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Original Article

Detecting Glucose Concentration in Biological Fluids

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Abstract *- Microfluidics paper-based diagnostics are novel and inexpensive point-of-care devices with significant potential for use in developing countries. These devices work on the principle of capillary action in a porous substrate in which the wicking distance is proportional to the square root of the time taken to travel across the porous substrate, illustrated by the Lucas-Washburn equation. These can be used to diagnose multiple diseases by measuring parameters like glucose levels and lipid levels in biological fluids. This paper focuses on an innovative and simple point-of-care diagnostic to measure glucose concentration in different solutions. The experimental device has a testing region coated with glucose oxidase-peroxidase mixed with potassium iodide. On reaching the testing zone, the glucose solution reacts with glucose oxidase to yield gluconic acid and hydrogen peroxide. In the next stage, hydrogen peroxide reacts with potassium iodide, liberating iodine. [The iodine develops colorimetric assays ranging from light pink to brown depending on the glucose concentration. A smartphone camera was used to capture the colorimetric assay, and the software ImageJ to convert it into an 8-bit image. The mean grey intensity of each colorimetric assay and the respective glucose concentrations were calculated using ImageJ. The results show a linear relationship, y=0.264x+67.3 (y: mean grey intensity, x: glucose concentration), between glucose concentration and mean grey intensity with a correlation coefficient of 0.97 (2 s.f), indicating a strong relationship between these variables. This innovation can be further developed to diagnose diabetes in low-income and rural communities and substitute for expensive and unaffordable diagnostic tools.*

Keywords *- Microfluidics, Paper-based diagnostics, Capillary action, Porous substrate, Diabetes.*

1. Introduction

Diabetes is a prevalent disease in India, with approximately 11.4% of the population suffering from it. This number is likely to increase in the coming years due to growing rates of obesity and unhealthy lifestyles, as stated by the NCBI. [1] In 2019, around 77 million people in India suffered from diabetes. By 2045, this number will surpass 134 million, and about 57% of these patients remain undiagnosed. [1] As of November 2023, the number of diabetes patients in India exceeded 100 million and it is probable that the number of patients will double over the next two decades. [2]

There are two types of diabetes: Type 1 and Type 2. Type 1 diabetes is a chronic condition where the pancreas makes limited quantities of insulin. This happens because it is an autoimmune disease that attacks the islet cells of the pancreas. Insulin is a hormone that allows sugar to permeate the cell wall for energy. However, in Type 1 Diabetes, blood sugar cannot enter the cells, making it build up in the bloodstream. [3] Type 1 diabetes can lead to a multitude of symptoms like excessive thirst and hunger, persistent urination, fatigue, blurry vision, and mood swings. [4] In Type 2 diabetes the body is not making enough insulin or making faulty insulin. [5] Type 2 diabetes also has an increased risk of insulin resistance. [6] Untreated type 2 diabetes can lead to multiorgan complications, leading to premature morbidity and mortality. [1] In turn, this will impose high costs on patients and burden the Indian Healthcare system. The need for preventative care is crucial for India to handle the crisis of diabetes. Rural areas have 3.5% to 8.7% of diabetic patients and 4.5% to 14.7% of pre-diabetic cases, while urban areas have 5.8% to 14.7% of diabetic patients and 7.2% to 16.2% of pre-diabetic patients. [1] Therefore, timely diagnosis is a massive challenge that requires a fit-for-context solution.

However, in rural communities, inaccessible point-of-care diagnosis poses a challenge to timely diagnosis of diseases like diabetes. This is because of limited healthcare infrastructure in these communities, travel costs, technical skills, and lack of the manpower required. Typically, glucometers, often used by diabetic patients to measure sugar levels in a blood sample, cost 149 INR to 3269 INR, [7] and the cost of one blood test can range from 300 INR to 1000 INR in India. [8] This adds additional hurdles to extensive use. Often, getting these results promptly is a challenge because of the travel time from the blood collection room to the laboratory. The total turnaround time for these results is greater than 120 minutes. [9] This further delays the

commencement of treatment and the handling of diabetes. Furthermore, quality assurance, technical skills, and laboratory capacity play a significant role in hindering prompt diagnosis of diseases. Therefore, there is a considerable gap in timely and accurate diagnosis of diabetes in these communities.

Microfluidics paper-based diagnostics are a novel invention in the field of point-of-care diagnostics, which can address the aforementioned concerns. These devices can be used in developing countries for the diagnosis of a multitude of diseases. It is pivotal that point-of-care diagnostic tools for emergent nations should be ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users. [15] With a diagnostic test costing about 2.49 INR, it is an ideal way to diagnose diabetes in low-income and rural communities. [10] Furthermore, these tests take up to 20 minutes to give test results, significantly reducing the total turnaround time. [11] These tests can also be done at home with little to no technical expertise required. Unlike conventional tools, these tests fulfill the ASSURED criteria, proving it to be an apt solution for diabetes diagnosis.

The aim of this experiment is to use paper-based microfluidics to develop low-cost point-of-care diagnostics to measure glucose levels in solutions. This idea can be developed further to diagnose diabetes for lower-income patients accurately and can be used in settings with underdeveloped healthcare infrastructure. These devices rely on the fundamental principle of capillary action in an inexpensive porous media: paper. Paper is essentially irregularly distributed cellulose fibres with pores in between these fibres. The fluid flows when it is sucked into these pores through adhesive forces between water molecules and cellulose. The fluid itself exerts cohesive forces on its molecules, pulling itself through the capillary network. Eventually, the fluid gets distributed through the capillary network. [12]

Paper-based diagnostics are created by creating hydrophilic channels of paper bound by hydrophobic barriers (wax) to ensure the flow of the fluid in a lateral fashion. Using this principle, a microPAD diagnostic tool is created consisting of three main parts: the loading zone, the hydrophilic channel, and the testing zone. The test fluid is added to the loading zone, wicks across the hydrophilic zone, and eventually reaches the testing zone.

The testing zone is coated with a reagent that reacts with the test fluid to give different colour intensities based on the concentration of the test fluid. [7] Through image analysis of the colorimetric assays, one is able to detect the concentration of the test fluid.

This property could apply to the detection of diabetes with urine as the test fluid. The accepted threshold for glucose in urine is within 0.8 mmol/L [13], and a higher concentration could be a credible indicator of diabetes. These diagnostic tools can be used in developing countries for accessible and accurate diagnosis of the same.

This principle can be applied to biological fluids like blood and urine. Figure 1 shows how blood separates into RBCs and plasma by prohibiting RBCs from reaching the testing zone, and Figure 2 depicts a demonstration of the plasma in blood reacting with a reagent in the testing zone to produce a colorimetric assay. RBCs have a larger diameter than the pore size.

This prohibits the RBCs from reaching the testing zone. However, plasma can easily pass through the pores. The reagent reacts with the glucose present in the plasma to produce a colorimetric assay. This is an example of how glucose levels can be detected in blood using paper-based diagnostic devices.

Table 1. Comparing solutions for diabetes detection

Criteria	Paper-based Diagnostic Devices	Blood Tests
Affordability	2.49 INR[10]	300-1000 INR[8]
Sensitivity	It is sensitive and able to detect small differences in the given parameters and give a corresponding colorimetric assay.	It is sensitive. Blood tests can accurately measure different parameters.
Specificity	It is specific. It can report information about lipid levels, RBC count, and glucose concentration, catering to the diagnosis of various diseases[15].	It is specific. Different blood tests can be carried out to measure cholesterol levels, sugar levels, iron concentrations, etc.
User-friendliness	No skilled labour is required	Technical skill is required
Rapid and Robust	20 minutes[11]	120 minutes or more [9]
Infrastructure requirements	No skilled labour is required	Sophisticated and expensive instruments and lab setups are required
Deliverable to end to end users	Unaffected by a shortage in manpower. The test can be conducted by the users themselves.	Highly dependent on the demand of the test and available manpower

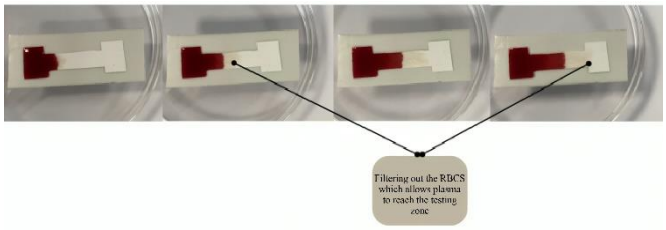

Fig. 1 Separation of RBCs and plasma

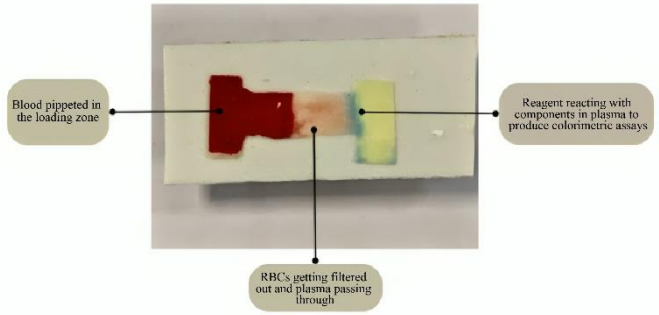

Fig. 2 Demonstrating the use of paper-based diagnostic devices to detect glucose levels in blood

In this experiment, 2 microlitres of glucose oxidase-peroxidase mixed with potassium iodide were added three times on the testing zone with an interval of 2 mins to dry. When the glucose solution reaches from the loading to the testing zone, glucose oxidase reacts with glucose to yield gluconic acid and hydrogen peroxide.

Hydrogen peroxide then oxidises potassium iodide to produce iodine, which is catalysed by peroxidase. [12] Thus, iodine develops colorimetric assays ranging from light pink and brown depending on the concentration of the glucose solution.

2. Methodology

2.1. Research Aim

This research paper aims to develop paper diagnostic devices that work on the principle of microfluidics to determine the concentration of glucose.

2.2. Research Design

The research experiment was designed using the following methodology:

2.2.1. Fabrication of Paper-Based Centre

A rudimentary paper-based diagnostic device was developed using the Origami method. [14] Whatman-graded chromatography paper was cut in the following dimensions - the testing and loading zones were both 6mm x 6mm, and the lateral column connecting the two zones was 3mm x 15mm, permitting the liquid to flow. This device was stuck on a double-sided adhesive tape which acted as a hydrophobic barrier, preventing spillage of liquid outside the diagnostic device.

Table 2. Concentration level and number of trials for each experiment

Serial Number	Number of trials	Concentration
1	3	50mg/dL
2	3	100mg/dL
3	3	150mg/dL
4	3	250mg/dL
5	3	400mg/dL

A glucose oxidase-peroxidase solution was prepared using a glucose oxidase-peroxidase reagent with a concentration of glucose oxidase ≥ 15 000 U L^{-1} and peroxidase with a concentration of ≥ 1600 U L^{-1}. [12] Then this solution was mixed with 2.0gm per 50 ml of potassium iodide, which was experimentally standardized as the working reagent to achieve the best colour resolution. [9] 2 microlitres of this reagent was pipetted onto the testing zone three times with an interval of ~5mins.

2.2.2. Preparation of Glucose Solutions

The glucose solutions of 200 microlitres were used for each of the experiments. The glucose solution concentrations used for the experiment were 50mg/dL, 100mg/dL, 150mg/dL, 250mg/dL and 400mg/dL. These were prepared by SERIAL dilution of a standard solution of concentration 400mg/dL. The solutions were modified to their respective concentrations by using the Phosphate Buffer Solution.

2.2.3. Data Collection

10 microliters of a glucose solution were pipetted onto the loading zone. The paper was left to sit for 30 minutes in order for colorimetric assays to develop. A picture of the colorimetric was taken with the paper placed on a white surface with an incandescent light source above.

2.2.4. Data Analysis

The images of colorimetric assays were converted to 8-bit images, and the mean grey intensity was measured using a software, ImageJ. Taking the background to be white in colour, the formula 255- mean grey intensity was used to calculate the average colour intensity.

2.2.5. Instrumentation

To create the paper, a basic origami method was used which required a ruler to measure the dimensions and scissors to cut the paper.

3. Results and discussion

The current section consists of an evaluation of the data collected.

The laminar flow of liquid can be modelled through the Lucas-Washburn equation, which states that the distance taken for the liquid to flow is directly proportional to the square root of the time taken to travel across the capillary. The equation is $x(t)=\sqrt{(r\sigma t \cos\theta / 2\mu)}$, σ is the surface tension

between the liquid sample and vapour, μ the dynamic viscosity of the fluid, r the capillary radius, and θ the contact angle between the capillary wall and the fluid. [15]

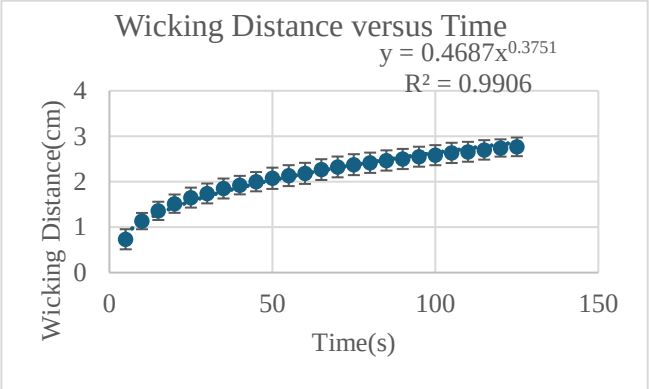

Fig. 3 Depicting the Lucas-Washburn equation of wicking distance versus time

To model the Lucas-Washburn equation, ink was pipetted on the loading zone of a similar paper-based diagnostic device constructed of Whatman filter paper. Through this experiment, the following equation of wicking distance, x, was deduced to be $x=0.469t^{0.375}$, with a correlation coefficient of 0.991. However, there are other factors that the aforementioned equation does not factor in, including liquid-vapour surface tension, dynamic viscosity, the radius of the capillary, and the angle of contact between the fluid and capillary walls. In order to develop an efficient diagnostic device, these factors can be altered to suit the purpose of the diagnostic device.

The colorimetric assays developed show a linear relationship with glucose concentration. [12] As glucose concentration in a solution increases, the colour intensity increases. This is visible in Figures 4 and 5.

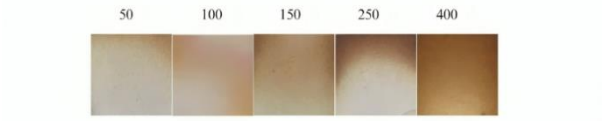

Fig. 4 Colorimetric assays developed with different concentrations of glucose(mg/dL)

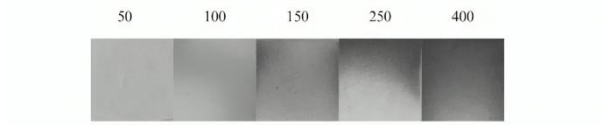

Fig. 5 Colorimetric assays developed with different concentrations of glucose(mg/dL) in greyscale

Figure 6 depicts the grey colour intensity vs glucose concentration. The equation y=0.264x+67.3, derived from this experiment, can be used to calculate the glucose concentration in the test solution. The correlation coefficient is 0.97(2 s.f), indicating a strong correlation between colour intensity and glucose concentration. [9] A similar process with biological fluids can be used to derive the equation to calculate the glucose concentration in biological fluids and inform users about their respective concentrations, warning them if they are potential patients of diabetes. Furthermore, additional developments can be done through multiple trials and machine learning to improve the accuracy.

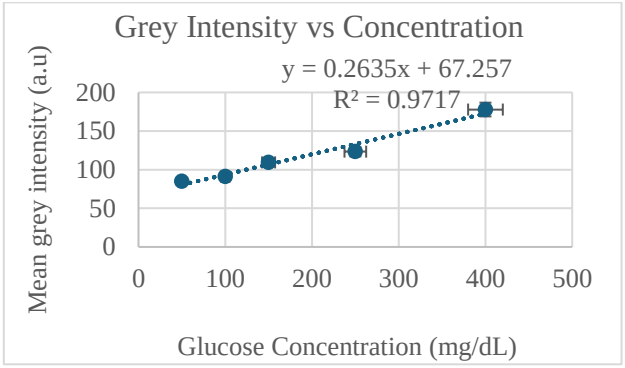

Fig. 6 The linear relationship between mean grey intensity and glucose concentration

4. Conclusion

The present study explored a novel method, paper-based diagnostics- to diagnose diabetes, which fit the ASSURED criteria. The tool developed can measure glucose concentration based on colorimetric assays. This device can be further refined to detect diabetes: a higher number of concentration levels for the glucose level can be taken to improve sensitivity and further trials can be conducted for each concentration level.

Paper-based point-of-care diagnostic devices work on the fundamental process of capillary action through porous mediums. The matrix of pores in the paper allows for the unobstructed flow of a specimen through the paper. The loading zone is coated with a reagent, which yields a colorimetric assay, varying depending on the concentration of glucose. Through the experiment conducted, it is evident that the colour intensity has a positive linear relationship with glucose concentration.

Accurate and timely diagnosis of diseases like diabetes still poses a great challenge. Currently, typical diagnostic devices are unable to resolve this issue. However, paper-based diagnostic devices show promise in mitigating this issue. These devices can be used to develop affordable and accessible point-of-care devices for society, allowing for timely diagnosis of chronic illnesses like the one detailed in this paper, diabetes. Further developments are being made to make these devices ASSURED.

By using paper as the porous substrate, researchers are able to fulfil the aforementioned criteria successfully. The criteria are further being fulfilled by using a smartphone app

to give an accurate diagnosis. The colorimetric results of the test can be scanned using a regular smartphone and uploaded on a smartphone app. This app can be built using machine learning and can be used to analyze the results and provide accurate information on their glucose concentration. It fulfils the ASSURED criteria and could have a monumental impact on the timely diagnosis and better management of chronic illnesses in all sections of society, especially in rural communities. These devices reduce costs of point-of-care diagnostics, reduce total turnaround time, require little to no technical skill, and can easily be done at home.

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