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*Article*

# Paper-Based Device for the Colorimetric Determination of Glucose in Whole Blood Samples Using a Smartphone

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**Abstract**

In many clinical settings, there is a great need for rapid, simple, and reliable diagnostic tools for the detection and quantification of various biomarkers. These tools enable early medical decisions, which can significantly influence patient recovery. Paper-based analytical devices (PADs) have become promising platforms for rapid and low-cost diagnostic testing in recent years. Among the most important biomarkers is glucose, a key metabolite involved in numerous physiological processes, which allows for the diagnosis and control of diabetes, the prevention of serious long-term complications such as cardiovascular disease, and the monitoring of the effect of medication, diet, and exercise on sugar levels in these patients. A fundamental step in detecting this marker in laboratories is the separation of plasma from whole blood. Several studies have demonstrated the successful integration of plasma separation in μPADs. This work presents the development of a paper-based device for the colorimetric detection of glucose in whole blood samples, allowing plasma separation and using a smartphone to perform a quantitative determination.

**Keywords:** POC devices; paper-based biosensors

## 1. Introduction

Whole blood analysis is widely recognized as the gold standard in clinical diagnostics, offering a comprehensive assessment of a patient's health status. However, the intense red coloration of hemoglobin can interfere with diagnostic techniques that rely on optical or colorimetric detection. Consequently, plasma separation from whole blood is often necessary - a process traditionally achieved through centrifugation. While effective, centrifugation requires specialized and bulky equipment, which limits its use in low-resource settings where laboratory infrastructure is not available or for Point of Care (POC) testing.

In many clinical scenarios, there is a pressing need for diagnostic tools that are rapid, simple, and reliable for the detection and quantification of biomarkers. These tools enable early medical decision-making, which can significantly influence patient outcomes. Delays in biomarker analysis may lead to increased risks of complications, long-term disability, or even death. Within this context, biosensors designed for Point-of-Care (POC) testing have gained considerable attention due to their ability to support timely and informed clinical decisions [1].

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Among the most critical biomarkers is glucose, a key metabolite involved in numerous physiological processes. Blood glucose levels are essential indicators in diseases such as diabetes, where continuous monitoring is crucial for effective disease management. Additionally, low glucose levels can result in hypoglycemia, a condition triggered by extended fasting or intense physical activity. Therefore, accurate and frequent glucose monitoring is vital for both diagnosis and the prevention of complications related to hyperglycemia and hypoglycemia [2].

To support the development of accessible and effective diagnostic tools, the World Health Organization (WHO) established the ASSURED criteria for POC technologies: Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable to end users. These guidelines are particularly important in developing regions, where cost and ease of use are key factors driving the adoption of diagnostic innovations [3,4].

In this context, paper-based analytical devices (PADs) have emerged as promising platforms for low-cost, rapid diagnostic testing. Thanks to their porous structure and the ability to transport fluids through capillary action, paper is an ideal material for creating devices that operate without external pumps or instrumentation. Since their introduction by the Whitesides group in 2007—using photolithographic patterning techniques—microfluidic paper-based analytical devices (μPADs) have demonstrated significant potential in fields ranging from clinical diagnostics and environmental monitoring to food safety and biohazard detection [5,6].

Among the detection methods integrated into PADs, colorimetric assays are particularly appealing due to their ability to visually display results without requiring complex equipment. Although commercial test strips offer qualitative or semi-quantitative analysis, they often lack the precision and reproducibility needed for clinical reliability. While colorimeters can enhance measurement accuracy, they are typically expensive and require trained personnel to operate [7,8].

Recent advances in technology have enabled the use of smartphones as powerful diagnostic tools at the point-of-care [9,10]. With high-resolution cameras and robust processing capabilities, smartphones have facilitated the development of mobile biosensors that overcome many limitations of traditional colorimetric devices—providing more affordable, portable, and user-friendly solutions for detecting biomarkers in blood and other biological fluids[11,12].

A critical step in many diagnostic assays is the separation of plasma from whole blood, as plasma contains essential biomarkers that reflect physiological and pathological states. Studies have demonstrated the successful integration of plasma separation into μPADs through different mechanisms[13,14]. One approach involves using blood separation membranes, such as LF1, which effectively trap blood cells while allowing plasma to wick into detection zones, enabling protein quantification in under two minutes [15]. Another strategy leverages red blood cell (RBC) agglutination, where antibodies immobilized on the paper matrix retain RBCs at the sample site, allowing clean plasma to migrate toward colorimetric readout zones [16,17]. Both methods have demonstrated the ability to deliver plasma with high purity and sufficient yield for accurate detection, achieving results comparable to conventional laboratory techniques. These integrated designs, combined with smartphone-based detection, represent a significant advancement in the development of fully self-contained, low-cost diagnostic tools suitable for resource-limited settings.

Another major challenge in the development of μPADs for electrochemical biomarker detection is the ability to collect sufficient plasma volume while preserving the biomarker integrity. A solution to this was proposed in a μPAD designed for the quantification of the S100B protein biomarker, relevant in neurological diagnostics[18]. Using NaCl-functionalized VF2 paper for blood collection and MF1 paper for plasma retention, the device achieved efficient plasma separation (50 μL from 300 μL of whole blood) in under four minutes[19].

This work presents the development of a paper-based device for colorimetric detection of glucose in whole blood samples, using a smartphone for a quantitative determination

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## 2. Materials and Methods

### *2.1. Substrate Selection and Characterization*

In order to select the most suitable material for the separation of plasma from whole blood, a comparative evaluation was carried out between different types of paper available in the laboratory and those reported in the literature [19,20].

The materials evaluated were as follows:

- Whatman Paper N°1: with a pore size of 11 µm and a thickness of 180 µm. Its intermediate porous structure allows partial retention of blood cells, especially leukocytes, and provides a good balance between flow and mechanical support.
- Polycarbonate membrane: with 3 µm pores and 22 µm thickness. Its small pore size makes it suitable for the retention of erythrocytes and platelets, allowing cleaner separation of plasma.
- Glass fiber membrane: with a pore size of 3 µm and a thickness of 785 µm. Despite sharing the same pore size as the previous one, its greater thickness provides higher absorption and retention capacity, making it suitable for complementing the separation process.

### *2.2. Paper Functionalization and Plasma Separation*

To facilitate selective plasma separation, the selected paper strips were functionalized with sodium chloride (NaCl) and sodium heparin (25,000 IU/5 mL).

The heparin solution was diluted 1:100 in distilled water to obtain a final concentration of 250 IU/mL, representing approximately 0.074 M sodium [21]. In parallel, a stock solution of 1M NaCl was prepared, and three different concentrations were obtained by serial dilution: 1 M, 0.68 M, and 0.154 M.

### *2.3. Colorimetric Enzymatic Assay for Glucose Detection.*

A colorimetric assay based on enzymatic reactions was used for glucose detection. The system included the enzymes glucose oxidase (GOx) from Aspergillus Níger type VII (100 units/g), horseradish peroxidase (HRP) type VI of Armoracia rusticana (≥250 U/mg), and the chromogenic reagent TMB (3,3',5,5'-tetramethylbenzidine)[22].

The reactions involved were as follows:

$$Glucosa + O_2 \rightarrow Gluconic\ Acid + H_2O_2 \quad (1)$$

$$H_2O_2 + TMB(colorless) \rightarrow [TMB]^{ox}(blue) + H_2O \quad (2)$$

A 1.5 µL solution of chitosan (1 mg/mL) in 0.25% (v/v) acetic acid was applied, and the paper was air-dried at room temperature for approximately 10 minutes. Subsequently, a bi-enzymatic colorimetric reaction system composed of glucose oxidase (GOx), horseradish peroxidase (HRP), and chromogenic reagents was employed for detection. For enzyme immobilization, a simple drop casting method was used. All necessary reagents were immobilized individually onto the chitosan-modified paper in a layer-by-layer manner, with a drying period of 10 minutes between each addition, in the following order:

- A 1.2 µL layer of TMB solution (pre-prepared).
- A 1.5 µL layer of HRP (7.11 U/mL) was prepared in phosphate-buffered saline (PBS, pH 7.4).
- A 1.5 µL layer of GOx (2 mg/mL) prepared in PBS (pH 7.4).
- A final 1.5 µL layer of TMB to complete the multilayer detection platform.

For glucose colorimetric analysis, specific enzymatic reactions were carried out between the analyte and the corresponding oxidases, generating hydrogen peroxide ($H_2O_2$). The resulting $H_2O_2$ further oxidized the co-immobilized chromogenic reagent (TMB), yielding colored products such as oxidized TMB, with HRP acting as the catalyst [23]. This allowed direct visual inspection and semi-quantitative analysis by the naked eye. For more sensitive quantitative analysis, color images of the

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test papers were captured using a smartphone via a specially designed app. Whole blood tests were conducted to determine the optimal device design with blood sample volumes of 20, 15, and 8 μL.

*2.4. Glucose Calibration*

To obtain a reference value, an enzymatic glucose kit (Winer Lab®, Rosario, Argentina) was used, providing a known glucose concentration, which was related to the color intensity obtained in the colorimetric analysis. This analysis allowed the creation of an equation that automated glucose measurement in the samples.

*2.5. Measurements Validation*

Whole blood samples were collected from participants by venipuncture between 8 and 10 AM, after an 8-hour fast. The blood was drawn into heparinized tubes (TUBLOOD, CABA, Argentina). The plasma was then separated by centrifugation and assayed for fasting blood glucose using a Mindray BS-380 chemistry analyzer (Mindray Bio-Medical Electronics CO., Ltd., Shenzhen, China). All specimens were analyzed within a maximum of 2 hours from collection and were qualitatively assessed for hemolysis. Parallel measurements of whole blood samples and plasma from the same samples (centrifuged blood) were performed to validate that the measurements obtained with the chips are equivalent to glucose measurements normally performed in biochemical laboratories. The experiments were approved by the Ethical Committee of the Faculty of Medicine, Tucumán University, Tucumán, Argentina (Exp. No. 80509/2025).

*2.6. Software Design for Glucose Detection*

To use the app, the user must access the URL through the browser: https://detector-glucosa.netlify.app/, where they must register to create a personal account and securely manage their glucose data. Once registered, the user logs in and can access their glucose records, as well as view a graph showing the evolution of their levels over time. In case of consistently high glucose levels, the user should consult a specialist so that the application can determine the appropriate and adjusted ranges for each user.

After logging in, the user can measure their glucose by uploading a photo of the test chip. The app allows capturing the image with the device's camera or selecting an image from the gallery. The captured photo will be automatically analyzed, and the glucose level will be calculated, updating the corresponding graph.

The app classifies glucose levels into “Low Glucose” (yellow), “Normal” (green), and “High Glucose” (red), and displays the graph with the corresponding concentration, as can be seen in Figure 1(b). The system also allows viewing representative colors of glucose concentrations and downloading a PDF file with this information for use outside the application. The device allows for a visual comparison between the detected color and a reference scale that represents different glucose concentration ranges. This way, the user can interpret the results even without a Wi-Fi connection or access to a mobile phone.

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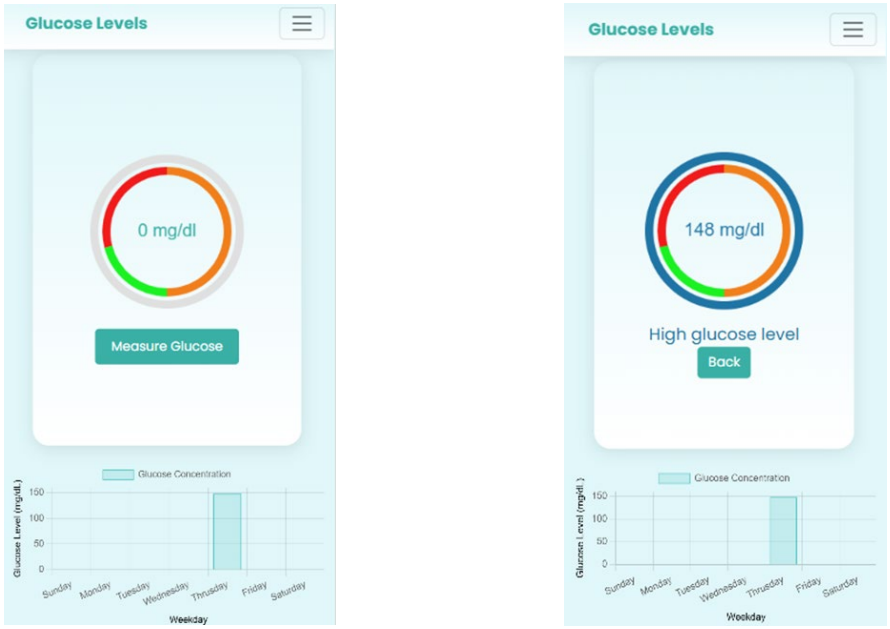


(**a**) (**b**)

**Figure 1. (a)** Home page; **(b)** Sample analysis and chart update.

*2.7. Image Processing and Colorimetric Analysis*

The software includes a module to detect the color resulting from the reaction between the glucose present in serum and the chromogenic reagent. For this purpose, the HSV (Hue, Saturation, Value) color model was used, which allows the isolation of specific shades associated with the presence of glucose [24,25].

2.7.1. Step 1: Definition of HSV Ranges

The following color ranges were established for the detection of blue and green, expressed as HSV matrices: Blue: low range = ([90, 50, 50]), upper range = ([130, 255, 255]); Green: low range = ([35, 50, 50]), upper range = ([85, 255, 255]). These ranges allow the identification of both intense and soft shades of the target colors.

2.7.2. Step 2: Object Segmentation and Mask Generation

The image uploaded by the user is processed to identify the region of interest. Once the object is segmented, a binary (grayscale) mask is generated based on the defined HSV ranges.

2.7.3. Step 3: Color Intensity Calculation

The intensity of the colorimetric reaction was quantified by counting the white pixels in the generated mask. This number is proportional to the presence of the desired color and, therefore, to the glucose concentration in the sample.

2.7.4. Step 4: Quantitative Analysis

A linear regression was constructed to correlate the intensity values obtained with known glucose concentrations, using an enzymatic glucose kit as a reference. This relationship allows the interpretation of the results of the visual analysis in terms of actual concentration, facilitating the quantification of plasma glucose.

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## 3. Results

For the comparative evaluation of the different types of paper, the main criterion was the ratio of paper pore size to the cellular dimensions of the blood components, primarily erythrocytes (~7 μm), leukocytes (8–20 μm), and platelets (~3 μm). Both pore size and paper thickness were considered critical parameters to ensure efficient cell retention without compromising capillary flow.

As a result, Whatman No. 1 paper was chosen for its optimal thickness for absorbing the sample without requiring large volumes of blood. This material proved to be the most efficient compared to the other materials evaluated.

The combined action of both agents, NaCl and sodium heparin, seeks to induce cellular deformation and prevent coagulation [26]. NaCl generates an osmotic gradient that causes erythrocyte crenation, favoring their aggregation and arrest in the initial areas of the paper; meanwhile, sodium heparin acts as an anticoagulant by inhibiting the action of thrombin[20].

The drop casting method was selected for enzyme immobilization. Although there are advanced methods, such as covalent immobilization of enzymes, this simple procedure was chosen to avoid possible loss of enzyme activity that such complex processes can cause. The use of chitosan as a biopolymer was also explored to improve the distribution of enzymes on paper. Its ability to form uniform films and its compatibility with proteins position it as a promising candidate for further optimization [27].

One of the main challenges associated with paper-based colorimetric assays is the poor color uniformity across detection zones, which is mainly attributed to the uncontrolled flow of reagents [26]. Several strategies have been reported to enhance color uniformity in these colorimetric assays, such as the covalent immobilization of enzymes. Despite the advantages offered by these strategies, their application has not been universal due to potential enzyme inactivation and the complexity of multiple experimental steps. Chitosan is a biocompatible and biodegradable substance with excellent film-forming ability and a high specific surface area. Paper modification with chitosan has been shown to improve color uniformity in such assays [20].

TMB is a chromogenic substrate commonly used in biochemical assays such as ELISA. Upon oxidation, it produces a blue color, making it an ideal choice for differentiation from blood. However, TMB is sensitive to environmental factors such as pH and the presence of certain metal ions, which can act as catalysts or inhibitors in redox reactions.

To improve performance, the cellulose-based detection zones were modified with chitosan, leveraging electrostatic interactions between the negatively charged cellulose and the positively charged chitosan, as well as additional interactions such as hydrogen bonding.

For the microfluidic chip design different papers were tested, including polycarbonate and fiberglass membranes along with Whatman No. 1 paper. The initial configurations, using a polycarbonate membrane, were unsatisfactory due to platelet accumulation, which blocked blood flow. A configuration that included only salt-functionalized Whatman No. 1 paper was then selected.

The osmotic action induced by NaCl favors the physical retention of red blood cells in the paper fibers, facilitated by the compression of the electrical double layer surrounding the cells, which increases interactions with the cellulose matrix. Concentrations were selected so as not to compromise the integrity of cellular components. This passive serum separation occurs by capillarity in less than five minutes and without the need for external centrifugation devices. The following figure shows the result of the separation:

The final design included a 5 mm diameter sample collection zone and an analysis zone, connected by a 10 mm long and 3 mm wide channel, but the plasma distribution in the analysis zone was uneven. To improve this distribution, the diameter of the test zone was reduced to 4 mm, and the channel was narrowed to 2 mm, allowing for greater uniformity in plasma distribution for colorimetric analysis, as shown in Figure 2.

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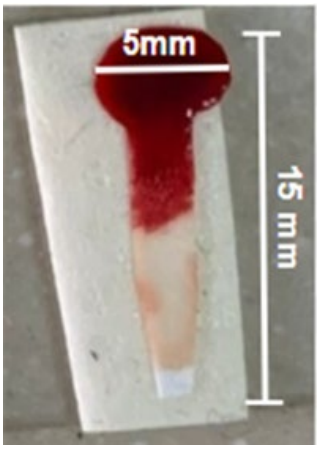


**Figure 2.** The result of the serum separation process.

The final design included a 5 mm diameter sample collection zone and an analysis zone, connected by a 10 mm long and 3 mm wide channel, but the plasma distribution in the analysis zone was uneven. To improve this distribution, the diameter of the test zone was reduced to 4 mm, and the channel was narrowed to 2 mm, allowing for greater uniformity in plasma distribution for colorimetric analysis, as shown in Figure 2.

During whole blood tests, the effectiveness of different saline concentrations in separating blood plasma was evaluated. The 1 M concentration was the most effective, achieving adequate plasma separation without hemoglobin contamination. Additionally, sodium heparin was incorporated into the paper to improve plasma flow and prevent clotting. The tests also evaluated the use of smaller blood volumes, adapting the design to work with smaller samples, which is crucial for Point-of-Care applications. The best concentration was 1M, which was used for all subsequent measurements. These results can be seen in a congress communication of our group [28].

Glucose solutions with concentrations of 2 mM and 6 mM were tested, representing glucose levels under hypoglycemic and hyperglycemic conditions. The results demonstrated a direct correlation between glucose concentration and the intensity of the color generated in the enzymatic reaction, confirming the device's sensitivity in measuring glucose levels.

Following the measurement and color reaction, the results were evaluated by processing color images captured with the smartphone using the specially designed app. For optimal image acquisition, the test paper should be placed under good lighting without flash, significantly improving the distinction between the desired color areas. This increases the sensitivity and accuracy of the assay, demonstrating a correlation between the gray values of the color images and glucose concentrations.

As was mentioned in Step 2 of the Materials and Methods, once the areas are detected, those corresponding to the desired color are shown in white, and the areas that do not match are shown in black, as can be seen in Figure 3.

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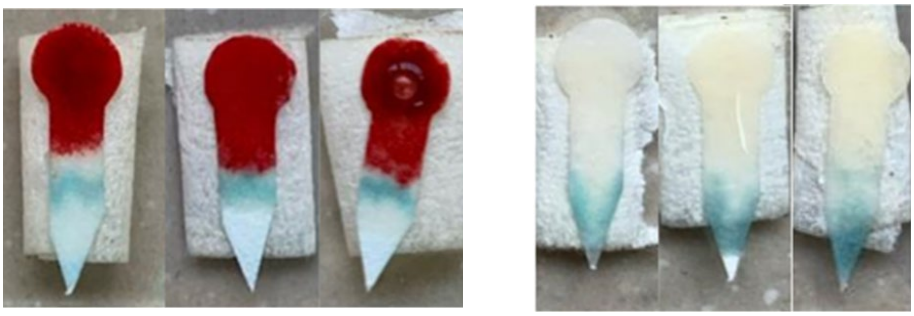
(a) (b)

**Figure 3. (a)** Outline of the desired object with the different color areas detected; **(b)** Gray mask.

The measurement protocol consists of: collecting an 8 µL blood sample; waiting 10 minutes; performing the test in a well-lit area, preferably under cold LED lighting or daylight; and taking the photo from a distance of 10 cm without using a flash.

Calibration is performed through a linear regression between the color intensity recorded by the smartphone and the glucose concentration obtained with the commercial blood glucose test kit. The obtained calibration equation allows the app to adjust an unknown glucose concentration based on color intensity. Glucose determination in the lab is normally determined by using plasma samples. To evaluate if the measurement and the application are consistent when using either whole blood or plasma, a parallel evaluation was conducted. Data collected from several samples (whole blood (a), and plasma (b) from the same sample, Figure 4) in triplicate are organized in a table that includes the glucose concentration measured with the blood glucose test kit, the average of the three color intensities detected for each sample, and the standard deviation of each measurement, as can be seen in Table 1. Each sample was analyzed using the smartphone application. A scatter plot and a calibration equation were obtained by linear regression. Figure 4 shows the resulting chips for each measurement.



(a) (b)

**Figure 4. (a)** Whole blood samples; **(b)** Plasma samples.

**Table 1.** Glucose concentrations and intensities with whole blood samples and blood plasma samples.

| N° | Glucose [mg/dl] (Commercial kit) | Average intensity of Whole Blood | Standard Dev. Whole Blood | Measured glucose [mg/dl] (Paper Chip) | Average of color intensity of Plasma | Standard Dev. Plasma | Measured glucose [mg/dl] (Paper Chip) |
|---|---|---|---|---|---|---|---|
| 1 | 50 | 140,3 | 12,7 | 48,9 | 160,3 | 14,0 | 50,6 |
| 2 | 71 | 159,3 | 2,1 | 76,8 | 158,0 | 3,8 | 47,0 |
| 3 | 87 | 148,7 | 1,5 | 61,2 | 165,0 | 3,0 | 57,7 |
| 4 | 88 | 158,3 | 1,5 | 75,4 | 187,3 | 5,0 | 91,9 |
| 5 | 128 | 174,3 | 8,1 | 98,9 | 167,0 | 2,1 | 60,8 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | 72,48 | 179,3 | 11,2 | 106,2 | 175,0 | 15,4 | 73,0 |
| 7 | 74,31 | 183,7 | 8,4 | 112,6 | 182,3 | 5,9 | 84,2 |
| 8 | 135 | 192,7 | 2,5 | 125,8 | 193,0 | 2,5 | 100,5 |

The resulting equation allows the application to predict the glucose concentration of a sample simply from the color intensity values, making the system self-contained and efficient for future analysis. Figure 5 shows the calibration curves for whole blood and plasma, respectively.

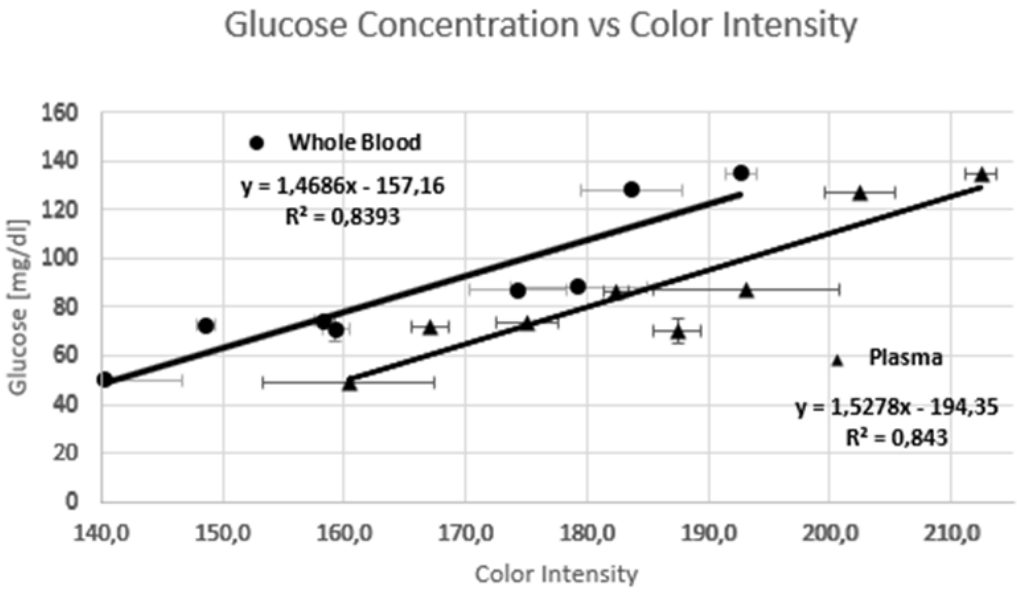


**Figure 5.** Calibration line of glucose concentration vs. color intensity measured with the whole blood samples and plasma samples.

The following equations describe the behavior of glucose concentration as a function of color intensity for whole blood (1) and plasma (2) samples.

$$Glucose\ [mg/dl] = 1.4686\ x\ Color\ Intensity\ Whole\ Blood - 157{,}16 \quad (1)$$

$$Glucose\ [mg/dl] = 1.5278\ x\ Color\ Intensity\ Plasma - 194{,}35 \quad (2)$$

As can be seen, the lines are parallel, i.e., they have almost the same slope, but there is a constant offset. It is noticeable that the chip overestimates the color intensity, possibly because, in the case of whole blood, the serum is not completely filtered. This issue could be related to the fact that the pore size of Whatman No. 1 paper is larger than the diameter of red blood cells, allowing some of them to pass through and mix with the serum. Additionally, it is important to note that at high salt concentrations, red blood cells could rupture, releasing hemoglobin and generating a faint pinkish color that interferes with the measurement by intensifying the final color.

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To evaluate the performance of the system, it was tested with an unknown blood sample. The separated plasma was analyzed using a commercial glucose determination kit, in order to compare the results with those obtained from the developed device. This comparison is shown in Figure 6.

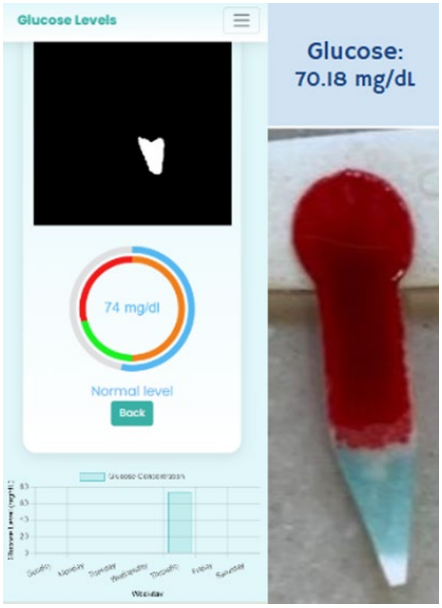


**Figure 6.** Control and analysis of the application using a new blood sample.

To evaluate the error in the curves, we used the RMSE (root mean square error), a metric widely used to measure the accuracy of predictions. It was calculated for both curves, and its value was approximately 10.83, while the standard deviations of the different subsets of data ranged from 1.5 to 15.4. Since the RMSE is lower than the maximum standard deviation (15.4), it can be inferred that the model presents a reasonable average error compared to the overall variability of the data. However, the RMSE exceeds some of the lower standard deviations, suggesting that the model may have less precise fitting for certain subsets with lower variability. Overall, these results indicate that the model has acceptable performance and adequate predictive capability, although it could benefit from further adjustment to improve accuracy in cases with lower dispersion.

A clear linear trend is observed in both calibration curves, with similar R^2 and RMSE values, confirming that the measurements obtained with the device using whole blood samples are comparable to those obtained with plasma.

## 4. Conclusions

In this work, an innovative method for the separation and analysis of plasma from whole blood was developed and validated, employing a paper-based chip with optimized geometry and surface functionalization. The final design incorporated a 4 mm diameter sample collection zone and an analysis zone connected by a 10 mm long and 2 mm wide channel. The system proved to be reproducible and enabled the formation of a suitable zone for colorimetric glucose detection. Calibration with a commercial kit yielded a correlation of $R^2 = 0.83$, indicating a reasonable agreement with standard analytical methods. Nevertheless, as this is a prototype, some variability was observed between whole blood and plasma measurements, underscoring the need for further optimization to reach the level of concordance expected in clinical applications. Additionally, a mobile application was developed to quantify glucose concentration from a minimal sample of 8 µL of whole blood, allowing for regular monitoring. Overall, the results demonstrate the feasibility of the proposed system as a practical, accessible, and low-cost alternative for glucose screening, particularly in low-resource settings.

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