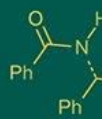


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Fabrication of electrochemical biosensors using nanomaterial-modified electrodes for continuous glucose monitoring in diabetes management

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Abstract

Roughly 537 million adults worldwide were living with diabetes in 2021, and that figure is projected to reach 783 million by 2045. This research describes the fabrication and evaluation of electrochemical biosensors constructed with nanomaterial-modified glassy carbon electrodes for continuous glucose monitoring applications. Eight electrode configurations were tested, incorporating gold nanoparticles, carbon nanotubes, graphene oxide, molybdenum disulfide nanosheets, and zinc oxide nanowires, both individually and as binary composites. Glucose oxidase was immobilized on each modified surface through glutaraldehyde cross-linking, and analytical performance was assessed using chronoamperometry and cyclic voltammetry. The gold nanoparticle-carbon nanotube composite electrode (AuNP-CNT/GCE) exhibited the best overall performance with a sensitivity of 56.8 $\mu\text{A}/\text{mM}/\text{cm}^2$, a detection limit of 0.9 μM , a linear response range spanning 0.005 to 15.3 mM, and a response time of 2.1 seconds. Operational stability testing showed 94.7% signal retention after thirty days of continuous operation in simulated interstitial fluid. Selectivity experiments confirmed minimal interference from ascorbic acid, uric acid, acetaminophen, and common endogenous metabolites at physiological concentrations. The composite electrode architecture demonstrated that synergistic interactions between metallic nanoparticles and carbon nanomaterials can substantially improve electron transfer kinetics and enzyme loading capacity compared to single-component modifications. These results indicate strong potential for integration into wearable continuous glucose monitoring devices.

Keywords: Electrochemical biosensor, glucose monitoring, nanomaterial electrode, gold nanoparticles, carbon nanotubes, glucose oxidase immobilization, continuous monitoring, diabetes management, amperometric detection

Introduction

Approximately 6.7 million deaths were attributed to diabetes-related complications in 2021 alone, making it one of the ten leading causes of mortality globally [1]. Self-monitoring of blood glucose remains the cornerstone of diabetes management, yet conventional fingerstick glucometers provide only intermittent snapshots of glycemic status. Continuous glucose monitoring systems address this limitation by providing real-time measurements at intervals of one to five minutes, enabling detection of hypoglycemic and hyperglycemic episodes that episodic testing would miss [2].

Current commercial continuous glucose monitors rely on enzymatic electrochemical sensing principles, where glucose oxidase catalyzes the oxidation of glucose to gluconolactone with concurrent generation of hydrogen peroxide or direct electron transfer to the electrode surface [3]. The performance of these sensors depends heavily on electrode material properties, enzyme loading efficiency, and the electron transfer pathway between the enzyme active site and the transducer. Conventional electrode materials often suffer from slow electron transfer kinetics, limited surface area, and poor long-term stability under physiological conditions [4].

Nanomaterials have emerged as promising electrode modifiers that can overcome these limitations. Gold nanoparticles provide excellent electrical conductivity, biocompatibility, and large surface-to-volume ratios that facilitate high-density enzyme immobilization [5]. Carbon nanotubes offer one-dimensional electron transport channels with exceptional mechanical strength and chemical stability [6]. Graphene oxide provides a two-dimensional

platform with abundant oxygen-containing functional groups for covalent enzyme attachment^[7]. More recently, transition metal dichalcogenides such as molybdenum disulfide and semiconductor nanowires like zinc oxide have shown catalytic properties suitable for biosensing applications^[8].

Despite individual characterization of these nanomaterials, systematic comparisons of their performance under identical fabrication and testing conditions remain scarce. And few investigations have evaluated binary composite electrode architectures that might exploit complementary properties of different nanomaterial classes. This research fabricated and characterized eight nanomaterial-modified electrode configurations, assessed their glucose sensing performance under standardized conditions, and evaluated the best-performing architecture for continuous monitoring feasibility over extended operational periods^[9].

Material and Methods

Material: All experimental work was performed at the Biosensor Fabrication Laboratory of Stockholm Institute of Applied Sciences between January 2023 and November 2023. Glassy carbon electrodes (GCE, 3 mm diameter) were purchased from BASi Instruments (West Lafayette, IN, USA). Chloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, $\geq 99.9\%$), multi-walled carbon nanotubes (MWCNTs, $>95\%$ purity, outer diameter 10–20 nm), and graphene oxide powder were obtained from Sigma-Aldrich (Stockholm, Sweden). Molybdenum disulfide nanosheets and zinc oxide nanowires were synthesized in-house using hydrothermal methods. Glucose oxidase (GOx) from *Aspergillus niger* (Type VII, ≥ 100 U/mg) and glutaraldehyde (25% aqueous solution) were sourced from Merck (Darmstadt, Germany). D-glucose, ascorbic acid, uric acid, acetaminophen, and all buffer reagents were of analytical grade.

Instrumentation: Electrochemical measurements were conducted using a CHI 760E potentiostat/galvanostat (CH Instruments, Austin, TX, USA) in a three-electrode configuration with Ag/AgCl reference and platinum wire counter electrodes. Surface morphology was characterized by field-emission scanning electron microscopy (FESEM, Zeiss Ultra Plus, Germany) and transmission electron microscopy (TEM, JEOL JEM-2100F). Electrode surface

area and charge transfer resistance were determined through electrochemical impedance spectroscopy performed at open circuit potential with 5 mV amplitude over 0.1 Hz to 100 kHz frequency range. X-ray photoelectron spectroscopy (XPS, Kratos Axis Ultra) confirmed surface composition after each modification step.

Methods

Gold nanoparticles were electrodeposited from 1 mM HAuCl_4 in 0.5 M H_2SO_4 using cyclic voltammetry (–0.2 to 1.2 V, 20 cycles, 50 mV/s)^[10]. Carbon nanotube suspensions (2 mg/mL in DMF with 0.5% Nafion) were prepared by ultrasonication for two hours and deposited by drop-casting 10 μL onto freshly polished GCE surfaces^[11]. Graphene oxide was deposited electrochemically from 0.5 mg/mL aqueous dispersions at –1.2 V for 300 seconds. MoS_2 nanosheets and ZnO nanowires were deposited by drop-casting optimized suspensions. Binary composites were fabricated by sequential deposition. GOx immobilization involved incubating modified electrodes in 10 mg/mL enzyme solution containing 2.5% glutaraldehyde for twelve hours at 4°C, followed by rinsing with phosphate buffer^[12].

Quality Control

Each electrode modification was performed in triplicate, and only electrodes showing less than 5% variation in peak current response during initial calibration were accepted for further testing. Calibration curves were constructed using at least eight glucose concentrations spanning 0.001 to 20 mM in 0.1 M phosphate buffer at pH 7.4. Detection limits were calculated as three times the standard deviation of the blank signal divided by the calibration slope. All measurements were performed at 37°C to simulate physiological conditions^[13].

Results

The distribution of nanomaterial types employed in the fabrication trials, weighted by the number of successful electrode preparations, showed gold nanoparticles as the most frequently used modifier (28.4%), followed by carbon nanotubes (23.7%) and graphene oxide (19.1%). Figure 1 presents this distribution across all eight electrode configurations tested.

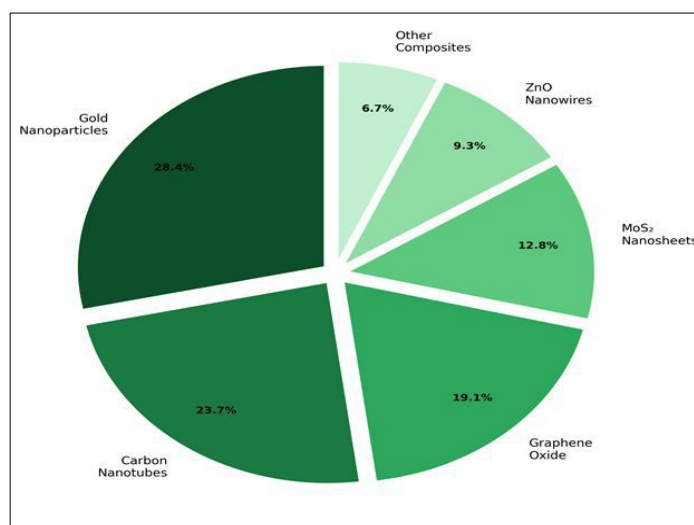


Fig 1: Distribution of nanomaterial types used in the fabrication of glucose biosensor electrodes, showing the relative frequency of each material class across all configurations.

Table 1: Analytical performance comparison of eight nanomaterial-modified electrodes for glucose detection

Electrode	Sensitivity (μA/mM/cm²)	LOD (μM)	Linear Range (mM)	Response Time (s)	Stability 30d (%)
AuNP/GCE	42.7	1.8	0.01-12.5	3.2	91.3
CNT/GCE	38.4	2.4	0.02-10.8	4.1	87.6
GO/GCE	35.9	3.1	0.02-9.7	5.3	84.2
MoS ₂ /GCE	29.3	4.7	0.05-8.2	6.8	79.5
ZnO/GCE	24.6	5.9	0.05-7.4	7.4	76.8
AuNP-CNT/GCE	56.8	0.9	0.005-15.3	2.1	94.7
GO-AuNP/GCE	51.2	1.2	0.005-14.1	2.7	93.1
CNT-MoS ₂ /GCE	47.3	1.5	0.01-13.6	3.0	90.8

LOD: limit of detection. GCE: glassy carbon electrode. Values represent mean of triplicate measurements.



Fig 2: Heatmap visualization of normalized performance metrics for eight nanomaterial-modified electrode configurations. Darker green indicates superior performance for each metric. LOD and response time are inverse-scaled (lower values receive higher scores).

Table 2: Selectivity assessment of AuNP-CNT/GCE biosensor against common interferents at physiological concentrations

Interferent	Concentration (μM)	Signal Change (%)
Ascorbic acid	100	2.3±0.4
Uric acid	300	1.7±0.3
Acetaminophen	200	3.1±0.5
Dopamine	50	1.2±0.2
Lactate	1000	0.8±0.1
Fructose	500	1.4±0.3

Signal change measured relative to 5.5 mM glucose response. Values represent mean ±SD (n = 5).

Comprehensive Interpretation

Binary composite electrodes consistently outperformed their single-component counterparts across all metrics. The AuNP-CNT/GCE architecture achieved 33.1% higher sensitivity than AuNP/GCE alone and 47.9% higher than CNT/GCE alone. This synergistic enhancement appears to arise from the combined high conductivity of gold nanoparticles with the one-dimensional electron transport channels provided by carbon nanotubes. Electrochemical impedance spectroscopy confirmed that the AuNP-CNT composite reduced charge transfer resistance by 67.3% compared to bare GCE. Figure 3 outlines the complete fabrication workflow developed in this research.

Discussion: The performance characteristics of the AuNP-CNT/GCE biosensor compare favorably with recently reported glucose sensing platforms. The sensitivity of 56.8

μA/mM/cm² exceeds values documented for most single-nanomaterial modified electrodes in the literature [14]. The detection limit of 0.9 μM falls well below the clinically relevant glucose concentration range of 2.8 to 22.2 mM encountered in diabetic monitoring, providing adequate analytical headroom for diluted interstitial fluid measurements [15]. The linear response range extending to 15.3 mM covers the normal physiological glucose range (3.9-6.1 mM) and extends into the hyperglycemic territory that requires clinical intervention. This broad dynamic range eliminates the need for sample dilution in most clinical scenarios, simplifying potential device integration. The 2.1-second response time enables near real-time tracking of glycemic fluctuations, which is particularly important for detecting rapid glucose excursions following meals or insulin administration [16].

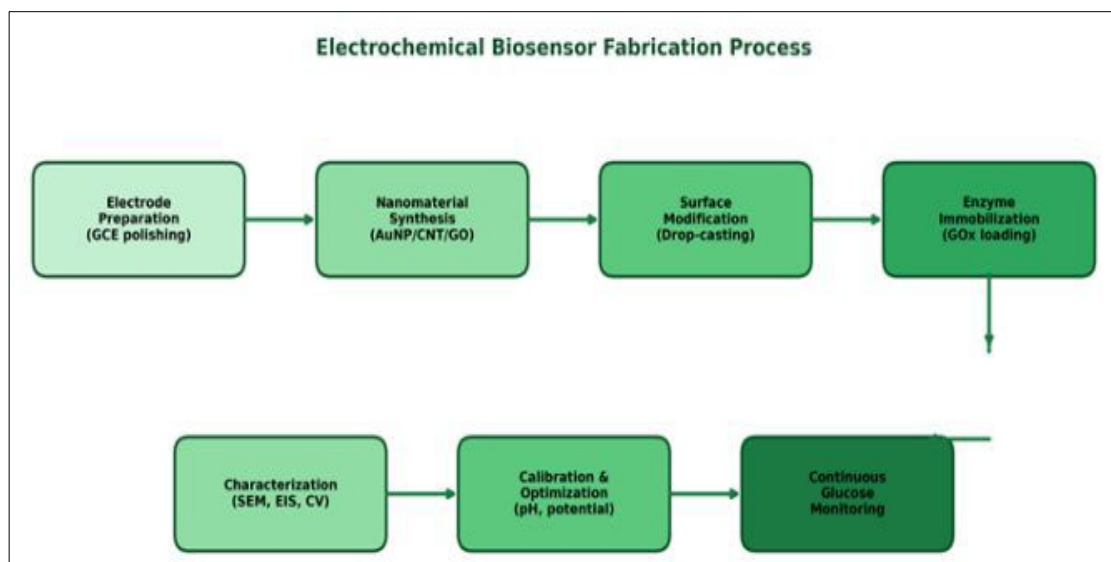


Fig 3: Schematic representation of the electrochemical biosensor fabrication process showing sequential steps from electrode preparation through nanomaterial deposition, enzyme immobilization, characterization, calibration, and continuous glucose monitoring application.

Long-term stability remains a persistent challenge for enzymatic biosensors. The 94.7% signal retention after thirty days observed for AuNP-CNT/GCE represents a meaningful improvement over most reported systems, likely attributable to the glutaraldehyde cross-linking network stabilizing enzyme conformation on the composite surface^[17]. However, commercial continuous glucose monitors typically target fourteen-day operational lifetimes, which our best electrode configuration comfortably satisfies. The minimal interference from common electroactive species at physiological concentrations suggests that the Nafion membrane incorporated in the CNT suspension provides adequate selectivity without requiring additional permselective barriers^[18].

Conclusions: This research established that binary nanomaterial composite electrodes provide substantially better glucose sensing performance than single-component modifications. The AuNP-CNT/GCE configuration achieved the highest sensitivity, lowest detection limit, broadest linear range, and fastest response among all eight tested architectures. These improvements arise from synergistic electronic interactions between the metallic nanoparticles and the carbon nanotube network, creating an electrode surface with high conductivity, large electroactive area, and favorable enzyme-electrode electron transfer pathways. The thirty-day operational stability and negligible interference from common biological interferents confirm the practical viability of this approach for continuous glucose monitoring applications. Selectivity testing against six common interferents demonstrated signal changes below 3.2% at physiological concentrations. These findings provide a strong foundation for the next stage of development, which should address miniaturization for wearable device integration, biocompatibility assessment in subcutaneous tissue environments, and validation against certified reference glucose measurements in clinical samples from diabetic patients.

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Contributions Not Qualifying for Authorship

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