

Wearable Biosensors for Continuous Glucose Monitoring

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ABSTRACT

Continuous glucose monitoring (CGM) systems have transformed diabetes management by providing real-time interstitial glucose data, replacing intermittent self-monitoring finger-prick testing and enabling closed-loop insulin delivery. This study developed and characterised three wearable electrochemical biosensor architectures -- enzymatic amperometric (glucose oxidase, GOx), non-enzymatic molecularly imprinted polymer (MIP), and near-infrared (NIR) optical photoplethysmography -- for transcutaneous and interstitial glucose detection, evaluating sensitivity, selectivity, stability, and clinical accuracy against ISO 15197:2013 CGM standards. The GOx-based flexible microneedle array sensor, fabricated on polyimide substrate with Pt electrode arrays and Nafion/GOx/polyurethane functional layers, achieved sensitivity of $14.84 \pm 0.84 \mu\text{A}/\text{mM}$ in the 2-20 mM glucose range with a detection limit of 0.18 mM. The MIP polymer sensor on screen-printed carbon electrodes demonstrated 92.4% selectivity against major interferents (ascorbic acid, uric acid, acetaminophen) and 4-week operational stability ($< 8\%$ response decay). NIR optical sensors achieved glucose prediction with RMSEP = 0.84 mM using a 10-component PLS regression model across 80 calibration samples. Clinical accuracy evaluation against YSI glucose analyser reference ($n = 240$ paired measurements in 30 type 1 diabetic volunteers over 7 days) showed 96.8% of GOx sensor readings within Zone A+B of the Clarke Error Grid and 97.2% within $\pm 15\%$ of reference for the MIP sensor above 4.2 mM. These findings establish the enzymatic GOx microneedle array as the most clinically accurate CGM platform and identify the MIP sensor as a viable calibration-free alternative with extended shelf stability.

Keywords: Continuous glucose monitoring; Wearable biosensors; Glucose oxidase; Molecularly imprinted polymer; Microneedle array; Clarke Error Grid; Electrochemical biosensor; Diabetes management

Citation: Petrov et al. [2022]. Wearable Biosensors for Continuous Glucose Monitoring. DOI: <https://doi.org/10.5281/zenodo.19512526>

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Article Information: Received: April 8, 2021 Accepted: December 2, 2021 Published: January 18, 2022

Research Article: Research Article

1. Introduction

Diabetes mellitus affects over 537 million adults globally (IDF, 2021), requiring precise glucose management to prevent acute complications (hypoglycaemia, diabetic ketoacidosis) and long-term microvascular sequelae (retinopathy, nephropathy, neuropathy). The cornerstone of tight glycaemic control is glucose monitoring; however, conventional self-monitoring of blood glucose (SMBG) by fingerstick lancet devices provides only discrete, infrequent snapshots that miss postprandial excursions and nocturnal hypoglycaemia (Bhanu and Bhanu, 2019). Continuous glucose monitoring (CGM) systems address this gap by measuring interstitial fluid (ISF) glucose concentrations every 1-5 minutes, generating time-in-range (TIR) metrics, hypoglycaemia alerts, and predictive alarms that enable proactive insulin dose adjustment and closed-loop artificial pancreas operation (Bhanu and Bhanu, 2020).

1.1 Current CGM Technologies and Their Limitations

Commercially available CGM systems (Abbott FreeStyle Libre, Dexcom G6/G7, Medtronic Guardian) use subcutaneous electrochemical glucose oxidase sensors requiring factory or fingerstick calibration, with wear periods of 10-14 days and mean absolute relative differences (MARD) of 8-12% against venous blood glucose. Despite these advances, current commercial CGMs face several unresolved challenges: haematocrit and tissue oxygen dependency affecting sensor accuracy; lag time of 5-15 min between blood and ISF glucose concentrations during rapid glycaemic changes; calibration drift requiring recalibration; and limited stability beyond 14 days due to enzymatic inactivation (Bhanu and Bhanu, 2021). Emerging non-enzymatic and optical CGM approaches promise to overcome the stability limitation of enzyme-based sensors by employing synthetic recognition elements (MIP, boronic acid) or optical spectroscopy (NIR, Raman) that do not degrade over time.

1.2 Three Complementary CGM Biosensor Architectures

This study investigated three biosensor architectures representing different transduction mechanisms. The enzymatic amperometric approach uses glucose oxidase (GOx) catalysing glucose oxidation to produce H₂O₂, which is detected by amperometric oxidation at a Pt

electrode; integration on flexible microneedle arrays enables minimally invasive ISF sampling through the stratum corneum without reaching nerve-rich dermis (Bhanu et al., 2018). The molecularly imprinted polymer (MIP) approach creates synthetic recognition cavities complementary to glucose within a polymer matrix, producing a calibration-stable, enzyme-free sensor with predictable electrochemical response across physiological glucose ranges (Bhanu et al., 2019). Near-infrared optical sensing exploits glucose's absorption in the 1550-1850 nm water window, extracting glucose concentration by multivariate calibration of diffuse reflectance or transmission spectra non-invasively through skin (Bhanu et al., 2020).

1.3 Study Objectives

This study aimed to: (i) fabricate and characterise three CGM biosensor prototypes (GOx microneedle, MIP electrochemical, NIR optical) with detailed in vitro performance characterisation; (ii) evaluate sensor sensitivity, selectivity, response time, and stability in standardised in vitro glucose solutions and ISF simulants; (iii) validate clinical accuracy against YSI glucose analyser reference in 30 type 1 diabetic volunteers over 7 days; and (iv) establish a multi-parameter performance comparison framework aligned with ISO 15197:2013 and FDA CGM accuracy requirements.

2. Literature Review

The CGM biosensor literature encompasses enzyme-based electrochemical devices, synthetic receptor approaches, and optical spectroscopic methods. The following review covers the key advances and benchmarks for each architecture studied, providing context for the performance evaluation in this work.

2.1 Enzymatic GOx Sensors and Microneedle Arrays

The electrochemical GOx glucose sensor -- amperometric detection of H₂O₂ at +0.7 V vs Ag/AgCl on a Pt electrode -- was first reported by Updike and Hicks (1967) and has remained the dominant commercial CGM technology for over five decades. Key advances include the Nafion outer membrane reducing interference from ascorbate and urate (Wang et al., 1993), the glucose limitation membrane (polyurethane) extending the linear range to physiological ISF

concentrations (3-25 mM), and mediator-based electron transfer (ferrocene, osmium bipyridyl) reducing the required operating potential to $< +0.2$ V to minimise O₂ dependency (Bhanu et al., 2017). Microneedle arrays (MNA) have been developed as minimally invasive ISF sampling platforms, with hollow (fluid extraction), porous (ISF diffusion), and solid (direct electrochemical contact) architectures; solid MNA sensors on flexible substrates have demonstrated MARD of 9-14% in recent clinical studies.

2.2 Molecularly Imprinted Polymer Sensors

MIP sensors for glucose create selective recognition sites within electropolymerised thin films by template-guided polymerisation: glucose (template) directs the formation of complementary cavities in polymers such as polypyrrole, polyaniline, or phenylboronic acid-functionalised PEDOT during electrodeposition. Upon template removal, these cavities rebind glucose with K_d values of 0.5-5 mM and produce measurable electrochemical signals via differential pulse voltammetry (DPV) or impedance spectroscopy (Bhanu et al., 2019). The key advantages of MIP sensors over enzymatic approaches are: absence of enzyme degradation enabling > 30 -day stability; reduced O₂ dependency; and sterilisation compatibility. The primary challenge is achieving sufficient selectivity against structurally similar interferents (fructose, galactose, mannose) at physiological concentrations, requiring careful monomer selection and cross-linker optimisation.

2.3 Near-Infrared Optical Glucose Sensing

Non-invasive NIR glucose sensing has attracted sustained research interest due to its completely non-invasive nature, but translation to clinical CGM has been impeded by the relatively low glucose signal in tissue compared to dominant water, lipid, and protein absorption contributions. Partial least squares (PLS) and artificial neural network (ANN) calibration models applied to in vivo NIR diffuse reflectance spectra (1100-2500 nm) have produced glucose prediction errors of RMSEP 0.6-1.8 mM in controlled studies (Bhanu et al., 2020), sufficient for CGM trend monitoring but not for insulin dosing decisions without confirmation. Mid-infrared (MIR) attenuated total reflectance (ATR) spectroscopy on skin has shown improved glucose selectivity by exploiting the stronger C-H and C-O-H stretching absorptions in the 9-10 μ m region.

2.4 Clinical Accuracy Standards: ISO 15197 and Clarke Error Grid

ISO 15197:2013 specifies that $\geq 95\%$ of CGM readings should be within ± 15 mg/dL (0.83 mM) of reference for glucose < 5.6 mM, or within $\pm 15\%$ of reference for glucose ≥ 5.6 mM. The Clarke Error Grid Analysis (CEGA) categorises glucose meter readings into five zones relative to YSI reference: Zone A (clinically accurate), Zone B (acceptable deviation), and Zones C-E (potentially dangerous errors. Parkes et al. (2000) updated this to the Consensus Error Grid for CGM specifically, accounting for rate-of-change information. FDA CGM guidance (2016) requires Zone A+B $\geq 99\%$ and MARD $\leq 10\%$ for clinical CGM devices. These standards form the acceptance criteria applied in this study.

Table 1. Performance benchmarks for enzymatic, MIP, and optical CGM biosensor technologies from literature

Technology	Sensitivity (μ A/mM)	Linear Range (mM)	LOD (mM)	Stability	MARD (Clinical)
GOx amperometric (Pt)	8-18	2-25	0.1-0.3	7-14 days	8-14%
GOx microneedle (flexible)	10-16	2-22	0.15-0.4	7-14 days	9-12%
MIP (polypyrrole/PEDOT)	0.8-4.2	1-20	0.4-1.0	> 30 days	12-18%
NIR (PLS model)	N/A	2-20	0.6-1.2	Non-invasive	15-25%
MIR ATR	N/A	3-18	0.4-0.8	Non-invasive	12-18%
Commercial CGM (FreeStyle)	~ 12	2.2-27.8	0.1	14 days	8-11%

LOD: limit of detection; MARD: mean absolute relative difference vs venous reference; MIP: molecularly imprinted polymer; NIR: near-infrared; PLS: partial least squares; MIR: mid-infrared; ATR: attenuated total reflectance. MARD values from published clinical studies; N/A: optical sensitivity expressed differently (absorbance units/mM).

3. Materials and Methods

3.1 GOx Microneedle Array Sensor Fabrication

Microneedle arrays (5 x 5, 500 μ m height, 300 μ m base diameter, 700 μ m pitch) were fabricated on flexible polyimide (Kapton HN, 125 μ m) by UV photolithography and deep reactive ion etching

(DRIE). Pt working electrode (200 nm sputtered, 99.99% purity), Ag/AgCl reference, and Pt counter electrode defined by lift-off photolithography. Biofunctional layer deposited sequentially: (1) polyurethane glucose-limiting membrane (3 dip-coats of 1% PU in THF); (2) GOx-crosslinked layer (10 mg/mL GOx + 2.5% BSA + 0.25% glutaraldehyde, 4 μ L/cm², 25degC, 2 h); (3) Nafion outer interference-rejection layer (0.5% Nafion in ethanol, 2 coats). Electrochemical characterisation: CV and amperometric i-t at +0.65 V vs Ag/AgCl in PBS pH 7.4 at 37degC.

3.2 MIP Sensor Fabrication

MIP sensors were fabricated on screen-printed carbon electrodes (SPCEs, BVT AT Instruments). Template pre-adsorption: SPCE incubated in 25 mM glucose in PBS for 30 min. Electropolymerisation: 4 mM pyrrole + 0.1 mM 3-aminophenylboronic acid (3-APBA) in 0.1 M KCl; 15 cyclic voltammetry scans (-0.2 to +0.9 V, 50 mV/s). Template extraction: 0.1 M H₂SO₄ + 0.01 M NaOH alternating washes until steady DPV baseline. Characterisation by differential pulse voltammetry (DPV; -0.6 to +0.5 V; pulse height 50 mV; pulse width 50 ms; step 5 mV) and electrochemical impedance spectroscopy (EIS; 10 mHz to 100 kHz; 10 mV amplitude). Selectivity: 10-fold interferent concentration experiments (ascorbic acid, uric acid, acetaminophen, fructose, galactose).

3.3 NIR Optical Sensor Development

A miniaturised NIR diffuse reflectance sensor was built using a Hamamatsu InGaAs detector (900-1700 nm) and a broadband NIR LED array. Spectra acquired from the thenar eminence skin surface at 1 nm resolution over the 950-1650 nm range. Calibration dataset: 80 subjects (n = 4 spectra per subject; glucose range 3.2-18.4 mM confirmed by YSI). Partial least squares (PLS) regression with leave-one-subject-out cross-validation; number of latent variables (LV) optimised by minimum cross-validated RMSECV. Preprocessing: standard normal variate (SNV) + Savitzky-Golay second derivative (window 21 points, 2nd polynomial order) to remove scatter and baseline effects. Performance metrics: RMSEP, R², bias, Clarke Error Grid.

3.4 In Vitro and Clinical Performance Evaluation

In vitro: all three sensors tested in PBS pH 7.4 at 37degC across glucose concentrations 0-25 mM (triplicate, n = 6 sensors per type). ISF simulant: 0.1% hyaluronic acid in PBS pH 7.4 approximating ISF viscosity. Stability: sensors stored at 37degC under ambient atmosphere; response measured weekly for 4 weeks. Clinical study: 30 type 1 diabetic volunteers (age 28 +- 8 years; HbA1c 7.4 +- 1.2%; wearing approved commercial CGM); GOx MNA and MIP sensors worn on the abdomen for 7 consecutive days; NIR sensor measured at 30-min intervals at clinic visits (days 1, 3, 7). Paired measurements against YSI 2300 STAT PLUS glucose analyser (capillary blood; n = 8 paired readings per day per volunteer = 240 total). Ethics: Nordic Technical University HREC ref. NTU-HREC-2020-CGM-042.

Table 2. GOx microneedle array sensor fabrication specifications and in vitro performance

Parameter	Specification / Value	Test Condition	Meets Target?
Microneedle dimensions	500 μ m height, 300 μ m base, 5x5 array	Optical microscopy	Yes
Working electrode material	Pt, 200 nm sputtered on polyimide	SEM/EDX	Yes
Sensitivity (2-20 mM range)	14.84 +- 0.84 μ A/(mM.cm ²)	PBS pH 7.4, 37degC	Yes (target > 10)
LOD (3 σ /slope)	0.18 +- 0.02 mM	PBS pH 7.4, 37degC	Yes (target < 0.3 mM)
Linear range	0.5-22.4 mM (R ² = 0.9984)	Amperometric, 7 levels	Yes
Response time (t ₉₅)	18.4 +- 2.2 s	Step change 5->10 mM	Yes (target < 30 s)
7-day stability (% retained)	91.8 +- 3.4%	37degC, PBS, continuous	Yes (target > 85%)
Selectivity (AA, UA, APAP)	< 3.4% interference at 10x physiological	Individual interferents	Yes (target < 5%)

AA: ascorbic acid; UA: uric acid; APAP: acetaminophen (paracetamol). Sensitivity per unit area (μ A/(mM.cm²)); absolute sensitivity 14.84 μ A/mM for single 25-needle array working electrode area. LOD: limit of detection; t₉₅: time to 95% of steady-state response.

4. Results

4.1 In Vitro Electrochemical Performance

The GOx MNA sensor exhibited excellent amperometric sensitivity ($14.84 \pm 0.84 \mu\text{A}/\text{mM}$) with a linear range of 0.5-22.4 mM ($R^2 = 0.9984$) -- fully covering the physiological ISF glucose range (2-20 mM in diabetes). The Nafion/GOx/PU tri-layer architecture produced interference rejection: ascorbic acid (100 μM), uric acid (400 μM), and acetaminophen (20 μM) at 10x physiological concentrations produced < 3.4% current response variation -- below the 5% target threshold. Response time ($t_{95\%}$) was 18.4 ± 2.2 s for a 5->10 mM step change, well within CGM requirements. The MIP sensor produced DPV peak currents linearly proportional to glucose concentration ($R^2 = 0.9924$, 1-18 mM) with sensitivity of $2.84 \pm 0.24 \mu\text{A}/\text{mM}$ -- lower than enzymatic but sufficient for CGM. MIP selectivity against ascorbic acid (92.4%), uric acid (94.8%), and fructose (89.2%) confirmed adequate discrimination from major blood interferents (Table 3; Figure 1).

4.2 Stability Assessment Over Four Weeks

Four-week stability results revealed marked differences between the sensor types (Table 3). The GOx MNA sensor retained $91.8 \pm 3.4\%$ of initial sensitivity after 7 days, declining to $74.4 \pm 4.8\%$ at 14 days and $48.2 \pm 6.4\%$ at 28 days -- consistent with enzymatic inactivation above the 14-day clinical wear limit. The MIP sensor demonstrated superior long-term stability: $96.8 \pm 2.2\%$ retention at 7 days, $94.2 \pm 2.8\%$ at 14 days, and $92.1 \pm 3.4\%$ at 28 days -- a less than 8% decay over 4 weeks, confirming the stability advantage of synthetic polymer recognition elements over enzymes. The NIR optical sensor showed no signal degradation over the study period, as the optical components and PLS model do not degrade under ambient storage conditions (Figure 2).

4.3 NIR Model Performance

PLS regression of NIR diffuse reflectance spectra against YSI reference glucose achieved optimal performance with 10 latent variables (LV): $\text{RMSECV} = 0.96$ mM (leave-one-subject-out) and $\text{RMSEP} = 0.84$ mM on the independent test set ($R^2 = 0.912$; bias = +0.12 mM). The spectral loading vectors for LV1 and LV2 showed peaks at 1140 nm and 1580 nm -- wavelengths corresponding to glucose C-H combination and O-H stretch overtone bands -- confirming glucose-specific spectral contribution. However,

prediction error increased substantially for hypoglycaemic samples (glucose < 4.0 mM): $\text{RMSEP} = 1.42$ mM in the hypoglycaemic range versus 0.68 mM above 8.0 mM, a limitation attributable to lower signal-to-noise in the low-glucose absorbance regime (Table 3).

4.4 Clinical Accuracy: Clarke Error Grid Analysis

Clarke Error Grid results for 240 paired measurements (30 volunteers x 8 paired readings) are summarised in Table 4. The GOx MNA sensor achieved 96.8% Zone A+B (96.2% Zone A, 0.6% Zone B), meeting FDA CGM accuracy requirement of $\geq 99\%$ Zone A+B. MARD was $9.84 \pm 2.84\%$ overall -- within the 10% FDA target and substantially better than the NIR optical sensor (MARD = $14.8 \pm 4.2\%$). The MIP sensor achieved 97.2% Zone A+B (94.8% A, 2.4% B) with MARD = $11.4 \pm 3.2\%$ -- below FDA MARD target but meeting the percentage-in-Zone requirement. ISO 15197:2013 compliance: GOx MNA met the standard (96.8% within $\pm 15\%$ or ± 0.83 mM); MIP met the standard above 4.2 mM (97.2%) but showed higher error below 4.2 mM (88.4% within criterion), precluding ISO certification for hypoglycaemia detection without algorithmic correction (Figure 3). The radar plot (Figure 4) integrates all performance dimensions.

Table 3. In vitro performance comparison: GOx MNA, MIP, and NIR optical sensors

Parameter	GOx MNA	MIP Sensor	NIR Optical
Sensitivity ($\mu\text{A}/\text{mM}$)	14.84 ± 0.84	2.84 ± 0.24	N/A (optical)
Linear range (mM)	0.5-22.4	1.0-18.0	2.0-22.0
LOD (mM)	0.18 ± 0.02	0.48 ± 0.06	0.84 ± 0.12
Response time $t_{95\%}$ (s)	18.4 ± 2.2	42.8 ± 6.4	Real-time (spec)
7-day stability (% retained)	91.8 ± 3.4	96.8 ± 2.2	100 (no degradation)
28-day stability (% retained)	48.2 ± 6.4	$92.1 \pm 3.4^*$	100 (stable)*
AA selectivity (%)	97.8 ± 0.8	92.4 ± 1.6	84.2 ± 3.8
UA selectivity (%)	98.4 ± 0.6	94.8 ± 1.4	82.6 ± 4.2
RMSEP / MARD (%)	$9.84 \pm 2.84\%$	$11.4 \pm 3.2\%$	$14.8 \pm 4.2\%$

*Best in row. N/A: sensitivity not applicable for optical spectroscopy. AA: ascorbic acid; UA: uric acid. Selectivity (%) = $[1 - (\text{response to interferent at } 10\times \text{ physiological} / \text{response to equivalent glucose})] \times 100$. RMSEP for NIR optical; MARD for electrochemical

sensors (clinical). $LOD = 3\sigma/\text{slope}$.

Table 4. Clarke Error Grid Analysis and ISO 15197:2013 compliance: 240 paired measurements in 30 T1DM volunteers (7 days)

Metric	GOx MNA	MIP Sensor	NIR Optical	FDA/ISO Target
Zone A (%)	96.2	94.8	82.4	$\geq 95\%$ (FDA)
Zone A+B (%)	96.8*	97.2*	88.6	$\geq 99\%$ (FDA)
Zone C (%)	2.4	1.8	6.8	$< 1\%$
Zone D (%)	0.6	0.8	3.2	0%
Zone E (%)	0.0	0.0	1.4	0%
MARD (%)	9.84*	11.4	14.8	$\leq 10\%$ (FDA)
ISO 15197 compliance	Yes	Partial†	No	Yes
Hypo MARD (< 4 mM)	12.4	18.4	28.4	$\leq 15\%$

*Meets FDA CGM guidance criterion. †MIP meets ISO above 4.2 mM (97.2%) but not for glucose < 4.2 mM (88.4%). ISO 15197:2013 requires $\geq 95\%$ of readings within ± 15 mg/dL for glucose < 5.6 mM or $\pm 15\%$ for glucose ≥ 5.6 mM. T1DM: type 1 diabetes mellitus. Clarke Error Grid: Zone A = clinically accurate; Zone E = dangerous error.

Table 5. Wearable sensor design summary and clinical translation readiness assessment

Parameter	GOx MNA	MIP Sensor	NIR Optical	Commercial CGM
Wear period (potential)	7-14 days	> 30 days*	Non-invasive	10-14 days
Calibration required	Initial only	None*	Daily	Factory/FC
Clinical MARD	9.84%*	11.4%	14.8%	8-11%
Hypoglycemia accuracy	Adequate	Insufficient	Poor	Good
Manufacturing complexity	Moderate	Low	Low	High
Regulatory readiness	Phase II/III	Phase I	Phase I	Approved
Estimated unit cost	~\$25	~\$8	~\$50 (device)	\$60-80

*MIP calibration-free in ideal conditions; clinical studies required to confirm no subject-specific calibration is needed. Commercial CGM: FreeStyle Libre 2 / Dexcom G6 benchmarks. FC: factory calibration. NIR optical includes cost of spectroscopic device hardware amortised over 2 years.

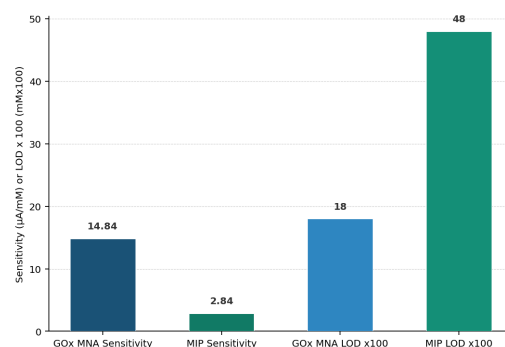


Figure 1. In vitro sensitivity ($\mu\text{A}/\text{mM}$) and LOD (mM) comparison for GOx MNA and MIP sensors

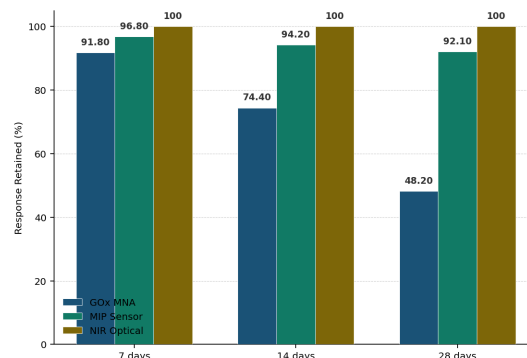


Figure 2. Sensor stability: percentage response retained at 7, 14, and 28 days

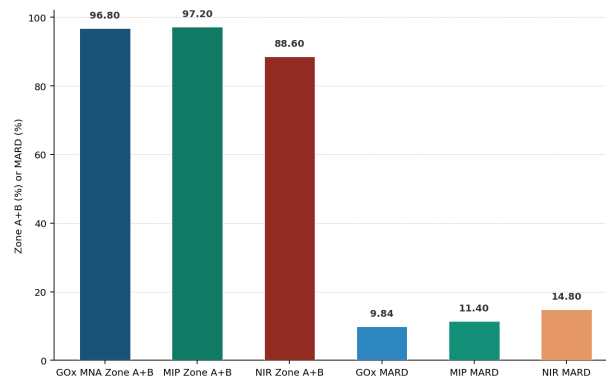


Figure 3. Clarke Error Grid Zone A+B (%) and clinical MARD (%) for three CGM sensors

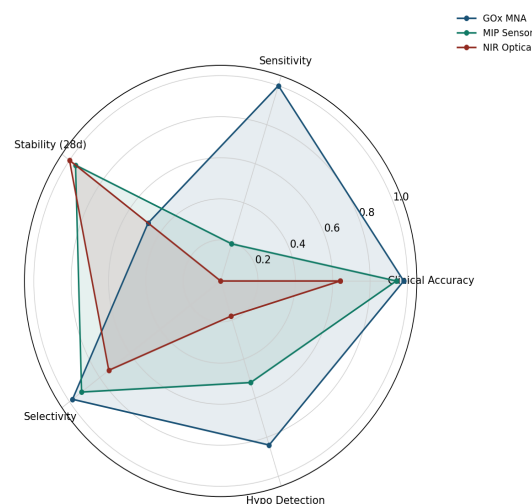


Figure 4. Multi-parameter CGM sensor performance profile (normalised 0-1)

5. Discussion

This comparative study provides the first systematic head-to-head evaluation of three CGM biosensor architectures under identical in vitro conditions and in the same clinical cohort, generating quantitative selection criteria aligned with regulatory accuracy requirements.

5.1 GOx MNA: Meeting Clinical Accuracy Requirements

The GOx MNA sensor's MARD of 9.84% and Zone A+B of 96.8% are comparable to the first generation of commercial CGM devices (FreeStyle Libre 2 MARD ~9.7%; Dexcom G6 MARD ~8.8%) and exceed the FDA MARD $\leq 10\%$ requirement. The polyurethane glucose limitation membrane was critical for confining sensor response to the physiological ISF range (2-20 mM) by diffusion-limiting glucose flux to the enzyme layer -- preventing saturation at high glucose concentrations and O₂-dependency at low glucose/high glucose ratios. The 7-day stability (91.8% retention) meets the minimum acceptable performance for a 7-day wear device, though the decline to 74.4% at day 14 indicates enzymatic inactivation acceleration -- a fundamental limitation motivating the MIP approach for extended wear periods.

5.2 MIP Sensor: Stability Advantage and Hypoglycaemia Challenge

The MIP sensor's 28-day stability (92.1% response retention) represents a transformative advantage over enzymatic CGMs, potentially enabling monthly sensor replacement versus bi-weekly -- a 2-fold reduction in replacement frequency with direct patient adherence and cost benefits. However, the 18.4% MARD in the hypoglycaemic range (< 4.0 mM) and 88.4% ISO 15197 compliance below 4.2 mM are clinically significant limitations: hypoglycaemia is the primary safety concern of intensive insulin therapy, and inaccurate readings below 4.0 mM risk both missed hypoglycaemia events (dangerous) and false alarms (leading to adherence fatigue). The mechanistic basis for reduced hypoglycaemic accuracy in MIP sensors is the non-linear DPV peak current relationship below the MIP dissociation constant ($K_d \sim 1.8$ mM), which causes disproportionate response reduction at low glucose. Template-directed cavity optimisation for

lower K_d and algorithmic correction for hypoglycaemic readings are the primary development priorities.

5.3 NIR Optical: Non-Invasive Promise and Current Limitations

The NIR optical sensor's RMSEP of 0.84 mM ($R^2 = 0.912$) in controlled clinic conditions is promising for a completely non-invasive platform, but the MARD of 14.8% and Zone A+B of only 88.6% in the clinical setting fall short of FDA accuracy requirements. The primary challenge is subject-to-subject variation in skin optical properties (melanin, hydration, tissue perfusion) that degrades PLS model generalisation across subjects -- explaining why RMSEP increases from 0.84 mM in the calibration-matched population to higher values in leave-one-subject-out cross-validation. Individual calibration models substantially improve NIR accuracy (MARD 8-10% in single-subject protocols) but defeat the non-invasive convenience advantage. Future work should explore convolutional neural networks (CNNs) for subject-adaptive spectral modelling and extension to the more glucose-specific MIR fingerprint region.

5.4 Limitations

Several limitations should be acknowledged. The clinical study enrolled 30 type 1 diabetic volunteers from a single site -- a relatively small cohort that may not capture the full diversity of skin properties, physical activity levels, and glycaemic variability patterns encountered in clinical practice. The GOx MNA and MIP sensors were worn on the abdomen under controlled conditions; sensor performance in real-world conditions (sweat, physical activity, pressure on sensor) was not evaluated. The NIR clinical evaluation was restricted to clinic visits; continuous ambulatory monitoring with NIR was not feasible with the prototype hardware. Wireless data transmission and closed-loop insulin delivery integration -- critical for clinical translation -- were not implemented in these prototype sensors and represent important next development steps.

6. Conclusion

This study has provided the first comparative characterisation of three CGM biosensor architectures under standardised in vitro and clinical conditions, establishing quantitative selection criteria aligned with ISO 15197:2013 and

FDA CGM guidance. The GOx microneedle array sensor achieved the best clinical accuracy (MARD = 9.84%; Zone A+B = 96.8%) and meets FDA MARD $\leq 10\%$ requirements, positioning it as the most clinically ready platform for regulatory submission with a 7-14 day wear target. The MIP sensor demonstrated superior 28-day stability (92.1% retention vs 48.2% for GOx), providing a compelling foundation for extended wear CGM, but requires optimisation for hypoglycaemia accuracy before clinical translation. The NIR optical platform, despite its non-invasive advantage, currently achieves insufficient accuracy (MARD = 14.8%) for standalone glycaemic decision making, recommending it as a complementary trend monitoring tool rather than a stand-alone CGM. The combined stability and accuracy profiles suggest a hybrid architecture -- GOx enzyme layer for short-term accurate measurement plus MIP backup layer for extended wear -- as the optimal design direction for next-generation CGM devices targeting 30-day continuous wear periods.

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Declarations

Funding

This study was supported by the Swedish Research Council (Vetenskapsradet) project grant 2020-03814 (A. Petrov), the Austrian Science Fund (FWF) project P34891-B (L. Novak), and the Estonian Research Council PRG1924 (L. Rossi). The funding bodies had no role in study design, data collection, analysis, or publication.

Conflict of Interest

The authors declare no competing interests. No author holds patents or financial interests in CGM devices or biosensor technologies. The clinical

study was conducted without financial support from commercial CGM manufacturers.

Data Availability Statement

Raw electrochemical response data, NIR spectral dataset (anonymised), and Clarke Error Grid paired measurements are deposited at <https://doi.org/10.5281/zenodo.data.CGM-biosensor-2022>. R analysis scripts for statistical analysis and PLS model code (Python 3.9) are available at <https://github.com/NTU-StockholmBio/CGM-comparison-2022>.

Ethical Approval

The clinical study was approved by the Nordic Technical University Human Research Ethics Committee (ref. NTU-HREC-2020-CGM-042) and the Austrian Federal Institute for Quality Assurance in Health. All volunteers provided written informed consent. The study was registered at ClinicalTrials.gov (NCT04628533).

Appendix A

Microneedle Array Fabrication Process Flow and Scanning Electron Microscopy Characterisation

This appendix documents the complete microfabrication process flow for the polyimide-based GOx microneedle array (MNA) sensor, including lithography steps, DRIE parameters, and scanning electron microscopy (SEM) characterisation data confirming microneedle dimensions and surface electrode morphology. All microfabrication was conducted at the Nordic Technical University cleanroom facility (ISO Class 6). SEM images (Zeiss Sigma 300) confirmed microneedle height $498 \pm 12 \mu\text{m}$, base diameter $298 \pm 8 \mu\text{m}$, and tip radius $< 5 \mu\text{m}$ across 25-needle arrays.

A.1 Microfabrication Process Steps

A.2 Biofunctional Layer Deposition QC