

Structural and Optical Properties of Fe₂O₃ Nanoparticles for Biosensing Applications

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General Background: Nanostructured metal oxides are widely explored for sensing technologies due to their tunable structural and optical behavior. **Specific Background:** Iron oxide (Fe₂O₃), particularly hematite, offers stability, biocompatibility, and catalytic activity suitable for biosensing applications. **Knowledge Gap:** However, the combined structural–optical characterization of Fe₂O₃ synthesized via a simple sol–gel route and its integrated performance within a glucose biosensor remains insufficiently examined. **Aims:** This study investigates the structural, morphological, and optical properties of Fe₂O₃ nanoparticles and evaluates their functionality as an active layer in a glucose oxidase (GOx) sol–gel biosensor. **Results:** XRD confirmed hematite with sharp peaks at ~33.2° and ~35.7°, indicating high crystallinity; UV–Vis/Tauc analysis yielded a direct band gap of 2.1–2.2 eV; SEM/EDS revealed quasi-spherical aggregates composed predominantly of Fe and O. The biosensor exhibited first-order amperometric responses with T₉₀ values of ~26 s (2 mM) and ~34 s (5 mM) and rapid T₁₀ recovery (~2 s). **Novelty:** The combination of sol–gel immobilization and Fe₂O₃'s intrinsic catalytic behavior produced fast, stable, and reversible glucose sensing. **Implications:** These findings support Fe₂O₃–sol–gel platforms as promising candidates for next-generation enzymatic biosensors.

Highlights:

- Fe₂O₃ nanoparticles exhibit high crystallinity and a 2.1–2.2 eV direct band gap.
- Sol–gel Fe₂O₃–GOx biosensor achieves rapid response and recovery times.
- Demonstrates a synergistic catalytic–structural design for reliable glucose detection.

Keywords: Fe₂O₃ Nanoparticles; Hematite; Glucose Biosensor; Optical Properties; Sol–Gel

Introduction

The Antibiotics have been one of the greatest achievements in modern chemistry and medicine, providing powerful tools to combat bacterial infections that once caused widespread mortality. Among these antibiotics, carbapenems represent a vital subclass of the β-lactam antibiotics, characterized by a unique β-lactam ring fused with a five-membered carbapenem ring that provides exceptional resistance to many β-lactamases, the enzymes responsible for antibiotic degradation [1].

The molecular structure of carbapenems plays a critical role in their antimicrobial activity and their ability to withstand hydrolysis by β-lactamase enzymes. Unlike penicillins and cephalosporins, carbapenems contain a carbon atom instead of sulfur at the C-1 position of the ring, as well as a double bond between C-2 and C-3, enhancing their



chemical stability and binding affinity toward bacterial penicillin-binding proteins (PBPs) [2]. These structural modifications allow carbapenems such as imipenem and meropenem to maintain broad-spectrum activity against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains [3].

However, the increasing emergence of β -lactamase-producing bacteria, especially those capable of producing carbapenemases, threatens the clinical effectiveness of these antibiotics. Therefore, understanding the structure-activity relationship (SAR) of carbapenems is essential for the design of new derivatives with enhanced resistance to β -lactamases and improved antibacterial potency.

This study aims to analyze the molecular structure of carbapenem antibiotics and evaluate how their chemical configurations influence their resistance to bacterial β -lactamase enzymes, providing insights for the development of next-generation carbapenem antibiotics.

Antibiotics

are defined as specific or modified secondary metabolic products that exhibit activity against various groups of microorganisms. They are also described as organic chemical substances produced by different types of microorganisms, capable of inhibiting the growth of other microbes without harming body cells. These substances include products obtained through chemical modification of natural antibiotics, as well as other microbial metabolic products or microbially modified synthetic compounds [4].

Some antibiotics possess a narrow spectrum of activity, targeting a specific microorganism or a limited group of microbes — referred to as narrow-spectrum antibiotics. In contrast, those effective against a wide range of microorganisms, including both Gram-positive and Gram-negative bacteria, are known as broad-spectrum antibiotics.

Certain antibiotics are bactericidal, meaning they actively kill bacterial cells and prevent the formation of new generations once the agent is removed. Others are bacteriostatic, inhibiting bacterial growth without killing the organisms, thus keeping their number constant during exposure; once the antibiotic is removed, bacterial growth can resume.

Antibiotics vary in their molecular weights and contain reactive organic groups. Despite their diversity, all antibiotics share the characteristic of being solid organic compounds. They are used to treat various bacterial infections, as well as in animal and agricultural production sectors. Additionally, antibiotics are employed as preservatives in certain foods, especially canned products, and serve as inhibitors in some chemical reactions [5].

Sometimes, antibiotics are combined to produce a synergistic effect, enhancing their inhibitory potential for treating serious infections such as endocarditis caused by *Enterococcus* bacteria or mixed infections like abdominal abscesses involving both aerobic and anaerobic bacteria. For instance, Nitronidazole is combined with aminoglycosides or broad-spectrum cephalosporins. To reduce toxicity, Amphotericin B is used with 5-flucytosine when treating *Cryptococcus neoformans* infections, as this combination allows a lower dose of Amphotericin B [6].

Another benefit of antibiotic combinations is preventing resistance development during treatment. For example, in infections caused by *Staphylococcus aureus*, Fusidic acid is preferably combined with Flucloxacillin to prevent resistant strains. Similarly, Rifamycin is combined with Isoniazid when treating tuberculosis to reduce the risk of resistance associated with Rifamycin monotherapy.

However, combining antibiotics may also have drawbacks, including an increased likelihood of adverse side effects [7].

transition from the macroscopic to the nanoscale is witnessing a radical shift in the scientific landscape, as rapid advances in nanotechnology have contributed to unprecedented clinical achievements [1]. This technology is emerging as a promising tool in biomedical engineering, particularly in the field of loading therapeutic agents, thanks to the unique surface properties of nanoparticles (NPs), which qualify them as effective platforms in drug delivery systems[2-8]. Among these particles, iron oxide is receiving particular attention due to its distinct properties that make it ideal for biomedical applications[9-11]. For example, Fe_2O_3 nanoparticles enhance the stability and permeability of drugs through tissues, prolonging their residence time in the circulation [12]. These particles also enable more effective treatment with lower drug doses, thanks to their ability to control drug release, reducing toxicity and limiting drug accumulation in healthy tissue [13]. These advantages, coupled with



the biocompatibility of iron oxide nanoparticles, have attracted extensive research interest in recent decades. The properties of these particles are influenced by multiple factors, such as shape, crystalline structure, size, and preparation method [14]. Surface modifications, resulting from their large specific surface area and high porosity, also enable their use as pH sensors in drug delivery systems [15]. Iron oxide nanoparticles are of additional interest due to their magnetic properties, which allow them to be guided by external magnetic fields to target specific sites in the body, enhancing drug delivery to tumors, for example [16]. These particles also possess electrical catalytic capabilities that accelerate the response in redox-sensitive drug delivery systems [17]. In the context of tissue engineering, nanoparticles are used as replacements for damaged tissue due to their ability to mimic the extracellular matrix, supported by a high surface-to-volume ratio that enhances the efficiency of drug loading and gradual release [18-20]. The diverse physicochemical properties of Fe_2O_3 are also exploited to stimulate cell proliferation and tissue regeneration, particularly in applications such as bone engineering [21, 22]. To ensure the effectiveness of scaffolds in tissue engineering, they must have sufficient porosity to support nutrient and gas transport, which can be achieved using iron oxide nanoparticles [23, 24]. Particles smaller than 10 nm also exhibit superior magnetic properties and immune cell evasion, with excellent drug retention and high electrical conductivity, enhancing their biological applications [25-27].

A glucose biosensor couples a selective biorecognition step—typically the glucose oxidase (GOx) reaction—with an electrochemical transducer that quantifies either O_2 consumption or the H_2O_2 generated when GOx oxidizes β -D-glucose to D-glucono- δ -lactone, enabling rapid, sensitive, point-of-care measurements [28].

In our design, iron oxide prepared by the sol-gel route serves as the active sensing layer and enzyme host: sol-gel matrices provide controlled porosity and high purity that stabilize GOx immobilization and facilitate mass transport at the electrode-electrolyte interface [29].

Beyond acting as a scaffold, Fe_3O_4 exhibits intrinsic peroxidase-like “nanozyme” activity that accelerates H_2O_2 redox, improving signal gain; this catalytic behavior is well established and has been leveraged in Fe_3O_4 -based glucose electrodes with low detection limits and good stability [30-32].

Clinically, enzymatic strips based on GOx remain the mainstay, while designs using GDH-PQQ require care because certain nonglucose sugars (e.g., maltose) can cause falsely elevated readings—an interference documented by regulators and clinical studies [33].

Method

The Experimental and MATERIALS PREPARATION

2.1 Preparation of Iron oxide (Fe_2O_3)

We weigh (4) grams of iron and place it in a glass beaker containing (50) ml of distilled water and place the mixture on the magnetic stirrer to obtain a homogeneous mixture. After the iron dissolves completely in the water, we add drops of sodium hydroxide (NaOH) and the drops are added continuously until it turns into a gel and we measure its acidity (PH) and it is about (9). We put the mixture in a tube, then put it in the centrifuge for (10) minutes, the device separates the substance from the solvent, as the substance collects at the bottom while the liquid remains at the top. Then we get rid of the liquid and throw it away. We add distilled water to the remaining substance at the bottom of the tube, and mix it well until the substance is homogeneous with the distilled water, and we put it in the device again, which is, we repeat the process twice. After separating the substance from the liquid, we get rid of the liquid, that is, we throw it away, so the substance remains in the tube. We pour it into a mold and place it in a convection oven at a temperature of (100°C) to dry. After the material dries, it becomes a powder. We grind it with a hand mortar to obtain a fine powder, and then put it in the oven again at a temperature of (550°C) degrees Celsius for two hours. After completing the process, we obtain iron oxide (Fe_2O_3). purpose of this cross-sectional study was to evaluate the immunological and metabolic states of pregnant women infected with *Lactobacillus* bacteria and healthy women 40 days postpartum. Setting: 2024, Al-Rifai Teaching Hospital, Dhi Qar Governorate, Iraq.

1. The Study Population:

This included a sample of 200 women: 100 with *Lactobacillus* infection during pregnancy and 100 as healthy controls.

- Women aged 35 to 45 years (inclusion criterion)

- o Two months (40 days postpartum).

- o A negative medical history for chronic illnesses and other endocrine disorders

- Exclusion criteria include underlying chronic, autoimmune, or hormonal diseases. Women were not allowed to participate in the study.



2. Data Collection:

- Basic demographic information such as age, weight, height, and past medical history was gathered using standard questionnaires.

Results and Discussion

XRD Analysis

X-ray diffraction (XRD) pattern of a sample of iron oxide, specifically (Fe_2O_3), as shown in Fig 1. The XRD pattern shows characteristic diffraction peaks of (Fe_2O_3). The main peaks are two peaks of maximum intensity at 33.24° (210) and 35.72° (10-1). Minor peaks are located at approximately 24.12° (110), 29.52° (202), 54.04° (312), 75° 44s (433), 62.44° (310), and 62.48° (2-1-1). Crystallinity Evaluation The sharp, well-defined peaks indicate that the sample is well crystalline. The background shows a minimally amorphous character, indicating a highly crystalline material. Phase Purity The diffraction pattern matches the standard pattern of Fe_2O_3 . Large, unindexed peaks appear, indicating relatively high phase purity. The peak positions are in good agreement with the JCPDS standard card (96-101-1241) for hematite. Crystal size analysis: The relatively narrow peak lengths indicate well-developed crystallinity of the main peaks, and XRD analysis confirms the successful synthesis of (Fe_2O_3) as the dominant iron oxide phase. The well-defined peaks and good crystallization density indicate a well-crystalline sample with minimal impurities or amorphous content.

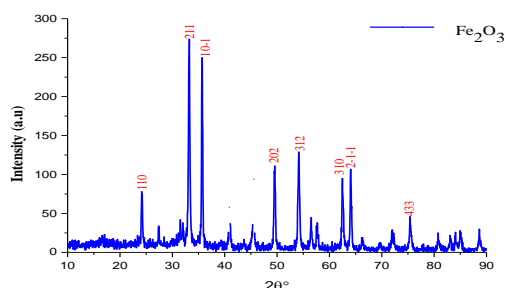


Figure 1: XRD spectra of Fe_2O_3 sample

Table1. Fabrication and Characterization of Fe_2O_3 Blends Nanoparticles.

2θ (Deg.)	FWHM	Crystallite size (nm)	Average Crystallite size (nm)
24.19	0.30	27.47	28.15
33.21	0.34	24.67	
35.68	0.25	34.03	
49.54	0.32	27.07	
54.15	0.37	24.05	
62.52	0.34	27.01	
64.08	0.30	31.76	
75.44	0.34	29.13	

3.2 The Morphological Investigations

Scanning Electron Microscope (SEM) image analysis of the Fe_2O_3 sample SEM images at different magnifications 200 nm, and 500 nm for the iron oxide sample show several important morphological properties that can be analyzed as These micrographs show a hierarchical structure of iron oxide particles with several notable features Particle morphology The primary structure consists of quasi-spherical or ovoid nanoparticles These particles show significant aggregation, forming larger secondary structures The edges of individual particles appear somewhat rounded rather than faceted These primary particles form aggregates ranging in diameter from submicron's to several microns The size distribution is relatively polydisperse, indicating possible differences in nuclear composition and growth during synthesis The particles show a strong tendency towards agglomeration, forming cluster-like aggregates Some regions show denser arrangements while other regions show more porous aggregates.

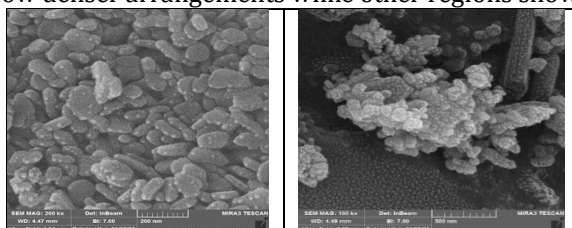


Figure 2: SEM image of Fe_2O_3 with different magnification

This a single-element Figure 3- showing the potassium-alpha emission of iron across the sample surface. The bright green spots indicate the presence and distribution of iron atoms. The distribution of iron appears relatively homogeneous but with some variations in concentration. Areas with dense clusters of green dots represent a higher concentration of iron. Some darker areas indicate areas with lower iron content. The overall coverage suggests that iron is the major component, consistent with the EDX spectrum. Fig3-b: Multi-element mapping. Elemental composite showing the spatial distribution of multiple elements on the sample surface. O (oxygen) is likely red. Zinc (Zn) is likely cyan. Fe (iron) is likely green. Cl (chlorine) is likely blue. Na (sodium) appears to be present, probably pink. Sample structure: The heterogeneous distribution suggests that this may be a mixture of different compounds (iron oxides, zinc compounds, chlorides).

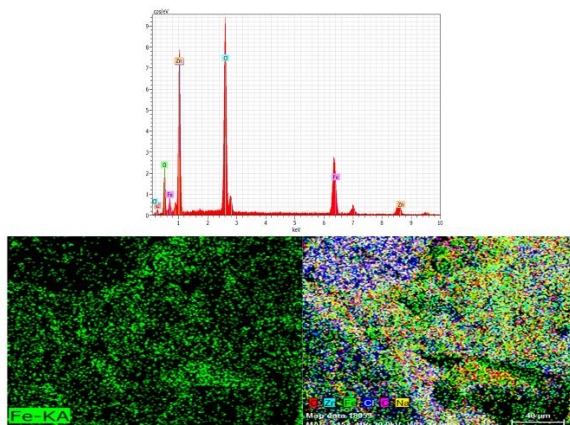


Figure 3: EDS /mapping images of Fe_2O_3

Table 2: Summarizes the map sum spectrum of the sample Fe_2O_3

Eiement	Linetype K-series	[wt.%]	[wt.%]
C	6	7.53	8.74
O	8	17.77	20.62
Na	11	13.62	15.8
Cl	17	20.37	23.64
Fe	26	17.6	20.42
Zn	30	9.3	10.79
Total:	86.19	100	100

3.3 The Optical Studies

This spectrum shows the relationship of absorbance to the wavelength of light in Figure 5-a. We can obtain the absorption spectrum from about 200 nm to 800 nm, covering the entire ultraviolet and visible region. The optical spectrum appears strongly in the ultraviolet region and along the visible optical wavelengths. The absorption decreases with the increase in the wavelength, especially after 550 nm. Also, the characteristic electronic transitions appear. Strong absorption appears in the 200-400 nm region. As a result, transfers occur from the O^{2-} (2p) orbitals to the Fe^{3+} (3d) orbitals. A broad peak is measured in the 400-550 nm region, due to the natural absorption in the 550-600 nm region. This represents the minimum flame required for electron traces from the sharing band to their bonding band. This location is responsible for the characteristic red-brown color of hematite. Figure 5-b shows the index between $(\alpha h\nu)^2$ on the vertical axis and the photon energy ($h\nu$) on the horizontal axis, where α is the absorption coefficient, h is an empty constant, and ν is the frequency of light. The use of the exponent 2 in $(\alpha h\nu)^2$ indicates the training in direct electronic transactions. Legitimate (direct transition allowed). Obtaining an energy of 2.1 to 2.2 eV. This value is assigned to all with the reference standard values for hematite- Fe_2O_3 (2.0-2.2 eV). Electronic transition properties: The clear linear part in 5-b (Figure) is part of the direct electron transition type. The overall growth indicates the crystal structure of the sample. The tendency towards the linear part reflects the quality of the crystals and the lack of crystal defects. The optical absorption properties and the clarity value clearly confirm that the sample is pure hematite Fe_2O_3 .

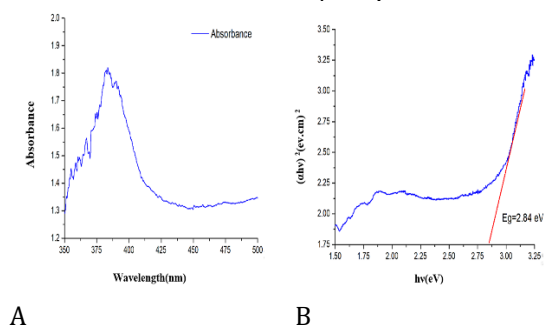


Figure 4:(A) the optical absorbance spectra against the wavelength of the as-prepared sample (Fe₂O₃), (B) Band gap energy curve of prepared (Fe₂O₃).

3.4 Biosensor application

Figure (6) and Table (3) show responses at 2 mM and 5 mM glucose that show the expected first-order rise for an enzymatic (GOx-based) amperometric sensor that transduces the H₂O₂ generated by glucose oxidation; by the IUPAC convention, the measured T₉₀ values of 26 s (2 mM) and 34 s (5 mM) denote the time to reach 90% of the new steady state after each concentration step, while the T₁₀ recovery values of \approx 2 s indicate the time to decay to 10% of the preceding plateau after switching back to PBS [34].

The slightly slower T₉₀ at the higher concentration is consistent with known kinetic/mass-transport constraints in first-generation GOx sensors—specifically, local oxygen deficit at elevated glucose and product-accumulation effects—which can slow the approach to steady state despite the larger driving force[35, 36].

The short, concentration-independent \sim 2 s recovery reflects efficient wash-out and minimal fouling within the sol-gel host, whose controlled porosity facilitates rapid diffusion of glucose and H₂O₂ to and from the enzyme layer[37, 38]. the iron-oxide phase (magnetite) possesses peroxidase-like nanozyme activity, which can accelerate H₂O₂ turnover and help both the rise and the clearance of the signal, contributing to fast, reversible transients[31, 39]. the 26–34 s response paired with \sim 2 s recovery is within the performance envelope reported for GOx glucose electrodes and indicates a well-coupled reaction-diffusion system in the sol-gel/iron-oxide architecture[40]

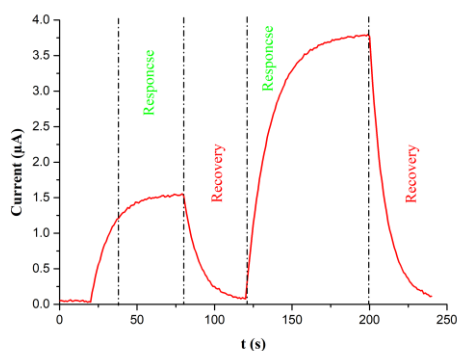


Figure 5:Current–time response and recovery of the glucose biosensor at different concentrations

Table 3: Response (T₉₀) and recovery (T₁₀) times of the biosensor for 2 mM and 5 mM glucose.

Step	Time	Value
2 mM	T ₉₀ (s)	26
2mM	T 10(s)	2
5 mM	T 90(s)	34
5 mM	T 10	2

Conclusion

The iron oxide (Fe_2O_3) expected structural (hematite) and optical (2.1–2.2 eV) signatures and, when embedded in a sol-gel host with GOx, enabled rapid, reversible amperometric glucose sensing ($T_{90} \approx 26\text{--}34\text{ s}$; $T_{10} \approx 2\text{ s}$). The short recovery and robust step responses point to a well-coupled reaction-diffusion process within the porous sol-gel/Fe-oxide layer and are consistent with state-of-the-art enzymatic glucose devices. Looking forward, work should quantify calibration performance (sensitivity, linear range, LOD/LOQ), interferences (ascorbate, urate, fructose), oxygen-dependence mitigation, and long-term stability, while leveraging Fe-oxide's established peroxidase-like activity to enhance H_2O_2 electrocatalysis.

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