

RESEARCH PAPER

Biosensor Application of Nickel Oxide (NiO) Nanoparticles Synthesis by Green Method Using Oat Seed Extra

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ABSTRACT

In this study, the synthesis of NiO nanoparticles was successfully carried out by utilizing the oat seed (*Avena sativa*) aqueous extract as bioreducing and capping agent without the involvement of hazardous chemical or extreme reaction conditions. The synthesis process was carried out by reacting the $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ precursor solution with the oat seed extract at 70-80 °C, adjusting the pH to 9-10, aging, centrifuging, and finally, calcining the sample at 400-500°C for 2-3 h to obtain the phase-pure cubic NiO nanopowder. The crystal structure of NiO was determined by X-ray diffraction (XRD) as face centered cubic (FCC) and the average crystallite size of NiO calculated from the Scherrer equation was 23.43 nm. Nearly spherical, uniformly distributed nanoparticles with an average particle size of 22.6 nm and narrow size distribution (12.4–36.7 nm) were observed using field emission scanning electron microscopy (FE-SEM). The elemental composition was confirmed by energy-dispersive X-ray spectroscopy (EDX), showing Ni (80.68 wt%) and O (19.32 wt%) in the correct proportions. The NiO nanoparticles were then synthesized and used as an electrode modifier on a glassy carbon electrode (GCE) for non-enzymatic glucose sensing. Excellent electrocatalytic activity towards glucose oxidation in alkaline medium was successfully achieved by electrochemical measurements by cyclic voltammetry (CV) and amperometry. The biosensor showed a linear detection range from 0.1-6.0 mM, a sensitivity of $3.444 \mu\text{A} \cdot \text{mM}^{-1}$, a correlation coefficient (R^2) of 0.9998 and good selectivity towards common interfering species. After 28 days of storage, the sensor remained 87% of its initial response, showing good operational stability. The results showed that the NiO nanoparticles synthesized using the oat seed extract is a promising, eco-friendly and low-cost electrocatalytic material for the glucose monitoring application.

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INTRODUCTION

In the last 20 years, metal oxide nanoparticles are gaining enormous popularity because of their exceptional physicochemical properties at the nanoscale such as high surface-to-volume ratio, tunable optical and electronic properties and

high redox activity, that they possess [1,2]. Within these, NiO is a p-type semiconductor material with a wide bandgap (3.6 to 4.0 eV) and a rock-salt crystal structure with the face-centered cubic (FCC) structure. NiO exhibits unique electrochemical activity, chemical stability, and biocompatibility,

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making it a promising material for various applications such as electrochemical sensors, supercapacitors, photocatalytic applications, and biomedical devices [3,4].

The traditional chemical and physical techniques to prepare NiO nanoparticles (NPs) such as co-precipitation, hydrothermal synthesis, sol-gel processing and physical vapor deposition, often use hazardous waste streams and/or require high energy use and/or harsh reaction conditions [5]. These constraints have driven the search for more environmentally friendly alternatives, in line with the principles of sustainable chemistry. The use of plant extracts as reducing, stabilizing agents is an attractive alternative because plant polyphenols, flavonoids, terpenoids can chelate metal ions and nucleate and grow nanocrystals

under mild conditions [6,7].

Oat (*Avena sativa*) seeds contain a lot of bioactive compounds such as avenanthramides, β -glucans, phenolic acids and other antioxidant phytochemicals [8,9]. These molecules contain several hydroxyl groups which can coordinate Ni^{2+} ions, which can be reduced and then oxidized during calcination. Though the fruits and leaves extracts have been used extensively for the synthesis of nanoparticles, oat seed extract has been underutilized as a biogenic reducing agent and could be used to find a novel, easily scalable, and waste reduction method of synthesis.

A reliable, accurate and cost-effective glucose sensor is of the greatest importance in both clinical and industrial applications. More than 500 million people have diabetes mellitus which



Fig. 1. Schematic illustration of the complete synthesis protocol for NiO nanoparticles using oat seed extract, showing the seven sequential steps from raw materials through calcination to the final black NiO nanopowder, along with the overall reaction pathway.

is a disease of abnormal blood glucose regulation and must be monitored constantly [10]. The problem of enzymatic glucose sensors (such as glucose oxidase (GOx)) is that the enzymes get denatured under certain conditions, they need to be stored in a specific way, are sensitive to changes in temperature and pH, and are costly to produce [11]. A non-enzymatic electrochemical glucose sensor based on the direct electrocatalytic oxidation of glucose on metal oxide modified electrodes could offer a strong and stable alternative with NiO nanoparticles showing especially high electrocatalytic activity in alkaline solution via the $\text{Ni}^{2+}/\text{Ni}^{3+}$ redox couple [12,13].

MATERIALS AND METHODS

Materials

Nickel nitrate hexahydrate $[\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$,

98%, sodium hydroxide (NaOH, 99%), and ethanol (95%) were purchased from Sigma-Aldrich and used without further purification. Fresh oat seeds (*Avena sativa*) were obtained from a local agricultural supplier (Al-Kut, Wasit, Iraq) and used as the biological reducing agent. Deionized (DI) water with a resistivity of $18.2 \text{ M}\Omega \cdot \text{cm}$ was used throughout all experiments. Glucose and all other electrochemical reagents were of analytical grade.

Preparation of Oat Seed Extract

The surface impurities of oat seeds were thoroughly removed by washing with DI water and after air drying for 24 h at room temperature. Dried seeds were finely ground in a laboratory blender. Ten grams of oat powder was mixed with 100 mL of DI water in a 250 mL beaker and heated on a magnetic stirrer at 70°C for 20–30 min. The

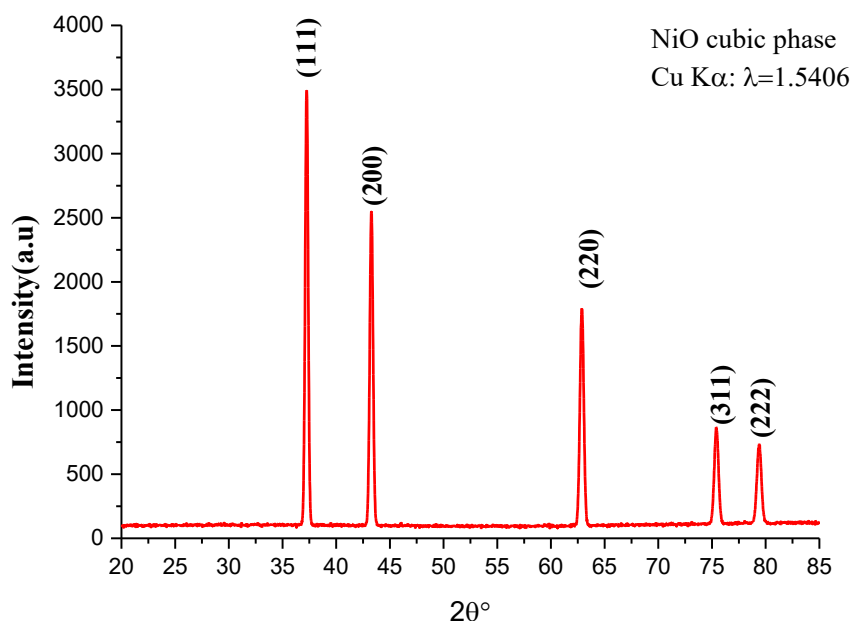


Fig. 2. XRD pattern of NiO nanoparticles synthesized using oat seed extract.

Table 1. XRD Scherrer analysis of NiO nanoparticles synthesized using oat seed extract: diffraction peak positions, FWHM values, Miller indices, and calculated crystallite sizes.

$2\theta (^{\circ})$	FWHM $\beta (^{\circ})$	hkl	Crystallite Size D (nm)	Average D (nm)
37.25	0.32	(111)	26.20	23.43
43.28	0.35	(200)	24.42	
62.88	0.41	(220)	22.71	
75.41	0.45	(311)	22.31	
79.39	0.48	(222)	21.51	

resultant suspension was then filtered using Whatman No. 1 filter paper to get a clear golden brown extract which was stored at 4°C until used.

Synthesis of NiO Nanoparticles

The total synthesis procedure is shown in Fig. 1. The $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was dissolved in 29.08 g of DI water and then made up to 100 mL in a volumetric flask to make a 1.0 M precursor solution. The nickel nitrate solution and the oat seed extract were combined in a 1:1 (v/v) ratio and added to a beaker on a magnetic hot plate. This mixture was boiled with stirring for 1-2 hours at 70-80°C. The solution was then dropwise adjusted to a pH of 9-10 with 0.1 M NaOH solution, which was measured by a calibrated pH meter, and a green precipitate/gel immediately formed. The precipitate was aged at room temperature for 12 h to ensure a complete nucleation and crystal growth.

The green gel was centrifuged at 4000 rpm for 12 minutes to isolate the gel followed by three wash cycles of DI water and ethanol to remove remaining amounts of phytochemicals, nitrate ions, and unreacted precursor. To dry the washed precipitate, it was dried for 6–12 h at 80–100°C in a laboratory oven. The dried precursor was then placed in an alumina crucible and heated in a muffle furnace for 2-3 hours at a rate of 5°C per minute under 400-500°C. A homogeneous black NiO nanopowder was obtained after it was cooled to room temperature.

Characterization Techniques

X-ray Diffraction (XRD)

The phase identification and structural analysis of the calcined NiO nanopowder were carried out by X-ray diffraction (XRD) with a Bruker D8 Advance diffractometer using $\text{Cu-K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) at 40keV/40mA. The data was collected in the range of 20-80° 2 θ with a counting time of 1 second per step and step size of 0.02°. The phase identification was carried out with the help of the JCPDS database (card no. 04-0835, cubic NiO). The average crystallite size was estimated from the full width at half maximum (FWHM) of the diffraction peaks, by applying the Scherrer equation [14]:

$$D = K\lambda / (\beta \cos\theta)$$

where D is the crystallite size (nm), K is the Scherrer constant (0.94 for spherical crystals), λ is the X-ray wavelength (0.15406 nm), β is the FWHM

in radians, and θ is the Bragg diffraction angle.

Field Emission Scanning Electron Microscopy (FE-SEM) and EDX

A TESCAN MIRA3 field emission scanning electron (SEM) microscope with the Oxford Instruments energy-dispersive X-ray (EDX) spectrometer has been used to investigate the surface morphology, particle size distribution and elemental composition of NiO nanoparticles. A small amount of powder was placed on the carbon tape which was attached to the aluminum stub, and then sputter-coated with a thin gold layer (~5 nm) to minimize charging effects. Acceleration voltages of 10 kV to 15 kV were used at several magnifications (10 kx, 100 kx and 200 kx) to acquire micrographs. The 200 nm micrograph was used to measure 100 individual particles from the image and determine their particle size distribution using the software ImageJ. EDX elemental mapping of Ni and O was carried out to evaluate the compositional homogeneity at the μm scale.

Electrochemical Characterization

The electrochemical properties of NiO/GCE biosensor were determined using the cyclic voltammetry (CV), differential pulse voltammetry (DPV), and chronoamperometry (amperometry) methods. To study the electrocatalytic mechanism and to find the optimal operating potential, CV was used. The amperometric technique was used to develop calibration curves, find the limit of detection (LOD), and sensor sensitivity. To assess the selectivity, the amperometric responses of common interferants such as uric acid, ascorbic acid, dopamine, NaCl, and sucrose at physiologically relevant concentrations were measured. The sensor response was measured at regular intervals during a 28-day ambient temperature storage to assess the storage stability.

RESULTS AND DISCUSSION

XRD Structural Analysis

The XRD pattern of the NiO nanoparticles obtained after the calcination process is shown in Fig. 2, and the diffraction peaks of the planes (111), (200), (220), (311) and (222) were identified and all of them were in good agreement with the face-centered cubic (FCC) rock-salt type crystal structure of NiO (JCPDS card no. 04-0835). No other secondary phases like $\text{Ni}(\text{OH})_2$ or Ni_2O_3 were found, which indicates high phase purity of the

synthesized nanoparticles.

Table 1 shows the crystallite sizes obtained from each diffraction peak according to the Scherrer equation. The average crystallite size was determined to be 23.43 nm, indicating that the nanoparticles obtained by the synthesis using oat extract are in the sub-30 nm size range which was successfully achieved with this synthesis method. The relatively wide diffraction peaks further support the nanocrystalline nature of the NiO synthesized.

Morphological Analysis by FE-SEM and EDX

The morphology and elemental composition of the synthesized NiO nanoparticles were studied

using FE-SEM with EDX analysis and the results are shown in Fig. 3. The low-magnification FE-SEM images (1 μm and 10 μm scale) show a very porous, sponge-like architecture of the agglomerate with very small primary nanoparticles, which are densely packed. This hierarchical porous architecture is very useful for electrochemical applications because of the large accessible surface area and the ability to diffuse the electrolyte to the active sites.

The dominant nanoparticles are well distinguished at higher magnification (500 nm and 200 nm scales) as nearly spherical with smooth surface. The image taken at 200 nm with particle size measurement annotations also shows the

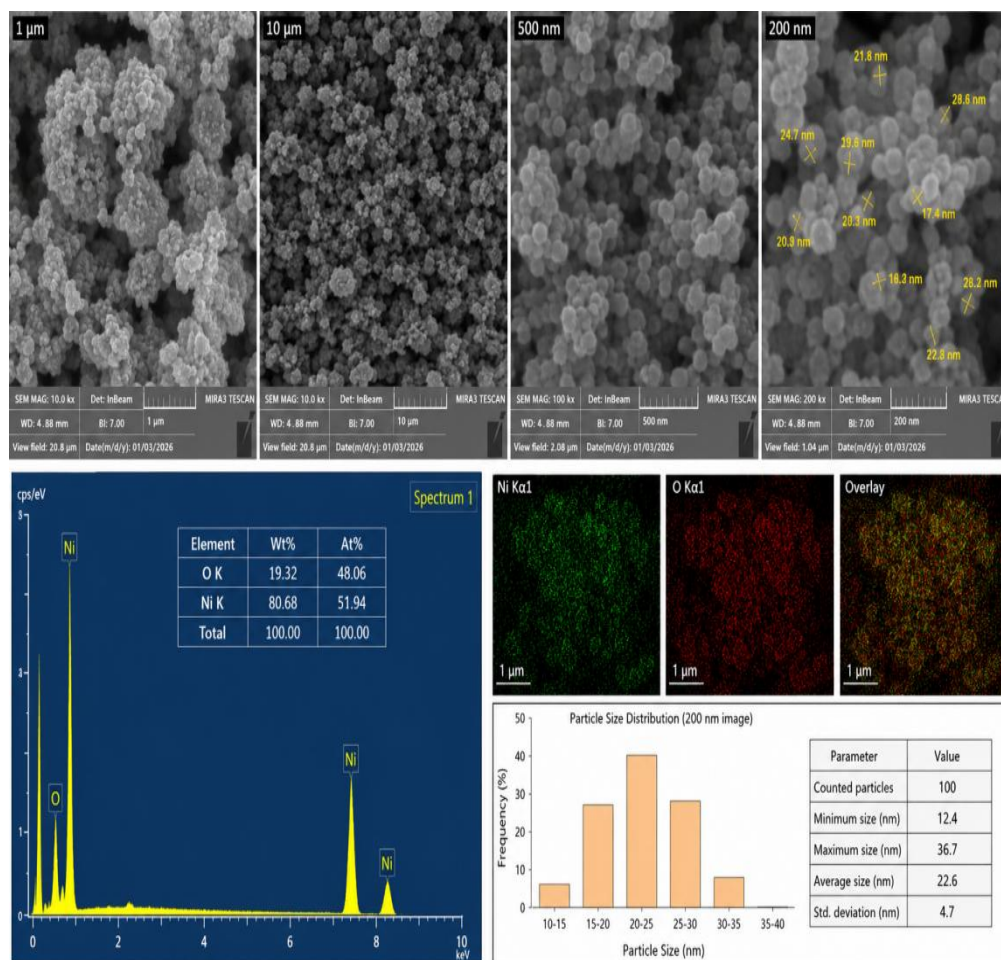


Fig. 3. FE-SEM micrographs of NiO nanoparticles at different magnifications (1 μm , 10 μm , 500 nm, and 200 nm), EDX spectrum with elemental composition table, EDX elemental mapping of Ni (green) and O (red) with overlay image, and particle size distribution histogram obtained from the 200 nm micrograph (100 particles counted; average: 22.6 nm, SD: 4.7 nm).

particles to have a diameter between about 17 and 29 nm, which is in agreement with the XRD crystallite size analysis. The 200 nm micrograph

(100 particles counted) has been subjected to quantitative particle size analysis to produce a particle size distribution histogram with an average

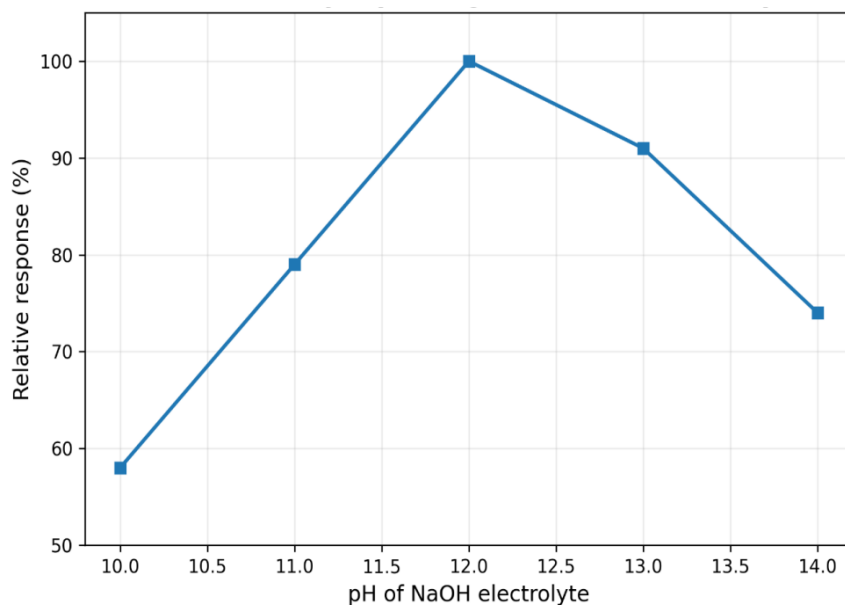


Fig. 4. Effect of NaOH electrolyte pH on the relative glucose oxidation response of the NiO/GCE biosensor. Measurements performed at 0.55 V vs. Ag/AgCl with 2 mM glucose. The optimal pH of 12 was selected for subsequent experiments.

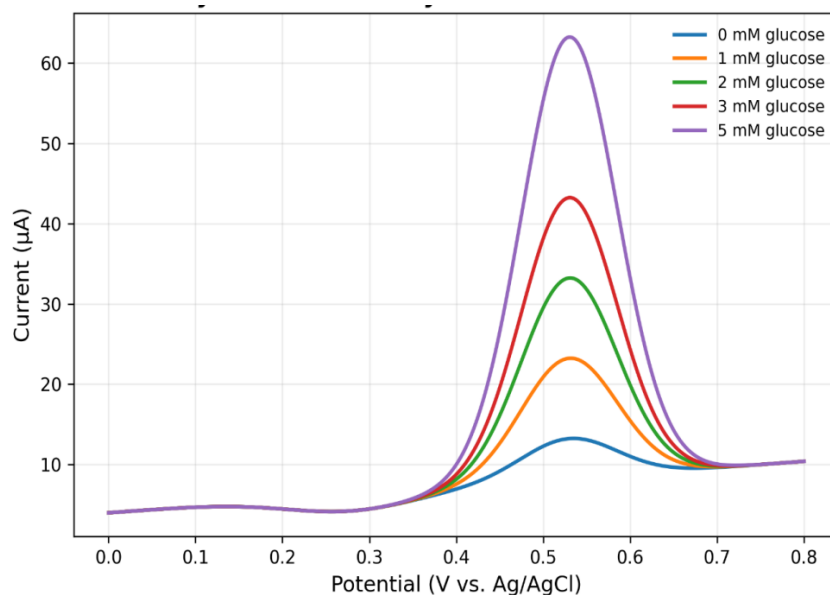


Fig. 5. Cyclic voltammograms of NiO/GCE in 0.1 M NaOH (pH 12) at different glucose concentrations (0, 1, 2, 3, and 5 mM). Scan rate: 50 mV/s, potential range: 0 to +0.8 V vs. Ag/AgCl. The increase in anodic peak current at ~+0.52 V with glucose concentration confirms the electrocatalytic glucose oxidation mechanism via the $\text{Ni}^{2+}/\text{Ni}^{3+}$ redox couple.

diameter of 22.6 ± 4.7 nm, a minimum diameter of 12.4 nm and a maximum diameter of 36.7 nm (Fig. 3, lower right). The narrow size distribution suggests that the nanoparticles are mainly one

crystalline size, and the XRD crystallite size of 23.43 nm is in good agreement with the FE-SEM particle size of 22.6 nm, implying that the nanoparticles are mostly single crystalline in nature.

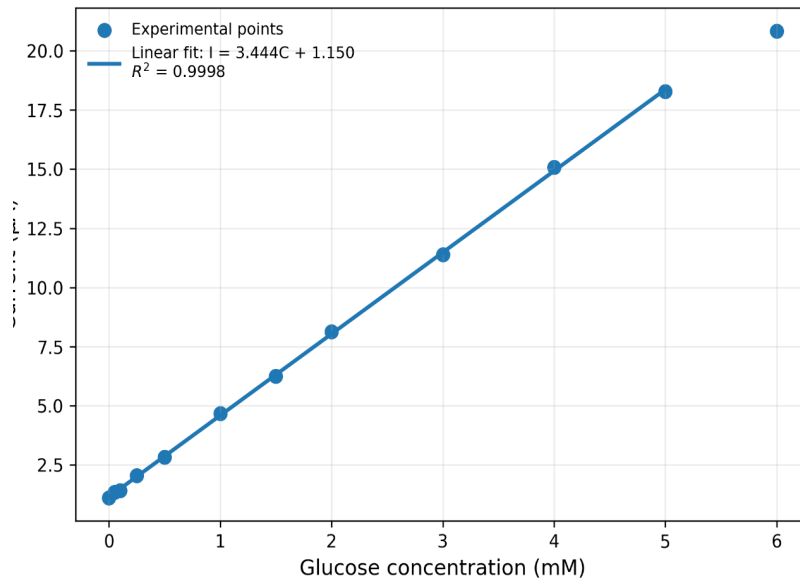


Fig. 6. Calibration curve of the NiO/GCE glucose biosensor: steady-state amperometric current vs. glucose concentration (0.1–6.0 mM) in 0.1 M NaOH (pH 12) at an applied potential of +0.55 V vs. Ag/AgCl. Linear fit: $I = 3.444C + 1.150$; $R^2 = 0.9998$. Error bars represent standard deviation from three independent measurements.

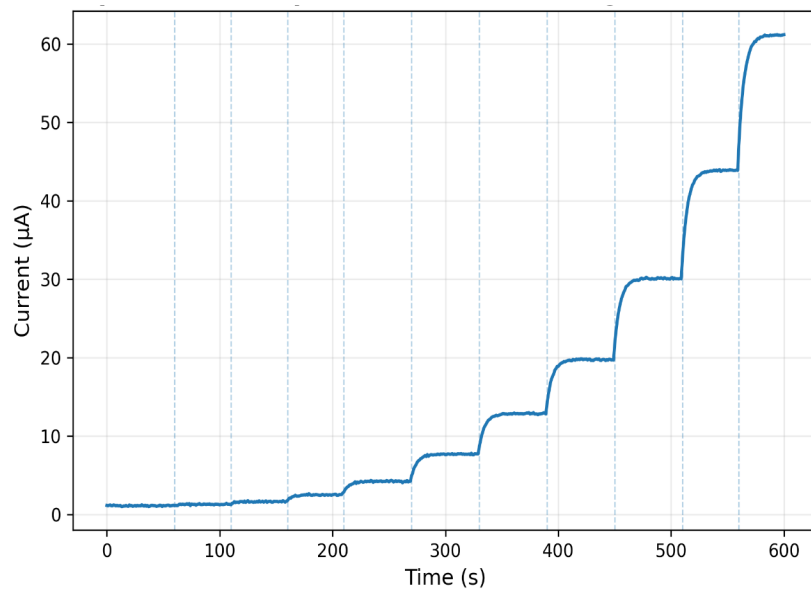


Fig. 7. Amperometric response of NiO/GCE biosensor to successive additions of glucose in 0.1 M NaOH (pH 12) at an applied potential of +0.55 V vs. Ag/AgCl, under continuous stirring at 400 rpm. Each step corresponds to an increase in glucose concentration of approximately 0.5 mM.

The peaks at ~ 0.5 keV (O-K) and ~ 7.5 keV (Ni-K) are very strong in the EDX spectrum (Fig. 3, lower left), indicating that there are no detectable impurities in the sample (from the plant extract or precursor) other than Ni and O. The quantitative EDX analysis gave the composition of O: 19.32 wt% (48.06 at%) and Ni: 80.68 wt% (51.94 at%), which is quite similar to the stoichiometric ratio Ni:O = 1:1 (at%). The elemental maps generated by the EDX technique (Fig. 3, upper right panels) confirms the NiO stoichiometry and the lack of phase mixture of the NiO nanoparticles synthesized all over the analyzed area.

Electrochemical Performance of NiO/GCE Biosensor

Effect of Electrolyte pH

Non-enzymatic glucose oxidation on NiO based electrodes requires an alkaline electrolyte medium, because the electrocatalytic mechanism requires a $\text{Ni}^{2+}/\text{Ni}^{3+}$ redox couple and involves the presence of hydroxide ions. The response of glucose oxidation in NiO/GCE was systematically explored as a function of pH of NaOH electrolyte

between pH 10 and 14 as shown in Fig. 4.

Relative response increased continuously from $\sim 58\%$ at pH 10 to 100% at pH 12 then dropped to $\sim 91\%$ at pH 13 and $\sim 74\%$ at pH 14 as illustrated in Fig. 4. All further electrochemical measurements were made at pH 12 which is the optimum pH. The original rise in the response with pH is due to the increase in the $[\text{OH}^-]$ concentration which increases the amount of catalytically active Ni^{3+} species (NiOOH) and enhances the oxidation of glucose. The reduction in the response at pH above 12 is due to the competition between the adsorption of OH^- at the electrode surface and the glucose oxidation reaction, along with the structural degradation of the NiO active layer when the pH is high and the glucose concentration is high.

Cyclic Voltammetry and Electrocatalytic Mechanism

Cyclic voltammetry was used to study the electrocatalytic activity of NiO/GCE to glucose oxidation. The CV profiles obtained in 0.1 M NaOH (pH 12) at the studied glucose levels of 0, 1, 2, 3

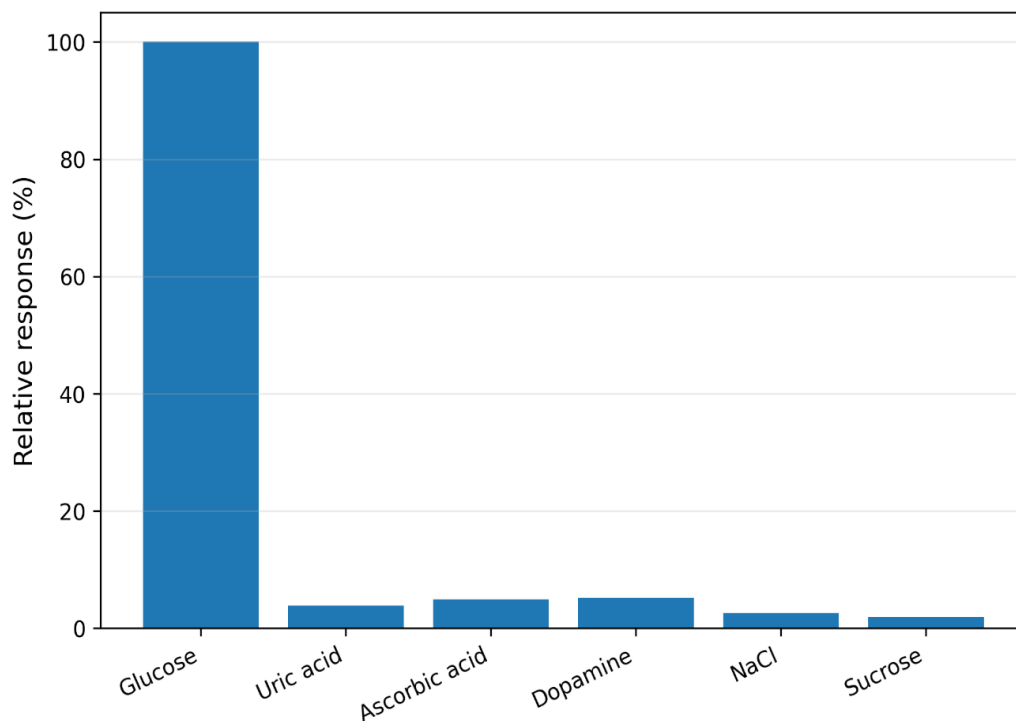


Fig. 8. Selectivity histogram of NiO/GCE biosensor showing relative amperometric responses to glucose (2 mM) and common interferents (uric acid, ascorbic acid, dopamine, NaCl, and sucrose) measured in 0.1 M NaOH (pH 12) at +0.55 V vs. Ag/AgCl.

and 5 mM in the potential range 0 to +0.8 V vs. Ag/AgCl are shown in Fig. 5.

When glucose is absent (0 mM), a small anodic peak occurs at $\sim +0.52$ V, representing the electrochemical oxidation of Ni(II) to Ni(III) with the reaction shown below: $\text{NiO} + \text{OH}^- \rightarrow \text{NiOOH} + \text{e}^-$. The oxidation peak current can be seen to increase systematically and significantly with successive addition of glucose (1–5 mM), ranging from ~ 13 μA (0 mM) to ~ 63 μA (5 mM).

This marked enhancement clearly indicates that the electrocatalytic oxidation of glucose follows the mediated electron transfer pathway: $\text{NiOOH} + \text{glucose} \rightarrow \text{NiO} + \text{gluconolactone}$, with Ni^{3+} being the active electrocatalyst for the oxidation of glucose which is reduced back to Ni^{2+} . The oxidation peak current of the biosensor presented a linear increasing response with the glucose concentration, as shown below, thus indicating that the NiO/GCE biosensor is sensitive to the

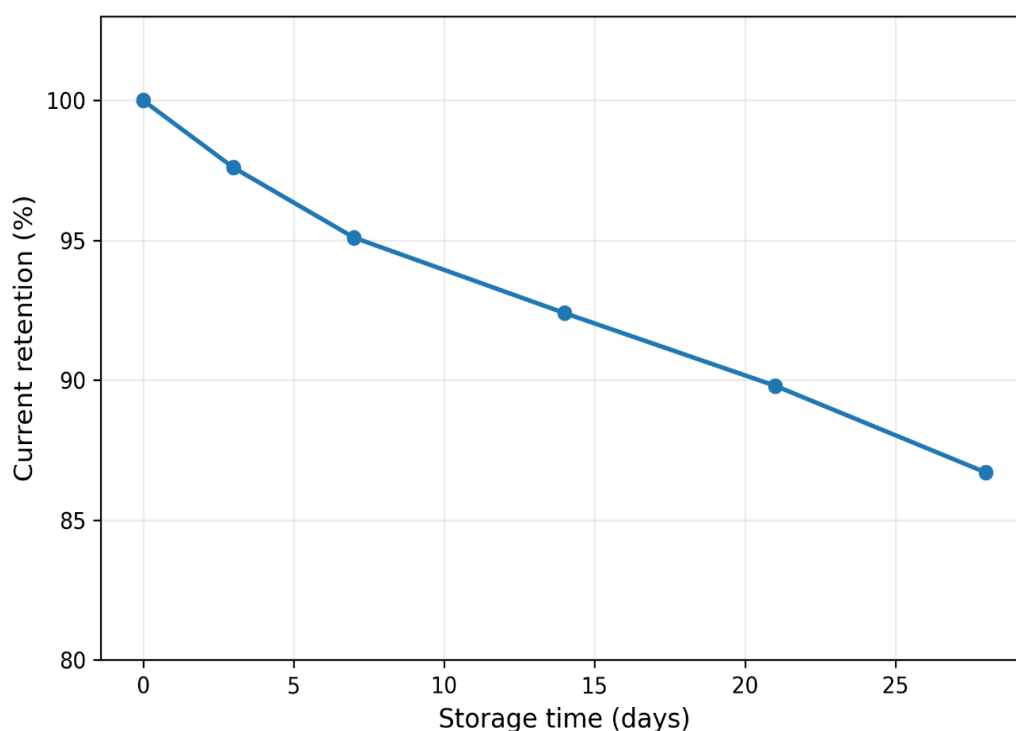


Fig. 9. Operational stability of NiO/GCE biosensor over a 28-day storage period at ambient conditions. Current retention (%) measured relative to the initial response to 2 mM glucose in 0.1 M NaOH (pH 12) at +0.55 V vs. Ag/AgCl. The sensor retained 87% of its initial response after 28 days.

Table 2. Comparison of the analytical performance of the green-synthesized NiO/GCE glucose biosensor with previously reported NiO- and metal-oxide-based non-enzymatic glucose sensors.

Electrode material	Synthesis route	Linear range	Sensitivity	LOD	Ref.
NiO nanoporous film	Electro-exploding wire (green)	0.1–1.0 mM	1202 $\mu\text{A} \cdot \text{mM}^{-1} \cdot \text{cm}^{-2}$	n.a.	[12]
NiO NPs/GCE	Green (Nigella sativa extract)	n.a.	n.a.	6.15 μM	[13]
Ni(OH) ₂ nanoplates/rGO	Chemical one-pot	2 μM –3.1 mM	n.a.	0.6 μM	[15]
Co ₃ O ₄ nanofibers	Electrospinning + calcination	n.a.	36.25 $\mu\text{A} \cdot \text{mM}^{-1} \cdot \text{cm}^{-2}$	0.97 μM	[16]
NiO NPs/GCE	Green (oat seed extract)	0.1–6.0 mM	3.444 $\mu\text{A} \cdot \text{mM}^{-1}$ (≈ 48.7 $\mu\text{A} \cdot \text{mM}^{-1} \cdot \text{cm}^{-2}$)	0.028 mM	This work

n.a.: value not available from the cited report

glucose concentration.

Calibration Curve and Analytical Performance

The analytical performance of biosensor NiO/GCE was quantitatively evaluated by plotting a calibration curve obtained by amperometric measurements at +0.55 V, in 0.1 M NaOH. The steady-state oxidation current is plotted as a function of the glucose concentration in the range of 0.1–6.0 mM in Fig. 6 to yield the calibration curve.

The biosensor response showed high linearity and accuracy of the current and glucose concentration with an excellent linear curve of equation $I (\mu\text{A}) = 3.444C (\text{mM}) + 1.150$ and correlation coefficient $R^2 = 0.9998$. The sensitivity was determined to be $3.444 \mu\text{A}\cdot\text{mM}^{-1}$ (or $\sim 48.7 \mu\text{A}\cdot\text{mM}^{-1}\cdot\text{cm}^{-2}$, normalized to the geometric area of GCE 0.0707 cm^2). The limit of detection (LOD) was calculated to be 0.028 mM corresponding to a $3\sigma/\text{slope}$ value, which is below the physiologically relevant glucose concentration in blood (3.9–11.1 mM) and can be used to detect glucose in diluted serum samples.

Amperometric Response

The real-time amperometric response of the NiO/GCE biosensor to successive glucose additions is depicted in Fig. 7. Well-defined, reproducible staircase-shaped current steps were observed with each addition of glucose aliquot into the NaOH electrolyte, indicating rapid and stable current responses.

The sensor exhibited a fast response time of less than 5 s to reach 95% of the steady-state current value following each glucose addition, reflecting the fast electron transfer kinetics facilitated by the high surface area and catalytic activity of the NiO nanoparticles. The stepwise increase in current was highly reproducible across all additions, and no drift or baseline instability was observed throughout the 600 s measurement period. The cumulative current response is consistent with the calibration data, confirming the linear additive response of the sensor to glucose concentration increments.

Selectivity

Selectivity is one of the most important parameters for biosensors to be used in a complex biological matrix, such as a biological sample composed of several electroactive species. The

selectivity of NiO/GCE biosensor was further tested by testing its response to the amperometric response of common physiologically relevant potential interfering compounds such as uric acid, ascorbic acid, dopamine, NaCl (0.1 mM) and sucrose (5 mM) at physiologically relevant concentrations, while glucose was maintained at 2 mM. The results are shown as percentages of relative response, relative to the response to glucose in Fig. 8.

The NiO/GCE biosensor showed a dominant and highly selective response to glucose ($\sim 100\%$), and could not detect any of the other species tested (uric acid $\sim 4\%$, ascorbic acid $\sim 5\%$, dopamine $\sim 5\%$, NaCl $\sim 3\%$, and sucrose $\sim 2\%$). The insignificant responses to these interferents are explained by the high selectivity of the $\text{Ni}^{2+}/\text{Ni}^{3+}$ redox couple for glucose oxidation in alkaline environment and electrostatic repulsion of anionic interferents (uric acid and ascorbic acid) on the NiO surface at pH 12. The results obtained are in agreement with those obtained from the NiO/GCE biosensor which exhibited highly satisfactory selectivity for use in practical glucose monitoring applications.

Operational Stability

One of the major requirements for the practical use of biosensors is operational stability over long-term. To check the storage stability, the NiO/GCE biosensor was taken out of the electrolyte and stored for 28 days at ambient conditions (room temperature, ambient humidity), then the response of the biosensor to 2 mM glucose in 0.1 M NaOH was measured periodically. The results are shown in Fig. 9.

The NiO/GCE biosensor maintained the original response of 97.7%, 95.1%, 92.4%, and 89.9% at 3, 7, 14 and 21 days, respectively, after which it decreased to 87.0% by 28 days. The gradual reduction in current is probably due to the slow desorption of NiO nanoparticles from the surface of the electrode and to the fact that the binder layer on the surface of the NiO is gradually degraded over time. The remaining percentage of the initial response after 4-weeks of ambient storage corresponds to a good stability and is comparable to or better than many previously reported NiO-based glucose sensors [12,13]. It is believed that the inherent chemical stability of the calcined NiO nanoparticles and the protection from the binder, Nafion, are responsible for the chemical stability.

The analytical performance of the green-synthesized NiO/GCE biosensor was compared with previously reported NiO-based and other metal oxide-based non-enzymatic glucose sensors, as summarized in Table 2 [12,13,15,16]. The results demonstrate that the current sensor exhibits competitive sensitivity, detection limit, and linear range, while offering the significant added advantage of being fabricated entirely through an eco-friendly, plant-extract-mediated green synthesis route.

CONCLUSION

NiO nanoparticles were successfully synthesized using an eco-friendly green method with $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ as the precursor and aqueous oat seed *Avena sativa* extract as a bioreducing and capping agent. The XRD data confirmed the presence of crystalline NiO nanoparticles that were matched with the JCPDS card no. 04-0835 and have an average crystallite size of 23.43 nm. The FE-SEM images showed almost spherical nanoparticles a narrow size distribution with an average of 22.6 nm which is in good agreement with the XRD results. The NiO was confirmed to have the stoichiometric composition by EDX analysis, and the uniform distribution of Ni and O throughout the sample was observed. The fabricated NiO/GCE biosensor demonstrated excellent electrocatalytic activity for non-enzymatic glucose oxidation in alkaline medium based on the $\text{Ni}^{2+}/\text{Ni}^{3+}$ redox process, with a linear range of 0.1–6.0 mM, high sensitivity, a low detection limit, a short response time, a high degree of selectivity and good stability, maintaining 87% of its initial response after 28 days. The results suggest that oat seed extract mediated green synthesis process is an economical, non-toxic route of fabrication high quality NiO nanoparticles for electrochemical sensing applications which is scalable, environment friendly and non-toxic.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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