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

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DOCTOR OF PHILOSOPHY

By

NEHA GAUTAM

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

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Chapter 6 Conclusion and future perspective of the Work

In the present thesis we have fabricated affordable paper based microfluidic device for blood-plasma fluidic property analysis and blood-plasma analyte measurement. These devices can be fabricated in few minutes with minimal infrastructure and serves as a point-of-care device for remote locations.

6.1 Concluding Remarks

This thesis provides insights into the fluid flow behaviour through an open microfluidics paper platform and its application for detecting pathological parameters. Various fabrication protocols were used to create microchannels on a hydrophilic paper surface to control the liquid flow rate.

An experimental study was conducted to analyze the effect of different fabrication processes on the liquid flow rate through porous paper channels. The analysis concluded that there are significant changes in the liquid flow rate depending on the fabrication process used. Fabrication processes where the entire paper surface comes into contact with the fabrication device exhibit slower velocities compared to methods with minimal exposure of the paper substrate to the fabrication device. In the wax printing process, the entire paper surface passes between the wax rollers. During this process, a few wax toner particles are deposited on the hydrophilic white surface, which are not visible to the naked eye and reduce the flow rate of liquid through the paper channel. In contrast, the laser cutting process uses a high-temperature flame (~400-500 ˚C) to cut the channel design from the paper surface. This high temperature alters the microfiber alignment along the cutting edges of the prepared channel, affecting the liquid flow rate through the paper. 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. Prior knowledge of the effects of fabrication processes on liquid flow rate helps manufacturers select the optimum fabrication process to control liquid flow rate for various applications. For example, the paper printing process shows a lower flow rate compared to laser-treated or simple scissor-cut prepared microchannels. However, after a threshold channel width (w > 4mm), there are no significant changes in the flow rate among the differently fabricated microchannels.

A simple paper-based microfluidic viscometer was fabricated using an office printer to measure the viscosity of plasma samples (Newtonian liquid) without violating the basic principles of measurement. In this work, a Brookfield viscometer, widely accepted for measuring the viscosity of Newtonian fluids, was used. Real human plasma samples were employed in the experiment. A linear correlation was found between the gold standard measurement and the fabricated device measurement, with ~96% accuracy Our developed paper viscometer is capable to measure the plasma viscosity using 50 µL sample in five-minute time duration with the help of a smartphone app, demonstrating its potential for further clinical deployment.

Additionally, a paper device was fabricated using the wax dipping method to separate plasma from whole human blood. This device utilized a combination of two different pore size membranes, LF 1 and Whatman grade 1, with pore sizes of ~2 µm and ~12 µm, respectively. Whole blood, containing both cellular particles (size above 3 µm) and plasma, when injected at the inlet made of LF1 membrane, traps the cell particles and allows only plasma to move forward. The Whatman grade 1 paper substrate shows a fast flow rate and provides a flexible surface for analyte measurement using various detection methods.

In Chapter 4, a simple paper-based platform was fabricated using the aforementioned wax dipping technique, achieving 98% reproducibility. Plasma creatinine measurement was performed using the Jaffee reaction, with a RGB sensor measuring color changes during the reaction. The device shows an excellent correlation between color intensity and creatinine concentration in blood-plasma samples, covering the entire clinical range. A validation study using 35 real samples shows ~96% accuracy compared to the gold standard measurement.

In Chapter 5, Ascorbic Acid (AA) measurement was performed on the same wax-dipped fabricated device using a Mg-Fe_2O_3 nanomaterial-modified screen-printed electrode (SPE) integrated with the device to measure changes in electrochemical signals due to the presence of AA. This device shows an excellent correlation between electrochemical signals and AA concentration, providing 98% accuracy against gold standard measurement.

6.2 Contributions

This thesis makes several critical contributions to both the scientific understanding and practical applications of fluid flow behaviour in open microfluidic paper platforms, especially concerning the detection of pathological parameters. Through a comprehensive study of various fabrication protocols, the research sheds light on how different methods significantly influence the liquid flow rates through porous paper channels. These insights are particularly valuable for designing and optimizing paper-based diagnostic devices, which are increasingly used in point-of-care testing and resource-limited settings.

Key contributions and real-life applications:

1. Understanding fabrication effects on flow rates:

The thesis explores the impact of different fabrication processes such as scissor cutting, laser cutting, single-side and double-side printing, and blank rolling on liquid flow rates in microchannels. For instance, it was found that fabrication processes like wax printing, where the entire paper surface is exposed to the fabrication device, tend to slow down the flow rate. This is because the deposition of wax toner particles, though invisible to the naked eye, creates resistance to fluid flow.

Real-Life Example: In a clinical setting, controlling the flow rate is crucial for accurate diagnostic tests. For example, in glucose testing for diabetes management, the timing and flow rate of blood through a test strip can affect the accuracy of the glucose concentration reading. Understanding how fabrication impacts flow can lead to more reliable test strips, where the flow rate is optimized to ensure consistent and accurate results.

2. Development of a Paper-Based Microfluidic Viscometer:

A significant contribution of the thesis is the development of a paper-based microfluidic viscometer using a standard office printer. This device, which can measure plasma viscosity with about 96% accuracy, requires only a small sample volume (50 µL) and delivers results within five minutes. The device's integration with a smartphone app further demonstrates its potential for clinical use, offering a low-cost, portable solution for viscosity measurements.

Real-Life Example:

Plasma viscosity is an important diagnostic marker for conditions such as blood clotting disorders and cardiovascular diseases. In rural or under-resourced healthcare settings, where access to advanced laboratory equipment is limited, this paper-based viscometer can provide a quick and affordable means to monitor plasma viscosity, aiding in timely diagnosis and treatment.

3. Plasma separation device using dual membranes:

The research also introduces a paper device fabricated using the wax dipping method for separating plasma from whole human blood. This device employs two different membranes LF1 and Whatman Grade 1 to trap cellular components while allowing plasma to flow through. This method ensures a fast and efficient plasma separation, which is essential for subsequent analyte detection.

Real-Life Example:

Plasma separation is a critical step in many diagnostic tests, such as those for infectious diseases, where the plasma is used to detect the presence of antibodies or antigens. In emergency situations or field testing, where quick results are needed, this paper-based device can simplify the plasma separation process, allowing healthcare workers to perform accurate tests on-site without the need for centrifuges or other complex equipment.

4. Electrochemical sensing for ascorbic acid (AA) detection:

The thesis extends the application of paper-based platforms to electrochemical sensing by integrating a Mg-Fe_2O_3 nanomaterial-modified screen-printed electrode (SPE). This innovation enabled the accurate measurement of ascorbic acid (AA) concentrations with 98% accuracy, demonstrating the device's potential for detecting small molecules in clinical samples.

Real-Life Example:

Ascorbic acid (vitamin C) levels are important in monitoring nutritional status and diagnosing certain health conditions. In remote or underserved areas, where laboratory facilities are not readily available, this paper-based electrochemical sensor can be used to quickly and accurately measure vitamin C levels from a small blood sample, assisting in the management of nutritional deficiencies or the monitoring of oxidative stress in patients.

Broader Impact on the Scientific Community:

The insights and innovations presented in this thesis contribute to the broader scientific community by advancing the knowledge of fluid dynamics in paper-based microfluidic systems. The practical applications demonstrated in this research highlight the potential for paper-based devices to revolutionize point-of-care diagnostics, particularly in settings with limited resources. The development of cost-effective, portable, and reliable diagnostic tools, as showcased in this work, aligns with global health priorities, where there is a growing need for accessible and rapid diagnostic solutions. The findings can also inspire further research into optimizing fabrication processes and exploring new materials and techniques for enhancing the performance of paper-based microfluidic devices in various healthcare applications.

6.3 Scope of Future Work

Although this thesis provides a simple microfluidic device as an alternative for measuring plasma analytes from whole human blood, further methods are needed to improve both the device fabrication processes and the electrochemical measurement methods. The following directions outline specific research problems for future work:

1. Automating the Fabrication Process with 3D Printing:

Research Problem: The current manual wax dipping method used to attach two different filter papers for blood plasma separation introduces fabrication errors.

Objective: Develop a 3D printing process to automate the fabrication of microfluidic devices, ensuring consistent and precise attachment of filter papers.

Approach: Design and optimize a 3D printer setup and protocol for fabricating microfluidic devices, including selecting appropriate materials and validating the performance through comparison with manually fabricated devices.

Expected Outcome: Reduction in fabrication errors, improved device reproducibility, and enhanced overall device performance.

2. Direct Fabrication of Electrodes on Whatman Filter Paper:

Research Problem: Integrating a commercially available SPE (Screen-Printed Electrode) with the paper surface introduces noise in the current signal measurement for AA (Ascorbic Acid).

Objective: Fabricate electrodes directly on Whatman filter paper to improve the current signal intensity and response time.

Approach: Investigate materials and techniques for electrode fabrication directly on filter paper, such as conductive ink printing or deposition methods. One has to optimize the electrode design and evaluate the electrochemical performance.

Expected Outcome: Enhanced signal-to-noise ratio, increased current signal intensity, and faster response times in electrochemical measurements.

3. Improving Ink Stability for Inkjet Printer Fabricated Devices:

Research Problem: The hydrophobic boundary of the ink used in inkjet printer fabrication fades after one month of channel storage, reducing the device's storage life.

Objective: Modify the ink properties to improve the long-term stability of the fabricated device.

Approach: Formulate new ink compositions with enhanced stability and test various additives or treatments to maintain the hydrophobicity of the boundaries. Conduct accelerated aging tests to evaluate the longevity of the modified ink.

Expected Outcome: Extended storage life of the fabricated microfluidic devices, maintaining functional hydrophobic boundaries for longer periods.

4. Integration with AI/ML for Enhanced Measurement and Data Visualization:

Research Problem: The current microfluidic devices lack advanced measurement and data visualization capabilities, limiting their clinical assay accuracy.

Objective: Integrate the portable microfluidic devices with AI/ML for measurement and data visualization using computer vision-enabled smartphone apps.

Approach: Develop a smartphone application with computer vision capabilities to process video data from the microfluidic devices. Train ML models to analyse the data and provide accurate measurements and visualizations.

Expected Outcome: Improved accuracy and usability of clinical assays, enabling real-time data processing and visualization through a user-friendly smartphone interface.

By addressing these specific research problems, significant advancements can be made in the fabrication and functionality of microfluidic devices for clinical applications.