

# Synthesis and Characterization of Ferrous Oxide Nano particles for Antimicrobial Activity

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## ABSTRACT

In this project, a facile convenient and Frugal method of sol-gel assisted hydrothermal method was used for the synthesis of pure FeO NPs. The structural and optical morphology are inspected using various techniques of the synthesized nanoparticles and at different molar concentrations of 1, 2 and 3 moles respectively. The crystallographic morphology of the synthesized particles was studied via X-ray diffraction which revealed Rhombohedral structure of the, pure FeO NPs and the crystal size is noted to be increasing with the increase in concentration. The Scanning Electron Microscope (SEM) demonstrates the crystalline growth being proportional to the concentration of nanoparticles. The Fourier Transform Infrared Spectroscopy (FTIR) confirmed the presence of various functional groups such as O-H, C-O, C=O, and C-N groups. The elemental composition studied using Energy-Dispersive X-Ray Spectroscopy (EDX) revealed the presence of Fe and O particles and the purity of FeO NPs were noted. Optical characterization of the sample using UV-Vis-NIR Spectroscopy revealed the band gap (E) being decreased from 6.2eV to 4.7eV with the increase in molar concentration of FeO NPs. Anti-Bactericidal of the NPs was assessed at pre-defined concentrations (25 and 100 µg /ml) against Gram +ve bacteria Staphylococcus aureus, Gram -ve bacteria Fungi Candida and Escherichia coli. Bacterial strains, which demonstrate the potential of NPs. The goal of this research was to see how the structure of the synthesized NPs affected their ability to guard against harmful bacterial activities.

**Key words:** bacteria, sol-gel assisted hydrothermal method, ferrous oxide, nanoparticles.

## I. INTRODUCTION

Recent study has focused on nanoparticles, which are a dimension midway between bulk materials and atoms/molecules. In recent years, the fields of nanoscience and nanotechnology have resulted in the manufacture of many types of antibacterial nanoparticles. Antimicrobial activity, ferro fluids, magnetic storage media, magnetic resonance imaging, cancer treatments, cell sorting, and targeted drug administration are just a few of the scientific and technological applications of ferrous oxide nanoparticles. Because of their biocompatibility and magnetic characteristics, ferrous oxide nanoparticles have also been frequently used in biomedical research. Sol-gel, hydrothermal, co-precipitation surfactant mediated/template synthesis, micro emulsion, electrochemical, and laser pyrolysis are all used to make these FeO nanoparticles. The advent of novel bacterium strains resistant to currently available antibiotics has become a serious public health concern, thus there's a strong incentive to find new bactericides from various sources. Nanotechnology advancements have given an interesting method for generating alternative antibacterial medications. Despite the fact that nanoparticles have long been known to affect a wide range of microorganisms, nothing is known about iron oxide nanoparticles' toxicity to these bacteria. The current study attempted to synthesise ferrous-oxide nanoparticles using the sol-gel method, and these particles were characterised using various techniques, as well as their antibacterial activity against human pathogenic Gram-positive bacteria, with the goal of

investigating their pharmaceutical applications.

## II EXPERIMENTAL SECTION

Here, Experimenting Ferrous Oxide Material for Anti microbial Activity. We have used few synthesizing process and also in Characteristic Technique.

### MATERIALS

All of the chemicals used in the experiment were of analytical grade and obtained from standard chemical sources. Bacillus subtilis, Pseudomonas (microbial type culture collection) were obtained from Eumic analytical Lab and Research Institute, Tiruchirappalli.

### SYNTHESIS OF FEO NANOPARTICLE

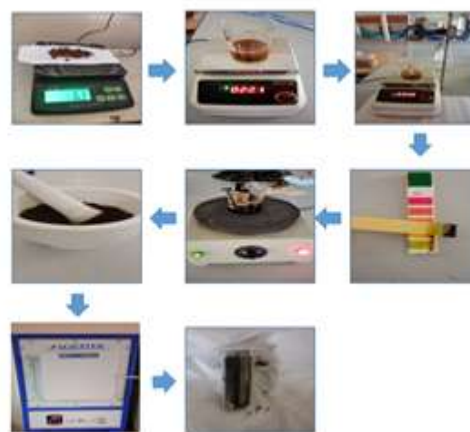
A definite amount of Raw Ferrous chloride is weighed in weighing machine with different mole concentrations such as 1, 2 and 3. The collected samples were stored in an air tight container for further synthesis. In a beaker of 100 ml, 50 ml of distilled water is measured and the sample was added then it was mixed well. The solution mixture of (Distilled water + ferrous chloride) was placed on the magnetic stirrer and then it was rotated at RPM of 650 to 750. After continuous stirring for 1 hour, a homogeneous solution was obtained. A few drops of ammonia were added to raise the Ph to 8. The influence of addition of ammonia in varying the Ph of the solution were tabulated in Table 4.1. Then the solution kept in the hot plate to maintain 80°C and solution converted into gel form. Then gel formed nano particles kept in hot temperature bath to maintain the temperature of 100°C for 6 hours.

**Table1 Influence of ammonia in varying the Ph**

Sample	Ammonia hydr oxide	Ph value approximately		Result
		Before adding ammonia	After adding ammonia	
1 Mole	25ml	10 <sup>-5</sup>	10 <sup>-8</sup>	Neutral
2 Mole	40ml	10 <sup>-5</sup>	10 <sup>-8</sup>	Neutral

organisms were introduced into molten nutrient agar plates which spread uniformly, then the wells were made on the agar plates with sterile borer and the different concentrations (25, 50, 75, and 100 µL) of the Fe (1, 2 and 3 mole) NPs and a positive

3 Mole	50ml	10 <sup>-5</sup>	10 <sup>-8</sup>	Neutral
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**Fig 1 Schematic diagram of preparative procedure**

### CHARACTERIZATION TECHNIQUES

The synthesized ferrous oxide nanoparticles are subjected to characterization. The structural, morphological, and optical examination of the prepared Feo nanoparticles is determined by scanning electron microscope. The SEM results indicate that nanoparticles are approximately spherical with the particle size in the range of 42, 27, 16 nm. EDX2 Energy Dispersive X-ray Analysis was used in order to determine the element composition that present in the samples. This result has confirmed to present in Fe and O. Fourier transform infrared (FT-IR) in the wavelength range 400–4000/cm, ultraviolet (UV)-visible spectroscopy in the wavelength range 200–1000/cm, X-ray diffraction (XRD), the average particle sizes were determined and XRD patterns with the data confirms the samples structure.

### ANTIBACTERIAL STUDY

The antibacterial activity of Fe (1, 2, and 3 mol) NPs was investigated using the well-known Kirby Bauer disc diffusion method. This technique applies to clinically isolated bacterial cultures such as Staphylococcus aureus (Gram-positive), Escherichia coli (Gram-negative), and the fungal Candida albicans obtained from the Umic Analysis Laboratory and Laboratories of Tiruchirappalli. Bacterial strains were maintained on nutrient agar slants (Hi media) at 4°C. About 100 µL of these control (reference standard Gentamicin antibiotic) were added by using a micropipette, followed by incubation at 37 °C for 24 hours. The diameter of the zone of inhibition (mm) and antibacterial

activity were measured for Fe (1, 2 and 3 mole) NPs

### III RESULT AND DISCUSSION CHARACTERIZATION OF FERROUS OXIDE

#### Scanning electron microscopy (SEM)

The nanoparticle of ferrous oxide with the size of few nanometers are clearly visible in figures suggesting a successful synthesis of ferrous oxide nanostructures. The SEM images of ferrous oxide at different morphological are shown in Figures 2 (a-c). To analyze morphological features of FeO field emission scanning electron microscope was used at an accelerating voltage of 10 KV. High resolution SEM images of as-grown low dimensional ferrous oxide NPs are presented in Figure 2 (a-c). It is clear from the SEM images that the synthesized products are NPs, which grown in a very high-density and possessed almost uniform shape presented in Figure 2 (a-c). Fig. 2 (b), exhibits the high-resolution SEM image of the synthesized NPs which reflected that most of the NPs possessing crystal shapes. The SEM results are also indicate that nanoparticles are approximately spherical with the particle size in the range of 42, 27, 16 nm. SEM images of ferrous oxide nanoparticles in which the smaller nanoparticles are observed in higher mole concentrations this results well matched in XRD results.

#### Energy Dispersive X-ray Analysis (EDX):

The purity of FeO NPs was determined via the EDX analysis. In this fig. 3 shows the EDX spectrum of FeO NPs. EDX was used in order to determine the element composition that present in the samples. Some weight errors are occur during the result revealed, because the unwanted impurities in Fe and O were  $\pm 3.93$  and  $\pm 1.83$ . Results revealed that the EDX data was composed of two elements which are Fe (69.20%) and O (30.80%). This result has confirmed to present in Fe and O. In the previous studies the mass percentage of Fe and O were 70.90% and 34.10% respectively. Finally, we get the conceptual expected mass percentage of Fe and O were 39.16% and 60.84%. Thus the EDX result revealed that the synthesized FeO NPs were of high purity, which contain high Fe and O element composition

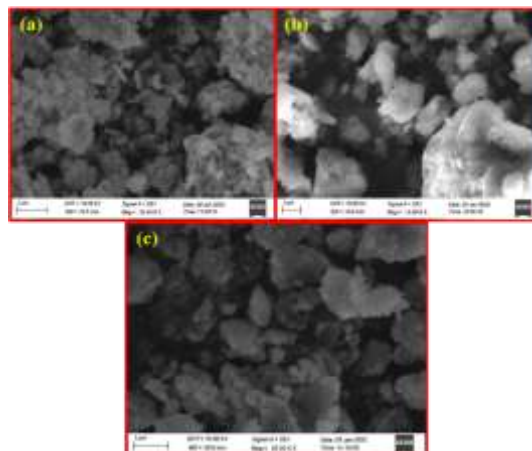


Fig 2 SEM images of the FeO nano particles with different mole concentrations. (a) 1 mole (b) 2 mole (c) 3 mole

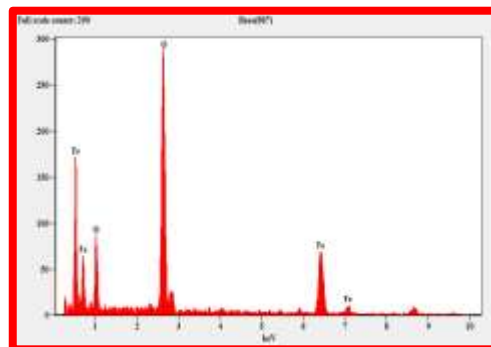


Fig 3 Elemental analysis of the FeO nano particles with 3 mole concentration

#### X – Ray Diffraction analysis

Fig 4 (a-c) shows that XRD pattern of FeO nano particles with different mole concentrations such as 1, 2, and 3 mole. The particle size of synthesized samples was measured from the peak broadening were in good agreement with the values obtained by SEM. Comparison of the XRD patterns with the data confirms the samples are with rhombohedral structure. The observed diffraction peaks at (012), (110), (104), (130), (113), (122), (214) and (441) respectively. Fig 1 (a) showed that the highest peak was positioned at  $2\theta$  ( $35.39^\circ$ ) with the intensity of 310.49 cts. Fig 1 (b) showed ferrous oxide (FeO) had a peak of  $2\theta$  ( $35.60^\circ$ ) with the intensity of 578.28 cts and also fig 1 (c) showed ferrous oxide (FeO) was positioned at  $2\theta$  ( $35.61^\circ$ ) with the intensity of 1155.06 cts. Due to the increasing intensity there was a slight shift in the highest mole concentrations of FeO NPs. From the XRD results, the average particle sizes are obtained using the Debye–Scherer equation,

$$D = 0.9\lambda/\beta\cos\theta$$

Average particles sizes of the prepared FeOnano particles were calculated as 42, 27 and 16 nm respectively for 1, 2 and 3 mole concentration. The annealing temperature one of the lead role to reducing the crystalline size for higher concentration. At lower mole concentrations, the iron oxide crystalline size became larger. The size growing and changing phase of formed ferrous oxide nanoparticles were confirmed by the SEM images. The XRD results were well agreement with SEM images

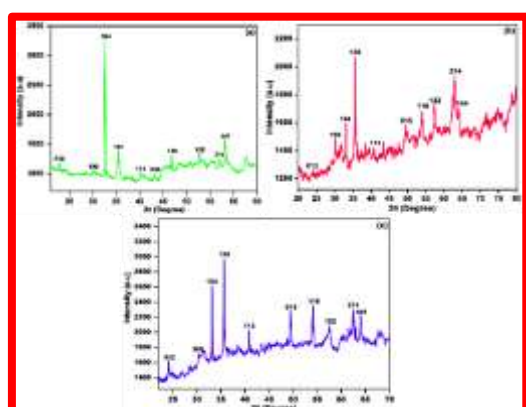


Fig 4 (a-c) XRD Patterns of FeOnano particles for different mole concentration

#### FTIR (Fourier Transform Infrared Spectroscopy)

FT-IR was performed in order to study and determine the functional groups of synthesized FeO NPs. Figure 5 (a-c) showed the FT-IR spectrum of synthesized FeO NPs. The FTIR spectra are recorded in the range of 4000 – 400  $\text{cm}^{-1}$ . Fig (a) the highest peak of 3133.36 showed the OH alcohol stretching bond vibration. Peaks found at 1733 and 1780 assigned to symmetric and asymmetric vibration of  $\text{C}=\text{O}$  of conjugated acid halides. The peaks at and 1401 and 1018  $\text{cm}^{-1}$  that indicate the vibration of C-F alkyl and aryl halides. Where a broad absorption band was observed at 581  $\text{cm}^{-1}$  that attribute to Fe-O stretching vibration. The ferrous oxide (FeO). Fig 4 (b) the highest peak of 3390 and 3212 showed the OH alcohol stretching bond vibration. A band near at 2000 - 3000  $\text{cm}^{-1}$  corresponds to stretching mode of CH alkanes group. Peaks found at 1733 and 1780 assigned to symmetric and asymmetric vibration of  $\text{C}=\text{O}$  of conjugated acid halides. The peaks at 1401 and 1294  $\text{cm}^{-1}$  that indicate the vibration of C-F alkyl and aryl halides. The peaks at 581 and 478 C-Cl alkyl and aryl halides, where a broad absorption band was observed at 581  $\text{cm}^{-1}$  that attribute to Fe-O stretching vibration. The ferrous oxide (FeO)

.Fig 3 the highest peak of 3370 and 3212 showed the OH alcohol stretching bond vibration. A band near at 2924 and 2854  $\text{cm}^{-1}$  corresponds to stretching mode of CH alkanes group. Peaks above at 1700 assigned to symmetric and asymmetric vibration of  $\text{C}=\text{O}$  of conjugated acid halides and ketones. The peaks at and 1401-1019  $\text{cm}^{-1}$  that indicate the vibration of C-F alkyl and aryl halides. The peaks at 555 and 477 C-Cl alkyl and aryl halides, where a broad absorption band was observed at 555  $\text{cm}^{-1}$  that attribute to Fe-O stretching vibration.

#### UV - Vis Spectroscopy Analysis

UV-Vis absorption spectroscopy was used to study the optical properties of Ferrous oxide (FeO) Nanoparticles. Figure 6 (a-c) reveals the absorption spectra of pure FeO film at different molar concentration of 1 mole, 2 mole and 3 moles respectively. From the figures, a strong optical absorption peak is observed below 300 nm. It is clear that the rise is molar concentration cause the wavelength to shift to slightly higher values. The maximum absorbance is observed for 3 mole FeO NPs. The absorption present near the visible range confirm the existence of some defect energy levels in the synthesized FeO Nanoparticles. The analysis of the transmission spectra allows us to access the calculation of the optical gap of FeO nanoparticles using the following relation of Tauc

$$\text{Band gap } (\alpha h\nu) = A(h\nu - E)^2$$

where  $\alpha$  is the absorption coefficient, A is a parameter depending on the transition probability, h is Planck constant,  $\nu$  the frequency of the incident photons.  $\alpha$  is determined from the relationship. In the plot of the relation (2) in Fig. 7 (a-c), we determine the gap by extrapolation of the linear part of the curve  $(\alpha h\nu)^2$  and its intersection with the energy axis gives the value of the band gap. Using this equation, the band gap energy  $E_g$  was found to be 6.2 eV, 5.8 eV and 4.7 eV for different mole concentration

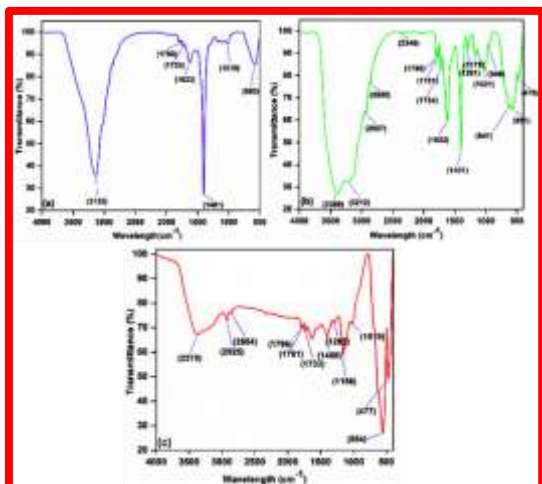


Fig 5 (a-c). FT-IR spectrum of the FeO prepared nano particles

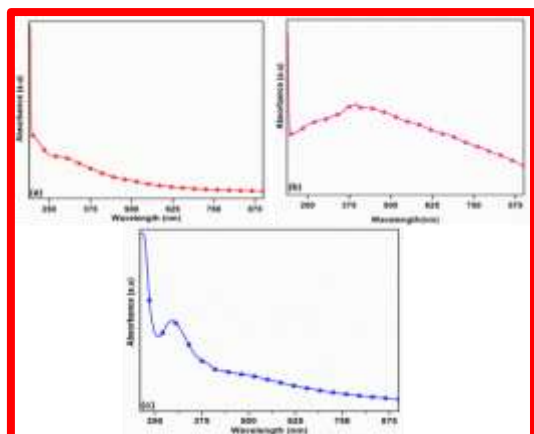


Fig 6 (a-c) Absorbance Spectrum of FeO nanoparticles obtained at different mole concentrations

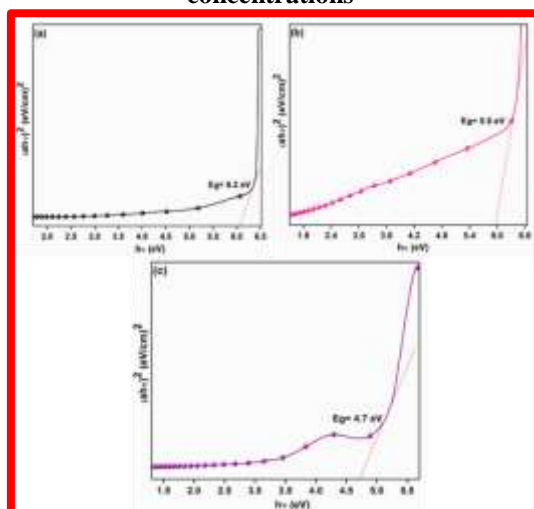


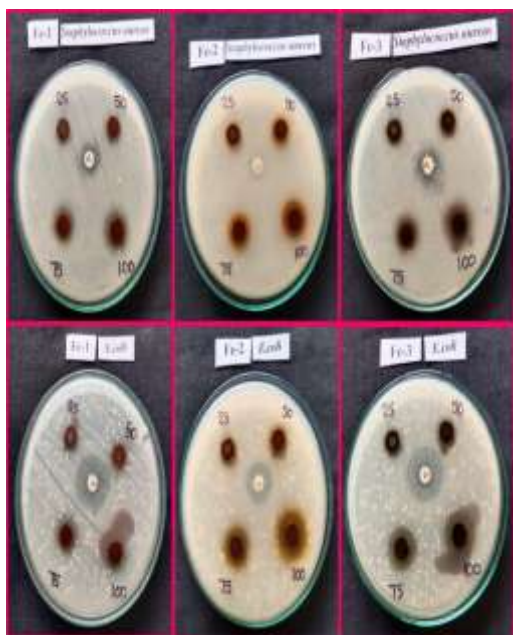
Fig 7 (a-c) Plots of  $(ah\nu)^2$  vs  $h\nu$  of FeO nanoparticles obtained at different mole concentration

### ANTIMICROBIAL ACTIVITY

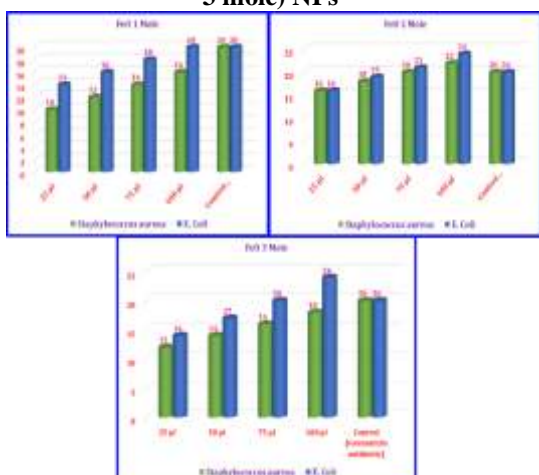
The antibacterial activity of synthesised Fe (1, 2 and 3 mole) NPs was investigated by the Kirby Bauer disc diffusion method and shown in **Figure-8**. The zone of inhibition values of Fe (1, 2 and 3 mole) NPs against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*) are shown in **Table-2**. The synthesised compounds Fe-1, Fe-2 and Fe-3 NPs are moderately effective against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*). With an increase in concentration, the activity also proportionately increases

**Table – 2**  
**Zone of inhibition values of Fe-1, Fe-2 and Fe-3 NPs against various microorganisms of *Staphylococcus aureus* and *E.coli***

FeO Mole	DMSO Extract 100 µl added and Zone of inhibition (mm/ml)				Control (Gentamicin antibiotic)
	25 µl	50 µl	75 µl	100 µl	
FeO 1 Mole	10	12	14	16	20
	14	16	18	20	
FeO 2 Mole	16	18	20	22	20
	16	19	21	24	
FeO 3 Mole	12	14	16	18	20
	14	17	20	24	



**Figure -8 : Antibacterial activity of Fe (1, 2 and 3 mole) NPs**



**Figure -9: Zone of Inhibition formed in the antibacterial activity comparison against various microorganisms of Staphylococcus aureus, and E. coli for Fe (1, 2 and 3 mole) NPs.**

#### IV CONCLUSION

We conclude that the FeO with various weight percentages was successfully demonstrated at a different concentration (1 mole, 2 mole, and mole 3) and it is effectively prepared on a large scale using the sol gel assisted hydrothermal method. The structural, optical morphology, and biological activities were investigated using various techniques like XRD, SEM, UV, FTIR, and EDX. The XRD revealed the pure FeO and rhombohedral structure of the FeO nanoparticle. No impurities were observed during XRD's detection limit. Scanning Electron Microscope (SEM)

analysis revealed that it alters the surface morphology of the pure particles when compared to the FeO Nano particles. The elemental compositions study approves the presence of Fe and O. Optical characterization of the sample revealed that the transmittance and energy band gap ( $E_g$ ) decreased from 6.2 eV to 4.7 with an increase in concentration as determined by UV-visible spectra. The Fourier-transform pure FeO nanoparticles were obtained at different functional groups. (FTIR) confirmed the presence of various functional groups such as C-O, O-H, C=O, and C-N. The compounds Fe (1, 2 and 3 mole) NPs showed highly active against gram positive bacteria (*Bacillus subtilis*) and Gram negative bacteria (*E. coli*). Fe (1, 2 and 3 mole) NPs showed high activity against to with an increase in concentration, the antibacterial activity also proportionately increases. The compounds Fe (1, 2 and 3 mole) NPs associated with antimicrobial activity agents can be a therapeutic option for the treatment of bacterial infections

#### SCOPE AND FUTURE WORK

Different mole concentrations of various doping materials utilised with FeO for the upcoming process. Advanced techniques such as TEM and XPS in morphological and structural examination, followed by reflectance in optical analysis, were used to describe the produced nanoparticles. These nanoparticles were found to be ineffective in the medical area in fighting diseases such as malignant development cancer.

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