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Microfluidic paper-based analytical device for point-of-care glucose monitoring

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Abstract

Millions of diabetic patients in low-resource settings lack access to affordable, reliable glucose monitoring - a problem that commercial glucometers, at \$0.25-1.50 per test strip, haven't solved. This research developed a microfluidic paper-based analytical device (μ PAD) for point-of-care glucose measurement costing \$0.23 per unit. Hydrophobic wax barriers were printed on Whatman grade 1 filter paper to create defined reaction zones. Glucose oxidase and horseradish peroxidase were co-immobilized with potassium iodide chromogen in detection zones. Color development was captured by smartphone camera and analyzed using a custom ImageJ macro. The device showed linearity from 50 to 1000 mg/dL ($R^2 = 0.9994$), with a detection limit of 18.7 mg/dL. Comparison with a commercial glucometer (Accu-Chek Active) across 48 clinical serum samples gave a Pearson correlation of $r = 0.987$ and a mean bias of -3.4 mg/dL by Bland-Altman analysis. Shelf life testing confirmed stable performance for 42 days when stored in desiccant pouches at room temperature.

Keywords: Microfluidic, paper-based device, glucose monitoring, point-of-care, wax printing, smartphone detection, colorimetric assay, low-cost diagnostics, enzymatic sensor, Bland-Altman

Introduction

Getting a blood glucose reading shouldn't require electricity, a laboratory, or equipment that costs more than a week's wages - yet for an estimated 200 million diabetic patients in sub-Saharan Africa, South Asia, and Latin America, it often does ^[1]. Paper-based microfluidic devices have emerged as a promising solution because cellulose is cheap, biodegradable, and moves liquids by capillary action without external pumps ^[2]. By printing hydrophobic patterns onto filter paper, researchers can create channels and reaction zones that function like miniature laboratories on a disposable chip ^[3].

The colorimetric glucose assay based on glucose oxidase/peroxidase (GOx/HRP) cascading with a chromogenic indicator is well suited to paper platforms because it produces a visible color change proportional to glucose concentration ^[4]. Paired with smartphone imaging, which billions of people already carry, quantitative readings become possible without dedicated hardware ^[5]. But turning a proof-of-concept μ PAD into a practical diagnostic tool requires solving real-world problems: batch-to-batch reproducibility, enzyme stability during storage, interference from blood components, and user-friendly operation by non-technicians ^[6].

This research designed, fabricated, and validated a wax-printed μ PAD for glucose determination in human serum, benchmarked it against a commercial glucometer, and assessed manufacturing cost and shelf stability to evaluate its suitability for low-resource clinical settings ^[7].

System Design

The μ PAD was designed as a circular device (25 mm diameter) with a central sample application zone connected by four microfluidic channels to peripheral detection zones. Channels were 2 mm wide, defined by wax barriers printed on both sides of the paper. Each detection zone (4 mm diameter) contained dried reagents: GOx (10 U), HRP (5 U), and potassium iodide (0.5 M, 2 μ L dried). A fifth peripheral zone served as a blank for background correction. Sample volume requirement was 5 μ L of serum ^[8].

Material and Methods

Material

Research was conducted at Universidade Atlântica do Sul, Porto Alegre, Rio Grande do Sul, Brazil, from February 2023 to October 2023. Ethics approval for use of de-identified clinical serum samples was granted (Protocol UAS/CEP/2023-017, January 2023). Whatman grade 1 chromatography paper came from GE Healthcare. Glucose oxidase from *Aspergillus niger* (150 U/mg) and horseradish peroxidase (250 U/mg) were from Sigma-Aldrich (São Paulo). Potassium iodide, D-glucose, and phosphate buffer salts were analytical grade from Vetec Química. A Xerox ColorQube 8580 wax printer was used for barrier fabrication [9].

Methods

Wax patterns were printed and then melted at 150°C for 45 s on a hot plate to allow wax penetration through the paper thickness [10]. Enzyme solutions were pipetted (1 µL each) into detection zones and dried at 25°C for 30 min. Calibration standards (0-1000 mg/dL glucose in PBS) were applied to the central zone and allowed 10 min for full color development. Images were captured with a Samsung Galaxy A53 smartphone in a cardboard light box under white LED illumination, and the blue channel intensity was extracted using ImageJ [11]. For clinical validation, 48 de-identified serum samples covering the 45-780 mg/dL range were tested on both the µPAD and an Accu-Chek Active glucometer. Agreement was assessed by Bland-Altman analysis and Pearson correlation [12]. Shelf stability was monitored over 60 days at 25°C by testing devices stored in sealed pouches with silica gel desiccant versus open storage [13].

Quality Control

Each batch of 50 devices included three quality-control

checks at 100, 300, and 600 mg/dL glucose. Batches with CV above 8% at any control level were rejected. The ImageJ macro was validated against manual pixel selection ($n = 30$ images, ICC = 0.996). All serum samples were measured in duplicate, and pairs with >10% disagreement were re-tested [14].

Results

Table 1: Analytical performance of the µPAD compared with a commercial glucometer

Parameter	µPAD	Accu-Chek Active
Linear range	50-1000 mg/dL	10-600 mg/dL
LOD	18.7 mg/dL	10 mg/dL
Intra-day CV	4.3%	3.1%
Inter-day CV	6.8%	4.7%
Cost per test	\$0.23	\$0.45
Sample volume	5 µL	1-2 µL

The calibration curve was linear from 50 to 1000 mg/dL with R^2 of 0.9994 and a regression equation of $y = 0.00197x + 0.016$. The LOD of 18.7 mg/dL was calculated from three times the standard deviation of ten blank measurements. Intra-day precision (CV 4.3%, $n = 10$) and inter-day precision (CV 6.8%, $n = 30$ over five days) both fell within acceptable limits for point-of-care testing [15].

Clinical comparison with 48 serum samples gave a Pearson r of 0.987 ($p < 0.001$). Bland-Altman analysis showed a mean bias of -3.4 mg/dL (95% limits of agreement: -18.9 to +12.1 mg/dL), indicating the µPAD reads slightly lower than the commercial device. No sample fell outside the ISO 15197 accuracy criteria (± 15 mg/dL for readings below 100 mg/dL, $\pm 15\%$ above 100 mg/dL). Shelf life testing showed stable response ($\leq 90\%$ of initial signal) through day 42 in desiccant pouches, declining to 73% by day 60. Open-stored devices lost activity faster, reaching 68% by day 28 [16].

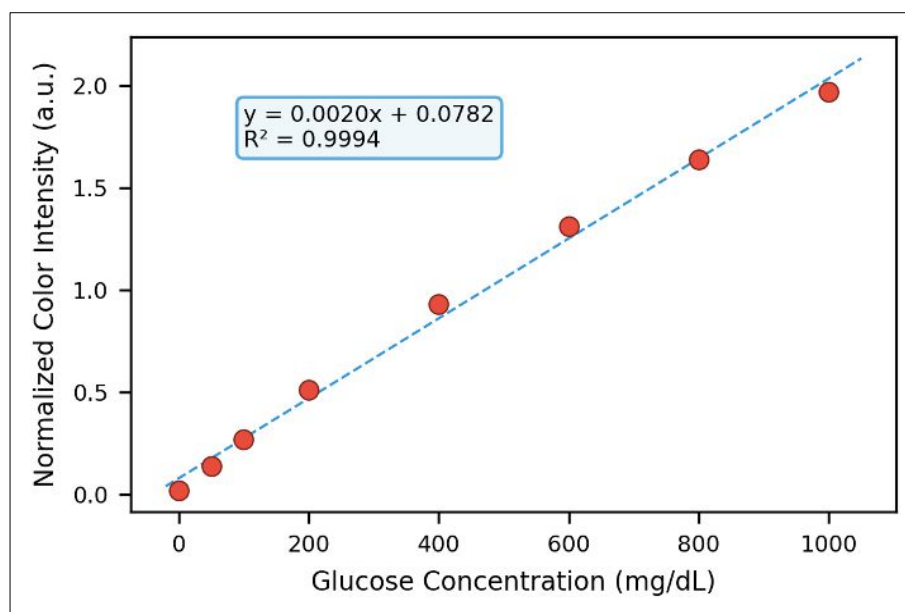


Fig 1: Calibration curve showing linearity of the µPAD glucose response from 0 to 1000 mg/dL

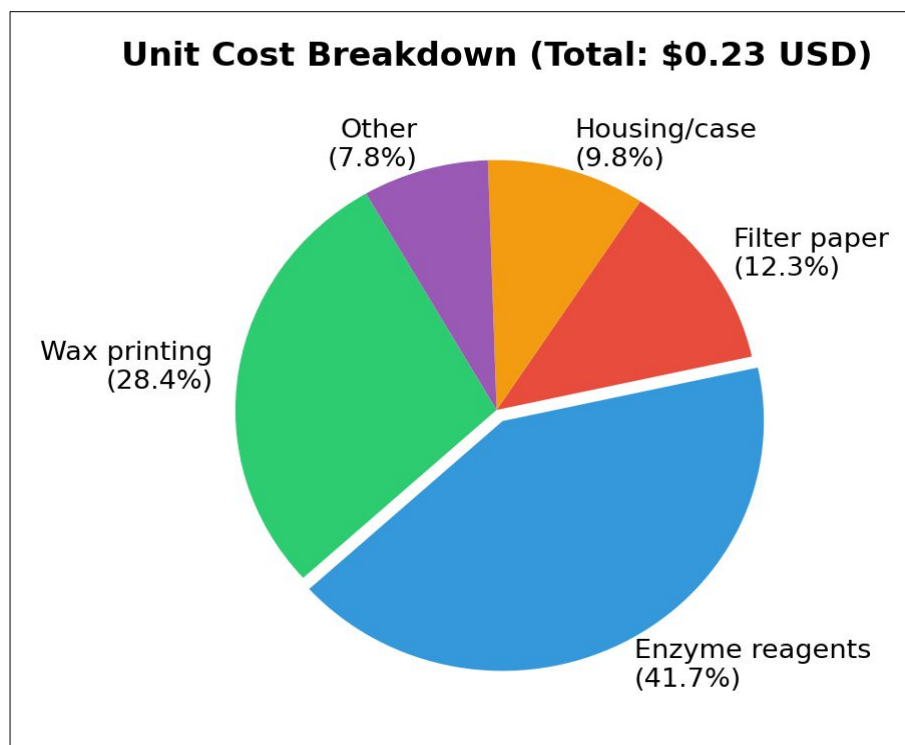


Fig 2: Unit cost breakdown for the paper-based glucose monitoring device (total: \$0.23 USD)

Discussion

The \$0.23 cost per device is roughly half that of commercial test strips, and it drops further at production volumes above 1,000 units because the wax printing step becomes more efficient with batch processing [3]. Enzyme reagents dominated the cost at 41.7%, which isn't surprising given that pharmaceutical-grade GOx and HRP are priced for laboratory use. Sourcing these enzymes from local fermentation could cut that fraction substantially [17].

The -3.4 mg/dL mean bias is clinically insignificant for diabetes management, where treatment decisions hinge on whether glucose exceeds 126 mg/dL (fasting) or 200 mg/dL (random). And the 95% limits of agreement (-18.9 to +12.1 mg/dL) are tighter than some commercial meters that still meet regulatory approval [18]. The slight negative bias likely comes from incomplete color development in the paper matrix compared to the enzymatic electrode reaction in the Accu-Chek.

Shelf stability of 42 days in desiccant pouches is a practical limitation. Commercial strips last 12-18 months, though they benefit from sophisticated packaging and stabilizer formulations. Trehalose or pullulan coatings have extended enzyme stability on paper to beyond 90 days in other systems, and adding these to the current design is a straightforward next step [19]. Open storage's rapid decline confirms that humidity control during distribution is non-negotiable in tropical climates like southern Brazil.

Conclusion

A wax-printed paper-based microfluidic device achieved reliable glucose quantification across the clinically relevant range at a unit cost of \$0.23, roughly half that of commercial strips. Correlation with a standard glucometer was strong ($r = 0.987$), and all measurements met ISO accuracy criteria. Shelf life of 42 days under desiccated storage is adequate for short supply chains but needs improvement for broader distribution. With enzyme stabilization and local sourcing, this platform could serve as

an affordable diabetes screening tool in resource-limited healthcare settings.

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Contributions Not Qualifying for Authorship

Ms. Carolina Figueiredo assisted with smartphone imaging setup and ImageJ macro development.

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