



Facile Synthesis and Comprehensive Characterisation of Nanoparticles of Fe_2O_3 and Further Study of their Antimicrobial and Antimalarial Efficacy

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Abstract

Due to their unique physicochemical properties, iron oxide nanoparticles have become an interesting class of materials that can be used in various technological processes. This study highlights the production of hematite nanoparticles (Fe_2O_3) by the simple co-precipitation method using primary reactants that are iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and ammonia solution. These nanomaterials were characterized by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The XRD analysis showed that the formation of crystalline Fe_2O_3 phase was successful and it had crystallite dimensions calculated as about 40 nm. FTIR analysis showed typical Fe-O bands vibrationally, which justified the creation of pure iron oxide structures. SEM micrographs exhibited the morphology of spherical particles and were made to behave as clusters at high temperatures of calcination. Also, the synthesized Fe_2O_3 nanoparticles possessed significant antimicrobial and antifungal efficacy against specific pathogenic strains, and this should suggest their future use in biomedical and environmental remediation processes.



Article History

Received: 24 November 2025

Accepted: 30 January 2026

Keywords

Antimalarial;
Antimicrobial;
 Fe_2O_3 Nanoparticles;
SEM;
XRD.

Introduction

The field of nanotechnology has emerged as a transformative discipline with substantial implications across multiple scientific domains, including environmental science, medicine, and advanced

materials development.¹⁻³ Nanoscale materials exhibit enhanced performance characteristics compared to their bulk counterparts, primarily attributed to their reduced dimensionality and resulting unique physicochemical properties.⁴⁻⁶

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Doi: <http://dx.doi.org/10.13005/msri/230107>

Contemporary research has particularly focused on applications in targeted drug delivery systems, biosensing platforms, diagnostic imaging, and heterogeneous catalysis support materials.⁶⁻⁸

Magnetic nanoparticles represent a specialized subset of nanomaterials that demonstrate exceptional magnetic behavior at the nanoscale, distinguishing them from conventional magnetic materials.⁹ The manipulation of matter at the atomic and molecular level, typically within the sub-100 nanometer range, has enabled the development of materials with unprecedented optical, magnetic, electronic, and catalytic characteristics.^{10,11} These distinctive properties render nanomaterials invaluable for applications in environmental restoration, energy conversion technologies, biomedical interventions, and electronic devices.¹²

Iron oxide nanoparticles have garnered considerable attention due to their inherent advantages, including natural abundance, economic feasibility, chemical inertness, biocompatibility, and magnetic responsiveness.^{11,13} The polymorphic nature of iron oxides encompasses several crystalline phases, including magnetite (Fe_3O_4), maghemite ($\gamma\text{-Fe}_2\text{O}_3$), and hematite ($\alpha\text{-Fe}_2\text{O}_3$)¹⁴ [8]. Hematite represents the thermodynamically most stable configuration under standard atmospheric conditions and exhibits environmental compatibility.¹⁵ As an n-type semiconductor material with a bandgap energy of approximately 2.1 eV, hematite demonstrates suitability for photocatalytic applications, energy storage systems, photoelectrochemical processes, and sensor technologies.¹⁶

Contemporary investigations have demonstrated the antimicrobial and antifungal efficacy of iron oxide nanoparticles, which is attributed to their capacity for generating reactive oxygen species (ROS), disrupting membranes, and interfering with metabolism.¹⁷ This functionality has stimulated interest in biomedical applications, including wound care products, pharmaceutical delivery vehicles, and antimicrobial surface treatments.^{18,19} However, the biological activity of nanoparticles demonstrates strong dependence on morphological parameters, surface characteristics, and crystalline structure.²⁰

Multiple synthetic approaches have been developed for iron oxide nanoparticle production, encompassing

sol-gel processes, hydrothermal synthesis, solvothermal methods, microemulsion techniques, and thermal decomposition procedures.²¹ Although these methodologies can produce high-quality nanomaterials, they frequently necessitate elevated temperatures, extended reaction periods, costly precursor materials, or sophisticated instrumentation, thereby limiting industrial scalability and economic viability.²²

The co-precipitation method offers significant advantages as a simple, cost-effective, and environmentally benign synthetic route that operates under mild conditions while maintaining excellent control over particle characteristics.²³ This approach facilitates the production of uniform nanoparticles with tunable properties through parameter optimization.²⁴ The present study aims to synthesize Fe_2O_3 nanoparticles via co-precipitation methodology and conduct comprehensive characterization to evaluate their structural, morphological, and biological properties.

Materials and Methods

Materials

Iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 99% purity) was obtained at Sigma Aldrich. The ammonia (NH_3 , 25% w/w) solution was purchased at Merck. All experimental procedures were performed with distilled water. The chemicals were taken as received without purification.

Synthesis of Fe_2O_3 Nanoparticles

A modified co-precipitation method was used to prepare Fe_2O_3 nanoparticles.²⁵⁻²⁷ Iron(III) chloride hexahydrate (5.4 g) was used in this procedure: 100 mL of distilled water was added to the entire mass of the compound, which was under consistent magnetic stirring at room temperature and was aimed at complete dissolution and homogeneity. The iron salt solution was then added slowly but vigorously in the presence of ammonia solution to achieve a pH of about 9-10. The instantaneous formation of a reddish-brown precipitate indicated the appearance of iron oxide.^{25,26} The mixture was stirred and kept at 60°C until further precipitation and growth of particles occurred, which took 2 more hours.

The resulting product was centrifuged at 4000 rpm for 15 minutes, and then washed repeatedly with distilled water and ethanol to remove any remained

ions and unreacted species. The dried product was left in an oven at 80°C. Lastly, the dried product was then subjected to 400°C temperature during 3 hours under the air to produce crystalline nanoparticle of Fe₂O₃.

The given method of synthesis is consistent with known co-precipitation procedures, with pH, temperature, and calcination parameters being vital in avoiding particle size, crystallinity, and phase purity.²⁵⁻²⁷ The procedure outlined is simple, reproducible and can be applied to the yield of Fe₂O₃ nanoparticles with the desired characteristics in different applications.

Characterization Techniques

X-ray diffraction (XRD) was used to examine the crystalline structure and phase purity of

the synthesized nanoparticles using a Rigaku MiniFlex 600 diffractometer with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) and operating at 40 kV and 15 mA. The XRD pattern was captured in the 2θ range (10-80°) and scan rate of 2°/min.

Fourier-transform infrared (FTIR) spectroscopy was done using a PerkinElmer Spectrum 100 spectrometer in a wavenumber range of 400-4000 cm⁻¹ to identify functional groups and chemical bonding using KBr pellet technique.

Scanning electron microscopy (SEM) was used to analyze the morphology of the surface, as well as particle size distribution on a JEOL JSM-7600F field emission microscope at 15 kV acceleration voltage. Gold sputtering of samples was performed to improve the conductivity of samples before SEM analysis.

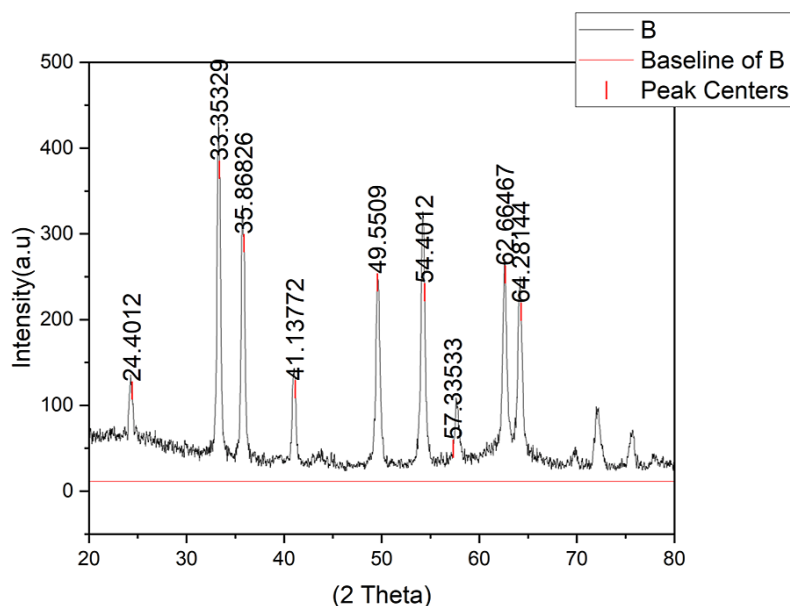


Fig. 1: XRD pattern of iron oxide nanoparticles

Antimicrobial Activity

Antimicrobial effect of the synthesized compounds was tested by using the broth microdilution method as per the guidelines of determining minimum inhibitory concentrations (MICs)^{28,29} 11017. The test compounds were produced as stock solutions in DMSO and finally showcased into the variety of broth-media to discount the variety of concentrations of interest in primary and secondary screening. To ascertain solubility of the compounds, the standard

bacterial strains were inoculated in each of the wells using DMSO as the diluent to guarantee the presence of the soluble compounds.^{29,30}

In the first screening, the compounds were tested with the concentration of 500, 250, and 200 $\mu\text{g}/\text{mL}$. Active compounds on this stage were further tested on further dilution (100, 62.5, 50, 40, 32, 25 and 12.5 $\mu\text{g}/\text{mL}$) in order to obtain accurate MIC

values. Positive controls (bacteria in the absence of antimicrobial agents) and negative controls (media only) were used in each of the assays to confirm the results.²⁸⁻³⁰ Inoculation of the microplates took place followed by incubation at 37C for 1624 hours. Defining of MIC was done in such a way that the least concentration of it did not actually show any growth of microbes at a particular point of time.²⁸⁻³⁰ Every test was conducted in a trio or at least in duplicate to provide good reproducibility. Control organisms with known susceptibility were used to test the accuracy of the drug concentrations.^{28,30} This standard method helps to compare the antimicrobial efficacy with fair

guarantees in a variety of compounds and strains of bacteria.

Results

X-ray Diffraction Analysis

XRD analysis of the synthesized material revealed the formation of pure hematite (α -Fe₂O₃) phase without detectable impurities.

FTIR Spectroscopy

FTIR analysis confirmed the formation of Fe₂O₃ nanoparticles through the identification of characteristic vibrational modes.

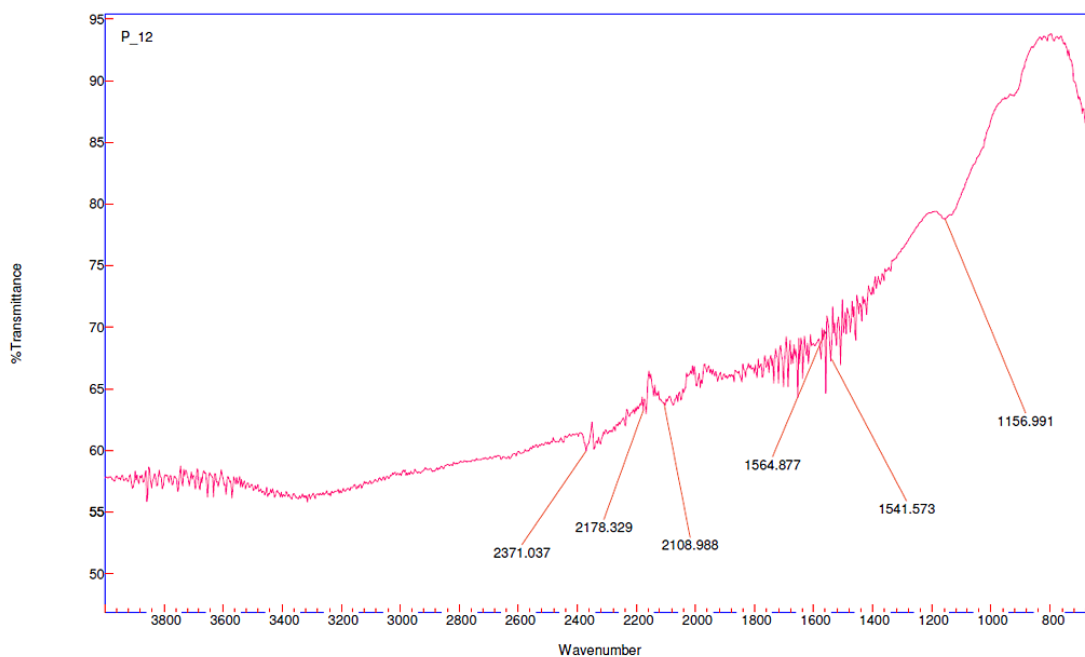


Fig. 2: FTIR of Fe₂O₃ nanoparticles

Antimicrobial Activity

The broth microdilution method was the method used to determine the antibacterial activity of the synthesized Fe₂O₃ nanoparticles, on the basis of the methods employed in our earlier study coumarin.^{31,32} The stock solutions of the nanoparticles were prepared through DMSO. The minimum inhibitory concentration values against *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1688), *S. aureus* (MTCC 96) and *S. pyogenus* (MTCC 442) were determined.

Discussion

X-ray Diffraction Analysis

XRD analysis of the synthesized material revealed the formation of pure hematite (α -Fe₂O₃) phase without detectable impurities. The diffraction pattern exhibited characteristic peaks at 2θ values of 24.1°, 33.2°, 35.6°, 40.9°, 49.5°, 54.1°, 57.6°, 62.4°, and 64.0°, corresponding to the (012), (104), (110), (113), (024), (116), (018), (214), and (300) crystal planes, respectively. These peaks match well with

the standard JCPDS card No. 33-0664 for hematite. The average crystallite size was calculated using the Debye-Scherrer equation:

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

where D is the crystallite size, K is the shape factor (0.9), λ is the X-ray wavelength (1.5406 Å), β is the full-width at half maximum (FWHM), and θ is the Bragg angle. The calculated average crystallite size was approximately 40 nm, indicating the successful synthesis of nanocrystalline hematite.

FTIR Spectroscopy

The Fourier transform infrared (FTIR) spectrum of the synthesized Fe_2O_3 nanoparticles recorded in the region of the frequency range of 4000 to 800 cm^{-1} is shown in Fig. X. The broad absorption band suggested in the region of 3400–3200 cm^{-1} is attributed to the O-H stretching vibration of surface hydroxyl and physically adsorbed water molecules. The weak band around 2371 cm^{-1} is associated with the CO_2 in the atmosphere. The bands at around 2178 cm^{-1} and around 2109 cm^{-1} could be combination or overtone vibrations from residual precursor species. The absorption bands appearing at about 1565 cm^{-1} and at about 1542 cm^{-1} are attributed to the bending vibration of the -OH groups or the traces left of nitrate residues from the synthesis process. The band at ~1157 cm^{-1} is attributed to Fe-O-H bending vibration. The characteristic Fe-O stretching vibrations of iron oxide nanoparticles are generally found below 700 cm^{-1} , thus confirming the formation of Fe_2O_3 nanoparticles. The characteristic Fe-O stretching vibrations below 600 cm^{-1} could not be clearly observed due to instrumental limitations.

Morphological Analysis

SEM examination revealed that the synthesized Fe_2O_3 nanoparticles exhibited predominantly spherical morphology with relatively uniform size distribution. The particles demonstrated a tendency to form small clusters, which can be attributed to magnetic interactions between individual nanoparticles. The average particle size observed from SEM images was consistent with the crystallite size calculated from XRD analysis. At higher calcination temperatures, increased agglomeration was observed, likely due to enhanced particle mobility and sintering effects.

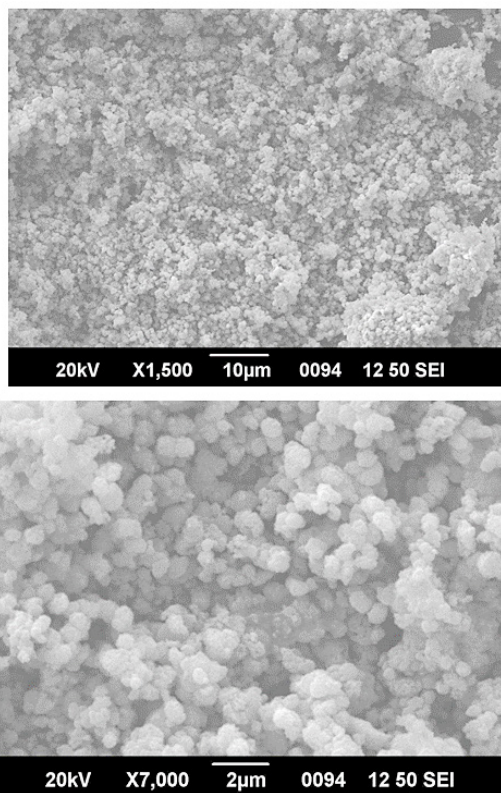


Fig. 3: SEM images of the Fe_2O_3 nanoparticles: (a) as prepared; (b) annealed sample

Antimicrobial Activity

The Fe_2O_3 nanoparticles were reported to have moderate antibacterial activity with MIC values of 100 $\mu\text{g/mL}$ towards *E. coli*, *S. aureus* and *S. pyogenus* as well as 200 $\mu\text{g/mL}$ towards *P. aeruginosa* as it was found in Table 1. Chloramphenicol, the classical antibiotic, presented lower values of MIC (50 $\mu\text{g/mL}$) against all the organisms tested, indicating that it is more potent. However, the results confirm that the ready to use Fe_2O_3 nanoparticles have enormous potential for use as antibacterial sources, and in particular when it comes to dealing with *E. coli* and *S. pyogenus*.

The broth microdilution method was used to measure the antifungal activity of the prepared Fe_2O_3 nanoparticles, and this method was reported in our previous papers.³² Nanoparticle suspensions were prepared with the help of DMSO being the solvent. The standard fungal strains (*Candida albicans*

(MTCC 227), *Aspergillus niger* (MTCC 282) and *Aspergillus clavatus* (MTCC 1323)) were compared against standard fungi to determine the minimum inhibitory concentration (MIC) values.

Table 1: Minimum inhibitory concentration of synthesized Fe₂O₃ nanoparticles against some bacterial strains

Entry	<i>E.coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S .pyogenus</i>
Fe ₂ O ₃ nanoparticles	MTCC 443 100 µg/mL	MTCC 1688 200 µg/mL	MTCC 96 100 µg/mL	MTCC 442 100 µg/mL
Chloramphenicol	50 µg/mL	50 µg/mL	50 µg/mL	50 µg/mL
Antibacterial efficiency (%)	50	25	50	50

Table 2 demonstrates that the Fe₂O₃ nanoparticles had a moderate antifungal activity with MIC values of 250 -1 g/mL against *C. albicans* and 500 -1 g/mL against *A. niger* and *A. clavatus*. Comparatively, the standard antifungal agents nystatin and griseofulvin had lower MIC values (100 µg/mL), which is more

active. However, the results observed indicate that the Fe₂O₃ nanoparticles have a strong the potential of antifungal, which can be explained by their nanoparticle size, surface reactivity, and potential interaction with fungal cell membranes to cause growth inhibition.

Table 2: Minimum inhibitory concentration of synthesized Fe₂O₃ nanoparticles against some fungal strains

Entry	<i>C. albicans</i>	<i>A. niger</i>	<i>A. clavatus</i>
Fe ₂ O ₃ nanoparticles	MTCC 227 250 µg/mL	MTCC 282 500 µg/mL	MTCC 1323 500 µg/mL
Nystatin	100 µg/mL	100 µg/mL	100 µg/mL
Greseofulvin	500 µg/mL	100 µg/mL	100 µg/mL
Antifungal efficiency(%)	40	20	20

Table 3: IC₅₀ value of synthesized Fe₂O₃ nanoparticles against *Plasmodium falciparum*

Entry	Name of compounds	Antimalarial activity (IC ₅₀ µg/ml)	Relative efficiency (%)
Fe ₂ O ₃ nanoparticles	Fe ₂ O ₃ nanoparticles	0.80 µg/mL	33.5
Standard	Quinine	0.268 µg/mL	100

Antimalarial Activity

Antimalarial activity of the synthesized Fe₂O₃ nanoparticles against *Plasmodium falciparum* cultures was tested and the values of antimalarial IC₅₀ were obtained. As indicated in Table 3 the Fe₂O₃ nanoparticles show good antimalarial activity with an IC 50 of 0.80 µg/mL as compared to the standard drug quinine, which had an IC 50 of 0.268 µg/mL.

The results showed a promising idea of the use of the nanoparticles as an antimalarial agent, despite the fact that Fe₂O₃ nanoparticles had less activity compared to the standard. This observed activity can be explained by the contact of nanoparticles with the parasitic cell membrane, which contributes to oxidative stress and damage to cellular metabolism. Increased surface area and the production of

reactive oxygen species by Fe₂O₃ nanoparticles may be crucial for preventing the development of Plasmodium species. Their effectiveness and the potential for therapeutic use in treating malaria can be improved through further research to elucidate and optimize their structures.

Conclusions

Co-precipitation of Fe₂O₃ was also achieved using a simple and affordable product co-precipitation technique that produced pure hematite phase with crystallites of nanoscale and spherical morphology. These nanoparticles have shown significant antimicrobial and antifungal properties against different pathogenic strains and this suggests that the nanoparticles have great potential in biomedical and environmental uses. The co-precipitation method was effective in the synthesis of Fe₂O₃ nanoparticles of high quality in mild conditions. Future studies must concentrate on the modification of surfaces in order to increase stability and biocompatibility and also to include expansion in the area of application, like catalysis, energy storage, and removal of contaminants in the environment.

Acknowledgments

The authors acknowledge the central instrumentation facility, SPPU, Pune, and the Sophisticated Test & Instrumentation Centre, Cochin University of Science and Technology, Cochin. The authors are grateful to the Microcare Research Laboratory, Surat, for antimicrobial evaluation.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

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Not applicable.

Author Contributions

- **Naba Shaikh:** Conceptualization, Methodology, Data Collection, Writing – Original Draft.
- **Nutan Sadgir:** Conceptualization, Methodology, Analysis, Writing Review & Editing,
- **Sunil Dhonnar:** Writing Manuscript, Writing, Visualization, Supervision

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