

Disposable Paper-Based Biosensor for multiplexed analysis

This chapter is divided into two parts. The first part studies the various designs of paper-based devices to optimize the flow rate for the purpose of multiplexed detection of biomarkers, while the second part deals with the the fabrication and utilization of a compact, handy, and smartphone-assisted microfluidic paper-based analytical device (μ PAD) fabricated through commercially available A4 paper for the multiplexed detection of three different analytes (glucose, lactate, and uric acid) by utilizing a novel trident shaped μ PAD.

5.1 DESIGN OPTIMIZATION OF TRIDENT SHAPED MICRO CHANNELS ON PAPER FOR MULTIPLEX DETECTION

5.1.1 Introduction

The surface characteristics of paper, i.e., surface area, pore size, porosity, capillary flow rate, and thickness, play an essential role in determining fluid flow through paper. Deposition of reagents, sensitivity, specificity, and reproducibility of paper-based sensors depend on the total effective area of the paper (Zhong et al., 2012). Each Whatman filter paper (WFP) has unique properties, particularly pore size. Whatman no. 1 (WN1) filter paper with a pore size of 11 μ m is the most commonly used. It has moderate retention and flow rates, while the Whatman no. 4 (WN4) has a pore size of 20-25 μ m and a very high filtration rate (Pratiwi et al., 2019). It was found that the rising rate of solution through the paper is slower for narrower channels due to hindrance to the solution flow (Evans et al., 2014). Liquid travels through the paper due to its capillary action. However, this feature does not allow for control over the flow's speed and direction, which may be its most significant downside. This often results in difficulties such as analyte loss during transport and, as a result, more significant detection limits than traditional approaches (M. P. Nguyen et al., 2018b). The volume of fluid transfer depends on the types of paper in paper-based biosensors. Inadequacy of tests in case of ultralow concentration of analytes is caused, as paper assimilates the liquid and spreads it consistently on the paper bed. The presence of a soaking pad required for the movement of the sample toward the detection zone causes less than half of the sample volume to reach the detection zone in paper-based strips. Thus, it requires more samples and increases the burden before detection (Ahmed et al., 2016). In the work, a 4 mm diameter was chosen for testing, and an uptake zone was used to detect uric acid and creatinine. The volume of sample and reagent required increases as the diameters of the zones increase. The selection of 4 mm diameter gave high color consistency and used less volume of reagent and sample, i.e., 2 μ l and 10 μ l respectively (Rossini et al., 2018). Paper-based sensors preferably used for colorimetric analysis of analytes due to its physical properties *viz.* capillary flow rate and porosity (Kishnani, Kumari, et al., 2022). These colorimetric results can be analyzed via naked eye or some sophisticated instruments. Further analysis of results can be enhanced towards more accuracy with the help of machine learning (Kishnani & Gupta, 2023d). The optimization of μ PADs is critical in assuring the fitness of chemical systems. Optimization, a commonly used word, is establishing the experimental conditions that will give the best possible analytical system response (Liana et al., 2015). Thus, flow control is still a challenge and an essential aspect of the

effectiveness of paper-based devices for utility, such as sample or reagent addition (Y. Yang et al., 2016).

5.1.2 Material and Methods

Whatman no. 1 and no. 4 Filter Paper (125mm diameter from GE Healthcare Life Sciences), Permanent Marker ink (Camlin), Ultra Gold reagent grade Triple Deionized water (DIW) from Organo Laboratories Chemical Pvt Ltd., Delhi, India (specific conductivity < 1). Glycerol from Sigma-Aldrich (St. Louis, MO, USA) ($\geq 99.0\%$), Methylene Blue (MB) dye from Fisher Scientific (Waltham, MA, USA) (Sp. Absorptivity $A\ 1\%/1\ \text{cm}$ max at 660–665 nm). COMSOL multi-physics 5.6 software for theoretical studies. HP Deskjet 1115 Printer, Drop Shape Analyzer DSA25b, Fluorescence Microscope.

It is well known fact that the normal blood viscosity lies between 3.5 to 5.5 cP (Nader et al., 2019). The viscosity of 40% glycerol is 3.7 cP, which lies within the range of blood viscosity values. Therefore, 0.2% of Methylene Blue dye was prepared in 40% glycerol. A volume range of 2 – 10 μl was chosen for volume optimization. 2, 3, 4, 5, 6, 7, 8, 9, and 10 μl of dye solution was pipetted in the sample inlet zone of different fabricated designs tapered at an angle of 5° . Similarly, it was performed for designs tapered at an angle of 10° , 15° , and 20° respectively. The time the dye solution took to travel through the paper from the sample inlet zone to the detection zone was also noted using a stopwatch in each of all the four different designs.

(a) Fabrication of PAD

Figure 5.1 shows the schematic representation of printing-based optimized design on WN1 and WN4 papers. Four different trident-shaped designs were first prepared using SolidWorks, which were tapered at all openings of the channels vertically and horizontally at an angle of 5° , 10° , 15° , and 20° respectively (see Figure 5.2). All four different designs were then printed on WN1 and WN4-filter paper with the help of printer. In this approach, hydrophobic barriers were created on WN1 and WN4 using permanent markers. It consists of a hydrophobic resin, a colorant, and a solvent usually, ethanol. The hydrophobic resin persists in the filter cellulose after the solvent has evaporated from the filter paper, generating hydrophobicity (Xu et al., 2014). Characterization of the hydrophobic barrier created using a marker was also performed by taking contact angle measurements of the hydrophobic layer and measuring the thickness of filter paper before and after the deposition of marker ink. Hydrophobic barriers and hydrophilic channels were created manually using Camlin permanent marker ink.

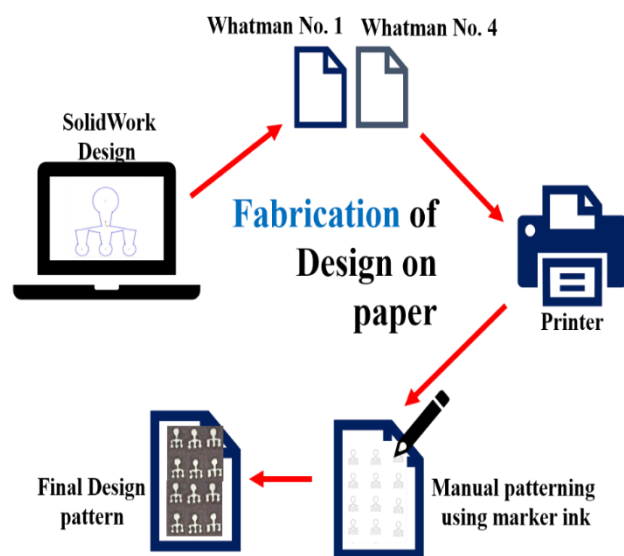


Figure 5.1 : Schematic representation of printing of optimized design on WN1 and WN4 papers

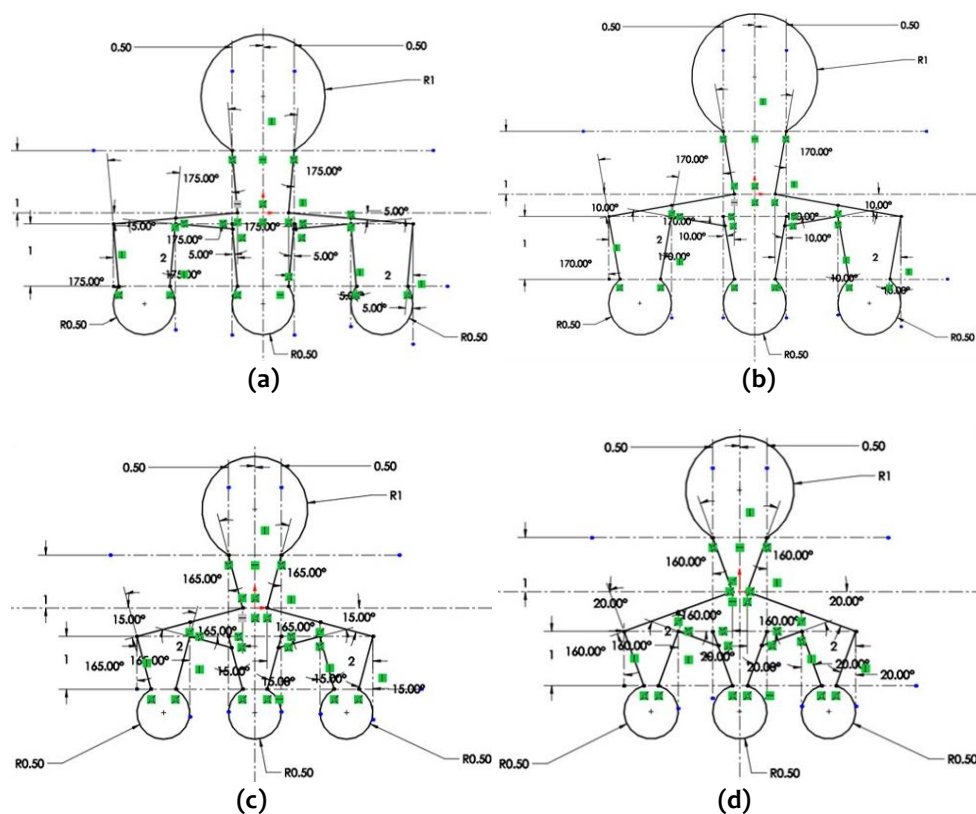


Figure 5.2 : Trident shaped designs tapered at (a) 5° (b) 10° (c) 15° (d) 20°

(b) Surface characterization

A Fluorescence Microscope was used to take microscopic images to show the hydrophobic barrier created using a permanent marker on paper. Images were also taken for measurement of the thickness of filter paper before and after the manual deposition of permanent marker ink.

5.1.3 Results and Discussion

(a) Fabrication of permanent marker ink-based barrier on paper

Trident-shaped designs were constructed by direct manual patterning using permanent marker ink. The ink of a permanent marker pen penetrates through the paper, blocks the pores between the fibers of the paper, and forms a hydrophobic barrier. This was confirmed in many ways. Blockage of paper pores by marker ink was confirmed by taking the microscopic image [Figure 5.3 (a)] of the boundary of the hydrophobic barrier and hydrophilic channel. It can be seen in Figure 5.3 (a) that the spaces between the fibers of the paper are filled by the marker's ink, creating a barrier. When dye solution was introduced into the hydrophilic channel, it was observed that the barrier created confines the solution, and it was also confirmed through the microscopic image. No leakage through the barrier or disruption of the barrier was observed, as can be visualized in Figure 5.3 (b).

The direct patterning of the paper-based device by manually depositing permanent marker ink increases the filter paper's thickness. It was also verified by measuring the thickness of filter paper before and after depositing marker ink on it using a microscope. Figure 5.4 depicts the thickness variation of WN4 filter paper before and after the deposition of marker ink. The thickness of filter paper was measured before and after the deposition of marker ink at five different positions, and the average thickness was found. The average thickness of filter paper before and after deposition was found to be 213.606 μm and 237.418 μm , respectively. Thus, the deposition of marker ink resulted in an increase in the thickness of filter paper by 23.812 μm , and this result shows the difference between the two surfaces.

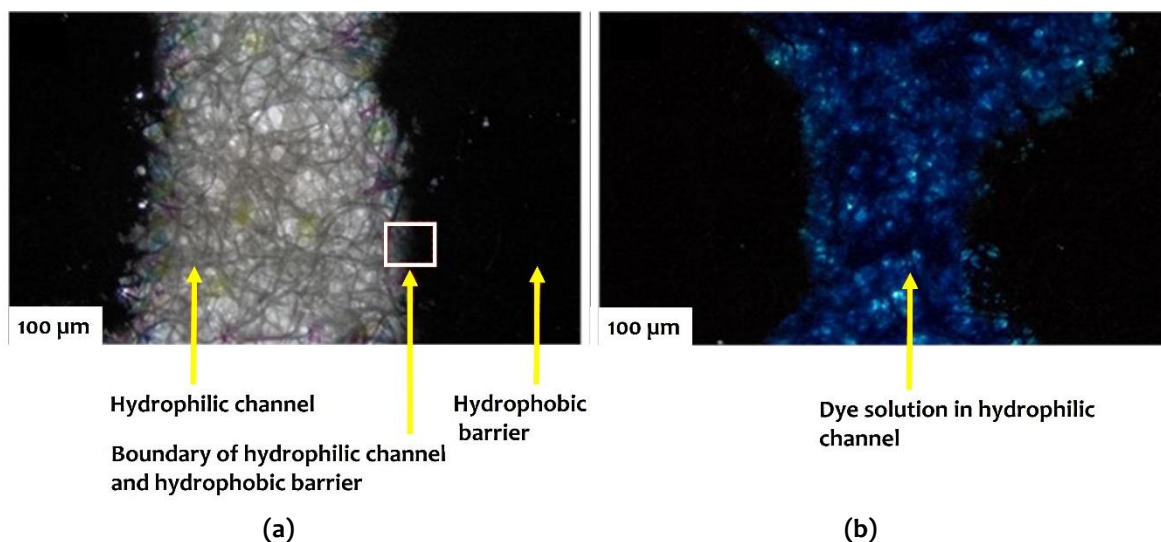


Figure 5.3 : Microscopic images of the boundary of hydrophilic channel and hydrophobic barrier (a) before and (b) after the flow of dye solution

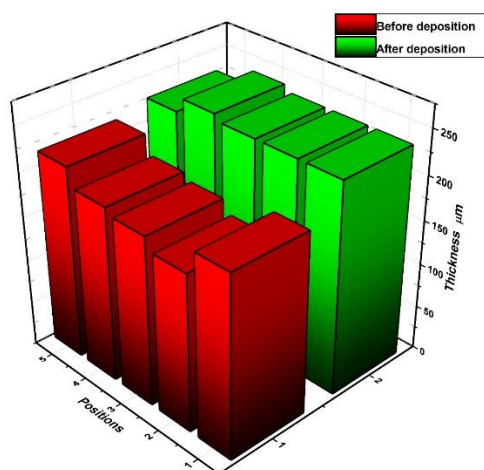


Figure 5.4 : The thickness of WN4 filter paper before and after deposition of marker ink

(b) Optimization of design, sample volume, and flow time

Optimization of sample volume and time taken by the sample to travel are other essential parameters studied along with design optimization. Sample volume and time optimization are essential to determine the minimum volume of sample that ultimately reaches the detection zone from the sample zone without any leakage or wastage in the least amount of time. Design optimization is first necessary to find the optimized sample volume and time. Design optimization was performed to determine the best design using the least sample volume and time. Four designs were made to establish the paper-based device's best analytical parameters. Two different WFP, i.e., Grade 1 and 4 with pore sizes 11 μm and 25 μm, respectively, were selected and tested to find the one that can provide the result in the least possible time. Four different designs tapered at different angles, i.e., 5°, 10°, 15°, and 20° were tested on both filter papers. On WN1 filter paper, 5 μl was the minimum volume for 5° tapering and 7 μl for 10° and 15° tapering to reach the detection zone, whereas it was 6 μl for 20°. It was found that 4 μl is the minimum volume of dye solution for all four designs reaching the detection zone on WN4 filter paper, as shown in Figure 5.5. Thus, the optimized volume determined was 4 μl on WN4 filter paper. It was observed from experiments that a large sample volume takes time to absorb on paper and leaks from the paper's backside, resulting in analyte loss.

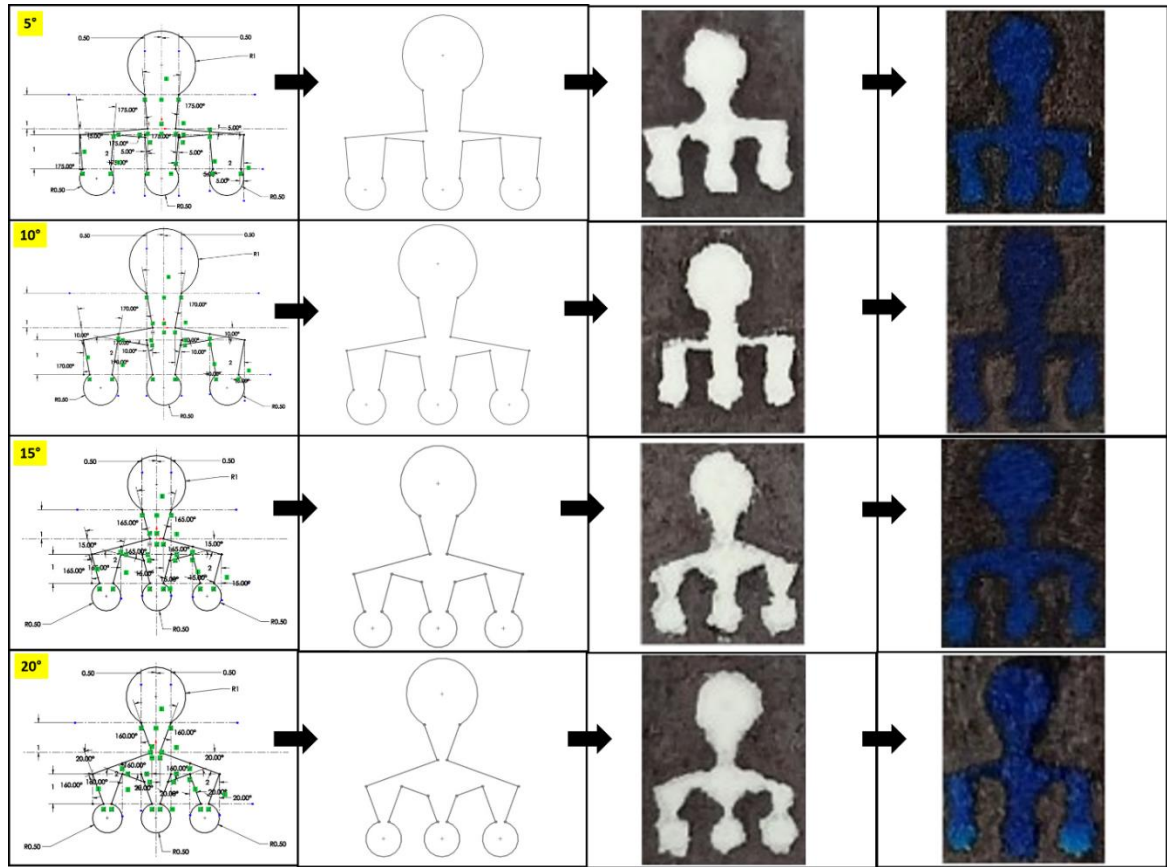


Figure 5.5 : 4 μ L volume of dye solution reaching completely till detection zone on WN4 for 5°, 10°, 15° and 20° tapering angled design

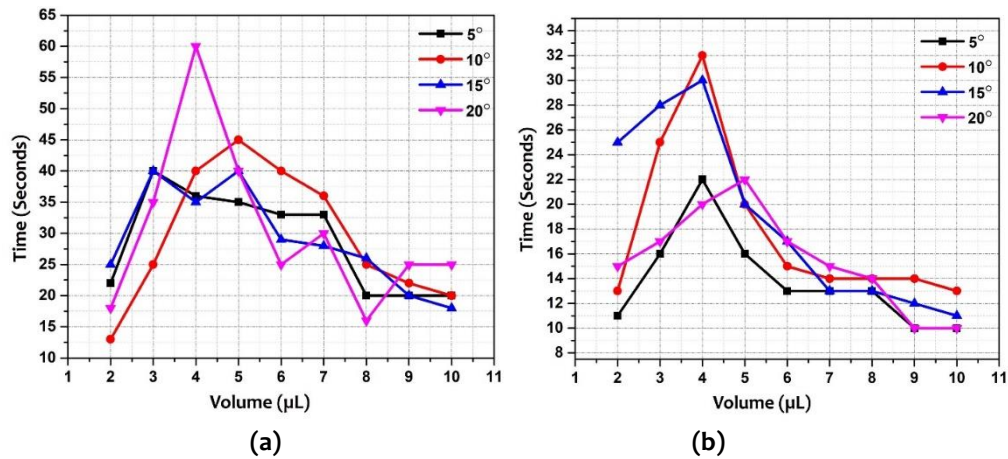


Figure 5.6 : Graph showing the time taken for different volumes of dye solution for designs tapered at 5°, 10°, 15°, and 20° on (a) WN1 and (b) WN4 filter paper

As it can be seen in Figure 5.6 for 2 μ L, the time taken is less on WN1 as the solution does not reach the detection zone, but on WN4, the time taken is more because the solution travels more compared to WN1. As the volume increases from 3-4 μ L, time also increases because the volume is less, and it requires more time to reach the detection zone. After 5 μ L, the time taken by the sample to reach the detection zone decreases with an increase in volume for all the designs on both filter papers because higher volume takes less time to travel through the hydrophilic channels. The time taken to travel through the channels is different for the same volume and on the same filter paper for different designs because of the different dimensions and the zones of different designs resulting from the tapering angle. The same sample volume travels less and takes more time on WN1 in contrast to WN4 because of its small pore size and low

permeability. Figure 5.7 represents the time taken by 4, 5, 6 and 7 μL of dye solution on WN1 and WN4 for all four designs. Time taken for optimized volume on WN4 was 22s, 32s, 30s, and 20s for designs tapered at an angle of 5° , 10° , 15° , and 20° respectively, as can be seen in Figure 5.8. Therefore, the optimized time and design were in the 20s for 20° -tapered design respectively.

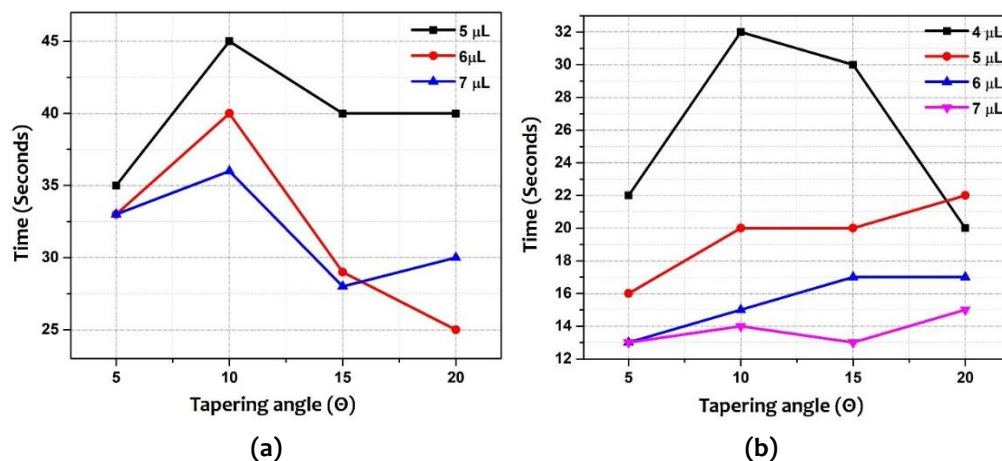


Figure 5.7 : Graph showing the time taken for each of the 4 different designs by 5, 6 and 7 μL of dye solution on (a) WN1 and by 4, 5, 6 and 7 μL of dye solution on (b) WN4 filter paper

(c) Reagent Volume Optimization

It was performed to determine the minimum volume of reagents that would be required to fill the detection zone. Immobilization of reagents should be done only in the detection zone, so the volume optimization of reagents is essential for an optimized trident-shaped design.

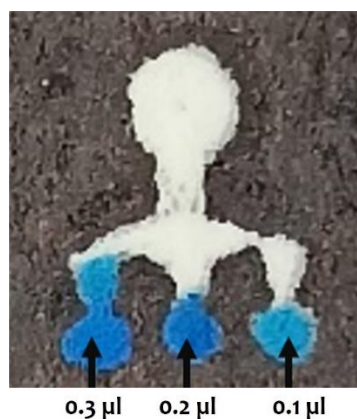


Figure 5.8 : Different volume of dye solution used in three limbs for reagent volume optimization

Reagent volume optimization was performed using a methylene blue dye solution. Figure 5.8 shows that 0.1 μL was the minimum volume of dye solution covering the detection zone, whereas 0.2 and 0.3 μL of dye solution spread out of the zone into the channels. Thus, this optimized design will use 0.1 μL of reagent and enzyme solution.

5.1.4 Summary

This work depicts that minimum two strokes of the marker ink on WN4 create a complete hydrophobic layer. This introduces a simple and easy method to generate hydrophobicity on paper. Design optimization is introduced as a primary focus before fabricating a paper-based device. Four different designs were prepared and tested to select the optimized design. The design in which the channels were tapered at an angle of 20° gave the best result on WN4 filter paper with an optimized sample volume of 4 μL and time taken at 20s. This design can be envisioned on WN4 for further applications, detection of biomarkers, and tissue engineering, as it requires less sample volume and time to reach the sample from the sample inlet zone to all three detection zones via hydrophilic channels.

5.2 A DEEP LEARNING ASSISTED MULTIPLE ANALYTES DETECTION ON INEXPENSIVE A4 PAPER USING IMAGE-BASED COLORIMETRIC ASSAYS

This study explores the fabrication and utilization of a compact, handy, and smartphone-assisted microfluidic paper-based analytical device (μ PAD) fabricated through commercial A4 paper for the multiplexed detection of three different analytes (glucose, lactate, and uric acid) by utilizing a novel trident shaped μ PAD. Herein, the entire μ PAD comprises chemically treated A4 paper, followed by creating microfluidic channels through hydrophobic ink. For the greater extent, the flow rate, sample/analytes volume, and design were optimized experimentally and theoretically. The rapid colorimetric method is utilized for the individual detection of three different analytes simultaneously using three individual indicators. To eliminate the influence of the illumination conditions and camera optics on the produced results, an image dataset was constructed at various illumination conditions by utilizing different smartphones; those image data sets were used to train deep learning algorithms to eliminate the influence of lighting conditions. This technology accurately extracts colour information from original photographs, regardless of the smartphone brand, time, and lighting angle. Furthermore, a bespoke Android application has been created for the purpose of analysis. The application's integrated image processing tools autonomously detect the region of interest (ROI) and minimize human error, enhancing the user-friendliness and precision of the platform. Figure 5.9 shows a schematic view of smartphone assisted microfluidic multiple analyte detection on inexpensive A4 paper using image-based colorimetric assays

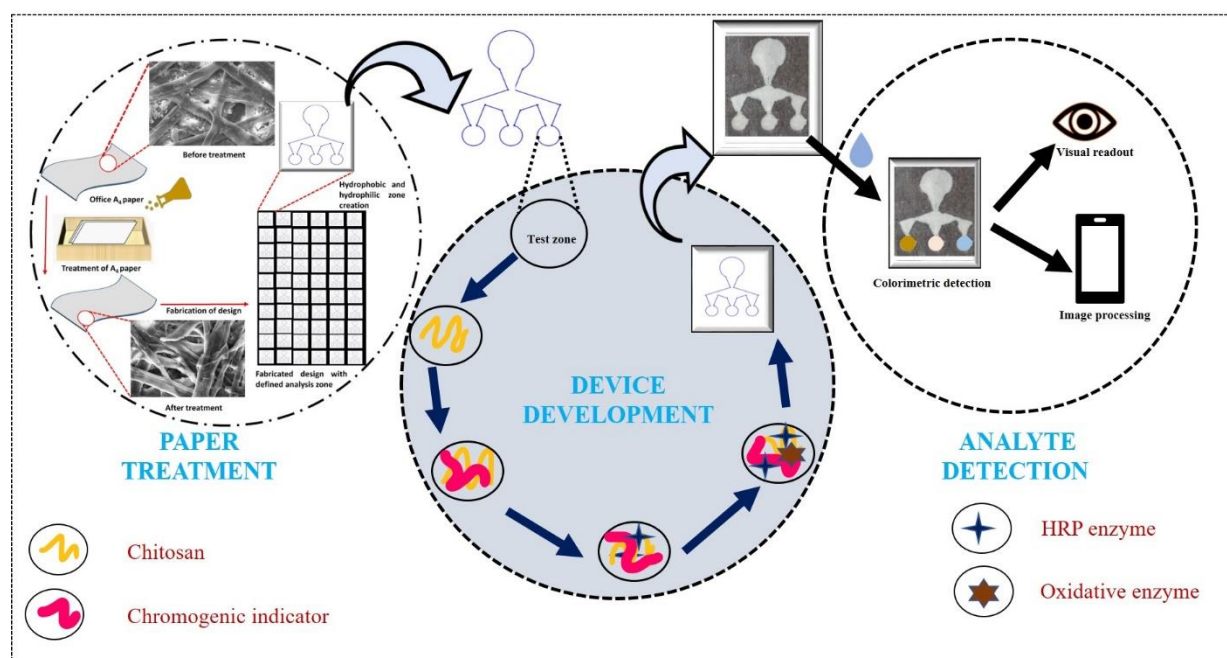


Figure 5.9 A schematic view of smartphone assisted microfluidic multiple analyte detection on inexpensive A4 paper using image-based colorimetric assays

5.2.1 Introduction

Cellulose-based membranes possess environmental-friendly characteristics, are cost-effective, can be recycled, are compatible with living organisms, and exhibit flexibility (Chun et al., 2012)(Carpenter et al., 2015). Cellulose-based membranes exhibit mechanical resilience due to the extensive network of hydrogen bonding between hydroxyl groups within and between glucose units. The applications of chemically modified cellulose functional membranes in energy storage devices and biomedical devices have been studied due to their physicochemical features. The industrial production of A4 paper includes several treatment steps, such as pulping to remove the lignocellulose complex. Reinforcing agents like starch and calcium carbonate are added to

improve the physical properties (such as printability and mechanical strength) and chemical properties (such as water-resistance and brightness) (*Pulp and Papermaking Additive*, 1977)(Gill & Sanders, 1988).

Microfluidic paper-based analytical devices (μ PADs) have demonstrated significant promise in the detection of disease-specific biomarkers during point-of-care testing (POCT) due to their affordability, user-friendly operation, and ability to facilitate fluid flow by capillary action (Gupta et al., 2019). Over the years, several biosensing methods, including electrochemistry, chemiluminescence, fluorescence immunoassay, and colorimetric, have been created and implemented on μ PADs. Colorimetric μ PADs are well-suited for deployment in resource-constrained environments, particularly when cell phones are utilized as colorimetric reading and analysis tools, eliminating the need for additional complex equipment. Recently, there has been significant research and advancement in the field of smartphone-based colorimetric μ PADs. These devices have been specifically designed to identify and measure several disease biomarkers, including glucose, cholesterol, and uric acid, etc. The colorimetric sensor generates colour signals that are quantitatively associated with the varying concentrations of the target molecules being sensed. These signals can be conveniently detected by smartphones (Kishnani, Kumari, et al., 2022)(Kishnani, Park, et al., 2021).

Conversely, multiplexed analyses are gaining more attention due to their ability to provide better clinical information while reducing the need for reagents, time, and expenses (Pollock et al., 2012)(Ge et al., 2012)(Kishnani & Gupta, 2023b). Multiplexed assays offer the capacity to detect many biomarkers simultaneously or specific combinations of biomarkers, hence enhancing the overall clinical utility of the test (Kishnani & Gupta, 2023a)(De Souza et al., 2012)(Jang et al., 2019). In addition, the prospective integration of these devices with nanomaterials could enhance their diagnostic capabilities by improving device performance, (Kumar et al., 2019), particularly in terms of sensitivity and long-term stability under normal conditions (Kishnani, Mondal, et al., 2022). The field of in vitro diagnostics has seen significant increase in the use of multiplexed point-of-care testing. This type of testing involves the simultaneous detection of many components from a single sample at the testing location. There are three primary methods used to achieve multiplexing: spatially separating detection sites using separate spots or wells, regionally separating them using distinct regions of a channel network or electrode arrays, or utilizing different labels such as enzymes, redox compounds, beads, and dyes. Optical and electrochemical detection technologies are employed specifically for the signal readout (Mitchell et al., 2021). Thus far, the predominant approach for on-site bioanalysis systems has been the spatial segregation of various analytes. However, the complexity brought on by device manufacture, assay setup, and consecutive signal readout is their main problem. Employing unique identifiers is another commonly employed method of multiplexing, which often results in elevated reading intricacy because to the increased quantity of analytes. Conversely, there has been an increasing use of regional separation of different detecting sites in recent applications, such as electrodes or channel portions with varying functionalities.

Smartphones have a large user base, an easy-to-use user interface (UI), and excellent data transfer capabilities. This computational platform has a high level of capability in automating the process of collecting, analysing, and presenting colorimetric information. It accomplishes this by utilizing its camera, processing unit, and screen (X. Huang et al., 2018)(Mercan et al., 2021). Furthermore, it typically costs less than the specialized medical-grade colorimetric reader does. By offering cost-effective, prompt, and precise illness screening, it has the potential to enhanced global healthcare significantly. Smartphone cameras are affected by various camera setups and, more importantly, by different lighting conditions in the area. As a result, the accuracy and responsiveness of the colorimetric μ PADs utilized in smartphones are greatly diminished.

Furthermore, as compared to conventional colorimetric μ PADs analysis techniques such as the RGB sensor and RGB scanner, the smartphone exhibits the lowest level of sensing efficacy,

regardless of the implementation of ambient lighting mitigation strategies (H. Fu et al., 2019)(Murdock et al., 2013). Therefore, it is highly beneficial to successfully reduce the impact of internal camera optics/settings and external ambient light conditions in order to enhance the analytical capabilities of colorimetric μ PADs that depend on cell phones. This will aid in promoting the progress of POCT in areas that have limited resources.

Machine learning (ML) and deep learning (DL) algorithms have been incorporated into healthcare research to enhance disease diagnosis and prognosis methods. ML and DL algorithms have the capability to offer intelligent forecasts, leading to enhanced illness management and expedited decision-making processes (Davenport & Kalakota, 2019). Moreover, it may be effortlessly implemented on smartphone platforms. Several research have employed ML algorithms to evaluate the colorimetric signal in lateral flow assays (LFA), resulting in highly precise qualitative and quantitative results (Mutlu et al., 2017)(Hoque Tania et al., 2020)(Min et al., 2021)(Solmaz et al., 2018). Machine learning has been employed to enhance the accuracy of colorimetric μ PADs on smartphones (Mercan et al., 2021)(Biswas et al., 2021). A deep learning algorithm was recently employed to accurately identify C-reactive protein (CRP) using colorimetric methods, achieving a 96% accuracy rate (Ning et al., 2022). This tendency has also promoted the integration of these sophisticated ML and DL algorithms into the development of biosensors (F. Cui et al., 2020).

This study presents a technique for creating cellulose-based membranes that are both extremely efficient and adjustable. The process begins with readily accessible A4 paper and involves simple chemical alterations. These findings indicate that by undergoing a series of purification and functionalization procedures, A4 paper can be converted into cellulose membranes that are both customizable by the user and capable of performing several functions. Therefore, a novel trident-shaped microfluidic channels were created with the help of hydrophobic ink to generate μ PAD for the detection of three different analytes simultaneously. These μ PADs serve as a platform for simultaneously detecting and quantifying three distinct analytes using colorimetric sensing. This was accomplished by utilizing a smartphone colorimetric μ PADs platform that incorporates DL algorithms to deliver fast, highly sensitive, and precise detection of three different markers by colorimetric sensing.

In addition, a user-friendly android application has been developed to facilitate analysis of the results. This application allows for a fully automated and efficient method of colorimetric sensing, where a picture has input results in an answer's output. The deep learning algorithms are solely responsible for the entire automation process. More precisely, we initially created a dataset consisting of 6160 samples by employing various analytes for colorimetric sensing on μ PADs. Subsequently, a diverse array of light sources was employed in conjunction with numerous smartphones to enhance the intricacy of the dataset utilized for training the algorithm. The primary aim was to augment the program's resilience and flexibility. By subjecting all the photos of experimental findings to training under various lighting situations, the deep learning system may autonomously acquire knowledge and mitigate the impact of illumination on the experimental outcomes. In addition, this research introduces a deep learning model called ReSENet-18, which utilizes the attention mechanism and deep transfer residual convolutional neural network structure to accurately identify concentration of different analytes based on colour intensity with a precision level of $\sim 97\%$.

5.2.2 Experimental

(a) Material and reagents

For the detection of the multiple analytes- D-glucose (anhydrous), uric acid (99%), and sodium L- lactate (98%) were purchased from Thermo Fisher Scientific Private Limited, India. For enzymatic reactions, Uric oxidase (UOx) from *Bacillus fastidious*, lyophilized powder ($\sim 9\text{U}/\text{mg}$), Glucose oxidase (Gox) from *Aspergillus niger* Type X-S, lyophilized powder, 100,000–250,000 units/g solid, and L-lactate oxidase (LOx) from *Aerococcus viridian*, lyophilized powder, 1.1 mg

solid and Horseradish Peroxidase (HRP) (solubility: 0.1 M PBS soluble 10 mg/mL) were purchased from Sigma Aldrich. For the colorimetric indication, Potassium Iodide (FTIR Grade, $\geq 99\%$ trace metals basis) used for the detection of glucose. 3,5-Dichloro-2-hydroxy-benzenesulfonic acid sodium salt (98%) from Thermo Fisher Scientific (Heysham), and 4-Amino antipyrine ($C_{11}H_{13}N_3O$) (AAP) ($>99\%$) from TCI development Co. Ltd (shanghai) for uric acid detection. 3,3',5,5'-tetramethyl benzidine (TMB) 99% from Loba for the detection of the sodium L-lactate. For device development, an A4 paper sheet (67, 100, and 120 GSM) has been purchased from a local vendor of the Jodhpur, permanent marker (Camlin, local vendor), Ultra Gold reagent grade Triple Deionized water (DIW) from Organo Laboratories Chemical Pvt Ltd., Delhi, India. Trehalose from Molychem, Mumbai, India (minimum assay- 99%), and phosphate-buffered saline (PBS) from Sigma Aldrich, Water Soluble, pH: 7.2–7.6 (one tablet/200 mL). Chitosan (0.5% in 2% acetic acid) from sigma Aldrich. Smartphones, printer (HP LaserJet M1005, HP, Delhi, India), and a commercially available laminator. Unless otherwise noted, all chemicals were acquired at the highest analytical grade and used without any purification. For the efficient flow and working in modified A4 paper, size of the proposed design was increased up to three times to the original which summarize as: sample zone to 6 mm and detection zone to 3 mm in diameter. For better presentation of the images in the document some images are masked.

(b) Measurement and characterizations

Scanning electron microscopy (SEM, Carl Zeiss-EVO 18) was used to examine the surface morphology of the treated and untreated paper surface. The elemental analysis was confirmed with energy dispersive spectrometer (EDS-Oxford instruments-51-ADDD-0048). The water contact angle were measured by instrument Drop shape analyser, contact angle goniometer (Kruss DSA25B) in ambient temperature. Thermal Analyzer (Perkin Elmer, STA6000) was used to determine simultaneous change of mass in treated and non-treated paper substrate. Porosity/pore size analysis was performed using BET Physisorption (Quanta chrome instruments, Autosorb iQ3).

(c) Surface modification of A4 paper

To utilize A4 paper as a base substrate for paper-based sensor, various commercial A4 papers have been taken with different GSM size *viz.* 67, 100, and 120, GSM. The cellulose fibre in printing paper is mixed with a sizable amount of filler. Natural minerals like limestone, clay, talc, and synthetic substitutes. The kind, quantity, and composition of the filler ingredients determine the paper's structure, thickness, and appearance (Hubbe & Gill, 2016) for the removal of filler material like calcium carbonate ($CaCO_3$), which is excessively found in commercial A4 paper. A slightly modified process was used (Ahn et al., 2020), which comprises acid/base treatment of A4 paper (0.4 M HCl and NaOH base solutions) for various time intervals. During processing, the A4 paper adapted its high porosity and adjustable pore sizes. The finished product displayed membrane performance that was comparable to that of commercial cellulose membranes and the chemical treatment processes on the A4 paper were tracked using elemental, morphological, and mechanical tests.

(d) Design and preparation of A4 paper substrate for diagnostic

For the development of the multiple analyte sensing device, a novel trident shape microfluidic channels were created on a modified A4 paper substrate with the help of hydrophobic ink, as described in Figure 5.10. The sample zone and detection zones are hydrophilic in nature, while the remaining surface is hydrophobic in nature. The detection zone from left to right is utilised for the detection of the glucose, uric acid, and lactate simultaneously. The nature of the paper was analysed using the drop surface analyser and SEM. For the diagnosis of glucose, uric acid, and lactate detection zone was impregnated with certain reagents. All these agents were prepared using the PBS except the uric acid. The preparation of uric acid was done using the NaOH.

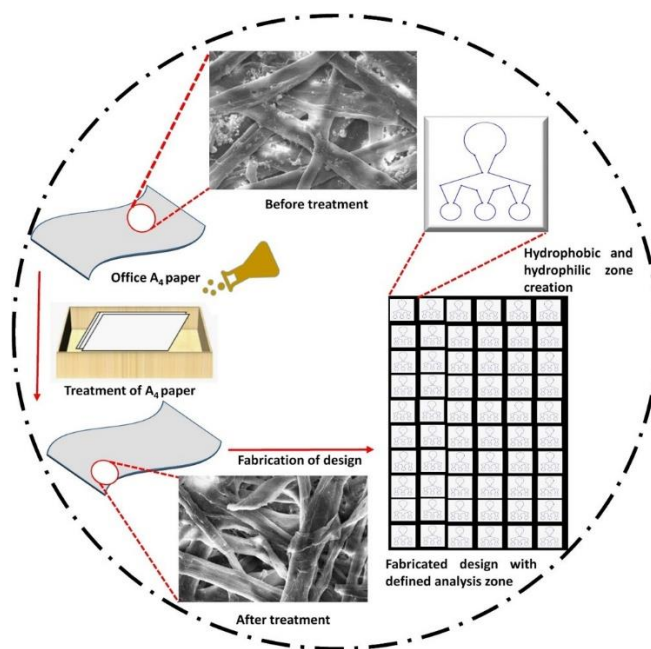


Figure 5.10 : Schematic representation of development of the multiple analyte sensing device

(e) Effect of reagent and sample volume

The volume size was studied to optimise the volume for the reagent as well as the whole sample. Various volumes of Rhodamine (Rd) dye (0.5, 1, 1.5, and 2 μL) were dropped into the detection zone. The effect of the sample volumes was studied by dropping 10 and 12 μL of MB dye solution into the centre of the device using the micropipette. Afterward, a mixture of solution (glucose, uric acid, and lactate) was used to understand the analysis based on colour. Images of fully developed tests were captured with the smartphone for further characterization.

(f) Theoretical analysis

COMSOL Multiphysics was chosen to analyse concentration profile in a paper-based substrate using physics models that account for phase transport in porous media and Darcy law. This choice is based on the fact that paper, being a porous material composed of cellulose fibres, allows flow in multiple directions within its structure. This physics study modified and analysed various parameters such as capillary pressure, permeability, pore radius, and porosity.

5.2.3 Image capturing and data analysis

(a) Data set and Image processing Method

The accuracy of paper-based colorimetric testing can be significantly influenced by various ambient lighting and camera optics, when using a smartphone as a reader (Hoque Tania et al., 2020). Deep learning algorithms can reduce the impact of those influences and achieve precise predictions of colorimetric testing results by training with a comprehensive dataset that includes colorimetric images (labelled with known sensing target concentration) captured under various lighting and phone conditions (Mercan et al., 2021). The quantification of images is influenced by factors such as the resolution and optics of the smartphone camera (Tania, 2018). To account for the impact of various smartphones, this work employed seven widely-used smartphones to capture colorimetric photos. To attain superior classification accuracy, deep learning methods necessitate training with an appropriate dataset. The content and quantity of the data set directly influences the classifier's performance. To enhance performance, one might increase the volume of input data. Nevertheless, this also brings about negative consequences, such as fluctuations in surrounding lighting conditions, angles of incident light, and camera optics. To tackle these difficulties, a dataset was generated by utilizing various smartphones in diverse lighting situations and angles, thereby replicating the unfavourable impacts in controlled lighting circumstances as described in our previous work (Kishnani & Gupta, 2023d).

Concisely, the light sources employed encompassed white (W), yellow (Y), room conditions, and sunlight (S), either separately or in conjunction. Multiple lighting conditions were established, including W, Y, YW, S, room condition (with light, without light, with concentrated flash), closed container (with flash), and sunlight (with and without shade), to capture images of each multiplexed μ PAD for concentration ranges ranging from 0.1 mM to 1 mM, with a consistent increment of 0.1 mM. We sought to include a broad spectrum of lighting circumstances that an average individual may face, although more sources of illumination may be incorporated to augment the dataset. The μ PADs were photographed under uniformly lighted conditions from multiple angles (0–360°, with a 45° variation) while keeping the smartphones and multiplexed μ PAD at a fixed distance of 150 mm. The positions of the μ PADs and smartphones were stationary. At the same time, the light sources were consistently adjusted at a 45° angle to maintain a uniform distance between the smartphones and μ PADs across all test groups while altering the angle of illumination. To mitigate errors, we sought to expand the dataset for DL by gathering images from various angles and under varying lighting circumstances, considering that individuals have distinct photography styles. The smartphone was configured to the default setting, hence minimizing user intervention. The dataset comprises 6,160 photos; these images were obtained from seven different smartphones. Due to different cameras, lenses, and imaging algorithms in smartphones, the resulting photographs show a wide range of variations, even when taken under-regulated lighting circumstances.

Table 5.1 presents the precise technical details of the smartphones utilized to capture the photos of the PADs for subsequent investigation. The smartphones were consistently positioned and set at the same height, when capturing photographs of each μ PAD, regardless of the illumination conditions and concentrations. The embedded image software's automated mode calibrated various settings, including colour, temperature, ISO, exposure time, and shutter speed.

Table 5.1 : Technical specification of smartphones used to capture the image for machine learning

S. No	Smartphone Brand	Resolution	Aperture Size	Megapixels
1.	Samsung Galaxy M30	1080*2340	1/1.9	13
2.	iPhone 11	828*1792	1/1.8	12
3.	Realme 8	1080*2400	1/1.8	64
4.	Samsung M51	1080*2400	1/1.8	64
5.	Motorola G Power	1080*2300	1/1.7	16
6.	Redmi 10	1080*2400	1/1.8	50
7.	OnePlus 8	1080*2400	1/1.8	48

(b) Deep learning analysis:

Due to its uncomplicated fundamental structure, the neural network has the potential to accommodate diverse datasets in principle. Therefore, a comprehensive integration of neural networks on a broad scale is required. The need for computer vision technology based on CNN (convolutional neural network) is steadily rising due to advancements in telemedicine and related fields. Hence, the neural network retains significant potential for future applications. In the realm of colorimetric biosensing, precise identification of color intensity requires capturing images using a camera and subsequently analyzing them through image processing and artificial intelligence techniques. In order to precisely determine the concentration of the sample by using photographs, it is necessary to first extract the features of the image. Deep learning has demonstrated strong signal prediction capacity in recent studies by effectively eliminating noise, particularly in picture identification and classification tasks (Krizhevsky et al., 2012)(Affonso et al., 2017).

This work utilized the ResNet18 deep learning method to measure the quantities of glucose, lactate, and uric acid using colorimetric analysis through the μ PADs. The acquired photos are automatically processed before training the deep learning system with this dataset. The image processing algorithm can quickly distinguish the reaction region of μ PADs by setting the right threshold due to the light color of the reaction area, which contrasts well with the dark surrounding area. Image processing is used to identify the Region of Interest (ROI). The contours of the test well are extracted, and the final data set is created by cropping along these contours to maintain the same size.

(c) Smartphone application

The Android system offers the benefits of cost-effective development and extensive adaptability (Mutlu et al., 2017). Consequently, an android application was developed featuring uncomplicated and intuitive interfaces that can automatically process images and analyse the results. This application also offers a more comfortable platform for data analysis. Furthermore, this program can fulfil the requirements of diverse users in a multitude of situations.

5.2.4 Results and Discussion

(a) Development of A4 paper (67 GSM) to hydrophilic substrate

The A4 paper was purified and chemically modified using an acid-base procedure to remove calcium carbonate and other impurities present in the market A4 paper. The cellulose membrane, which was modified using A4 paper, maintained a consistently high degree of porosity with the ability to vary the size of its pores during the entire process. The chemical treatment of A4 paper was monitored using elemental, morphological, and mechanical analyses. The final product exhibited membrane performance comparable to that of commercially available cellulose membranes.

Figure 5.11 shows SEM images and EDX analysis of A4 paper before and after treatment. Figure 5.11 (a) shows SEM image of untreated A4 paper consist of filler material like Ca as shown in EDX analysis (inset). While after the acid-base treatment, it was found that all the filler material was removed as shown in Figure 5.11 (b), which was further confirmed through EDX analysis (inset). The acid base treatment done for the different time interval for the complete removal of the filler material. Figure 5.12 shows SEM images and there EDX analysis of the treated paper for the different time interval. It was observed that for the complete removal of calcium as a filer material from A4 paper it required approx. 5 minutes of treatment time. Table 5.2 shows EDX analysis of treated A4 paper at different time interval. To determine the compatibility of the A4 paper, three different types of A4 paper were utilized, which consist of different GSM Viz., (1) 67 GSM, (2) 100 GSM, and (3) 120 GSM. These three paper with varied GSM value were tested for their compatibility and utilization. After the treatment, it was observed that thickness of the A4 paper decreases, which was analysed through the microscopic view of the treated paper.

Further, to analyse the surface morphology and absorptivity of the treated paper, contact angle was observed before and after the treatment for all the paper substrates. It was observed that, thickness and contact angle of the paper substrate decreases continuously and absorptivity increases. For 67 GSM paper water droplet absorbed completely, while for 100 and 120 GSM shows partial absorption as shown in Figure 5.11 (c). From SEM, EDX, and contact angle analysis it was confirmed that 67 GSM paper would be the suitable one. Further, thermal analysis was done to determine simultaneous change of mass in treated and non-treated paper substrate. A4 paper's findings demonstrated the thermal breakdown of CaCO_3 into CaO and CO_2 in a temperature range of roughly 640–680°C, as shown in Figure 5.11 (d). Following the treatment, the weight percentage of the final residue reduced significantly from 16.577 to 0.025 due to the elimination of CaCO_3 . According to the TGA analysis the A4 papers were found to contain approximately 11.4 weight percentage of CaCO_3 . The SEM pictures and EDX spectra confirm the

elimination of CaCO_3 following the treatment, as they demonstrate the absence of calcium ions
Figure 5.11

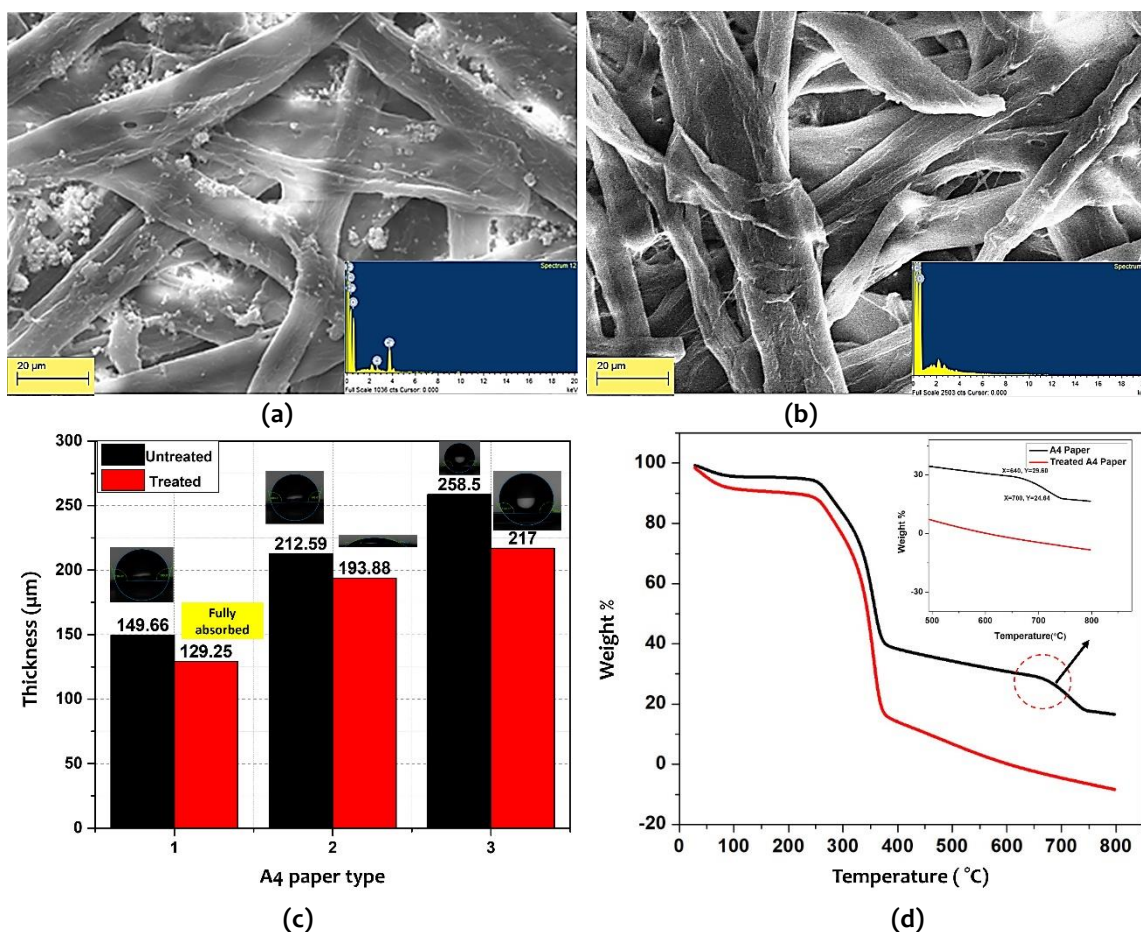


Figure 5.11 : Elemental and morphological analysis of treated and untreated paper. SEM images of the (a) untreated and (b) treated paper, (c) Thickness and contact angle variation of various A4 paper (d) TGA analysis.

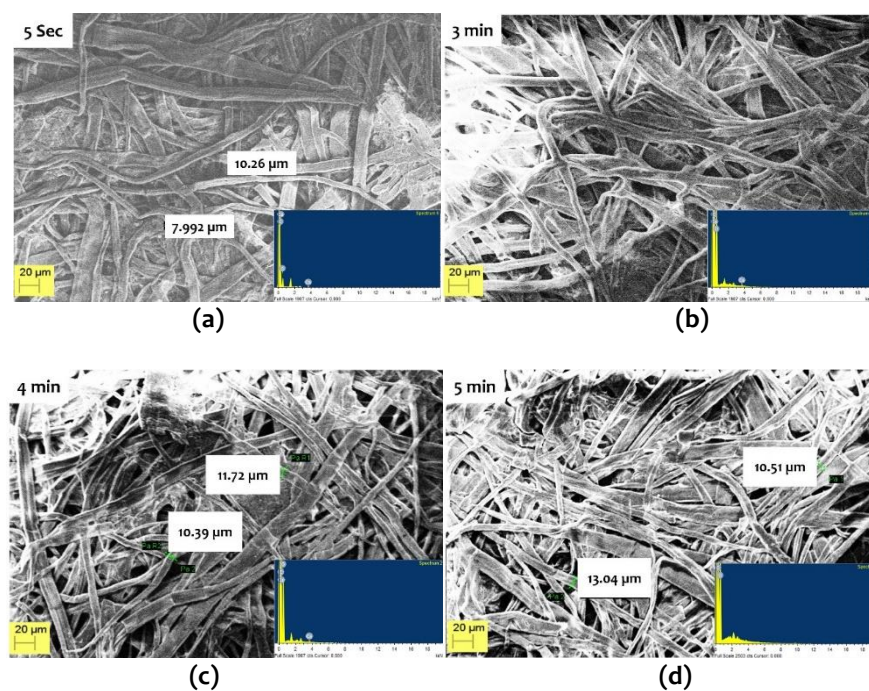


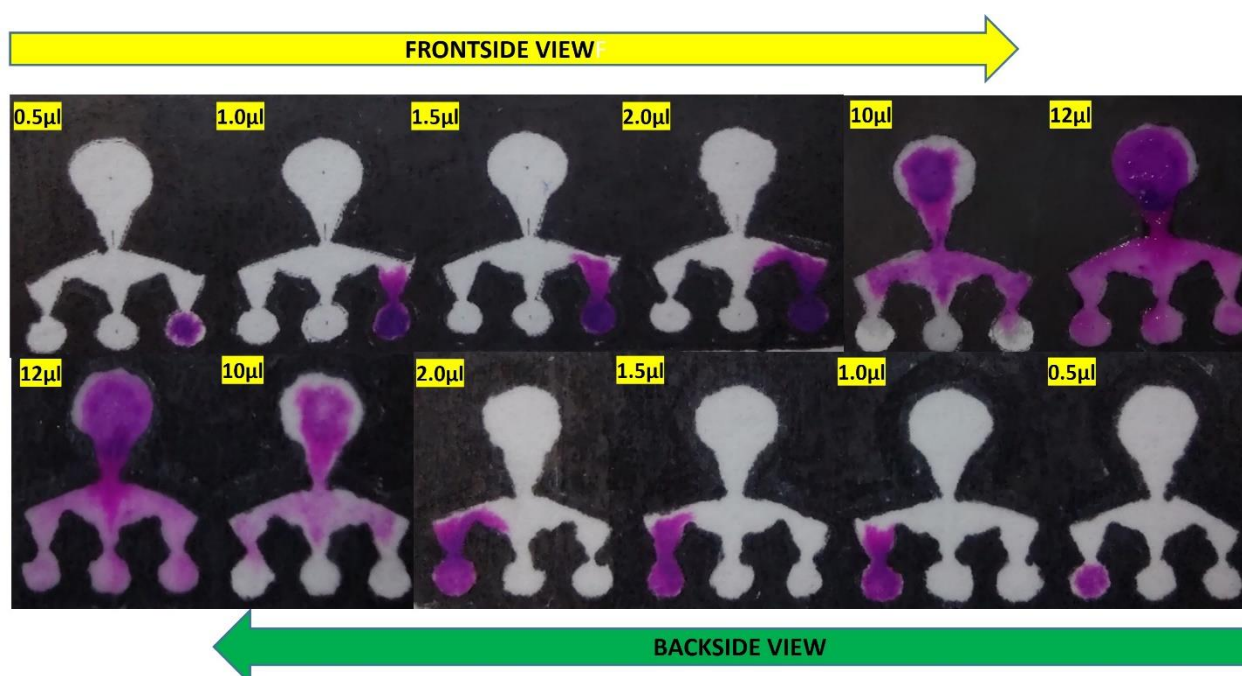
Figure 5.12 : Comparative SEM images with EDX data for different time of treatment

Table 5.2 : Elemental analysis of treated paper at different time intervals

Element	Non-Treated A4 Paper		Treated Paper (5 Sec)		Treated Paper (3 min)		Treated Paper (4 min)		Treated Paper (5 min)	
	Wt. (%)	Atomic (%)	Wt. (%)	Atomic (%)	Wt. (%)	Atomic (%)	Wt. (%)	Atomic (%)	Wt. (%)	Atomic (%)
C K	34.76	43.12	44.29	51.60	44.21	51.40	43.26	50.42	46.09	53.25
O K	58.24	54.24	55.10	48.19	55.59	48.53	56.61	49.53	53.91	46.75
Ca K	6.22	2.31	0.61	0.21	0.21	0.07	0.13	0.04		
Cl K	0.78	0.33								
Total	100.00		100.00		100.00		100.00		100.00	

(b) Sample and reagent volume optimization

It is required to optimize the sample and reagent volume for the development of cost-effective point of care diagnostics device. In this work, a 3 mm diameter reaction zones were designed in such a manner that it will consume less amount of reagents and produce results as sufficient as it can be visible with the naked eyes. To optimize the sample volume in sample zone and analytes volume in detection zone, a coloured dye (Rd) was used for the analysis by dropping a volume range of 0.5, 1, 1.5, and 2 μL for the analytes zone, while 10 and 12 μL for the sample zone. It was observed that 0.5 μL of the Rd cannot completely cover the detection zones, whereas 1.5 and 2 μL shows overflow of the detection zone. Therefore, 1 μL of reagent solution was selected for the further studies. Further, it was observed that the minimum sample volume required for complete immobilization of the sample in the entire device was 12.0 μL to fill all detection zones as shown in Figure 5.13.

**Figure 5.13 :** Sample and reagent volume optimization in the proposed multiplexed device (front and backside view after the sample flow)**(c) Theoretical analysis of design**

To validate the experimental results for the proposed design, its sample and reagent volume optimization. It was proposed to do theoretical Multiphysics analysis of the device. A finite element study was performed using COMSOL multi-physics 6.0 phase transport in porous media and Darcy law. The model solves the water flow through a porous substrate; all the parameters were user defined. By resolving Darcy's law for an incompressible fluid in two

dimensions and three dimensions, the flow fields were determined. All the modules taken were time dependent in controlled physics.

Further mathematical calculation was done to know the basic properties of the treated paper. Porosity of the treated paper is calculated using the Eq.(5.1) (Ohzawa et al., 1998), where ϵ denotes the porosity value, whereas D_f and d reflect the fibre thickness and size of the pores, respectively. The difference in capillary pressure between different points in the paper substrate is what causes the flow of liquid within the paper. The capillary pressure drop (P_c) is determined by the Eq.(5.2). The Surface tension (γ) of the air-water system at 25° C taken as 72.0 mN/m and the contact angle as zero. The paper's pore radius is represented as ' r '. Further, linear flow rate can be determined by Eq.(5.3), and velocity (ϑ) can be determined through the Eq.(5.4), here P_c , μ , r , l , and ϑ denoted as capillary pressure drop, dynamic viscosity of the fluid, pore radius, length of the substrate and velocity, respectively. The table 5.3 shows basic parameters calculated for the treated A4 paper and further utilized for the theoretical calculation.

$$1 - \epsilon = \frac{3\pi D_f^2}{4(d + D_f)^2} \quad (5.1)$$

$$P_c = \frac{2\gamma \cos\theta}{r} \quad (5.2)$$

$$\frac{dl}{dt} = \frac{r^2 P_c}{8\mu l} \quad (5.3)$$

$$\vartheta = \frac{dl}{dt} \quad (5.4)$$

Table 5.3 : Basic parameters used for the theoretical analysis

S. No.	Parameter	Value
1.	Contact angle (θ)	0
2.	Entry capillary pressure (P_c)	11250 N/m
3.	Paper strip length (l)	$18*10^{-3}$ m
4.	Paper strip thickness (t)	$1.29*10^{-4}$ m
5.	Paper strip width (w)	$17*10^{-3}$ m
6.	Permeability (K)	$1.5688*10^{-11}$ m
7.	Pore radius (r)	$12.8*10^{-6}$ m
8.	Porosity (ϵ)	0.766
9.	Surface tension (γ)	0.072 N/m
10.	Fibre thickness (d)	$11.77*10^{-6}$ m

Further, a complete flow analysis of trident shape model was executed via COMSOL 6.0, two processes were analysed for the flow analysis in 160-degree design model. First model defined for the injection flow analysis, while the second was defined for the boundary wicking flow. In order to replicate original condition, it was ensured that it would be tested for 2D and 3D design structure. The boundary condition for both the case chosen with no flow constraint.

Figure 5.14 shows velocity profile and pressure contour for the 2D trident shape module. A partial fluid flow was observed at the detection zone as shown in velocity profile of the 2D trident shape Figure 5.14 (a). While a low pressure was spotted for the fluid flow at the detection zone as shown in pressure contour for the 2D trident shape Figure 5.14 (b). Figure. 5.15 shows a complete velocity profile view of the 2D trident shape for injection input.

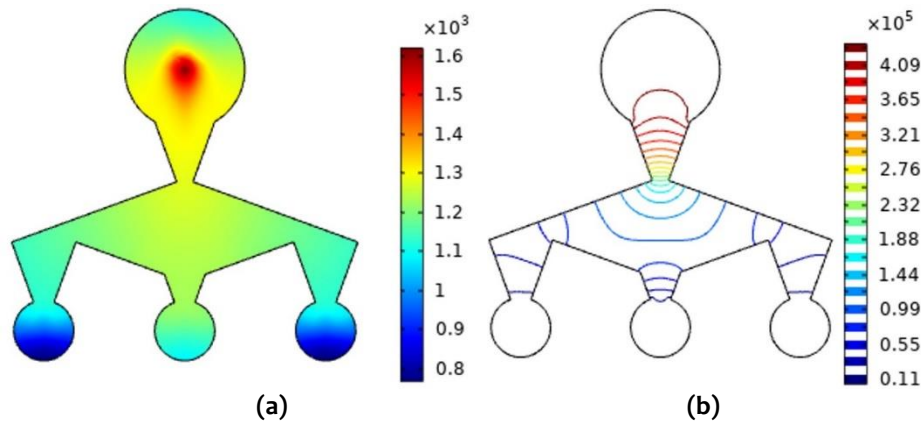


Figure 5.14 : (a) Velocity profile for 2D trident shape for injection input (b) Pressure contour for 2D trident shape for injection input.

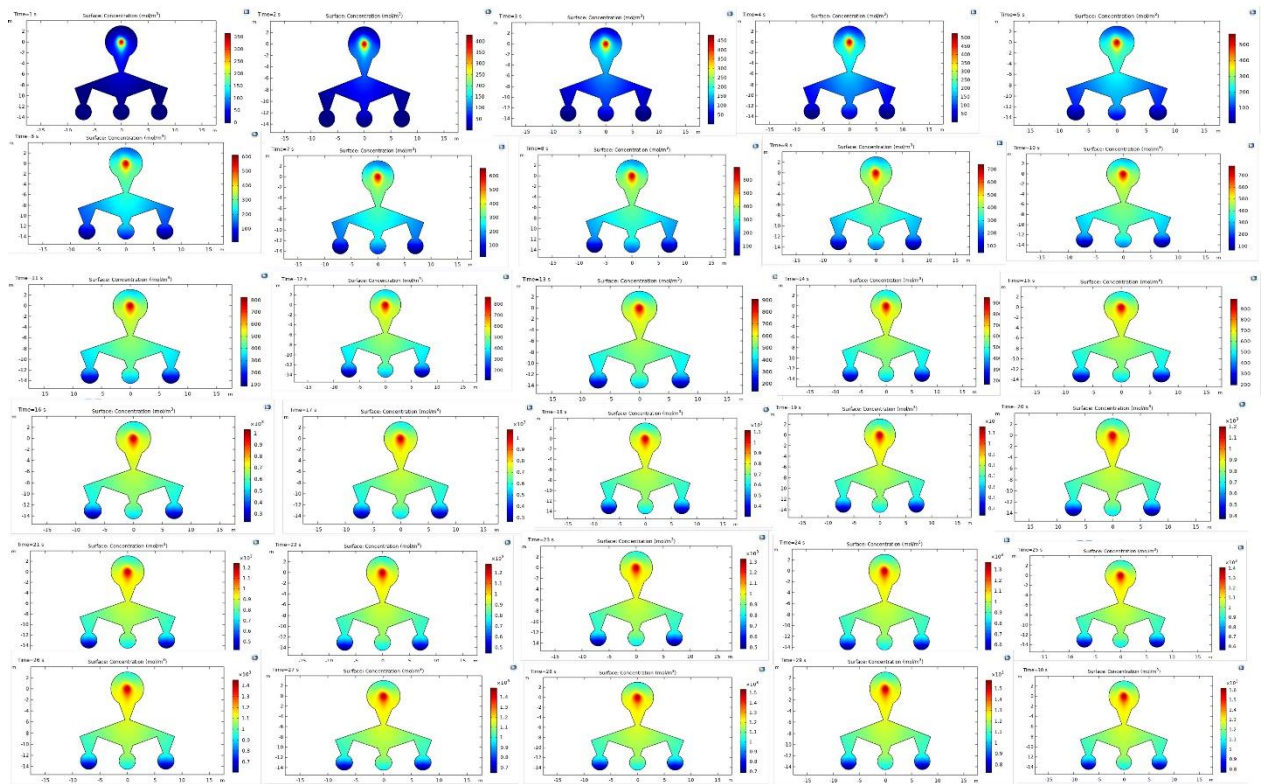


Figure 5.15: Velocity profile for 2D trident shape for injection input

Further, to obtain the complete flow 3D trident shape was tested for the boundary wicking condition. After the computation, it was observed that a complete volumetric flow towards the detection zone from the sample zone as shown in Figure 5.16 (a). While the pressure streamline shows complete pressure streamline in entire section as shown in Figure 5.16 (b). Figure 5.17 (a) and (b) shows a complete view of surface pressure streamline and volume fraction for 3D trident shape for boundary wicking

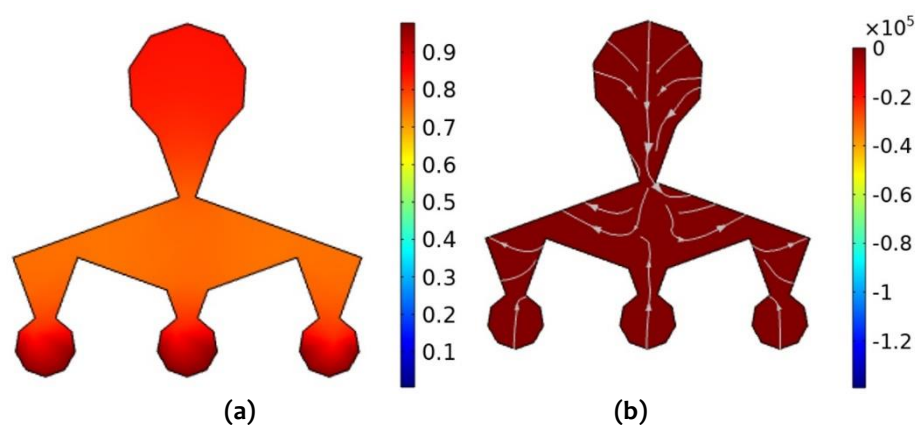


Figure 5.16 : (a) Surface volume fraction for 3D trident shape for boundary wicking (b) Surface pressure stream line for 3D trident shape for boundary wicking

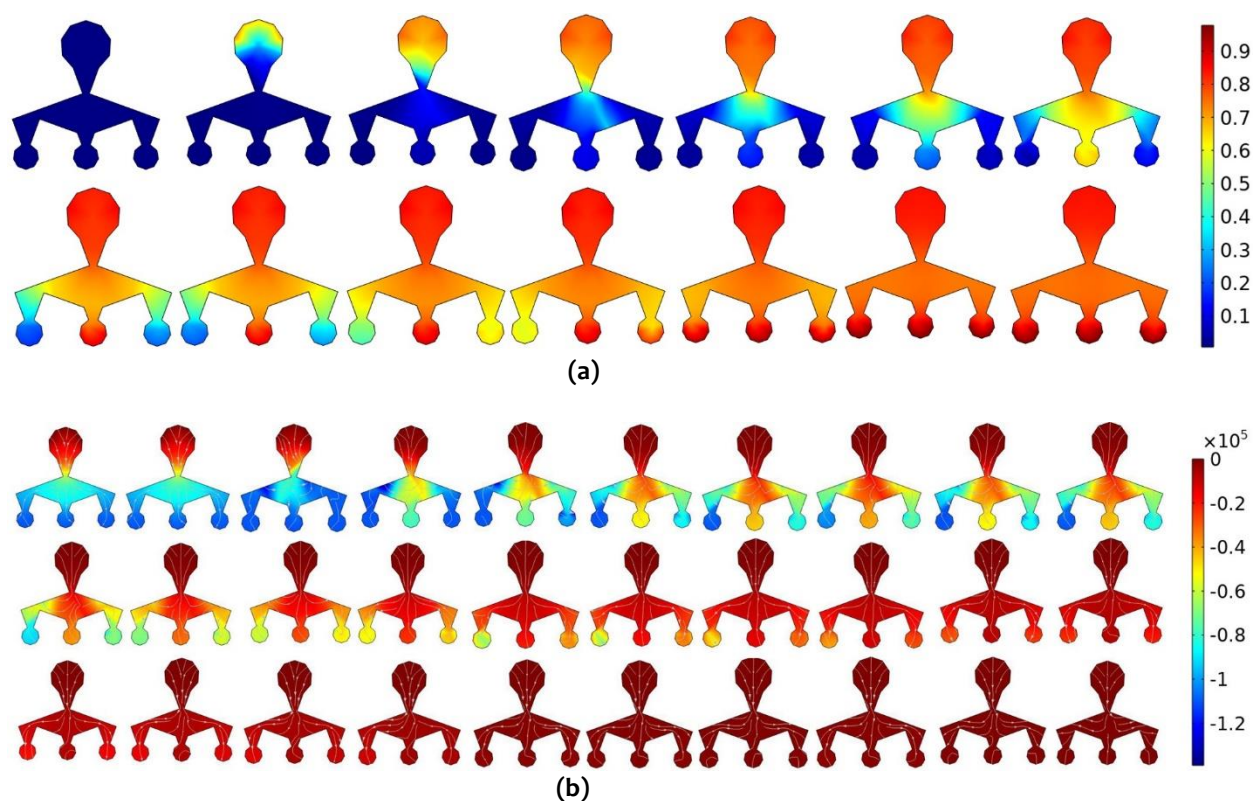


Figure 5.17 : Surface volume and pressure stream line in 3D trident shape for boundary wicking (a) Surface volume fraction (b) Surface pressure stream line

(d) Multiple analyte detection on paper sensor

For the colorimetric detection, the reagents were immobilised as sequential order described in the Figure 5.18. The sample zone and detection zones are hydrophilic in nature, while the remaining surface made hydrophobic in nature via the hydrophobic ink. The detection zone from left to right side utilised for the detection of the glucose, uric acid and lactate, respectively. For the diagnosis of glucose, uric acid and sodium L lactate, the detection zone were impregnated with certain reagents. All the reagents were prepared using the phosphate buffered saline except the uric acid. The preparation of uric acid was done using the NaOH.

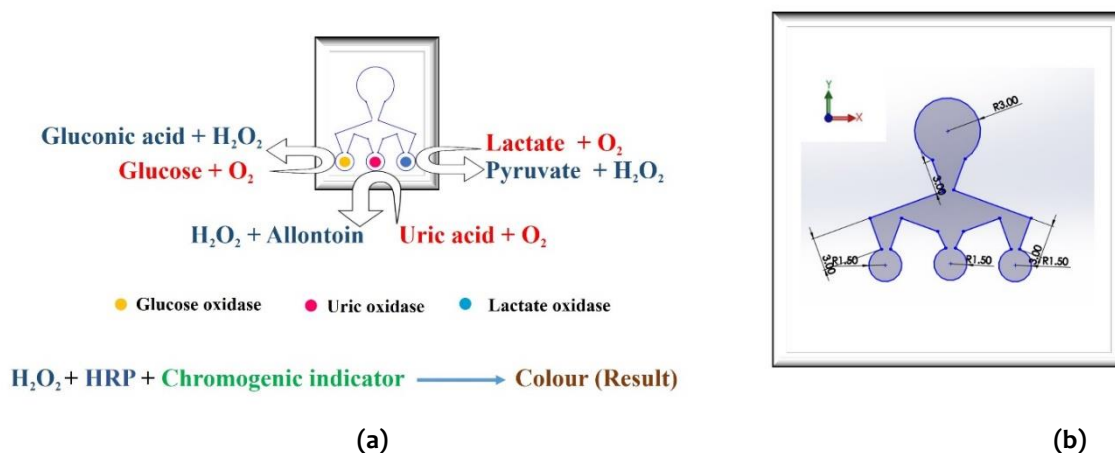
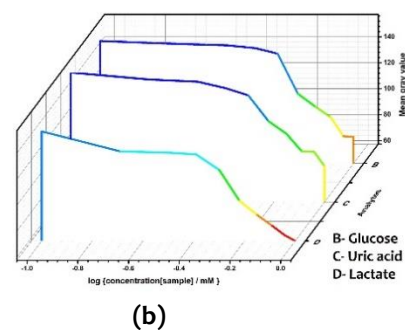
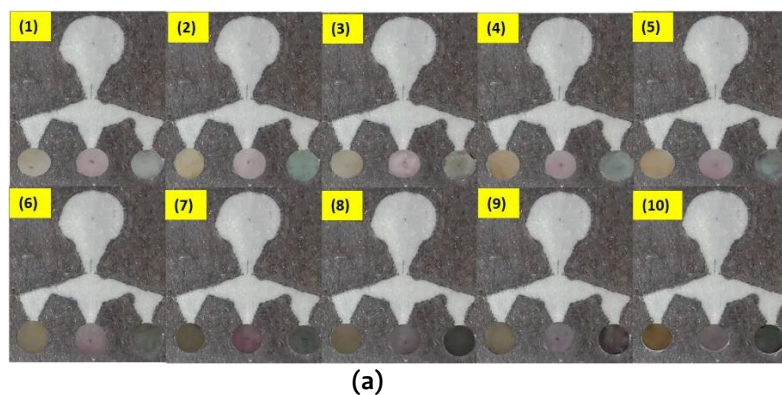
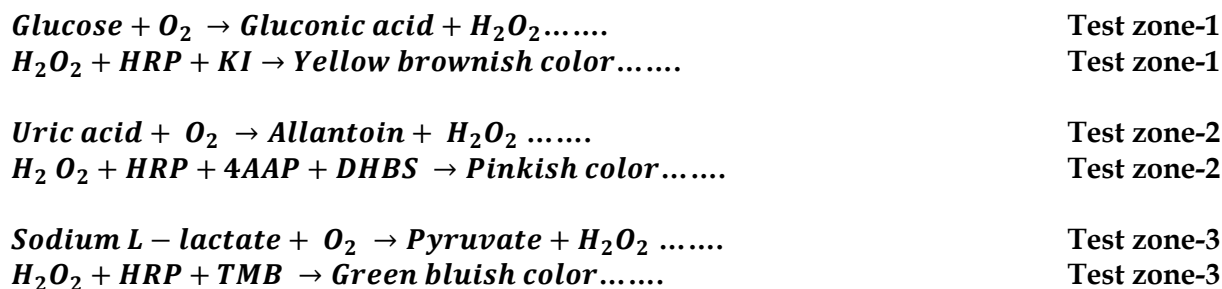


Figure 5.18 : Schematic diagram for the multiple analytes sensing over three different detection zones (a) analytes reaction at individual zone (b) outline and size of the detection zone

For multiple detection, different molarity mixed solution (0.1-1 mM) of glucose, uric acid and lactate samples were prepared in phosphate buffer at pH 7.2. For colorimetric detection, 12 μ l mixed solution of glucose; uric acid, and lactate were added on the sample zones. As the sample flow through the channel (via capillary action), it reacts with respective oxidative enzymes and reagents, results into the formation of colour for visual readout. Afterwards, the smartphone captured colour photographs of the paper sensor, which were then analysed for quantification. The generation of the colour undergoes the several chemical reactions, which are described as following equation:



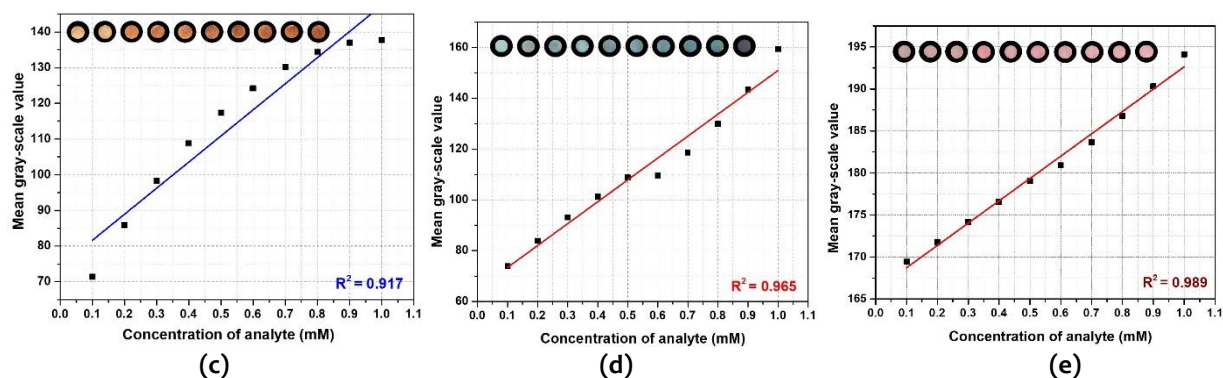


Figure 5.19 : (a) Simultaneous detection of glucose, lactate, and uric acid using a μ PAD (b) Concentration vs mean grey value graph for simultaneous detection of glucose, uric and lactate mean grey value for different concentration of (c) Glucose (R^2 0.98) (d) lactate (R^2 0.965), and (e) uric acid (R^2 0.989)

The paper-based sensor platform was fabricated using a multilayer modification method. The cellulose paper in the detecting zones underwent modification with chitosan through electrostatic interactions between negatively charged cellulose and positively charged chitosan, as well as other interactions such as hydrogen bonding. This resulted in the creation of a chitosan-modified paper substrate. Followed by HRP, and chromogenic reagents, which serve as catalysts for the oxidation of diverse organic substrates by hydrogen peroxide then oxidase enzymes. After every functionalization, there was 10-minute buffer zone. After functionalization, the prepared device was incubated at 4° C for further utilization.

For the analysis, different molarity mixed solution pipetted at the sample zone. After the complete wicking action towards the detection zone, it reacts with respective oxidative enzymes and results into the formation of colour for visual readout. As the concentration of the sample is increasing the intensity of the colour on the paper substrate were also increasing. The sample easily distinguishable based on different colour produced by the different analytes on the paper substrate. Figure 5.19 (a) shows multiplexed detection of glucose, lactate, and uric acid over the μ PAD after complete enzymatic reaction. While, Figure 5.19 (b) shows log of concentration vs mean grey value graph for simultaneous detection of glucose, uric and lactate, it shows as the concentration increases mean grey value of the captured images decreases. Further, an analytical curve was constructed between mean grey value (RGB intensity) vs concentration in the range of 0.1 mM- 1.0 mM with a limit of detection (LOD) and coefficient of determination (R^2) of 0.28 mM and 0.98 for glucose as shown in Figure 5.19(c). Same procedure was followed for the detection of the lactic acid and uric acid except there chromogenic reagents and enzyme specified, the LOD and R^2 value obtained for the uric acid and L-lactate concentration of 0.22 mM and 0.989 Figure 5.19 (d) and 0.40 mM and 0.965 Figure 5.19 (e), respectively. The detection analytes were easily distinguishable because of their different colour produced by the different analytes on the simple paper substrate.

(e) Data Pre-processing and Augmentation

ResNet-18 comprises a single convolutional layer and eight residual building units. The residual building unit serves as the fundamental architecture of the ResNet-18 network (Zhu et al., 2020)(. et al., 2021). Initially, the dataset containing images of all concentrations at different atmospheric lightning conditions and smartphones is obtained and subjected to pre-processing. Next, the ResNet-18 model is presented. The dataset of multiple colorimetric analytes with 6160 images were divided into training and a testing set according to the ratio of 70:30, means 4312 training images, and 1848 testing images, respectively. The convolutional neural network's strong generalization capability relies on a substantial volume of data. Consequently, when the data quantity is insufficient, the model is prone to experiencing the overfitting issue, significantly constraining its generalization capacity (H.-C. Kim & Kang, 2020)(W. Zhang, Li, et al., 2020).

To analyze the data set through deep learning, it was classified in two parts, part I includes all image data set into three sub classification glucose, lactate and uric acid. While part II includes all image data set in to ten sub classification from 0.1 mM to 1.0 mM. These classifications went under the analysis in two different conditions normalization and without normalization. Part I data set shows the remarkable accuracy of 99.35 % and the loss approximately 0.019. Additionally, throughout the testing phase, the overall accuracy was found up to the 99.59 % as shown in Figure 5.20 (a) and Figure 5.20 (b). Which shows training and validating dataset for part I. Where, all image data set classified into three different classes “0” for glucose, “1” for lactate, and “2” for uric acid.

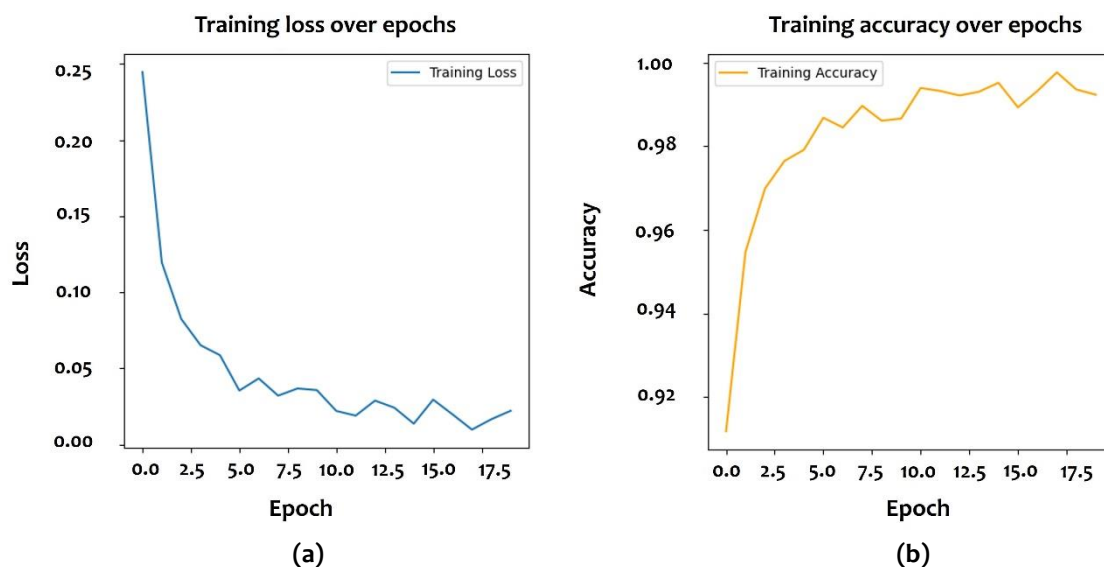


Figure 5.20 : The model underwent training using a dataset specifically designed for part I, which included both (a) a loss value and (b) an accuracy value

Part II data set shows the best accuracy of 98.81 % and the loss approximately 0.044. Additionally, throughout the testing phase, the overall accuracy was found up to the 97.12 % as shown in Figure. 5.21 (a) and (b). Which shows training and validating dataset for part II. Where, all image data set classified into ten different classes “0.1 mM” to “1.0 mM”.

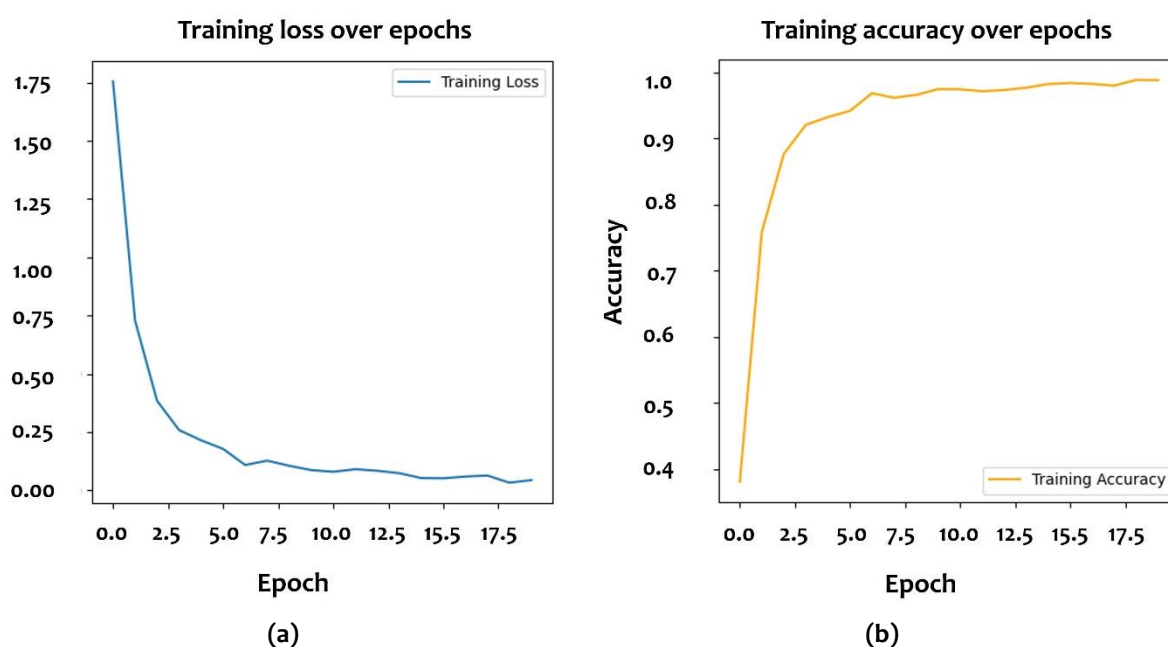


Figure 5.21 : The model underwent training using a dataset specifically designed for part I, which included both (a) a loss value and (b) an accuracy value

(f) Model Performance

The deep learning system will utilize classification techniques to detect the concentration classes associated with the pictures in the test dataset. The evaluation of the deep learning algorithm's performance is determined by measuring its accuracy and f1 score. Accuracy in biosensor terminology refers to the combined measurement of true positive and true negative rates, as represented by Eq.(5.5). In the field of biosensors, true positives (TP) are defined as instances where sensors correctly identify samples as positive, while false positives (FP) occur when sensors incorrectly identify negative samples as positive. The true negatives (TN) and false negatives (FN) display comparable patterns. The F1 score is computed by taking the harmonic mean of precision and recall. Precision measures the accuracy of positive class predictions, while recall represents the ratio of actual positive samples to all samples classified as positive, as shown in Eq.(5.6).

$$Accuracy = \frac{TP+TN}{TP+FP+TN+FN} \quad (5.5)$$

$$F1 = \frac{2TP}{2TP+FP+FN} \quad (5.6)$$

Figure 5.22 shows the recognition accuracy of the model for glucose, lactate, and uric acid for part I. It was observed that it was 100% for lactate in the case of without normalization Figure 5.22 (a), while for normalized condition lactate and uric acid were 100% Figure 5.22 (b). In the testing set, the total number of images was 3156.

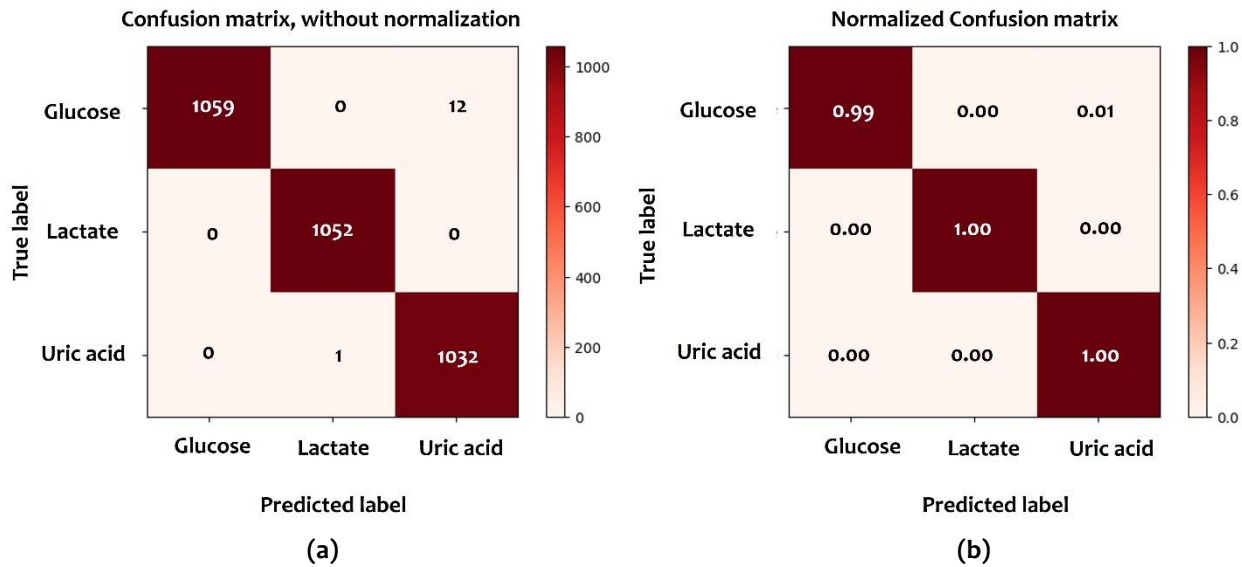


Figure 5.22 : Confusion matrices of ReSNet-18 for part I. The x-axis of the confusion matrix shows the predicted analytes, while the y-axis denotes actual analytes. The confusion matrix displays correct guess with more intensity in darker color (a) confusion matrix without normalization and (b) confusion matrix with normalization

The confusion matrix for the concentration range (0.1 mM-1.0 mM) is shown in Figure 5.23 for two conditions without Figure 5.23 (a) and with normalization Figure 5.23 (b). This visual representation illustrates the relationship between the real and anticipated labels (concentration class) of the test dataset. The confusion matrix is utilized to evaluate the predicted and actual values of concentration of glucose, lactate, and uric acid for the ReSENet-18 model, specifically for the test dataset, which has 1853 instances or 30% of the entire dataset.

Here, the confusion matrix displays correct guess with more intensity in darker colour. The ReSENet-18 algorithm provides high accuracy (99%) for classifying 0.1, 0.3, 0.5, and 0.9 mM concentrations Figure 5.23 (b).

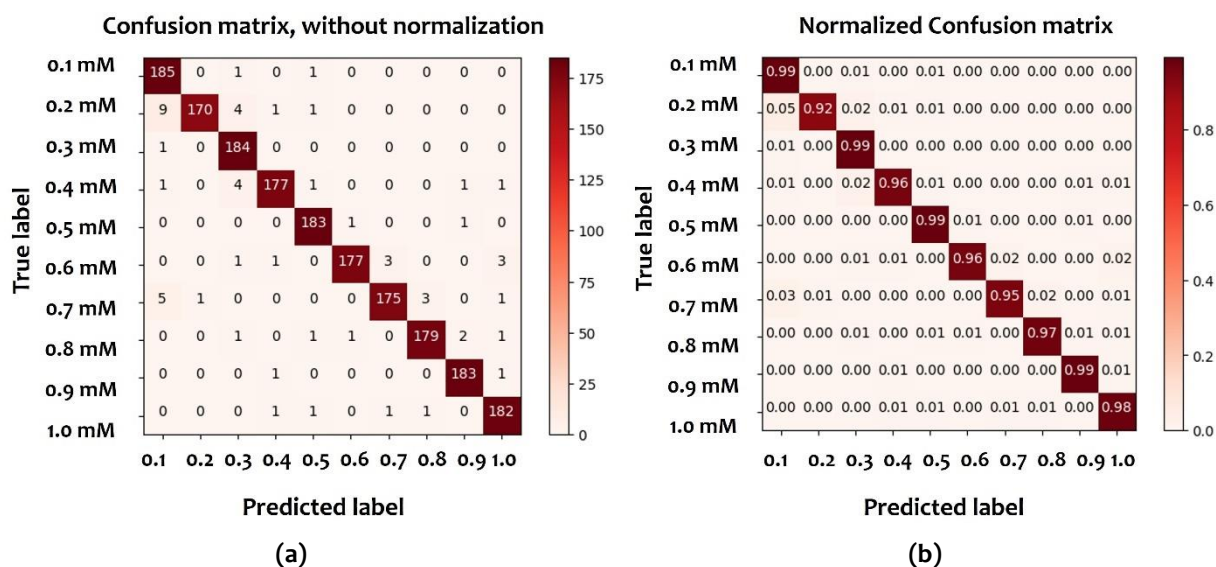


Figure 5.23 : Confusion matrices of ReSNet-18 for part II. The x-axis of the confusion matrix shows the predicted concentration, while the y-axis denotes actual concentration. The confusion matrix displays correct guess with more intensity in darker color (a) confusion matrix without normalization and (b) confusion matrix with normalization

(g) Smartphone Application

An Android based software was created for smartphones as per our previous work (Kishnani & Gupta, 2023d). In addition, this work consists selection of three ROI as an addition to this app. It has all the necessary features for capturing images and analysing them using a ML algorithm. The application additionally performs pre-processing on captured images to eliminate undesirable noise and normalizes the derived features to enhance the accuracy of the results. As an Android-based smartphone application, it carries out three essential tasks of a quantitative diagnostic assay: capturing images, processing and analysing images, and displaying test results (as shown in Figure 5.24). The camera's features that were taken into account while capturing photographs in auto image capturing mode were mode, ISO, white balance, exposure duration, shutter speed, and others. This application necessitates a fundamental patient registration process, which is stored in the phone's memory with a maximum capacity of 1000 entries. Following the registration process, users have the option to capture photographs either straight from the camera or select from their existing image library. Once the image is obtained, a process of cross-verification will be conducted specifically for the three different region of interest (ROI). If the ROI is not recorded accurately, the image might be retaken to obtain a flawless ROI. The images obtained are subjected to four distinct phases of processing: pre-processing (involving adjustments for white balance, correction of light gradients, and removal of colour masking), ROI segmentation (precise identification of colorimetric signals irrespective of variations), extraction of picture features, and finally, analysis of these features to estimate the sample value.

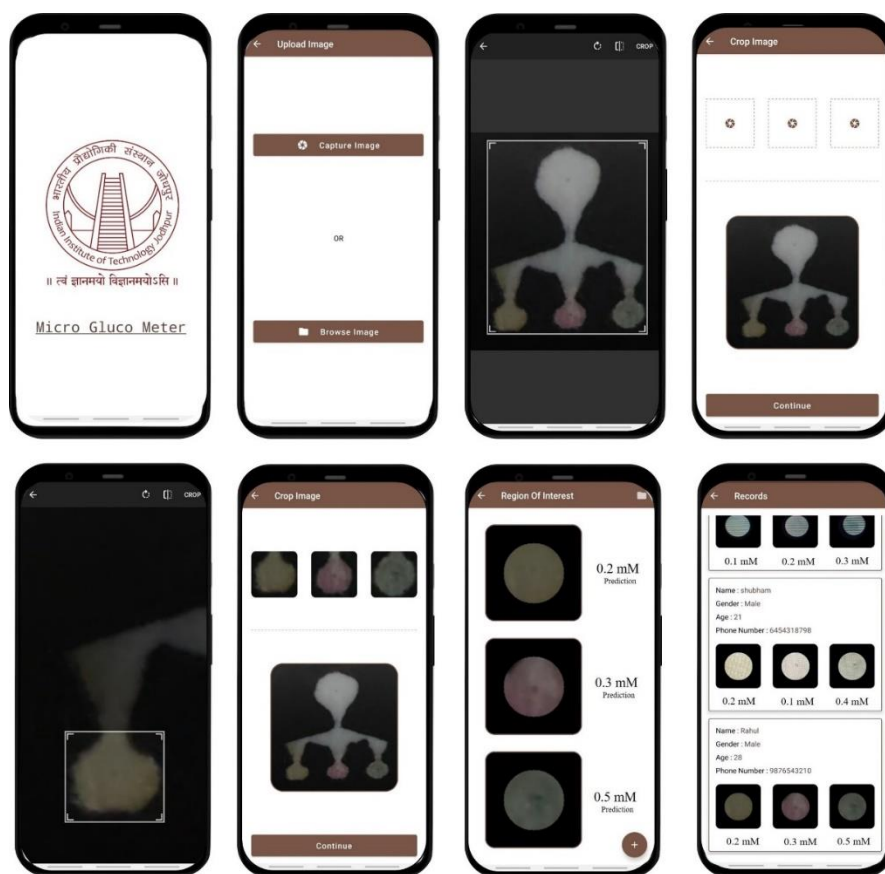


Figure 5.24 : Schematic representation of the mobile app

5.2.5 Summary

This study presents a platform that use deep learning to accurately identify three distinct biomarkers (Glucose, lactate, and uric acid). The detection is achieved using a unique trident-shaped μ PAD fabricated on a chemically modified A4 paper. The A4 paper was modified through chemical treatment to remove filler material and make it comparatively more porous to generate microfluidic flow. TGA results shows 11.4 weight percentage removal of CaCO_3 , while contact angle measurement shows complete absorption of water droplet over treated paper. A novel trident shape design was fabricated over modified A4 paper for the three-channel analytes flow, which was further analysed through COMSOL analysis. Three different biomarkers, glucose, lactate, and uric acid were detected through colorimetric enzymatic reaction on the novel trident-shaped μ PAD, within a range of 0.1 mM to 1.0 mM with LOD of 0.28 mM (R^2 0.98), 0.40 mM (R^2 0.965), and 0.22 mM (R^2 0.989), respectively. The colour generated through the enzymatic reaction was captured through seven different smartphones at most of the possible lightning conditions available to improve the platform's robustness to lightning variations, incident light angle variation, and camera optics. This platform accurately extracts colour information from original photographs, regardless of the smartphone brand, time, and lighting conditions. It achieves a high accuracy rate of approximately 97% in predicting three different colorimetric findings, both quantitatively and qualitatively. Furthermore, a bespoke Android application capable of doing picture processing has been created. The application's integrated image processing tools autonomously identify the region of interest (ROI) and minimize human error, enhancing the user-friendliness and precision of the platform. The qualities of this platform amplify the benefits of μ PAD for widespread application in resource-limited regions and hold significant potential for practical implementation. In this study, we employed ResNet18, which is a type of deep neural network (DNN) model. In our forthcoming endeavours, we shall persist in investigating and implementing the novel algorithms in our upcoming trials.

5.3 RESEARCH OUTCOMES

- Kishnani, V., Gupta, A., A deep learning assisted multiple analytes detection on inexpensive A4 paper using image-based colorimetric assays, manuscript completed.
- Kishnani, V., & Gupta, A. (2023, December). Design optimization of trident shaped micro channels on paper for multiplex detection. In *2023 16th International Conference on Sensing Technology (ICST)* (pp. 1-6). IEEE, Doi: [10.1109/ICST59744.2023.10460852](https://doi.org/10.1109/ICST59744.2023.10460852)