प्रौद्योगिकी संस्थान / Indian Institute of Technology

(काठिंडी / B.H.U.)

वाराणसी-221005 / Varanasi-221005

Sameer
Head of the Department

विभागाध्यक्ष / HEAD

एन.आई.टी. (आई.आई.टी.) / Dept. of Mechanical Engg.

भारतीय प्रौद्योगिकी संस्थान / Indian Institute of Technology

(काठिंडी / B.H.U.)

वाराणसी-221005 / Varanasi-221005

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: **Development of Affordable Paper-based Sensors for Blood-plasma Related Microfluidic Analysis and Pathological Diagnostics**

Candidate's Name: **Neha Gautam**

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (Banaras Hindu University) Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the Ph.D. degree.



Date:

Place: **Varanasi**

(Neha Gautam)

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for author's personal use provided that the source and the Institute's copyright notice are indicated.

Acknowledgement

First and foremost, I would like to thank my supervisor, Prof. Arnab Sarkar, for his guidance, patience, faith, and continued enthusiasm. He is an inspiration to me as a researcher and an example of what it means to be a good person and mentor. I have felt privileged to work in his lab over the years. He has opened so many doors for my development and future, and I have tried to walk through as many as possible. His meticulous and valuable review and constructive criticism have greatly improved the quality of the work. For all of this, I shall be forever grateful to him.

I also thank Dr. Vijay Shinde and Dr. Binita Pathak for serving on my research progress evaluation committee (RPEC). I thank all of them for sparing their valuable time and assisting me throughout my research and completion of this thesis. I wish to extend my sincere thanks to Prof. Santosh Kumar, Head, Department of Mechanical Engineering, IIT (BHU) Varanasi, for providing me necessary resources to enable me to complete this research work. I also thank to Dr. Jay Singh, Assistant Professor, Department of Chemistry, Institute of Science, Banaras Hindu University, for allowing me to learn and perform my experiments in his laboratory. I also thank Dr. Priya Ranjan Muduli, Assistant Professor, Department of Electronics Engineering, IIT (BHU) for allowing me to use sensors in his lab. At the same time, I also convey my deep gratitude to Prof. P. Ghosh for permitting me to use instruments in his lab.

Along with IIT (BHU) I express my deep gratitude to Prof. Suman Chakraborty, IIT Kharagpur to motivate me to carry out my research in the broad domain of bio-microfluidics. At the same time, I am also obliged to Dr. Shantimoy Kar, NIPER Hyderabad to motivate me to continue collaborative research with his group.

During this research journey, I have met many people who have made this period of my life memorable and very pleasant. Among them, I would like to sincerely acknowledge the assistance and motivation provided by Dr. Ranjana Verma, Dr. Rahul Verma, Dr. Chandra Prakash Singh, Dr. Rishi Ram, and Mr. Vikas Bishnoi.

I express my thankfulness to my friends Vinay Kumar Yadav, Dr. Dhanraj Meena, Manisha Meena and Sonam for their help during my stay at IIT (BHU), Varanasi.

Along with my collaborators and colleagues from India I also express my sincere gratitude to Prof. Christophe Ley and Prof. Stephane Bordas for inviting me in the university of Luxembourg. It was really a great experience along with another faculty member, Prof. Anupam Sengupta where I present my current and future research plan.

Finally, my sincere and heartfelt gratitude to my mother, father, sister and brother-in-law for their immense support and showing belief in me, which enabled me to pursue my dreams. This journey cannot be completed without their support.

List of Equation

1.3.1.....	5
1.3.2.....	6
1.3.3.....	6
1.3.4.....	6
1.3.5.....	7
1.3.6.....	7
1.4.1.....	9
1.4.2.....	9
1.4.3.....	9
1.4.4.....	10
1.4.5.....	10
2.3.1.....	33
2.3.2.....	34
2.3.3.....	34
2.3.4.....	34
2.3.5.....	34
2.3.6.....	35
2.4.1.....	37
2.4.2.....	37
2.4.3.....	37
2.4.4.....	37
2.4.5.....	37
2.4.6.....	38
2.4.7.....	38
3.3.1.....	55
3.4.1.....	64

List of Figure

Figure 1.1 (a) Blood plasma separation through LF1 membrane filter paper (b) FESEM image of interface of varying pore diameter LF1 and Whatman grade 1 filter paper.	4
Figure 1.2 (a) Darcy model for liquid transport through porous structure (b) L-W equation for liquid transfer through porous structure.....	7
Figure 1.3 Recent advancements in the fabrication techniques for microfluidic paper-based devices build upon the foundational principles set by initial methodologies, incorporating modern technologies and innovative approaches. These advancements are directed towards enhancing the efficiency of the manufacturing process, substantially reducing both the production time and costs associated with these devices[43].	16
Figure 1.4 (A) Experimental phases of the wax device printing process included: (i) the session dedicated to the creation of the device, (ii) optimization of flow rates by varying channel widths, (iii) analysis of flow rate versus time across different channel dimensions, and (iv) the final device configuration. (B) Schematic representation of the detection device based on aptamer-functionalized lateral flow assays (LFAs). (C) Design and development of the paper-based sensor encompassing: (i) steps for constructing the sensor featuring one inlet and six reaction chambers, (ii) scanning electron microscopy (SEM) images displaying the structural details of glass microfiber (top panel) and PES membrane (bottom panel), and (iii) the completed device version. (D) Assembly and modification of electronic paper-based analytical devices (ePADs) integrated within microfluidic channels for biotin detection. (E) Development process of an aptamer-based sensor for cortisol detection. These descriptions and methodologies are used with permission from references [51], [52], [53], [54], [55].....	19
Figure 2.1 Schematic representation of different fabrication processes laser cutting, both side printing, single side printing, and blank rolling.	30

Figure 2.2 Schematic of paper-based device fabrication using in house laser jet printer : (a) Initial design is drawn using Inkscape software (b) Double sided printing is carried out using office printer (c) Heating process of the printed paper is performed inside a hot oven for 5 mins at 150 °C (d) Final device with hydrophobic boundary is fabricated for further utilisation.....	32
Figure 2.3 The schematic representation of experimental methodology for liquid flow rate measurement.	33
Figure 2.4 Schematic of capillary filling through a one-dimensional capillary.....	35
Figure 2.5 FESEM images of the hydrophilic surface of the Whatman grade 4 filter paper after different fabrication processes.	39
Figure 2.6 Transient variation of imbibition length depicting difference in flow rate different fabrication protocols for (a) Whatman Grade 4 and (b) Whatman Grade 1.	41
Figure 2.7 Time derivative of contact angle vs imbibition time for different fabrication processes.	43
Figure 2.8 Transient variation of imbibition length in different fabrication processes for Whatman Grade 4 filter paper with varying channel widths: (a) 2, (b) 4, (c) 8, (d) 12-mm. ..	45
Figure 3.1 A schematic illustration depicts a paper-based chip designed for measuring plasma viscosity. In the initial step, plasma is pipetted into the inlet zone. Subsequently, the user opens the viscometer application on a mobile device and presses the start button as the liquid reaches point P. As the liquid arrives at point Q, the user then presses the stop button. The application calculates and displays the numerical value of the viscosity on the mobile screen.....	49
Figure 3.2 Schematic representation of μ PAD. The design was printed on Whatman cellulose filter paper and heated at 150° C for 4-5 minutes to create a hydrophobic boundary. The μ PAD is a rectangular chip with length and width of 80 mm and 20 mm respectively. The μ PAD have	

a microchannel in the middle which is a combination of a rectangle of 4 mm in width and a circle of diameter of 12 mm.....57

Figure 3.3 Variation of the flow rate for 3mm, 4mm, and 5 mm wide channels at 25 °C and 70 % RH for (a) DI water, (b) 20% glycerol solution. Each data set represents the mean value and error-bar shows the standard deviation of all five repetitive experiments60

Figure 3.4 Variation of travel time with samples of different viscosities (DI, 20 and 40 % glycerol solution) to travel a fixed 30 mm imbibition length (25 °C, 70% RH) in (a) 3, 4, and 5 mm wide channels, and (b) 5 mm wide channel. Each data set represents the mean value and error-bar shows the standard deviation of all five repetitive experiments.61

Figure 3.5 Sequential representation of experiment steps: (a) Fabricated device placed horizontally, (b) Injection of sample on the circular reservoir of the device, (c) Measurement of time travelled by the sample from point P to Q using Visc-Sens app.65

Figure 3.6 (a) A calibration curve was constructed by plotting observed time against the viscosity of standard Glycerol solution at 25° C. Each set of experiments was repeated five times. The average value was used for the calibration curve and the error was shown by standard deviation. Comparison of the viscosity measured by μ PV with viscosity reported by Brookfield viscometer (n = 20 samples): (b) Linear least-square regression analysis of the correlation between the viscosities values measured by both the methods, (c) Bland-Altman plot was constructed by plotting difference of the two methods against the mean of the two methods. The bias (mean) and standard deviation of the differences are - 0.0462 cP and 0.0553 cP. The lower limit of agreement (LLOA) and upper limit of agreement (ULOAA) are - 0.1545 and 0.0622, respectively. (d) The viscosity of the 15 % glycerol solution was measured at 25, 30, 35, and 40° C using both a standard Brookfield viscometer and our proposed device. Each measurement was repeated five times. The error bar is shown for five data points of every sample.67

Figure 4.1 Schematic representation of wax dipping method (a) side view of the device assembly prior to dipping inside molten wax container. (b) Dip the whole assembly inside melted wax at 120 °C for 1 s. (c) Finally fabricated paper device with precise wax hydrophobic boundary and a combination of LF1 and Whatman filter paper.	78
Figure 4.2 The schematic representation of overall colorimetric assay.	79
Figure 4.3 (a) FESEM image of sample inlet zone made of LF1 membrane (b) FESEM image of reaction zone made of Whatman grade 1 filter paper (c) a linear correlation between fabricated channel diameter and mold inlet diameter and error bar shows standard deviation for five measurements.	83
Figure 4.4 Blood-plasma separation and reaction mechanism on fabricated paper-based device.	84
Figure 4.5 Graph between plasma separation time vs different hematocrit blood sample	85
Figure 4.6 (a) depicts a scanning electron microscope (SEM) image of interface of Glass fiber LF1 and Whatman filter paper (b) wax coated hydrophobic surface (c) LF1 filter membrane image at 20 µm scale (d) Whatman grade 1 filter paper at 20 µm scale.	86
Figure 4.7 (a) 3D projection of LF1 filter paper and (b) Whatman grade 1 filter paper.....	87
Figure 4.8 (a) Surface height histogram of LF1 (b) Whatman grade 1	88
Figure 4.9 Schematic showing Jaffé reaction between creatinine and picric acid in NaOH ..	89
Figure 4.10 (a) Calibration curve for different concentration of creatinine by keeping grey scale intensity on y axis vs creatinine concentration on x axis (b) validation of our fabricated device with gold standard measurement (c) interference analysis of various bio analyte presents in plasma sample.	91
Figure 5.1 Schematic representation of wax dipping method to develop microfluidic channel. (a) Dumbbell shape iron mould for microfluidic channel fabrication (b) Side view of LF1 and Whatman grade 1 filter paper assembly (c) Top view of filter paper assembly kept on a glass	

slide (d) Permanent magnet attached at the back side of the glass slide (e) Dumbbell shape Iron mould kept at the top of the filter paper (f) Whole assembly dip inside the hot melted wax (110 °C) for 1 sec.	105
Figure 5.2 Schematic representation of complete fabrication process of E μ PAD from material synthesis to integration of microfluidic platform with n-MgF/SPE.	106
Figure 5.3 (a) A bar graph between different volume of 50 % Hct blood sample injected from both ends and time taken by the plasma to completely fill the detection zone; (b) A line graph between a fixed volume (80 μ L) of different Hct samples vs time taken by plasma to completely fill the detection zone.	109
Figure 5.4 (a) FTIR analysis of n-MgF; (b) XRD analysis of n-MgF.....	110
Figure 5.5 Micrographs of TEM analysis of n-MgF (a), (b), and (c); SEM and EDX analysis of n-MgF (d), (e) , and (f) ; AFM two dimensional (2D), three dimensional (3D), and roughness parameter analysis of n-MgF/SPE (g), (h), and (i).	111
Figure 5.6 CV analysis of unmodified SPE and n-MgF/SPE; (b) pH study of n-MgF/SPE; (c) Scan rate analysis of n-MgF/SPE for 10 to 100 scan rate; (d) peak current values plotted against the square root of the scan rate ($v^{1/2}$).	114
Figure 5.7 DPV analysis of unmodified SPE and n-MgF/SPE; (b) Response time study of n-MgF/SPE.....	115
Figure 5.8 (a) Stability analysis of n-MgF/SPE; (b) reusability analysis of n-MgF/SPE.....	117
Figure 5.9 (a) The CV plot for different concentration of AA on n-MgF/SPE; (b) A linear curve plotted between change in current ($\Delta I = I_b - I_a$) on y axis vs AA concentration (μ M) on x-axis; (c) Interferents analysis of n-MgF/SPE.	120

List of Table

Table 2-1 Basic properties of Whatman Grade 1 and Grade 4 filter paper	29
Table 2-2 Weibull parameters of pore size for different fabrication processes.	40
Table 3-1 Uncertainty analysis due to device fabrication process	56
Table 3-2 Values of viscosity of standard glycerol solutions obtained from Brookfield viscometer at 25° C	58
Table 3-3 Sample volume optimisation data for a 4 mm wide channel at a 30 mm distance from the beginning of the rectangular section	63
Table 3-4 Accuracy table for blood plasma viscosity measurement using our developed device and gold standard data	68
Table 3-5 Viscosities of 15 % Glycerol solution at different temperatures measured by both Brookfield viscometer and paper-based viscometer	70
Table 4-1 <i>Comparison between proposed device and available microfluidic paper device for creatinine measurement</i>	94
Table 5-1 A comparison between previously reported modified SPE with our developed n-MgF sensor for AA detection.	122
Table 5-2 Whole blood sample spiked with standard AA solution and concentration value measured in real spiked sample.	123

Abstract

Microfluidics has gained huge popularity due to its several promising advantages such as miniaturization, affordability, transformability, easy handling, minimum fluid consumption and so on. Out of several microfluidic devices micro-paper based analytical devices (μ PAD) has shown its promising growth due to smooth flow of fluid in paper based porous substrate without any external pump where capillary actions provide self-pumping. Over last few decades paper based microfluidic platforms have manifested its huge applicability in food safety monitoring, environmental health monitoring as well as medical diagnostics. This thesis mainly focusses on the study of medical diagnostics using promising paper based microfluidic devices.

Among medical diagnostics blood related pathological diagnostics are very common while detecting any disease ranging from ordinary fever to cancer. Blood is a complex rheological fluid composed of plasma and several cellular components which can easily flow through porous paper substrate. On the other hand, in most of blood related liquid biopsy we have to add reagent to detect a particular analyte in blood and it is worthy to mention that the paper based microfluidic platform bears minimum consumption of such costly reagent along with that such paper-based substrates itself provides storage platform of such reagents which can be considered as an added advantage of using paper-based platform for blood related pathological diagnostics.

Though during last few decades lots of paper based microfluidic platforms have been used for pathological diagnostics, most of these platforms use separated plasma for further processing. There are few reported paper-based platforms which use directly blood, however, separation of plasma in such platforms either suffer from purity or require costly reagent as well as fabrication processes. Moreover, present paper-based microfluidic devices mainly concentrate

to estimate complete blood count (CBC) related parameters such as haematocrit, haemoglobin etc., however, due to increase of the disease burden in the society, estimation of proteins and vitamins from blood-plasma become need of the hour specially for underserved populations where sophisticated health care facilities are really scarce and for disabled and elderly people who cannot report to the nearest hospitals, for their regular health monitoring. In a nutshell, as of now there is no such paper based microfluidic diagnostic platform which uses whole blood and determine several blood-plasma analytes from sample-to-result integration via single step with the involvement of minimally trained human resources.

In light of the above-mentioned discussion, this thesis at first envisions flow imbibition mechanism through porous paper substrates. Reported findings pinpoint that flow rate through porous paper substrate is mainly governed by geometrical as well as environmental parameters. However, in sharp contrast of reported findings this thesis highlights that different fabrication protocols also play a significant role in controlling imbibition dynamics because of the accompanied changes in pore size diameter which is caused due to changes in microfiber alignment of a paper surface. Accordingly, transient variation of imbibition length as well as flow rate has been studied for five different fabrication processes such as: 1) scissor cutter, 2) laser cutter, 3) single side ink printing, 4) double side ink printing, and 5) Blank roll along the printer. The mean pore size distribution for channels fabricated using scissor cutting, laser cutting, single-side printing, double-side printing, and blank rolling on Whatman Grade 4 paper were found to be 24.72, 19.59, 15.46, 13.23, and 12.05 mm, respectively. This indicates that pore size distribution is significantly influenced by the fabrication process. The flow rates for channels fabricated using laser cutting, single-side printing, double-side printing, and blank rolling were 0.27, 0.43, 0.37, and 0.31 times slower than those fabricated using scissor cutting. Flow rate analysis revealed that scissor cutting provides the highest flow rate, likely due to minimal contact with the paper substrate during fabrication. In contrast, the lowest flow rates

were observed for channels fabricated by double-side printing or blank rolling, likely due to the complete exposure of the paper surface during these processes. These effects are significant only for microchannels with widths less than 4 mm; however, for wider channels, the flow rate remains consistent across different fabrication methods.

In line with this, a protocol has successfully implemented to determine plasma viscosity when flow of plasma is occurring in such porous substrate. The flow mechanism for such cases is governed by Lucas-Washburn equation and an optimum length has been chosen to observe duration of flow for different plasma samples having a wide variation of viscosity in a constant width channel for a fixed sample volume. This would lead to the ultimate value of plasma viscosity through a developed smartphone app. Plasma mimicking liquid solution glycerol were used to draw the calibration curve between viscosity against time duration. Brookfield viscometer is used to measure the viscosity of different liquid samples and validation is performed with the in house developed paper based capillary device. An excellent correlation was found between the values measured by both methods with a coefficient of determination (R^2) of 0.9644. The proposed device covers the entire clinical range of blood plasma viscosity (0.9~4 cP) and can easily distinguish even a small difference of 0.13 cP leading to high sensitivity in the clinical range of plasma viscosity.

In the next step, blood-plasma creatinine was estimated in paper based miniaturized platform through sample-in answer-out format using colorimetric assay through RGB sensor. The well-known Jaffe' reaction was used in which the yellow color of alkaline picrate changes from yellow to yellow-orange due to the reaction between plasma creatinine and alkaline picrate. The blood-plasma separation has also been integrated in this miniaturized platform where wax dipping technique was used and flow through two paper substrates with different porosities have been augmented. Moreover, color changes have been observed in a 3D printed box eliminating the effects of ambient lights and RGB analysis has been carried out in a RGB sensor

replacing the use of a smartphone where image analysis sometimes become platform dependent leading to inaccuracies. In a nutshell, the developed microfluidic paper device in this thesis provides a miniaturized platform for estimating plasma creatinine directly from whole blood sample considering sample-to-answer integration via single step mediated by spontaneous blood-plasma separation where sensor has been used to enhance the order of accuracy and bring the same in line with gold standard devices. The limit of detection (LOD) of our device is 0.219 mg/dL which is well below the normal physiological limits. Furthermore, we have validated the performance of our device with 35 clinical samples, delineating excellent agreement with the gold standard measurements.

Lastly, it has been envisaged that in the current society, vitamin testing is too costly affair and as of now, point-of-care devices have rarely been developed to estimate the concentration of vitamins. In view of this, in this thesis vitamin C (ascorbic acid) has been targeted to detect as an analyte from whole blood samples. We combined LF1 blood plasma separation membrane and Whatman grade 1 filter paper to separate plasma from whole blood through wax printing. A screen-printed electrode (SPE) was modified with spherical-shaped MgFe_2O_4 nanomaterial (n-MgF) to improve the catalytic properties of SPE. The n-MgF was prepared via hydrothermal method, and its material phase and morphology were confirmed via XRD, FTIR, TEM, SEM, and AFM analysis. The fabricated n-MgF/SPE/E μ PAD exhibited detection of AA ranging from 0 to 80 μM . The obtained value of the detection limit, limit of quantification, sensitivity, and response time are 2.44 μM , 8.135 μM , $5.71 \times 10^{-3} \text{ mA } \mu\text{M}^{-1} \text{ cm}^{-2}$, and 10 seconds, respectively. Our developed n-MgF/SPE/E μ PAD shows marginal interference with the common analytes present in plasma, such as uric acid, glutamic acid, glucose, urea, lactic acid, and their mixtures. Overall, our low-cost, portable device with its user-friendly design and efficient plasma separation capability offers a practical and effective solution for estimating AA concentration from whole human blood in a single step.

In earlier reported literature, real blood samples were rarely used whereas in this thesis we focus processing of real blood samples for detecting above-mentioned analytes. In each case, this thesis performed a sound statistical analysis in order to validate the efficacy of our device with the gold standard device. Our accuracy ranges have been found between 96-98%. Accordingly, Bland-Altman plots have been made for detection of each analyte. Along with this parametric test, non-parametric such as chi-square tests have also been carried out for further confirmation of the potential excellence of these developed devices to replace expensive gold standard devices in order to deploy our devices to resource-poor settings. The guidelines set by WHO for statistical validation as well as ASSURED criteria have also been followed to consider these devices as true candidates of POC devices. These diagnostic test kits have two-fold benefits: on one hand these will provide quick diagnostics reducing disease burden in the society and on the other hand easy operation of these devices create employment opportunities among underprivileged population in developing and underdeveloped nations.

Table of Contents

Dedication	ii
Thesis Certificate	iii
Declaration by the Candidate.....	iv
COPYRIGHT TRANSFER CERTIFICATE	v
Acknowledgement	vi
Abstract	viii
Table of Contents	xvi
List of Equation.....	xx
List of Figure.....	xxi
List of Table	xxvi
Chapter 1 Introduction.....	1
1.1 Brief Overview	1
1.2 Motivation	2
1.3 Liquid imbibition dynamics through porous filter paper: Overview	4
1.4 Factors Influencing Paper Substrate Selection in microfluidic devices	7
1.5 Paper based microfluidics: an overview.....	10
1.6 Research Gap.....	20
1.7 Outline of thesis	21
Chapter 2 How Flow characteristics of Paper-based Devices is Dependent of fabrication Methods? 24	
2.1 Overview	24
2.2 Introduction	25
2.3 Materials and Methods	28
2.3.1 Fabrication using Laser Cutting.....	30
2.3.2 Fabrication using Scissor	31
2.3.3 Fabrication using blank roll	31
2.3.4 Fabrication using laser Jet printer	31

2.3.5	Experimental Methodology	32
2.3.6	Physics of fluid flow through porous paper surface	33
2.4	Results and Discussion.....	36
2.4.1	Effect of Different Fabrication Process over the Pore Size of Paper Substrate	36
2.4.2	Flow Rate Variations in Differently Fabricated Microchannels	41
2.4.3	Influence of contact angle measurement of differently fabricated paper substrate	42
2.4.4	Effect of Different Width in Microchannel for Flow Rate Variation through Porous Substrate.....	44
2.5	Summary	46
Chapter 3	A low-cost and disposable capillary-based paper sensor for measuring blood-plasma viscosity using a smartphone app	48
3.1	Overview	48
3.2	Introduction	49
3.3	Materials and instruments	54
3.3.1	Selection of cellulose paper, design and fabrication of the device	54
3.3.2	Blood sample collection and processing.....	57
3.4	Experimental methodology	58
3.4.1	Measurement of viscosity with Brookfield viscometer	58
3.4.2	Optimization of geometric parameters.....	59
3.4.3	Measurement of viscosity using μ PV	63
3.5	Results and discussion.....	65
3.5.1	Performance analysis of the developed paper-based viscometer against the Brookfield viscometer.....	65
3.5.2	Effect of temperature on Plasma viscosity.....	69
3.6	Summary	70
Chapter 4	Quantification of Creatinine on Paper-based Devices from Whole Blood using RGB Sensor	73

4.1	Overview	73
4.2	Introduction	73
4.3	Experimental Methodology	77
4.3.1	Materials and Method	77
4.3.2	Device fabrication process	77
4.3.3	Creatinine estimation method	78
4.3.4	Image analysis through RGB sensor	79
4.4	Result and discussion	81
4.4.1	Reproducibility of fabricated channel	81
4.4.2	Blood plasma filtration mechanism through porous paper substrate	84
4.4.3	Advantage of combination of Whatman grade 1 and LF1 membrane	86
4.4.4	Performance assessment in relation to the conventional approach	89
4.4.5	Performance analysis of the fabricated device	92
4.4.6	Impact of co-analytes	92
4.4.7	Comparison with existing creatinine measurement microfluidic device	93
4.5	Summary	94
Chapter 5 Development of a Biodegradable Microfluidic Paper-based Device for Blood-plasma Separation Integrated with Non-enzymatic Electrochemical Detection of Ascorbic Acid		
97		
5.1	Overview	97
5.2	Introduction	98
5.3	Experiment	102
5.3.1	Chemical and materials	102
5.3.2	Instruments	103
5.3.3	Blood Sample collection	103
5.3.4	Microfluidic channel fabrication	104
5.3.5	Material synthesis and SPE modification	105
5.3.6	Measurement of AA using E μ PAD	107

5.4	Results and discussion.....	107
5.4.1	Microfluidic channel optimisation.....	107
5.4.2	LF1 Membrane and sample volume optimisation data.....	108
5.4.3	Time taken by various Hct sample to reach at detection zone.....	108
5.4.4	Material phase and morphology characterisation	109
5.4.5	Electrochemical analysis.....	112
5.4.6	Calibration curve and interference study of n-MgF/SPE sensor for AA	118
5.4.7	Comparison with another developed sensor	120
5.4.8	Real sample analysis	123
5.5	Summary	124
Chapter 6	Conclusion and future perspective of the Work.....	127
6.1	Concluding Remarks	127
6.2	Contributions.....	129
6.3	Scope of Future Work.....	132
References.....		135
List of paper based on thesis.....		156