

Electrochemical and Colorimetric Glucose Monitoring with Portable Flexible Electrode

Beste Şimay Arslan , Mustafa Şen* 

İzmir Kâtip Çelebi University, Department of Biomedical Engineering, İzmir, Türkiye

*Corresponding author: mustafa.sen@ikcu.edu.tr

Abstract

In modern healthcare, particularly for the management of diabetes, the development of non-invasive, cost-effective, and reliable biosensors for glucose monitoring is a critical imperative. Herein, we present a dual-mode glucose biosensor featuring PET-based, screen-printed carbon electrodes supported by laminated Whatman filter paper. Functionalized with glucose oxidase (GOx) and horseradish peroxidase (HRP), the platform enables electrochemical detection via cyclic voltammetry and chronoamperometry, as well as colorimetric analysis through TMB oxidation. In artificial tear samples (0.1–1 mM glucose), the sensor achieved a detection limit (LOD) of 0.3 mM and demonstrated consistent performance across multiple electrodes. Its electrochemical performance ($R^2=0.99$) and reproducibility reflect recent advancements in tear glucose sensing platforms. Given that tear glucose levels typically remain around 0.5 mM, the device is highly suitable for real-time, non-invasive monitoring. Unlike many fluorescence- and nanoparticle-based systems, our device combines simplicity, low cost, and portability, making it exceptionally well-suited for point-of-care and home-use applications. The platform's robust design, reproducible fabrication process, and dual detection methods collectively position it as a promising candidate for non-invasive, user-friendly glucose monitoring in diabetes management. This work represents a significant stride in biosensor technology.

Keywords: Electrochemical, tear, colorimetric, glucose, wearable sensor

Introduction

Diabetes mellitus is a chronic metabolic disorder with profound human, social, and economic implications. It arises from insufficient insulin production or impaired insulin action, preventing glucose uptake by cells. According to the World Health Organization (WHO), approximately seven million people are diagnosed with diabetes annually, and diabetes-related deaths are projected to rise by 50% in the coming decade [1]. The major concern with diabetes lies in the secondary complications it can induce, such as vision loss, cardiovascular disease, renal dysfunction, and chronic non-healing wounds that may result in limb amputations due to microvascular damage [2]. Despite technological advances, traditional glucose monitoring methods often require multiple daily finger-prick tests, leading to pain and poor patient compliance. In response, there have been numerous efforts to develop painless, non-invasive glucose sensing methods to enhance patient comfort and long-term disease management [3]. Particularly in the wake of the COVID-19 pandemic, the global demand for rapid, reliable, and portable biomedical diagnostic tools has intensified, attracting considerable attention from both academia and industry. Among these, glucose biosensors hold special significance due to the increasing prevalence of diabetes worldwide [4]. In recent years, tear fluid has emerged as a promising non-invasive alternative for glucose monitoring. While the glucose concentration in tears is relatively low, it remains within a detectable range, making it suitable for biosensor applications. Tear sampling offers several advantages over blood sampling, including painless collection, easy accessibility, and minimal infection risk—making it especially attractive for home-based monitoring systems. Within this context, hybrid biosensor platforms that combine electrochemical and colorimetric methods have gained attention in the literature for their ability to deliver both quantitative and visual outputs. Electrochemical detection methods are widely used due to their high sensitivity, rapid response, low cost, ease of use, and suitability for point-of-care diagnostics without requiring specialized personnel [5,6]. Furthermore, soft, flexible, disposable, and portable electrochemical sensors are especially relevant in the field of wearable technologies. However, most existing systems rely on a single detection modality, which may limit analytical reliability. To address these limitations, this study presents a novel dual-mode biosensor capable of both electrochemical and colorimetric glucose detection. The platform features screen-printed carbon electrodes on a PET substrate integrated with Whatman filter paper, and is functionalized

with glucose oxidase and horseradish peroxidase. This design enables accurate measurement of glucose concentrations with high sensitivity. The integration of electrochemical and colorimetric modalities within the same platform allows for cross-validation of results, thereby significantly enhancing the accuracy and reliability of the measurements.

In addition, the use of the GalvanoPlot system—featuring portability, Android compatibility, multichannel measurement, wireless communication, and a customizable interface—greatly contributes to the system's real-time usability in academic and industrial applications. These innovations collectively demonstrate the platform's potential as a flexible and robust tool for non-invasive glucose monitoring.

Materials and Methods

Materials

D-(+)-Glucose ($\geq 99.5\%$), 3,3',5,5'-Tetramethylbenzidine (TMB, $\geq 99\%$), horseradish peroxidase (HRP, Type VI-A, salt-free), and glucose oxidase (GOx) from *Aspergillus niger* (100,000–250,000 U g⁻¹, Type XS) were purchased from Sigma-Aldrich (USA). Tween 20, poly(sodium 4-styrenesulfonate) (PSS, 30% in water), poly(ethylene glycol) 2-mercaptoethyl ether acetic acid (PEG-COOH, $M_n \approx 2100$), trisodium citrate dihydrate ($\geq 99.0\%$), and gold(III) chloride hydrate ($\geq 99.995\%$) were also obtained from Sigma-Aldrich. Whatman filter papers of Grade 6 (3 μm pore size, cellulose, ashless), Grade 1 (11 μm), and Grade 41 (20 μm) were used throughout the experiments. Bovine serum albumin (BSA, protease-free) was purchased from VWR (Belgium). Phosphate-buffered saline (PBS) containing 1 mg mL⁻¹ BSA and 0.05% Tween 20 was prepared following standard protocols. Polyethylene glycol-coated gold nanoparticles (PEG-AuNPs, 20 nm) were synthesized as described previously in the literature

Development of Screen Printed Electrode and Fixing Filter Paper by Lamination

Screen-printed electrodes (SPEs) were fabricated on polyethylene terephthalate (PET) substrates using three custom-designed screen-printing templates. A commercial conductive silver (Ag) ink was used for the reference electrode (RE), while conductive carbon ink was employed for the working (WE) and counter electrodes (CE). A small piece of Whatman Grade 1 filter paper was placed on the detection zone, and the entire surface was insulated by laminating an additional PET layer. Finally, the SPEs were precisely cut for use in individual analyses.

Evaluation of Lamination Quality by Electrochemical Characterization of Screen Printed Electrode

In this stage, the effects of the lamination process on electrode performance were evaluated, and the integrity of the lamination was verified. To achieve this, cyclic voltammetry (CV) measurements were conducted using 1 mM Ferrocenemethanol (FcMeOH) solution applied onto the laminated filter paper positioned over the working electrode. The measurements were performed using a GalvanoPlot potentiostat at varying scan rates ranging from 20 mV/s to 1000 mV/s, in order to analyze the relationship between anodic and cathodic peak currents. A near-unity ratio of anodic to cathodic current was considered indicative of successful lamination and proper electrochemical functionality of the electrode. Additionally, three different electrode designs were comparatively analyzed using cyclic voltammetry to assess their electrochemical performance. Based on the CV results, the most suitable electrode configuration was identified. This comparative evaluation enabled the selection of an optimal electrode design that ensured enhanced electrochemical characteristics and overall platform performance.

Preparation and Analysis of Flexible Printed Electrode for Colorimetric and Electrochemical Measurement

In this stage, screen-printed electrodes were surface-functionalized with glucose oxidase (GOx) and horseradish peroxidase (HRP) enzymes to develop a dual-mode sensing platform capable of both colorimetric and electrochemical detection. Initially, a 1 mg/mL GOx solution was drop-cast onto filter paper placed on the working electrode, followed by a drying step to allow enzyme immobilization. After drying, 40 μL of HRP and 10 μL of GOx were re-applied to the same filter paper. The modified electrode was then kept in a dark environment for a short period to complete the immobilization process. Subsequently, 20 mM of 3,3',5,5'-Tetramethylbenzidine (TMB) was added, rendering the electrode ready for glucose measurement. Artificial tear samples with different glucose concentrations (0.1, 0.25, 0.50, 0.75, and 1 mM) were prepared and analyzed via chronoamperometry using a GalvanoPlot

potentiostat within a potential window of 0 V to 0.4 V and a scan rate of 50 mV/s. Upon oxidation of glucose by GOx, hydrogen peroxide (H₂O₂) was generated and subsequently utilized by HRP to oxidize TMB, resulting in the formation of a blue-colored product. For colorimetric analysis, a reference color strip was created using the same concentration levels, and the corresponding color changes were visually observed. Quantitative analysis of blue color intensity was performed using ImageJ software by measuring the blue channel pixel values.

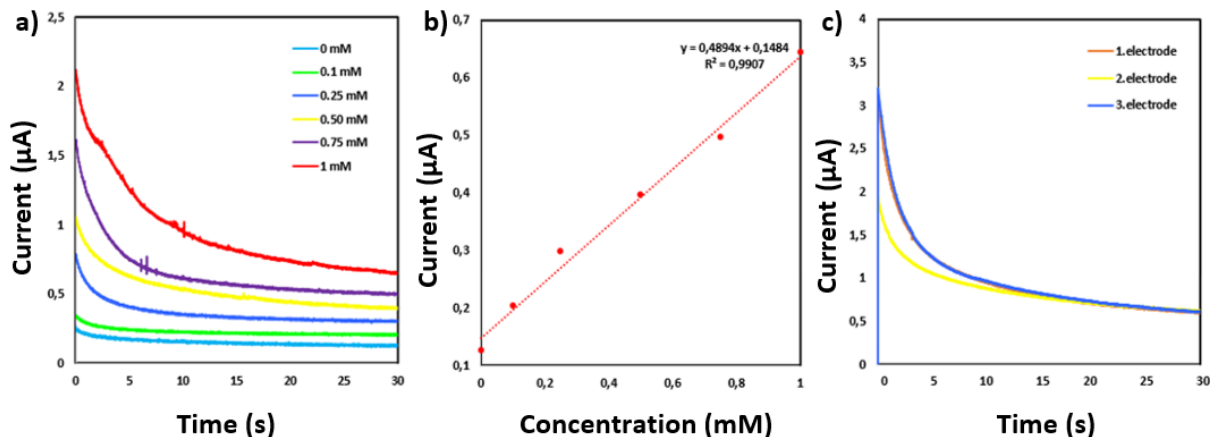


Figure 1. Chronoamperometry curves obtained from tears with different glucose concentrations between 0.1 mM and 1 mM, (a) calibration curve (b) glucose measurement results obtained from three different electrodes in tears containing 0.5 mM glucose (c).

Experimental Results and Discussion

The use of screen-printed carbon electrodes supported by a filter paper layer enabled effective immobilization of glucose oxidase (GOx) enzyme, which significantly enhanced the measurement sensitivity. As presented in Figure 1a, glucose measurements were performed in artificial tear samples containing different concentrations (0.1 mM to 1 mM) within a potential range of 0 V to 0.4 V at a scan rate of 50 mV/s. According to Figure 1b, a strong linear relationship was observed between current response and glucose concentration, with a correlation coefficient (R^2) of 0.99. This near-perfect R^2 value demonstrates the high accuracy and reliability of the sensor in detecting glucose levels, confirming that the current signal changes proportionally with variations in glucose concentration. Such performance highlights the biosensor's robustness and potential for real-world biomedical applications. Furthermore, the limit of detection (LOD) of the developed glucose biosensor was calculated using the standard equation $LOD = 3.3 \times (STD/slope)$, resulting in a value of 0.3 mM. This level of sensitivity is sufficient for monitoring glucose levels in tear fluid, particularly for diabetic patients. As illustrated in Figure 1c, glucose detection in 0.5 mM artificial tear solution was conducted using three different electrodes, and all measurements yielded consistent and overlapping results. This excellent agreement confirms the system's high repeatability and reliability. In Figure 2, cyclic voltammetry (CV) measurements conducted with 1 mM Ferrocenemethanol (FcMeOH) on the filter paper laminated onto the working electrode showed clear anodic and cathodic peaks associated with the redox behavior of the compound. Subsequently, the electrochemical responses of three different electrode designs were compared via CV analysis. The anodic-to-cathodic peak current ratios being close to 1 confirmed both the successful lamination process and the proper electrochemical performance of the electrodes.

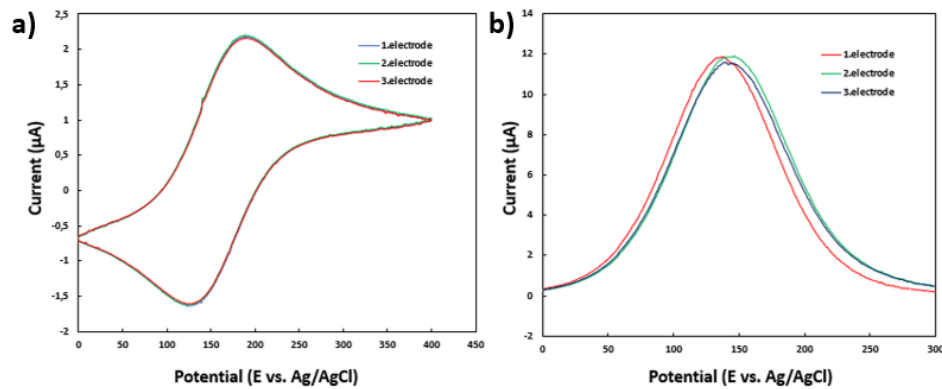


Figure 2. Cyclic voltammetry (a) and differential pulse voltammetry (b) curves of three different electrodes in 1 mM FcMeOH solution.

As shown in Figure 3, colorimetric measurements were carried out on enzyme-modified screen-printed electrodes by applying varying glucose concentrations. The glucose oxidase enzyme catalyzed the oxidation of glucose, generating hydrogen peroxide (H_2O_2), which was subsequently utilized by horseradish peroxidase (HRP) to oxidize TMB, resulting in the formation of a distinct blue-colored product. This visible color change enabled qualitative detection of glucose levels, and the intensity of the blue coloration was quantitatively analyzed. Using ImageJ software, blue channel intensities were measured and found to exhibit a clear increasing trend in correlation with glucose concentration, demonstrating the effectiveness of the colorimetric sensing platform.



Figure 3. Calibration curve between glucose concentration and blue color intensity (a) Blue color change in glucose colorimetric measurement (b)

Conclusions

A dual-mode glucose biosensor was successfully developed using enzyme-modified, screen-printed carbon electrodes on PET supported by Whatman filter paper. The sensor provided reliable electrochemical and colorimetric glucose detection in artificial tears with consistent performance across electrodes. Its LOD of 0.3 mM indicates sufficient sensitivity for noninvasive tear-based monitoring, which is especially important for diabetic patients. It shows that the developed biosensor platform can be a low-cost, portable, and effective tool for tear-based glucose monitoring. In conclusion, the obtained findings demonstrate the design and manufacturing quality of the sensor and clearly demonstrate its potential in the field of glucose monitoring technologies. This study is a significant achievement in biosensor technology.

References

- [1] Gabriel, E. F. M., Garcia, P. T., Lopes, F. M., & Coltro, W. K. T. (n.d.). Gözyaşı glikoz ölçümleri için kağıt tabanlı kolorimetrik biyosensör. In F. A. Gomez & C. D. Garcia (Eds.)
- [2] Liu, L., Zhan, K., Kilpijärvi, J., Kinnunen, M., Zhang, Y., Yaltaye, M., Li, Y., Zhyvolozhnyi, A., Samoylenko, A., Vainio, S., & Huang, J. (n.d.). Bridging optical sensing and wearable health monitoring: A functionalized plasmonic nanopillar for non-invasive sweat glucose detection
- [3] Sajjadi, S., Keihan, A. H., Norouzi, P., Habibi, M. M., Eskandari, K., & Hadizadeh Shirazi, N. (2017). Fabrication of an amperometric glucose biosensor based on a Prussian blue/carbon nanotube/ionic liquid modified glassy carbon electrode. *Journal of Applied Biotechnology Reports*, 4(2), 603–608.

- [4] Witkowska Nery, E., Kundys, M., Jeleń, P. S., & Jönsson-Niedziółka, M. (2016). Electrochemical glucose sensing: Is there still room for improvement? *Analytical Chemistry*, 88(22), 11271–11282. <https://doi.org/10.1021/acs.analchem.6b03219>
- [5] Liu, L., Zhan, K., Kilpijärvi, J., Kinnunen, M., Zhang, Y., Yaltaye, M., Li, Y., Zhyvolozhnyi, A., Samoylenko, A., Vainio, S., & Huang, J. (2021). Bridging optical sensing and wearable health monitoring: A functionalized plasmonic nanopillar for non-invasive sweat glucose detection. *Journal of Alloys and Compounds*, 886, 161226. <https://doi.org/10.1016/j.jallcom.2021.161226>