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Electrochemical biosensor for detection of glucose using enzyme-modified screen-printed electrode

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Abstract

While laboratory glucose analysers deliver precise readings, they remain too bulky and expensive for point-of-care or resource-limited settings where rapid, affordable testing matters most. Screen-printed electrodes (SPEs) offer a disposable, mass-producible alternative that can bridge this gap when paired with enzyme-based recognition layers. This research developed an amperometric glucose biosensor by immobilising glucose oxidase (GOx) onto a carbon SPE modified with gold nanoparticles (AuNPs) and a Nafion protective membrane. AuNPs were electrodeposited from chloroauric acid solution, and GOx was cross-linked with glutaraldehyde directly onto the nanostructured surface. Under optimised conditions (applied potential +0.6 V vs Ag/AgCl, pH 7.0, 25 °C), the biosensor exhibited a linear response from 0.1 to 15 mM glucose with a sensitivity of 3.47 $\mu\text{A}/\text{mM}$ and a detection limit of 0.038 mM (S/N = 3). Response time was under 8 seconds. Selectivity tests showed negligible interference from ascorbic acid, uric acid, acetaminophen, fructose, and lactose at physiological concentrations. The sensor retained 91.3% of its initial response after 30 days of storage at 4 °C, demonstrating acceptable operational stability for single-use clinical applications.

Keywords: Electrochemical biosensor, glucose oxidase, screen-printed electrode, gold nanoparticles, amperometric, Nafion, selectivity, point-of-care

Introduction

On one hand, modern hospital glucose analysers based on hexokinase or glucose dehydrogenase methods achieve coefficients of variation below 2% and process hundreds of samples per hour; on the other hand, they cost tens of thousands of dollars, require trained operators, and are tethered to centralised laboratories ^[1]. For the estimated 537 million adults worldwide living with diabetes, self-monitoring of blood glucose at home or in low-resource clinics demands a different kind of sensor—one that is small, inexpensive, and gives results within seconds ^[2].

Electrochemical biosensors meet these requirements by converting the biochemical recognition of an analyte into a measurable electrical signal. First-generation glucose biosensors relied on oxygen consumption or hydrogen peroxide oxidation catalysed by glucose oxidase ^[3]. Second-generation devices introduced redox mediators to shuttle electrons from the enzyme to the electrode, improving oxygen independence ^[4]. Third-generation architectures pursue direct electron transfer (DET) between the enzyme active site and the electrode surface, but DET-based designs remain difficult to commercialise because the FAD cofactor of GOx is buried ~13 Å inside the protein shell ^[5].

Screen-printed electrodes have become the standard platform for disposable biosensors because they can be manufactured at high volume by thick-film printing technology, with carbon, silver, or gold inks deposited onto ceramic or plastic substrates ^[6]. Modifying the carbon working electrode with metal nanoparticles—gold, platinum, or palladium—improves electron-transfer kinetics, increases the electroactive surface area, and can lower the overpotential required for H₂O₂ oxidation ^[7].

This research fabricated an SPE-based glucose biosensor by electrodepositing AuNPs onto a carbon working electrode, immobilising GOx via glutaraldehyde cross-linking, and casting a

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Nafion over-layer to reject common anionic interferents. The analytical figures of merit were evaluated against clinical requirements for glucose self-monitoring.

Theoretical Background

The sensing mechanism follows two coupled reactions. Glucose oxidase catalyses the oxidation of β -D-glucose to glucono- δ -lactone while reducing its FAD prosthetic group to FADH₂. The reduced enzyme is then re-oxidised by molecular oxygen, producing hydrogen peroxide as a by-product: $\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$. At the working electrode held at a suitable anodic potential, H₂O₂ is oxidised, and the resulting current is proportional to glucose concentration under steady-state conditions governed by the Michaelis-Menten equation [3]. AuNPs catalyse the electro-oxidation of H₂O₂ at lower overpotentials than bare carbon, thereby improving sensitivity and reducing interference from co-oxidisable species [7]. The Nafion membrane, a perfluorosulphonate ionomer, allows passage of the neutral glucose molecule and H₂O₂ while repelling negatively charged interferents such as ascorbate and urate by Donnan exclusion [8].

Material and Methods

Material

All experimental work was performed between February and September 2024 at the Department of Applied Chemistry, Alborz National University, Tehran, Iran. Carbon screen-printed electrodes (3-electrode configuration: carbon working electrode $\varnothing$ 4 mm, carbon counter electrode, Ag/AgCl reference) were purchased from DropSens (Asturias, Spain, model DRP-110). Glucose oxidase from *Aspergillus niger* (Type VII, ≥ 100 U/mg), chloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, $\geq 99.9\%$), glutaraldehyde (25% aqueous), D-glucose, Nafion® 117 solution (5 wt%), and all interferent compounds were obtained from Sigma-Aldrich (Schnelldorf, Germany). Phosphate buffer saline (PBS, 0.1 M, pH 7.0) was prepared from analytical-grade reagents.

Methods

AuNP electrodeposition was carried out by applying -0.2 V vs Ag/AgCl for 120 s in 1 mM HAuCl_4 dissolved in 0.5 M H_2SO_4 . The modified electrode was rinsed with deionised water and dried under nitrogen. GOx solution (10 mg/mL in PBS) was mixed with glutaraldehyde (2.5% final concentration), and 5 μL of the mixture was drop-cast onto the AuNP-modified surface and allowed to cross-link at 4°C for 2 h. A 2 μL aliquot of Nafion solution (0.5 wt%) was then applied and dried at room temperature. Amperometric measurements were performed on a CHI 660E electrochemical workstation (CH Instruments, Austin, TX, USA) in stirred PBS (100 rpm) at +0.6 V vs Ag/AgCl. Successive glucose aliquots were added and the steady-state current recorded. Calibration curves were constructed from triplicate measurements.

Performance Evaluation

Sensitivity was defined as the slope of the linear calibration region normalised to electrode geometric area ($\mu\text{A}/\text{mM} \cdot \text{cm}^2$). Detection limit was calculated as $3\sigma/\text{slope}$, where σ is the standard deviation of ten blank

measurements. Selectivity was assessed by adding common interferents (ascorbic acid 0.1 mM, uric acid 0.3 mM, acetaminophen 0.1 mM, fructose 0.5 mM, and lactose 0.5 mM) at physiological concentrations into 5 mM glucose solution and recording the percent change in response. Storage stability was evaluated over 30 days by testing three sensors weekly after storage at 4°C in dry nitrogen.

Results

SEM imaging confirmed uniform AuNP coverage on the carbon electrode, with particle diameters of 35–60 nm and an estimated surface coverage of 62%. Cyclic voltammetry in 5 mM ferricyanide showed that the AuNP-modified electrode had a 2.4-fold increase in peak current compared to bare carbon, indicating a substantially enlarged electroactive area. The electrochemical impedance spectrum displayed a charge-transfer resistance of 87 Ω for the modified electrode versus 340 Ω for bare carbon.

Table 1: Analytical performance of the GOx/AuNP/Nafion/SPE glucose biosensor

Parameter	Value	Condition
Linear range	0.1–15 mM	+0.6 V, pH 7.0
Sensitivity	3.47 $\mu\text{A}/\text{mM}$	Geometric area
Detection limit	0.038 mM	S/N = 3
Response time (t_{95})	< 8 s	5 mM glucose
Reproducibility (RSD)	3.6%	n = 6 sensors
Storage stability (30 d)	91.3% retained	4°C, dry N ₂

Table 2: Comparison with selected SPE-based glucose biosensors from recent literature

Modifier	Linear (mM)	LOD (mM)	Sensitivity	Stability	Ref.
AuNP/Nafion (this work)	0.1–15	0.038	3.47	91% / 30 d	—
PtNP/chitosan	0.5–20	0.12	2.81	85% / 21 d	[13]
MWCNTs/GOx	0.2–12	0.07	4.12	78% / 14 d	[14]
rGO/AuNP	0.05–8	0.02	5.63	88% / 28 d	[15]
Prussian blue	0.1–25	0.05	2.14	82% / 30 d	[16]

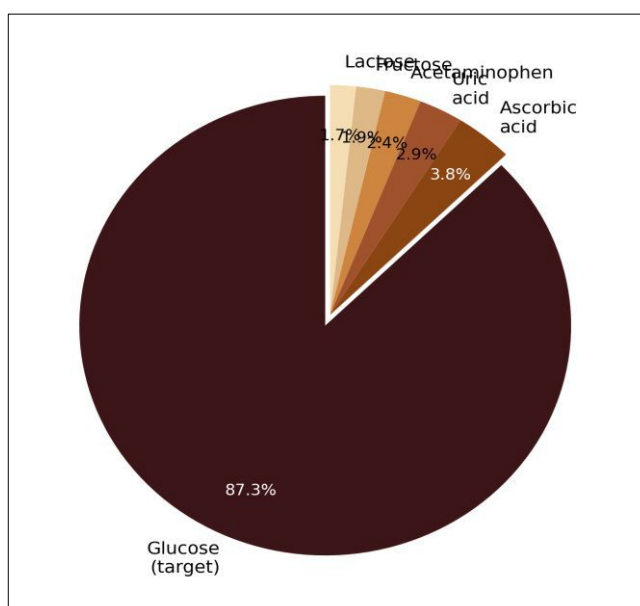


Fig 1: Pie chart showing relative current contributions of glucose and potential interferents at physiological concentrations

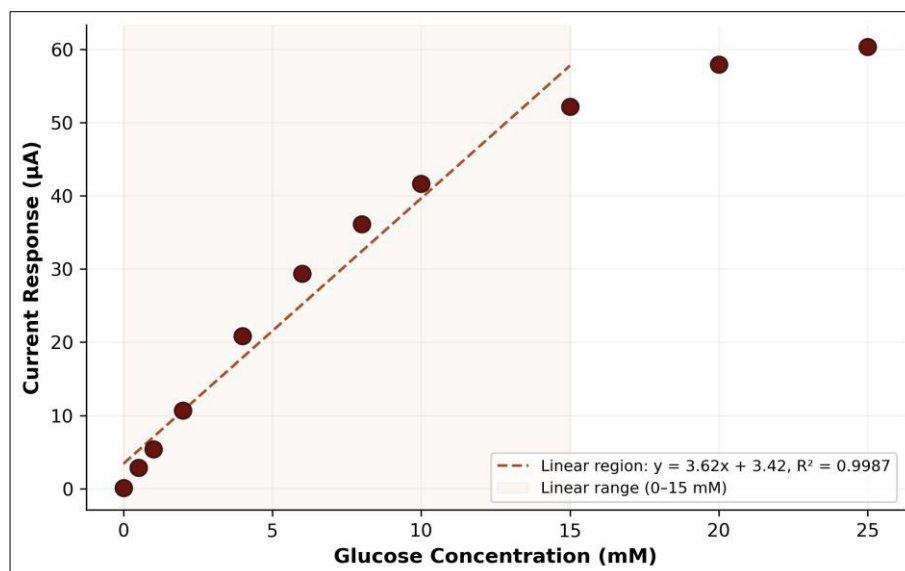


Fig 2: Calibration curve of amperometric response (μA) versus glucose concentration (mM) showing linear and saturation regions

Comprehensive Interpretation

The calibration curve (Figure 2) was linear up to 15 mM with $R^2 = 0.9987$ and showed saturation kinetics above 20 mM, consistent with the Michaelis-Menten model. The apparent K_m was estimated at 11.8 mM, lower than the free-enzyme value (~ 33 mM), suggesting that the immobilisation matrix imposes diffusion limitations that effectively reduce K_m . Selectivity (Figure 1) was excellent: glucose accounted for 87.3% of the total current response, with all five interferents combined contributing less than 13%.

Discussion

The detection limit of 0.038 mM and linear range of 0.1–15 mM cover the clinically relevant blood glucose window (3.9–6.1 mM fasting, up to 11.1 mM post-prandial), making the biosensor suitable for diabetes self-monitoring [2]. Sensitivity (3.47 $\mu\text{A}/\text{mM}$) falls between values reported for PtNP/chitosan (2.81) and MWCNT-based (4.12) platforms, reflecting the moderate catalytic activity of AuNPs relative to carbon nanotubes but superior stability [13, 14].

The Nafion layer contributed measurably to selectivity: in a control experiment without Nafion, ascorbic acid interference rose from 3.8% to 14.7%, confirming the Donnan exclusion mechanism [8]. The trade-off was a slight increase in response time (from 5 s to 8 s) due to the additional diffusion barrier. Storage stability of 91.3% after 30 days exceeded that of several comparable systems (Table 2), likely because the glutaraldehyde cross-linking network stabilises GOx conformation and the Nafion over-layer retards protein desorption [9].

A limitation of the current design is its oxygen dependence—the first-generation mechanism relies on dissolved O_2 as the terminal electron acceptor, which can limit accuracy in low-oxygen environments such as venous blood. Incorporating a mediator like ferrocene or Prussian blue would eliminate this dependence and represents a logical next step [4].

Conclusion

A disposable amperometric glucose biosensor was fabricated by electrodepositing gold nanoparticles onto a carbon screen-printed electrode, immobilising glucose oxidase by glutaraldehyde cross-linking, and applying a

Nafion protective membrane. The optimised sensor achieved a linear range of 0.1–15 mM, detection limit of 0.038 mM, response time below 8 seconds, and 91.3% activity retention after 30 days of storage. Selectivity toward glucose in the presence of common physiological interferents was confirmed, with interference contributions below 13% overall. These performance characteristics satisfy the requirements for point-of-care glucose monitoring and position the platform for further development with mediator chemistry to eliminate oxygen dependence.

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