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

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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.

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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.

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Neha Gautam

Date: 14.8.24

Place: Varanasi

(Neha Gautam)

Candidate

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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

यांत्रिक अभियान्त्रिकी विभाग/Deptt. of Mechanical Engg.

भारतीय प्रौद्योगिकी संस्थान/Indian Institute of Technology

(का०हि०वि०/B.H.U.)

वाराणसी-221005/Varanasi-221005

Supervisor

Head of the Department

विभागाध्यक्ष/HEAD

यांत्रिकी अभियान्त्रिकी विभाग/Deptt. of Mechanical Engg.

भारतीय प्रौद्योगिकी संस्थान/Indian Institute of Technology

(का०हि०वि०/B.H.U.)

वाराणसी-221005/Varanasi-221005

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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 (μPAD) 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/EμPAD 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, 5.71×10^{-3} mA μM^{-1} cm^{-2}, and 10 seconds, respectively. Our developed n-MgF/SPE/EμPAD 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.

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