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Development and Preliminary Analytical Evaluation of a ZIF-8/rGO/MWCNTs-Modified Electrochemical Sensor for Metformin Determination in Human Serum and Exploratory Correlation in Women with Polycystic Ovary Syndrome

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Abstract

Metformin is an approved treatment for polycystic ovary syndrome, particularly in patients with insulin resistance. This study developed a modified glassy carbon electrode (ZIF-8/rGO/MWCNT/GCE) via drop-casting for the electrochemical detection of metformin in serum samples from women with PCOS. Under optimized conditions—including a pH 5.5 phosphate-buffered solution, a 2:1:1 nanocomposite ratio, a 40-minute drying time, and a 100 mV/s scan rate—the sensor exhibited a linear response from 2 to 28 µM (R = 0.99671), with a limit of detection of 0.14 µM and a limit of quantification of 0.42 µM. High recovery rates (95.65%–99.68%) confirmed the accuracy and suitability of the method for serum analysis. When applied to serum samples from treated PCOS patients, the sensor successfully measured variable metformin concentrations; however, exploratory correlation analysis revealed no direct associations between these serum levels and the evaluated biochemical or hormonal clinical indicators.

Keywords: Metformin; ZIF8/ rGO/MWCNTs, polycystic ovary, Electrochemical sensor

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age, with a global prevalence ranging between 10% and 13% (1). It manifests in many different ways, including reproductive, endocrine, and metabolic abnormalities, such as menstrual dysfunction, hyperandrogenism, and polycystic ovarian morphology (2). PCOS is associated not only with reproductive complications but also with

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insulin resistance (IR), obesity, dyslipidemia, and an increased risk of developing type 2 diabetes mellitus and cardiovascular disease (3). These metabolic disturbances significantly contribute to disease progression and have deleterious effects on fertility and long-term health outcomes.

Insulin resistance is a major pathogenetic trait of PCOS and an important contributor to metabolic and hormonal abnormalities (4-6). Hyperinsulinemia leads to increased androgen production, disturbances in normal follicular development, and ovulation-related disorders (7-8). Consequently, therapeutic approaches that enhance insulin sensitivity have emerged as the cornerstone of PCOS management (4). Among the currently approved therapeutic agents, metformin has been used for its ability to decrease hepatic glucose production, enhance peripheral insulin sensitivity, and relieve a range of metabolic and reproduction-related symptoms associated with PCOS (9-10). Other studies have also reported improvements in hormonal balance after metformin administration, including reduced luteinizing hormone levels and enhanced ovarian function (11-12). Beneficial effects on prolactin levels, body mass index, and other metabolic parameters have been documented (13-16). Despite its widespread clinical use, substantial interindividual variability exists in the pharmacokinetics and therapeutic responses of metformin (17). Figure 1 shows the chemical structure of the metformin hydrochloride.

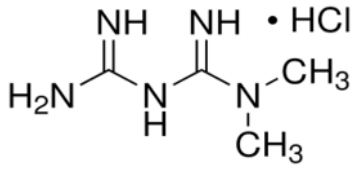


Figure. 1: Chemical structure of metformin hydrochloride.

Differences in renal function, patient characteristics, and drug transport mechanisms may significantly influence circulating drug concentrations and therapeutic outcomes in different patient populations (18-20). Accurate determination of metformin levels in biological fluids is of paramount importance for assessing treatment efficacy, optimizing dosage regimens, and minimizing the risk of adverse effects. Therefore, therapeutic drug monitoring (TDM) is a beneficial option for evaluating drug exposure and guiding individualized therapy (21).

Numerous analytical procedures have been proposed to determine metformin in drugs and biological samples, including spectrophotometric, spectrofluorimetric, chromatographic, electrophoretic, and electrochemical methods. (22-24). Among low-cost analytical approaches, charge-transfer spectrophotometry has recently been applied for the determination of metformin hydrochloride in pure form and pharmaceutical preparations, supporting the continued value of simple spectroscopic strategies for metformin assays outside complex biological monitoring applications (25). Related design-assisted charge-transfer spectrophotometric studies on basic pharmaceutical compounds have also demonstrated the usefulness of systematic optimization and validation in developing low-cost analytical methods (26). The determination of drugs using chromatographic techniques (e.g., HPLC and LC-MS/MS) provides good sensitivity and selectivity but usually entails high-cost instruments, long sample preparation times, and the need for trained personnel (27-28). Although these techniques are valuable reference methods, their routine use for rapid clinical monitoring is often constrained.

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Owing to their simplicity, rapid response, portability, low cost, and high sensitivity, electrochemical sensors have been widely studied as alternative analytical platforms (29). Recent advances in nanomaterials have further improved the analytical performance of electrochemical sensors by improving the electron-transfer kinetics, surface area, and analyte adsorption capacity. Zeolitic imidazolate framework-8 (ZIF-8) is one such material, exhibiting high porosity and adsorption capacity, whereas reduced graphene oxide (rGO) and multi-walled carbon nanotubes (MWCNTs) exhibit excellent electrical conductivity and facilitate rapid electron transfer (30). Combining these materials in a single sensing system is anticipated to produce synergistic effects that enhance the analytical sensitivity and overall sensor performance (31).

Despite extensive efforts to develop metformin analytical techniques, most studies have focused on analytical performance characteristics rather than clinical relevance. (32) Studies assessing serum metformin concentrations and their associations with biochemical and hormonal parameters in women with PCOS are still scarce (33). Therefore, the purpose of the present study was to develop a ZIF-8/rGO/MWCNT-modified electrochemical sensor for the sensitive detection of metformin in human serum and to examine the relationship between serum metformin concentration and specific clinical, biochemical, and hormonal parameters in women with PCOS. The proposed sensor may offer a convenient analytical tool for measuring serum metformin levels; however, its clinical applicability for therapeutic monitoring needs to be validated under controlled sampling and dosing conditions.

Materials and Methods

Chemicals and reagents

The ZIF-8 powder was obtained from fluorochem. Reduced graphene oxide (rGO), multi-walled carbon nanotubes (MWCNTs), dimethylformamide (DMF, 99%), methanol (99%), and triethylamine (TEA, 99%) were purchased from Sigma-Aldrich (Darmstadt, Germany). A standard metformin solution was prepared in deionized water. Ethanol, deionized water, and phosphate buffer solution (PBS) were used to prepare nanomaterial suspensions and for electrochemical measurements. HPLC-grade acetonitrile was used for protein precipitation and metformin extraction from the human serum. Commercial diagnostic kits for glucose, total cholesterol, triglycerides, and HDL-C were obtained from Biolabo (France), and LH, FSH, and prolactin ELISA kits were obtained from Monobind Inc. (USA).

Apparatus and electrochemical measurements

Electrochemical measurements were performed using a Corrtest electrochemical workstation controlled by CS Studio software. All experiments were conducted in a conventional three-electrode cell with a glassy carbon electrode (GCE) as the working electrode, Ag/AgCl/KCl electrode as the reference electrode, and platinum wire as the counter electrode. The modified electrode prepared with ZIF-8/rGO/MWCNTs was used as the working electrode for the determination of metformin.

Cyclic voltammetry (CV) was performed for the electrochemical characterization of the bare and modified electrodes, and differential pulse voltammetry (DPV) was used for the

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quantitative determination of metformin. DPV measurements were performed under the optimized experimental conditions, and the peak current obtained was used for calibration curve construction and for the quantitative determination of metformin in extracted serum samples.

The main DPV instrumental parameters used for the metformin determination are summarized in Table 1. The same DPV settings were applied to the metformin standard solutions, spiked serum samples, and extracted serum samples.

Table .1: DPV instrumental parameters used for the metformin determination.

Parameter	Value
Initial E (V)	−0.5
Final E (V)	0.59
Low E (V)	−0.4
Quiet time (s)	10
Scan rate (mV/s)	100
pulse amplitude (mV)	50
pulse width (ms)	50
step potential (mV)	5
sampling width/time(ms)	20

Preparation of the ZIF-8/rGO/MWCNTs-modified GCE

Prior to modification, the glassy carbon electrode (GCE) surface was polished to obtain a clean and smooth surface. The polishing procedure is shown in Figure 2. ZIF-8, rGO, and MWCNTs suspensions were prepared separately in aqueous/ethanolic media and sonicated to obtain homogeneous dispersions. Optimized composite suspensions were prepared using a 2:1:1 mixture of ZIF-8, rGO, and MWCNTs and sonicated for 30 min to obtain a uniform hybrid suspension. Subsequently, 6 µL of the ZIF-8/rGO/MWCNT composite suspension was drop-cast onto the polished GCE surface and dried at room temperature for 40 min. The prepared electrode was designated as ZIF-8/rGO/MWCNTs/GCE.

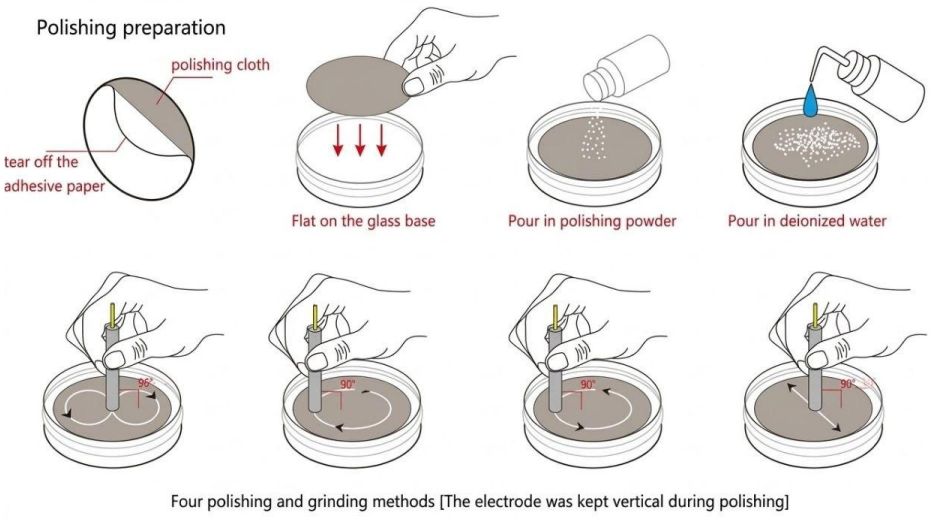


Figure. 2: Polishing procedure for the glassy carbon electrode (GCE).

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Optimization of experimental conditions

The experimental conditions for the electrochemical response of metformin were optimized prior to the analytical application. The evaluated parameters were the pH of the supporting electrolyte, ZIF-8/rGO/MWCNTs modifier composition and ratio, electrode drying time, and scan rate. Under the optimized conditions, the supporting electrolyte was a phosphate buffer solution with a pH of 5.5. The concentrations of ZIF-8, rGO, and MWCNTs were set at 1.0, 0.5, and 0.5 mg/mL, respectively, with an optimized ZIF-8:rGO:MWCNTs ratio of 2:1:1. In addition, the modified electrode was dried at room temperature for 40 min before use, and the scan rate was set to 100 mV/s for electrochemical measurements.

Serum sample collection and study design

This study included 120 blood samples. Seventy samples were obtained from women diagnosed with polycystic ovary syndrome (PCOS) according to the Rotterdam criteria. The patients were divided into two groups: 35 women receiving metformin therapy and 35 women not receiving pharmacotherapy. In addition, 50 apparently healthy women were recruited as the control group. All participants were aged 18–45 years. Samples were obtained from external laboratories in Salah Al-Din Governorate between November 1, 2025, and February 28, 2026. The study protocol was approved by the Research Ethics Committee of the University of Samarra, College of Education for Pure Sciences, Department of Chemistry (approval dated October 15, 2025), and all participants provided written informed consent before sample collection.

Serum preparation and metformin extraction

Venous blood samples were collected in gel tubes and allowed to clot at room temperature. The samples were then centrifuged at 3000 rpm for 10 min, and the separated serum was placed in Eppendorf tubes and stored at −20 °C until analysis. For metformin extraction, 100 µL of serum was added to 300 µL of acetonitrile, vortexed for 30 s, and centrifuged at 10,000 rpm for 10 min at 4 °C. Prior to the DPV measurement, 100 µL of the clear supernatant was diluted to 1.0 mL with PBS (pH 5.5). Therefore, no net dilution factor was applied. Metformin concentration was calculated directly from the aqueous external calibration equation and expressed as serum metformin concentration.

Biochemical and hormonal measurements

Serum glucose, total cholesterol, triglyceride, and HDL-C levels were determined using enzymatic colorimetric methods according to the manufacturer's instructions. The LDL-C and VLDL-C concentrations were calculated using standard equations. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin levels were measured using enzyme-linked immunosorbent assay (ELISA) kits. The body mass index (BMI) was calculated for all participants based on body weight and height measurements.

Analytical performance evaluation

The analytical performance of the ZIF-8/rGO/MWCNT-GCE sensor was evaluated under optimized experimental conditions. Linearity was assessed using metformin standard solutions over the 2–28 µM concentration range. A calibration curve of peak current versus

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metformin concentration was constructed, and the slope, intercept, and correlation coefficient were determined using regression analyses. The LOD and LOQ were calculated using a regression-based method according to the equations LOD = 3.3 × Sa/b and LOQ = 10 × Sa/b, where Sa is the standard error of the regression and b is the slope of the calibration curve. Accuracy and recovery were assessed by analyzing serum spiked with known concentrations of metformin at low, medium, and high levels. Freshly spiked serum samples were extracted using the same acetonitrile extraction procedure applied to real serum. The analytical performance was evaluated using the recovery, relative error, and relative standard deviation. Repeatability was examined by repeated measurements of metformin using the same modified electrode.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Differences among the study groups were evaluated using one-way analysis of variance (ANOVA), followed by post-hoc multiple-comparison testing. Pearson's correlation analysis was used to assess the relationships among the selected biochemical and hormonal parameters. Spearman's correlation analysis was used to explore the relationship between serum metformin concentration and selected clinical parameters. Only quantifiable samples with metformin concentrations equal to or higher than the LOQ were included in the correlation analysis; samples reported as <LOD or <LOQ were excluded and not treated as zero concentration. Receiver operating characteristic (ROC) curve analysis was used to evaluate the discriminatory performance of the selected hormonal markers. Statistical significance was set at $p < 0.05$.

Results

Electrochemical characterization of the modified electrode

The electrochemical behavior of the bare and modified glassy carbon electrodes was investigated using cyclic voltammetry to evaluate the effect of surface modification on the electron transfer performance. As shown in Figure 3, the bare GCE exhibited a relatively weak redox response, indicating a limited electroactive surface area and slow electron transfer kinetics. After modification with rGO or MWCNTs, the voltammetric response increased, which can be attributed to the high electrical conductivity and improved charge transfer properties of these carbon-based nanomaterials.

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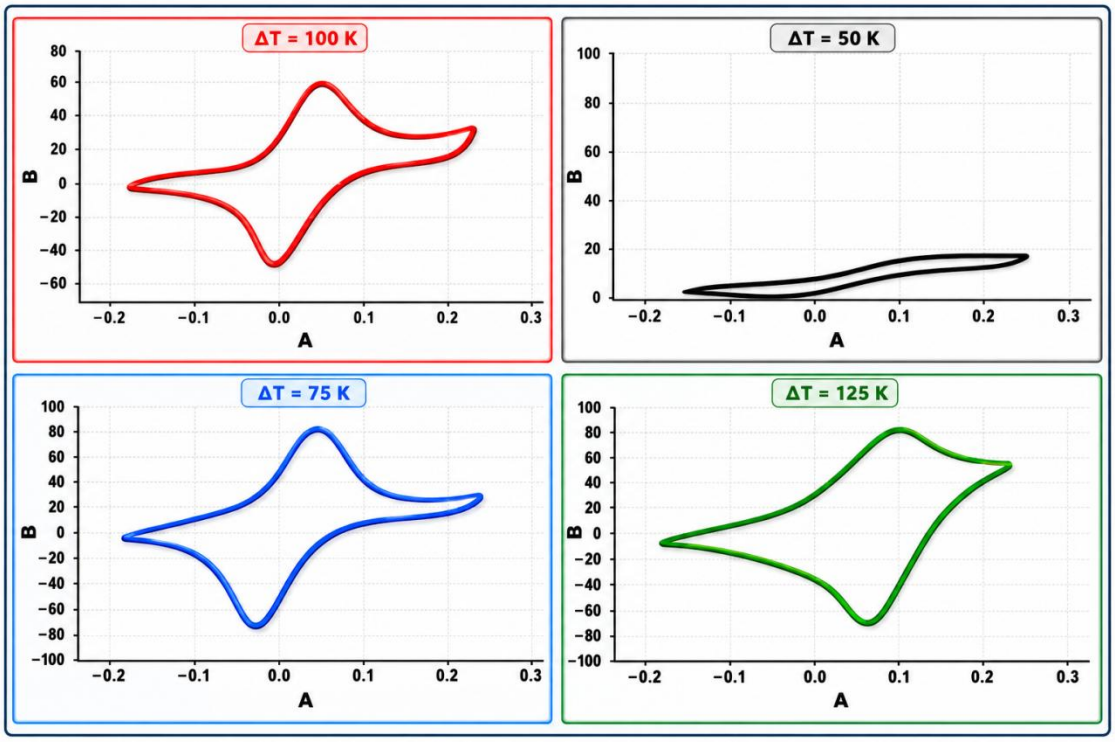
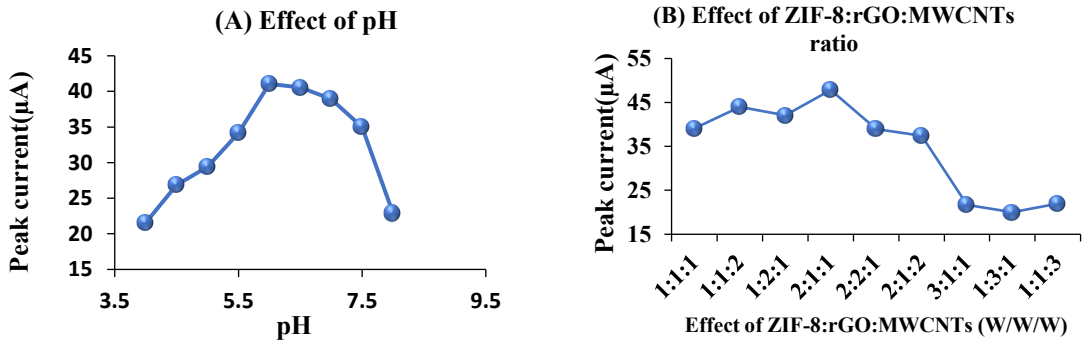


Figure. 3: Cyclic voltammograms of the bare GCE (black), MWCNTs/GCE (green), rGO/GCE (red), and ZIF-8/rGO/MWCNTs/GCE (blue).

Optimization of experimental conditions

The main experimental parameters influencing the metformin electrochemical response were studied to select the appropriate operating conditions for the analytical measurements. The parameters investigated were the pH of the supporting electrolyte, ZIF-8:rGO:MWCNTs modifier ratio, electrode drying time, and scan rate. Figure 4 shows the influence of these parameters on the metformin peak current.



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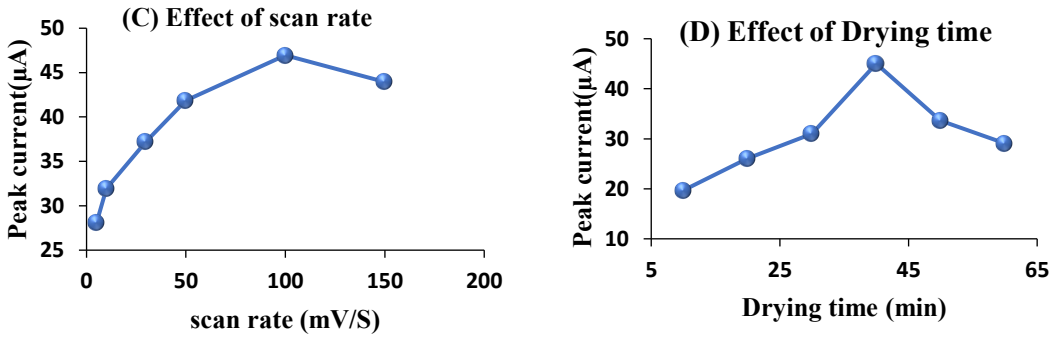


Figure. 4: Optimization of the experimental parameters affecting the electrochemical response of metformin at the ZIF-8/rGO/MWCNTs/GCE: (A) effect of pH, (B) effect of ZIF-8:rGO:MWCNTs ratio, (C) effect of scan rate, and (D) effect of drying time.

The selected experimental conditions are listed in Table 2.

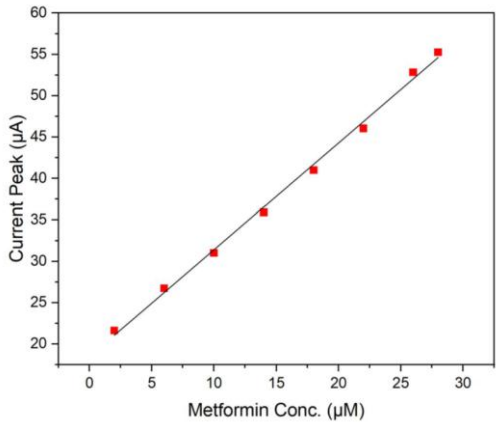
Table .2: Optimized experimental conditions for metformin determination using ZIF-8/rGO/MWCNTs/GCE.

Parameter	Optimized condition
Supporting electrolyte	Phosphate buffer solution
pH	5.5
Modifier ratio	ZIF-8:rGO:MWCNTs = 2:1:1
ZIF-8 concentration	1.0 mg/mL
rGO concentration	0.5 mg/mL
MWCNTs concentration	0.5 mg/mL
Drying time	40 min
Scan rate	100 mV/s

Analytical performance parameters

Linearity, LOD, and LOQ

The linearity of the ZIF-8/rGO/MWCNTs/GCE sensor for metformin was assessed under the optimized experimental conditions. A linear response was obtained over the 2–28 µM range, and the regression parameters were used to quantitatively determine metformin in the extracted serum samples. Figure 5 shows the calibration curves.



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Figure.5: Calibration curve for metformin determination using ZIF8/rGO/MWCNTs/GCE.

The LOD and LOQ were calculated using a regression-based method. The regression equation, correlation coefficient, LOD, and LOQ are presented in Table 3.

Table.3:Regression parameters, LOD, and LOQ of the proposed ZIF-8/rGO/MWCNTs/GCE sensor for metformin determination.

Parameter	Value
Linear range	2–28 µM
Regression equation	Ip = 1.29008 C + 18.4878
Slope	1.29008
Intercept	18.4878
Correlation coefficient, R^2	0.99671
LOD (µM)	0.14
LOQ (µM)	0.42

Accuracy and recovery

Serum samples spiked with low, medium, and high concentrations of metformin were analyzed to assess the accuracy and recovery. The samples were processed using the same acetonitrile-based extraction procedure applied to the real serum samples. The results are presented in Table 4.

Table .4 : Accuracy and recovery of metformin determination in spiked serum samples using the ZIF-8/rGO/MWCNTs/GCE.

Drug	Conc. Taken (µM)	Conc. found (µM)	REC (%)	RE (%)	RSD (%)
Metformin	2	1.974	98.70	1.30	0.017
	10	9.565	95.65	4.35	0.049
	25	24.920	99.68	0.32	0.024

Repeatability and reproducibility

The repeatability of the modified electrode was determined by performing repeated metformin measurements using the same ZIF-8/rGO/MWCNTs/GCE, whereas the reproducibility was assessed using independently prepared modified electrodes. The obtained RSD values are summarized in Table 5.

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Table.5 : Repeatability and reproducibility of the modified electrode.

Test	Condition	Metformin conc. (µM)	Number	RSD (%)
Repeatability	Same electrode	10	3	0.049
Reproducibility	Different electrodes	10	3	0.050

Determination of metformin in extracted serum samples

The applicability of the ZIF-8/rGO/MWCNTs/GCE sensor was evaluated by determining the concentration of metformin in serum samples extracted from patients with PCOS undergoing metformin therapy. (34-35) The samples were analyzed under optimized experimental conditions, and metformin concentrations were calculated using the established calibration equation, applying the dilution factor introduced during serum extraction and sample preparation.

Metformin was quantified in several patient serum samples; some samples did not exhibit a measurable metformin peak at the corresponding oxidation potential or produced a response below the calculated LOD, and these were reported as <LOD.(36) Samples that displayed a response above the LOD but below the LOQ were reported as <LOQ and were not considered quantitatively reliable. Samples with concentrations equal to or greater than the LOQ were considered quantifiable and were used in concentration-based statistical analyses.

Table.6: Final serum metformin concentrations were determined in extracted serum samples from metformin-treated patients with PCOS.

Sample No.	Final serum metformin concentration (µM)	Status
S1	3.26	Quantifiable
S2	7.07	Quantifiable
S3	7.82	Quantifiable
S4	3.79	Quantifiable
S5	<LOD	Below detection limit
S6	4.09	Quantifiable
S7	11.894	Quantifiable
S8	6.93	Quantifiable
S9	3.31	Quantifiable
S10	7.26	Quantifiable
S11	6.81	Quantifiable
S12	<LOD	Below detection limit
S13	8.72	Quantifiable
S14	9.94	Quantifiable
S15	11.04	Quantifiable
S16	7.29	Quantifiable
S17	3.88	Quantifiable
S18	3.91	Quantifiable
S19	<LOD	Below detection limit

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Sample No.	Final serum metformin concentration (µM)	Status
S20	9.76	Quantifiable
S21	5.08	Quantifiable
S22	8.25	Quantifiable
S23	4.01	Quantifiable
S24	9.79	Quantifiable
S25	12.06	Quantifiable
S26	7.44	Quantifiable
S27	<LOD	Below detection limit
S28	<LOD	Below detection limit
S29	3.84	Quantifiable
S30	5.85	Quantifiable
S31	6.55	Quantifiable
S32	7.86	Quantifiable
S33	11.84	Quantifiable
S34	<LOD	Below detection limit
S35	3.65	Quantifiable

Note. The LOD = 0.14 µM and 0.42 µM, respectively, in the measured solution. Samples reported as <LOD showed no measurable metformin peak at the expected oxidation potential or a response below the calculated limit of detection (LOD). Samples reported as <LOQ showed a detectable response above the LOD but below the LOQ and were not considered to be quantitatively reliable. Values <LOD or <LOQ were excluded from the correlation analysis and were not treated as zero concentration.

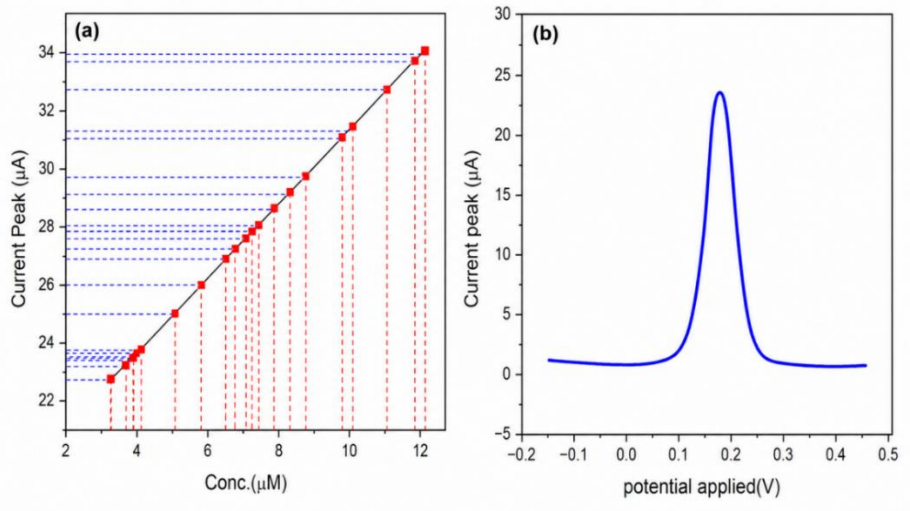


Figure. 6: (a) Projection of the serum-sample peak current values onto the calibration curve for metformin-concentration determination; (b) representative DPV responses of selected extracted serum samples.

Comparison with reported methods

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The analytical performance of the developed ZIF-8/rGO/MWCNTs/GCE sensor was compared with previously reported methods for metformin determination, including chromatographic, spectroscopic, and voltammetric techniques. All reported linear ranges and limits of detection (LODs) were converted to micromolar (µM) units to facilitate direct comparison, as presented in Table 7.

Table.7: Comparison of the proposed method with previously reported methods for metformin determination.

Method/ electrode	**Technique**	**Sample**	**Linear range (µM)**	**LOD (µM)**	**Rec. (%)**	**Ref.**
RP-HPLC	HPLC–UV	Human serum	3.1–94.5	14.9	99.78	(36)
UHPLC-QTOF/MS	LC–MS	Human serum	0.013–0.77	0.003	—	(37)
Colorimetric (AuNPs)	UV–Vis	Human serum	48.4–1032	13.9	96.36 – 99.61	(38)
P-g-C_3N_4/MOF-199/CPE	DPV	Pharmace-utical	0.0005–1.2	0.000 15	—	(40)
$ZnFe_2O_4$–CuO/AuNPs/GCE	Voltammetry	Standard solution	0.001–1.0	0.000 3	—	(41)
Cu-loaded charcoal/CPE	DPV	Real matrices	0.05–60	0.009	—	(42)
N-CNT–chitosan/GCE	DPV	Standard solution	0.3–10	0.009 6	—	(43)
CuMoP/SPCE	DPV	Pharmaceutica l	0.99–13.82	0.85	—	(44)
Cu-BTC MOF/MWCNTs/GCE	Voltammetry	Pharmaceutica l	—	—	—	(45)
ZIF-8/rGO/MWCNTs/GCE	DPV	Human serum (patients)	2–28	0.14	95.65 – 99.68	This work

Biochemical and hormonal profile of the study groups

The biochemical and hormonal characteristics of the study groups are shown in Table 8. Compared to the control group, both PCOS groups showed significant metabolic and hormonal alterations. Blood sugar, total cholesterol, low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), triglyceride, luteinizing hormone (LH), and prolactin levels were higher in patients with PCOS, whereas high-density lipoprotein cholesterol (HDL-C) and follicle-stimulating hormone (FSH) levels were lower.

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Table .8: Biochemical and hormonal parameters of the study groups (mean ± SD).

Parameter	G1	G2	Control
Blood sugar (mg/dl)	101.570±6.527^{b}	112.430±6.171^{a}	91.100±21.036^{c}
Total cholesterol (mg/dl)	191.800±22.516^{b}	208.800±22.848^{a}	169.960±12.135^{c}
HDL-C (mg/dl)	43.740±4.003^{b}	39.710±5.068^{c}	61.920±4.780^{a}
LDL-C (mg/dl)	119.114±21.394^{b}	132.320±23.506^{a}	90.967±13.883^{c}
VLDL-C(mg/dl)	28.943±5.472^{b}	36.851±5.513^{a}	17.252±6.721^{c}
Triglycerides (mg/dl)	144.710±27.360^{b}	184.260±27.565^{a}	86.260±33.606^{c}
LH (m IU/mL)	10.051±2.489^{b}	14.874±3.220^{a}	6.268±2.123^{c}
FSH (m IU/mL)	5.140±0.855^{b}	5.563±0.864^{b}	6.318±1.489^{a}
Prolactin (ng/mL)	14.717±4.482^{b}	17.946±5.343^{a}	10.282±4.305^{c}
BMI (kg/m²)	29.999±4.104^{a}	27.009±5.977^{b}	26.690±5.979^{b}

Note. Values are expressed as the mean ± SD. Different superscript letters within the same row indicate significant differences at $p \leq 0.05$, whereas identical letters indicate non-significant differences at $p > 0.05$. G1: PCOS patients receiving metformin; G2: PCOS patients not receiving metformin.

Correlation and ROC analysis

Correlation and ROC analyses were performed to assess the associations between selected biochemical and hormonal variables and the discriminatory ability of the hormonal markers.

Table.9: Summary of the correlation analysis among selected biochemical and hormonal parameters in the metformin-treated PCOS group.

Correlation	Direction	Strength	Interpretation
LH vs FSH	Positive	Significant	Higher LH levels were associated with higher FSH levels in the metformin-treated PCOS group.
FSH vs VLDL-C	Positive	Weak	A weak positive association was observed between these variables.
FSH vs Triglycerides	Positive	Weak	A weak positive association was observed between these variables.
Other tested relationships	—	Not significant	No notable correlations were observed between the measured hormonal markers.

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Table.10: ROC analysis of selected hormonal markers for discrimination between the control and PCOS groups.

Comparison	Marker	Cut-off	Sensitivity (%)	Specificity (%)	AUC	p-value
Control vs G2	LH	>9.2	97.1	96.0	0.983	<0.001
Control vs G1	LH	>8.4	80.0	90.0	0.888	<0.001
Control vs G2	Prolactin	>13.1	85.7	86.0	0.874	<0.001
Control vs G1	Prolactin	>12.7	68.6	82.0	0.777	<0.001
Control vs G1	FSH	≤6.3	94.3	50.0	0.729	<0.001
Control vs G2	FSH	≤6.4	88.6	50.0	0.641	0.019

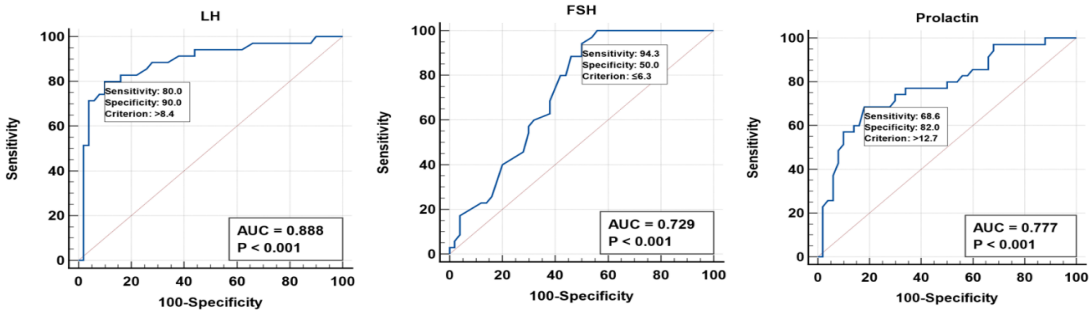


Figure. 7: ROC curves of LH, FSH, and prolactin for differentiating the control group from the metformin-treated PCOS group (G1).

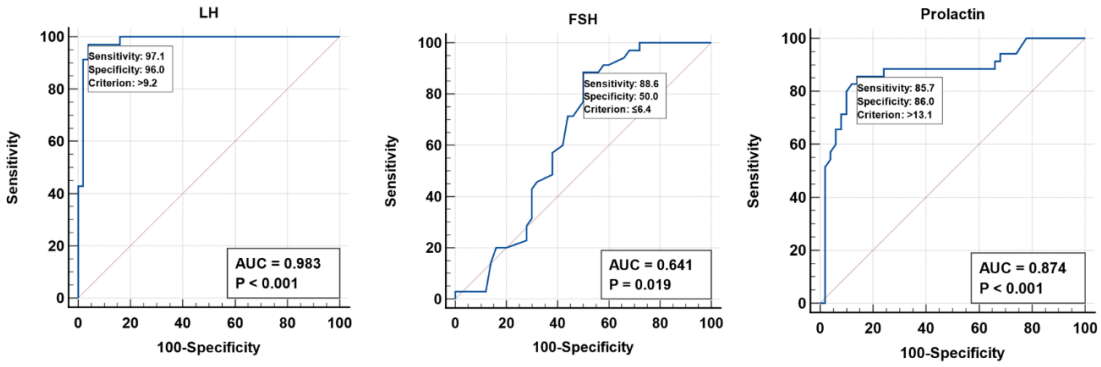


Figure. 8: ROC curves of LH, FSH, and prolactin for differentiating the control group from the untreated PCOS group (G2).

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Exploratory correlation between serum metformin concentration and clinical parameters

An exploratory correlation analysis was conducted in the metformin-treated PCOS group to evaluate the relationship between serum metformin concentration and selected biochemical, hormonal, and clinical parameters. Only quantifiable samples with metformin concentrations ≥ LOQ were included in the correlation analysis; samples reported as <LOD or <LOQ were excluded and not treated as zero concentration. None of the correlations between serum metformin concentration and assayed parameters were statistically significant (Table 11).

Table. 11:Correlation between serum metformin concentration and selected biochemical, hormonal, and clinical parameters in metformin-treated patients with PCOS.

Parameter	n	Spearman's ρ	p-value	Direction	Strength	Interpretation
Blood sugar (mg/dL)	29	−0.147	0.446	Negative	Very weak	Not significant
Total cholesterol (mg/dL)	29	−0.207	0.282	Negative	Weak	Not significant
HDL-C (mg/dL)	29	0.013	0.948	Positive	Very weak	Not significant
LDL-C (mg/dL)	29	−0.186	0.334	Negative	Very weak	Not significant
VLDL-C (mg/dL)	29	−0.243	0.205	Negative	Weak	Not significant
Triglycerides (mg/dL)	29	−0.243	0.205	Negative	Weak	Not significant
LH (m IU/mL)	29	−0.145	0.452	Negative	Very weak	Not significant
FSH (m IU/mL)	29	−0.125	0.518	Negative	Very weak	Not significant
Prolactin (ng/mL)	29	−0.270	0.156	Negative	Weak	Not significant
BMI (kg/m²)	29	−0.262	0.170	Negative	Weak	Not significant

Note. Samples reported as <LOD or <LOQ were excluded from the correlation analysis and were not treated as zero-concentration samples. Only quantifiable metformin concentrations ≥ LOQ were included in the study.

Discussion

The ZIF-8/rGO/MWCNTs-modified glassy carbon electrode exhibited the highest current response compared with the bare GCE and individually modified electrodes. This enhanced response can be attributed to the synergistic effect of the porous ZIF-8 framework, which provides adsorption sites, and the conductive properties of rGO and MWCNTs, which facilitate electron transfer across the electrode surface. These findings suggest that combining

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the three nanomaterials improves the electrochemical interface and supports the application of the modified electrode for metformin determination.

The optimization experiments identified pH 5.5, a ZIF-8:rGO:MWCNTs ratio of 2:1:1, a drying time of 40 min, and a scan rate of 100 mV/s as the conditions that produced the highest electrochemical response. These optimized conditions were subsequently used for the analytical determination of metformin. The sensor demonstrated a linear response over the concentration range of 2–28 µM, with a correlation coefficient (R^2) of 0.99671. The calculated limit of detection (LOD) and limit of quantification (LOQ) were 0.14 µM and 0.42 µM, respectively, indicating the capability of the developed sensor to detect and quantify metformin at relatively low concentrations under the optimized experimental conditions.

The comparison presented in Table 7 highlights the analytical advantages and limitations of the developed ZIF-8/rGO/MWCNTs/GCE sensor relative to previously reported methods. Compared with chromatographic and spectroscopic methods applied to human serum, the proposed sensor offers a lower detection limit than both RP-HPLC–UV and colorimetric procedures (37), without requiring chromatographic separation or a chromogenic derivatization step. Although the UHPLC-QTOF/MS method (38) provides substantially higher sensitivity, this performance is associated with the advanced instrumentation and technical expertise required for mass-spectrometric analysis. In this context, the proposed sensor offers a potentially more accessible alternative for laboratories equipped with basic electrochemical instrumentation.

Regarding previously reported voltammetric sensors, several modified electrodes achieved detection limits in the nanomolar range (41–44), which were lower than that obtained in the present study. Nevertheless, these platforms were characterized predominantly using pharmaceutical formulations or standard solutions, where matrix complexity is considerably lower than that of biological samples. In contrast, studies demonstrating metformin determination directly in authentic serum samples from treated patients remain relatively limited.

An important contribution of the present study is therefore the application of the developed sensor to serum samples obtained from patients with PCOS receiving metformin therapy. The successful quantification of metformin in 29 out of 35 samples demonstrates its practical applicability in a complex biological matrix, while allowing the measured concentrations to be considered alongside the corresponding biochemical and hormonal profiles. Furthermore, the observed linear range of 2–28 µM encompassed all quantified serum concentrations (3.26–12.06 µM), indicating that the established calibration range was suitable for the concentrations encountered in the analyzed samples. Therefore, the main contribution of the developed sensor lies not in achieving the lowest detection limit among previously reported techniques, but in demonstrating its applicability to authentic human serum samples within the concentration range encountered in the studied patients.

Values were converted to µM using the molar mass of metformin free base (129.16 g mol^{-1}) where originally reported in mass units. The untreated PCOS group (G2) showed the most pronounced metabolic disturbances, particularly in lipid-related parameters, including total cholesterol, LDL-C, VLDL-C, and triglyceride levels. In contrast, the metformin-treated PCOS group (G1) showed lower values for several metabolic and hormonal parameters than G2, although these values did not fully return to the control levels. This pattern may reflect partial improvement in the treated group, while also indicating persistent metabolic and endocrine imbalances. Broader biochemical disturbances have also been reported in patients

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with diabetes under therapy, supporting the interpretation of insulin-related metabolic disorders as systemic biochemical conditions rather than isolated glycemic abnormalities.

BMI was highest in the metformin-treated PCOS group compared with the control and untreated PCOS groups. This finding should be interpreted with caution, as the BMI differences may reflect baseline patient characteristics rather than the direct effect of metformin treatment. Overall, these results confirm the metabolic and hormonal alterations associated with PCOS and provide a clinical background for evaluating serum metformin concentrations in the treatment group. The correlation analysis revealed limited associations among the assessed biochemical and hormonal parameters in the metformin-treated PCOS group. ROC analysis indicated that LH was the most discriminating hormonal marker, particularly in the comparison between the untreated PCOS group and the control group, followed by prolactin. In contrast, FSH showed comparatively lower discriminatory performance. These findings suggest that LH and prolactin may provide useful discriminatory information regarding the hormonal alterations associated with PCOS in the studied population.

The exploratory correlation analysis did not reveal statistically significant associations between serum metformin concentrations and the assessed biochemical, hormonal, or clinical parameters. Therefore, no direct relationship could be established between circulating metformin concentrations and the evaluated biomarkers in the present dataset. Several factors may need to be considered when interpreting these findings, including variations in sampling time, dosing history, and individual pharmacokinetic characteristics. More extensive investigations involving controlled sampling times, detailed dosing histories, renal function evaluation, and larger sample sizes are needed to clarify these relationships.

Conclusion

A ZIF-8/rGO/MWCNT-modified glassy carbon electrode was constructed for the electrochemical determination of metformin in human serum. The modified electrode exhibited an improved electrochemical response and concentration-dependent analytical behavior, enabling the quantitative determination of metformin under optimized conditions. The developed method was applied to extract serum samples from metformin-treated patients with PCOS, and variable, detectable metformin concentrations were observed. Biochemical and hormonal differences were evident across the study groups and confirmed the clinical relevance of the selected PCOS patient subgroups. However, the exploratory correlation analysis found no relationship between serum metformin concentration and the assessed biochemical, hormonal, or clinical parameters. The developed ZIF-8/rGO/MWCNTs/GCE sensor provides a simple and inexpensive electrochemical interface for measuring metformin in extracted serum samples. Nevertheless, because a validated chromatographic reference method was not used in this study, the proposed method should be regarded as a preliminary analysis of a potential therapeutic drug-monitoring platform. This recommendation is consistent with LC–MS/MS analytical applications in complex matrices, where matrix-aware extraction and validation are essential for reliable quantitative interpretation (39). Future studies should directly compare it with HPLC or LC-MS/MS using matched patient serum samples with controlled sampling times, dosing information, renal function data, and a larger sample size to confirm its suitability for clinical TDM.

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Competing interests statement

The authors declare that they have no conflict of interest.

Author contributions

Zahraa Abdullah Ahmed was responsible for the conceptualization of the study, data analysis, interpretation of the findings, and preparation of the manuscript. Abdulsalam Tawfeeq Salih Al-Samarai and Mumin F. Al-Samarrai contributed substantially to data collection and analysis. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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