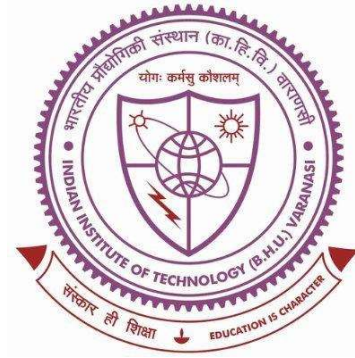


Development of Low-cost Portable Microfluidic Point-of-Care Devices for Estimation of Hematological and Biochemical Parameters



Thesis submitted in partial fulfillment for the
Award of Degree

Doctor of Philosophy

By

Rishi Ram

DEPARTMENT OF MECHANICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI-221005
INDIA

16131011

2022

CERTIFICATE

It is certified that the work contained in the thesis titled "**Development of Low-cost Portable Microfluidic Point-of-Care Devices for Estimation of Hematological and Biochemical Parameters**" by **Rishi Ram** has been carried out under my/our 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.



Dr. Arnab Sarkar
(Supervisor)

Department of Mechanical Engineering

IIT (BHU), Varansai-221005

Associate Professor

Deptt. of Mechanical Engg

Indian Institute of Technology (BHU)

Varansai-221005



Dr. Pradip Paik
(Co-Supervisor)

School of Biomedical Engineering

IIT (BHU), Varanasi-221005

सह-अध्यापक/ASSOCIATE PROFESSOR

संयुक्त प्रौद्योगिकी विभाग/संयुक्त प्रौद्योगिकी विभाग

SCHOOL OF BIOMEDICAL ENGG

भारतीय प्रौद्योगिकी संस्थान (का हि वि)


INDIAN INSTITUTE OF TECHNOLOGY (BHU)

वाराणसी-221005/Varanasi-221005

DECLARATION BY THE CANDIDATE


I, "**Rishi Ram**", certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of **Dr. Arnab Sarkar** and **Dr. Pradip Paik** from "2016" to "2022", 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 willfully 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.

Date:
Place: Varanasi


Rishi Ram
(Candidate)

CERTIFICATE BY THE SUPERVISOR(S)

It is certified that the above statement made by the student is correct to the best of my/our knowledge.


Dr. Arnab Sarkar
(Supervisor)
Associate Professor
Deptt. of Mechanical Engg.
Indian Institute of Technology (BHU)
Varanasi-221005


Head of the Department
विभागाध्यक्ष / HEAD
संस्कृत प्रौद्योगिकी विभाग / Deptt. of Mechanical Engg.
भारतीय प्रौद्योगिकी संस्थान (भारतीय प्रौद्योगिकी संस्थान)
(भारतीय प्रौद्योगिकी संस्थान)
ii
वाराणसी-221005


Dr. Pradip Paik
(Co-Supervisor)
सह-आचार्य/ASSOCIATE PROFESSOR
संस्कृत प्रौद्योगिकी अभियांत्रिकी स्कूल
SCHOOL OF BIOMECHANICAL ENGG.
भारतीय प्रौद्योगिकी संस्थान (भारतीय प्रौद्योगिकी संस्थान)
INDIAN INSTITUTE OF TECHNOLOGY
वाराणसी-221005/VARANASI-221005

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: **Development of Low-cost Portable Microfluidic Point-of-Care Devices for Estimation of Hematological and Biochemical Parameters**

Name of the Student: **Rishi Ram**

COPYRIGHT TRANSFER

The undersigned hereby assigns to the 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 PhD degree.

Date:

Place: **Varanasi**



(Rishi Ram)

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.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor, **Dr. 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 my co-supervisor, **Dr. Pradip Paik**, for his guidance and continuous motivation. I also thank **Dr. Amitesh Kumar** and **Dr. J. P. Chakraborty** 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 **Prof. Suman Chakraborty**, Professor, Department of Mechanical Engineering, Indian Institute of Technology Kharagpur, for allowing me to learn and perform my experiments in his laboratory.

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. Sumit Kumar, Dr. Sudip Chattopadhyay, Dr. Rahul Agarwal, Mr. Dharmendra Kumar, and Ms. Neha Gautam. Finally, I would like to thank my parents and relatives for providing moral and economic support whenever I needed it.

ABSTRACT

Blood is the most commonly used human body fluid for preliminary screening of health conditions of any patient in clinical settings. This may be thanks to the fact that a blood sample may provide a plethora of preliminary information that may help a healthcare professional prompt clinical decision-making regarding the further course of actions. Traditionally, pathological tests with blood samples rely on sophisticated laboratories equipped with high-throughput, efficient instrumentation and skilled technicians. However, these gold-standard medical devices suffer from several shortcomings, such as the elevated cost associated with their purchase, maintenance, and routine use; the requirement of large sample and reagent volume; non-portability; and accessibility and affordability to the patients residing in resource-limited settings. Therefore, point-of-care (POC) testing diagnostic technologies are advanced to circumvent these limitations. Microfluidic-based devices have, off-late, ushered great promises to translate the gold standard testing methods to POC settings. The work conducted within this dissertation focuses on the development of simple, inexpensive, and portable methods for detecting hemoglobin, hematocrit, and creatinine using whole human blood exploiting the paper-based and centrifugal microfluidic platforms.

The primary aim of this thesis is to exploit the advantages of paper-based and centrifugal microfluidic platforms for diagnostics and translate the same into prototype development, demonstrating proof of performance. Chapter 1 of the thesis describes the fundamental of paper-based microfluidics and centrifugal microfluidic platforms. In the subsequent sub-sections, the essential physical aspects driving the central theme of the present thesis are accordingly summarized first.

Chapter 2 of the thesis describes the development of a low-cost reagent-free method for hemoglobin estimation on a simple portable device that can be deployed among the under-served population in resources constrained settings without sacrificing the fundamental principle of direct evaluation of hemoglobin extracted from the human blood sample. Exploring the fundamental principle of osmotic hemolysis for extraction of hemoglobin from red blood cells, the method harnesses the dynamics of a blood drop on a rotating platform and simple imaging to come up with results in a turn-around time of about 13 minutes. The efficacy of the device has been justified by validating with established pathological gold standards. These results are likely to pave the pathway of establishing the clinical assay of a first-principle-based yet reagent-free evaluation of blood pathology in health care diagnostics.

Chapter 3 of the thesis describes the development of a low-cost and simply-fabricated μ PAD to estimate plasma creatinine from the finger-pricked whole human blood. A simple paper strip is proposed to accurately measure creatinine concentration using just 10 μ L of finger-pricked blood. The proposed device mainly involves three steps. The first step is the separation of plasma from the whole blood, the second step is the transportation of plasma to the detection by exploiting the capillary properties of the paper matrix, and the third step is the quantification of the creatinine concentration through a smartphone using an in-house developed app. The creatinine concentration of the same sample is then estimated by a biochemical auto-analyzer and compared with the values obtained from our proposed device. The results obtained by our device show a reasonable accuracy compared to the gold-standard method.

In Chapter 4 of the thesis, a novel paper-based device for simultaneous measurement of hematocrit and hemoglobin is put forth. To determine the hematocrit level,

a 20 μL of the whole human blood is deposited on the detection zone already immobilized with sodium chloride and Ethylenediaminetetraacetic acid (EDTA) solution. However, a 20 μL droplets of the mixture of blood and deionized water are deposited on the hemoglobin detection zone to determine hemoglobin concentration. The resulting stains were digitized with a scanner and analyzed using the freely available software ImageJ. The mean area of the stain is used to quantify the hematocrit level, while the mean grey color intensity is used to quantify hemoglobin concentration. The performance of the paper-based device is compared with an automated hematology analyzer. A high degree of correlation has been observed between the values measured by the paper-based device and automated hematology analyzer ($R^2 = 0.9651$ for hematocrit level; and $R^2 = 0.9701$ for hemoglobin concentration). The device provides a simple, fast, disposable, and inexpensive tool to determine the hematocrit level and hemoglobin concentration simultaneously.

Chapter 5 of the thesis describes the development of a simple, affordable, and portable spinning disc for measuring plasma-creatinine concentration with 10 μL of whole human blood. 5 μL of the alkaline picrate solution is loaded into the device and rotated at 1000 rpm to transport this solution to the periphery of the microchannel. Further, 10 μL whole blood is loaded in the same channel and spun at 1300 rpm for 10 minutes. The creatinine in the blood plasma reacts with alkaline picrate (Jaffe reaction), and the color of the mixture changes to yellow-orange color. The resulting color is captured with a smartphone, and creatinine concentration is estimated using an in-house developed app (CREA-SESE). The value of creatinine measured with the present device and the gold standard device is highly correlated ($R^2 = 0.998$). This study demonstrates the feasibility of a simple, inexpensive, and portable rotating device for measuring creatinine concentration using 10 μL of finger-pricked whole human blood, which can easily be

deployed among underserved population in resource-constrained settings to monitor renal diseases.

Finally, the conclusion of the thesis is presented in chapter 6. The novel contributions from the preceding chapters are summarized, and some possible extensions are also discussed. In a nutshell, our focus is to develop a novel diagnostic delivery model enabled by disruptive technology for better healthcare diagnostics at sufficiently low cost and establish the same through few studies combining the empowerment of underserved population as human resources with niche diagnostic technology such as paper and compact disc-based centrifugal microfluidics.

TABLE OF CONTENTS

CERTIFICATE	i
DECLARATION BY THE CANDIDATE	ii
COPYRIGHT TRANSFER CERTIFICATE.....	iii
ACKNOWLEDGEMENTS.....	iv
ABSTRACT.....	v
TABLE OF CONTENTS.....	ix
LIST OF TABLES.....	xiv
LIST OF FIGURES	xv
Chapter 1: Introduction.....	1
1.1 Motivation	1
1.2 Fundamental of centrifugal microfluidics	3
1.2.1 Centrifugal force.....	3
1.2.2 Coriolis force	4
1.2.3 Euler force.....	4
1.3 Fundamental of paper-based microfluidics	5
1.3.1 Mechanism of fluid transportation on paper substrate.....	5
1.4 Outline of the thesis.....	6
Chapter 2: Reagent-Free Hemoglobin Estimation on a Spinning Disc	9
2.1 Chapter overview.....	9
2.1.1 Introduction.....	9

2.2	Experimental details	12
2.2.1	Device design and fabrication.....	12
2.2.2	Blood sample collection and processing.....	16
2.2.3	Numerical modeling.....	17
2.2.4	Experimental setup and procedure.....	20
2.3	Results and Discussions.....	22
2.3.1	Extraction of Hb from RBC and optimization of the volume of DI Water .	22
2.3.2	Performance evaluation vis a vis gold standard.....	25
2.3.3	Detection of anemia	28
2.3.4	Comparison with other Hb measuring devices	29
2.4	Conclusions	29
Chapter 3: Smartphone-based Automated Estimation of Plasma Creatinine from Finger-pricked Blood on a Paper Strip via Single-user Step Sample-to-result Integration.....		
3.1	Chapter overview.....	32
3.1.1	Introduction.....	32
3.2	Experimental details	38
3.2.1	Chemicals and materials	38
3.2.2	Device design and fabrication.....	39
3.2.3	Collection of blood samples and processing.....	40
3.2.4	Method of estimation	41
3.2.5	Smartphone app (CREA-SENSE) development and image analysis	43
3.3	Results and discussion	45

3.3.1	Effect of salt concentration on the plasma separation efficacy.....	45
3.3.2	Principle for plasma separation and colorimetric reaction based on time-dependent phenomena	46
3.3.3	Technology translation to the extreme point-of-care application using modified Jaffe reaction	47
3.3.4	Performance evaluation compared to the conventional approach	48
3.3.5	Analytical performance of the proposed method.....	51
3.3.6	Chi-square test	53
3.3.7	Reagent stability and selectivity	53
3.3.8	Estimation of cost for creatinine test	55
3.4	Conclusion.....	57
Chapter 4: A Simple and Low-cost Paper-based Device for Simultaneous Determination of Hematocrit and Hemoglobin Levels in Point-of-care Settings.....		
		59
4.1	Chapter overview.....	59
4.2	Introduction	59
4.3	Materials and methods.....	63
4.3.1	Materials and equipments	63
4.3.2	Design and fabrication of μ PAD	63
4.3.3	Blood sample collection and processing.....	64
4.3.4	Experimental methodology.....	65
4.3.5	Statistical analysis.....	66
4.4	Results and discussion	66

4.4.1	Hematocrit determination	66
4.4.2	Hemoglobin estimation	69
4.5	Conclusion	73
Chapter 5: A Smartphone-integrated Portable Rotating Platform for Estimation of Concentration Level of Plasma-Creatinine using Whole Human blood.....		75
5.1	Chapter overview	75
5.2	Introduction	75
5.3	Materials and methods.....	78
5.3.1	Chemicals and materials	78
5.3.2	Device design and fabrication.....	78
5.3.3	Blood collection and processing	81
5.3.4	Experimental methodology	81
5.3.5	Development of smartphone app and image analysis.....	82
5.3.6	Image recording process	84
5.4	Results and discussion	85
5.4.1	Translation of modified Jaffe reaction for point-of-care settings	85
5.4.2	Performance evaluation of present device against the conventional approach.....	86
5.4.3	Analytical performance of the proposed method.....	87
5.4.4	Selectivity of creatinine	88
5.5	Conclusion	89
Chapter 6: Conclusions and Future Work.....		91

6.1	Summary and conclusions	91
6.2	Scope of future studies	93
	REFERENCES	95
	LIST OF PUBLICATIONS	110

LIST OF TABLES

Table 2.1 Comparison of performance of present work with reported methods for estimation of Hb concentration.....	30
Table 3.1 The various phases of kidney disease with representative blood plasma creatinine concentration values.....	34
Table 3.2 Accuracy measurement of proposed method (paper-based microfluidic device).....	52
Table 3.3 Comparison of estimation of creatinine concentration between developed app and scanner detection mode	53
Table 3.4 Cost-component break-up per test	56
Table 3.5 Assay performance comparison with other representative reported methods ..	57

LIST OF FIGURES

Fig. 1.1 Schematic presentation of the forces acting on a centrifugal microfluidic platform3

Fig. 2.1 Representation of the experimental process. (A) Design and specifications of the compact disc. Side view of the device showing thicknesses of the top, middle and bottom layers which are 1 mm, 20 μm , and 1 mm, respectively. The top view of the compact device showing the channel length which is 49.4789 mm, the angular span of each sector which is 50° , and the diameter of the disc which is 130 mm. (B) Stepwise procedure of sample processing in the spinning disc. Loading of DI water in the microchannel (Step I); rotating the disc (at 1000 rpm for 1 min) for transporting water to the outer periphery of the microchannel (Step II); loading of whole blood in the same channel (Step III); rotating the disc (at 2000 rpm for 13 min) to accomplish the separation of RBC from whole blood (Step IV) and mixing with DI water. A magnified view of the RBC lysis in a microchannel is shown as a blown-up figure. (C) Schematic illustration of the process flow of Hb estimation in the present POC device. The essential steps include a collection of finger pricked blood into a tube pre-coated with an anti-coagulant, loading of blood in the micro-channel of the disc, separation of RBC due to centrifugation, and mixing with DI water. The rotational speed is displayed using a display unit to attain a precise setting. After osmotic hemolysis leading to the release of Hb, the colored image is captured using a smartphone for rapid analysis..... 14

Fig. 2.2 Numerical simulation details. (A) Schematic representation of the computational domain used for the numerical simulation. The axis of rotation is along the z-axis at $r = 0$. The inlet of the micro-channel is at $r_1=10$. Initially, the whole blood is filled in the region ranges from r_1 to r_2 (25.30 mm in length), and DI water is filled in the region ranges from

r_2 to r_3 (24.18 mm in length). The height of the entire micro-channel is 20 μm . (B) Mixing of blood with DI water inside the channel where T is the total time taken by the blood for complete mixing. (C) Variation of separation time with angular speed. (D) Decaying trend of the normalized time with increasing value of rotational speed. 19

Fig. 2.3 Optimization of the volume of DI water for osmotic hemolysis of RBC. Blood has been mixed with DI water at 10:1, 10:2.5, 10:5, 10:10, 10:15, and 10:20 ratios (vol/vol) which are represented by images (A) to (F) respectively. 22

Fig. 2.4 Estimation of Hb on the proposed spinning disc. (a) Calibration curve showing the correlation between average color intensity measurements in our proposed device with the concentration of commercially available Hb standards. Every data point represents the mean \pm SD. Three replicates of each concentration are used to generate the calibration curve. (b) Comparison of Hb measurements in the proposed portable device (Measured) with the standard results obtained using the automated hematology analyzer (Actual) for 25 different blood samples that are different from the ones used to construct the calibration curve. Error bars indicate the standard deviations among three independent experimental data. The experimental data with Hb concentrations ranging from 8 g/dl to 16 g/dl demonstrate a good agreement between the present and gold standard methods with a correlation coefficient of 0.992. (c) Coefficient of variation of measured data of Hb for 25 blood samples using the present device. The coefficient of variation for every sample is $<7\%$, indicating good precision among the measurements. (d) The Bland–Altman analysis plot, comparing an average of the gold standard (Sysmex) and present (portable device) based predictions of Hb concentrations, highlighting the difference between the gold standard and measured Hb concentration values, to evaluate the accuracy of the device.

The red solid line shows a bias of -0.073 g/dl. Dotted red lines indicate 95% limits of agreement: 0.35 g/dl (upper limit) and -0.49 mg/dl (lower limit).24

Fig. 2.5 Clinical evaluation of the present Hb sensor in anemia detection. (A) Correlation between Hb concentration measurement results of 15 blood samples in the proposed portable device (Measured) and automated hematology analyzer (Actual). Each data point represents the mean \pm SD of three separate measurements. The experimental data with Hb concentrations ranging from 5 g/dl to 11.5 g/dl (in anemic range) demonstrates a good agreement between the two methods with a correlation coefficient of 0.98. (B) Coefficient of variation of measured data of Hb for 15 samples using the portable device. The coefficient of variation for each sample ranged from 0.95-3.36%, indicating very good precision among the measurements. (C) The Bland–Altman analysis results, comparing an average of the gold standard (Sysmex) and measured (present device) values of the Hb concentration, with the difference between actual and measured Hb concentrations to evaluate the accuracy of the device. The red solid line shows a bias of 0.531 g/dl. Dotted red lines indicate 95% limits of agreement: 1.33 g/dl (upper limit) and -0.26 g/dl (lower limit).....27

Fig. 3.1 Illustration of the aggregation of RBCs: (a) Whole blood containing RBC, WBC (not shown in the figure), Ca^{2+} etc. dispersed in plasma in vivo, (b) Addition of anti-coagulant (EDTA) that binds to Ca^{2+} to prevent RBCs aggregation in vitro, (c) Addition of excess Ca^{2+} initiates aggregation of RBCs and plasma separation in vitro.37

Fig. 3.2 Schematic representation of the printed paper device with the hydrophilic channel containing a sample inlet zone, blood-plasma separation zone, and detection zone, and the patterning of hydrophobic barriers by LaserJet printer. The magnified view shows the microscopic image of the Whatman cellulose paper (Grade-4) matrix. The paper matrix

consists of random distribution of cellulose fibers and forms non-uniform, microscopic inter-fiber pores for liquid flow.41

Fig. 3.3 Schematic illustration of paper-strip-based creatinine estimation. After immobilization of the sample inlet zone with CaCl_2 , the detection zone is immobilized with alkaline picrate. Further, after collection, the loading of whole blood in the salt-functionalized sample inlet zone of the paper-based device is carried out. Separation of plasma from the whole blood occurs due to aggregation of RBCs in presence of calcium ions and the separated plasma travels to the detection zone due to capillary effects and is resisted by viscous forces. Creatinine present in separated plasma is reacted with reagent (alkaline picrate) in the detection pad, leading to the formation of color at the detection zone. The image is captured in a smartphone followed by an image analysis using an in-house developed smartphone app (CREA-SENSE) for quantitative estimation of creatinine.42

Fig. 3.4 The smartphone-based image analysis for quantitative estimation of creatinine. (a) Flowchart for image processing and implemented in the “CREA-SENSE” app. (b) Screenshots of sequential operation of the app are (i) open the app (ii) start new test (iii) capturing the image (iv) select the image for analysis (v) view of the image from the gallery where it stored and (vi) displaying the outcome.44

Fig. 3.5 Effect of CaCl_2 concentrations on plasma separation. (a) Separation of plasma from EDTA-treated whole blood on μPADs functionalized without and with 50 mM CaCl_2 solutions (optimum), respectively. (b) The curve shows the distance of separated plasma traveled from the RBC front (mm) vs. concentrations of CaCl_2 solution. Every data point represents the mean values and error bars show the standard deviation among three independent experimental results.46

Fig. 3.6 Real-time images captured by microscope showing the separation of plasma from whole blood and transportation of the separated plasma to the detection zone for colorimetric assay at different time intervals. Step 1: loading of blood in salt functionalized sample inlet zone (t = 0 sec), Step 2: start separating plasma from whole blood after 30 seconds (t = 30 sec), Step 3: transportation of separated plasma through the hydrophilic channel and arriving at the detection zone (t = 90 sec), Step 4: reaction of creatinine in plasma with alkaline picrate takes place in the detection zone and formation of color (t = 120 sec) for quantitative estimation of creatinine.....49

Fig. 3.7 Results obtained from the integrated paper-based device. (a) Calibration curve of average color intensity with the concentration of commercial creatinine in the range of 0.1 to 15 mg/dl in the paper-based microfluidic device. (b) Comparison of creatinine measurement results obtained using the paper-based microfluidic device (Measured) and biochemical auto-analyzer (Actual) for 30 different blood samples. (c) Coefficient of variation of creatinine measurement data for 30 blood samples using the paper-based microfluidic device. (d) The Bland–Altman plot shows the mean of against difference between actual and measured creatinine concentration to evaluate the accuracy of the paper-based microfluidic device. The thick red line indicates the mean. Dotted red lines show 95% limits of agreement (LOA), (average difference \pm 1.96 SD).50

Fig. 3.8 Performance of storage stability of reagent (alkaline picrate) on salt functionalized paper with two different concentrations of commercial creatinine (0.5 and 1.5 mg/dl) at room temperature. The curves show that color intensities of the creatinine-picric acid complex are reduced very little after approximately 75 days.54

Fig. 4.1 Design of μ PADs on cellulose chromatography paper64

Fig. 4.2 (a) Immobilization of NaCl and EDTA on hematocrit detection zone and allow to dry for 10 minutes, (b) dispensing Whole blood on hematocrit detection zone and a mixture of blood and DI water on hemoglobin detection zone, (c) Radially spread of the sample on the μ PAD; (d) digitalization of the sheets of chromatographic paper containing μ PADs with the scanner, (e) Analyzing area of spread for Hematocrit and Grey color intensity for hemoglobin; (f) Comparison of the result for Hematocrit.65

Fig. 4.3 Dependency of the mean area of the stain on hematocrit: (a) Variation of the area of the stain with hematocrit level, (b) Calibration curve was constructed by plotting the hematocrit level along the horizontal axis and area of stain along the vertical axis. Comparison of hematocrit values measured with paper-based device and automated hematology analyzer (n = 19 samples): (c) The diagonal (black solid line) represents a 1:1 correspondence between actual and measured hematocrit values. The red dashed line represents linear regression analysis of the correlation between the hematocrit value measured by paper-based device and automated hematology analyzer, (d) Bland-Altman plot of the limit of agreement between the paper-based device and automated hematocrit analyzer. The solid black line is the mean of the difference between the hematocrit values measured by two methods (bias). Dashed red lines are the limits of agreements within 95% confidence interval.....67

Fig. 4.4 The osmotic hemolysis of RBCs for the different ratios of blood and DI water: (a) 1:0.5, (b) 1:1, (c) 1:1.5, (d) 1:2, (e) 1:2.5, (f) 1:3.....70

Fig. 4.5 Dependency of the mean color intensity of the stain on hemoglobin: (a) Variation of the color intensity of the stain with hemoglobin concentration, (b) Calibration curve was constructed by plotting the hemoglobin concentration along the horizontal axis and mean grey color intensity along the vertical axis. Comparison of hemoglobin values measured

with paper-based device and automated hematology analyzer (n = 31 samples): (c) The diagonal (black solid line) represents a 1:1 correspondence between actual and measured hemoglobin values. The red dashed line represent linear regression analysis of the correlation between the hemoglobin value measured by paper-based device and automated hematology analyzer, (d) Bland-Altman plot of the limit of agreement between the paper-based device and automated hematology analyzer. The solid black line is the mean of the difference between the hemoglobin levels measured by two methods (bias). Dashed red lines are the limits of agreements within 95% confidence interval. 72

Fig. 5.1 Fabrication of the device: (a) Top layer is an one mm PMMA disc containing holes for sample loading and pressure balancing; (b) The middle layer is fabricated by 20 μ L pressure-sensitive double-sided adhesives (PSA) incorporating the patterns for microfluidic channels in the form of a sector having a length of 25 mm and sector angle of 45°; (c) The bottom layer is also an one mm thick PMMA disc which provides base and structural support; (d) All three layers are manually aligned and compressed to obtain the final compact device. 80

Fig. 5.2 Step-by-step clinical assay of estimation of creatinine level on the developed device: loading of 5 μ L of alkaline picrate in the microchannel of the disc; rotating CD to transport the alkaline picrate to the periphery of the channel; loading of blood in the same microchannel of the CD; rotating CD for mixing of blood with alkaline picrate, and separation of RBCs and mixture of plasma and alkaline picrate; recording the image with smartphone camera inside a lightbox after the color of this mixture changes to yellow-orange; and quantification of creatinine level using CREA-SENSE app. 82

Fig. 5.3 Estimation of creatinine concentration using captured images inside the light box: (A) Algorithm implemented in the “CREA-SENSE” app for image processing; (B)

Sequential operation of the app: (a) launching, (b) selecting new test, (c) image recording, (d) selecting image from smartphone gallery for analysis, (e) display of the selected image in the app and (f) displaying creatinine concentration.....84

Fig. 5.4 Lightbox setup for image capture: (a) An opening is created in the top surface of the box to accommodate the smartphone camera lens; (b) Four LED light strips covered with paper are secured on the top surface with the help of double-sided tape, and after the development of the color, the CD is placed inside the light box at indicated placed; (c) All sides of the lightbox are closed with the lights on. The phone camera lens is placed over the opening, and the image is captured for analysis.85

Fig. 5.5 (a) Calibration curve which is a plot of average color intensity against the known concentrations of commercially available creatinine (0.1 to 15 mg/dl); (b) Comparison between the creatinine values obtained from our device (Measured value) and biochemical auto-analyzer (Actual value) for 30 blood samples; (c) The Bland–Altman plot shows the bias in the values of the creatinine concentration is 0.0135 mg/dl. The lower and upper limits with 95% limits of agreement are - 0.27 and 0.30 mg/dl, respectively.87

Fig. 5.6 Selectivity test of the device in the presence of creatinine and other interfering substances89