

***IN VITRO* ANTIMICROBIAL ACTIVITY OF COBALT FERRITE NANOPARTICLES SYNTHESIZED BY Co-PRECIPITATION METHOD**

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Abstract: This report presents the synthesis of cobalt ferrite with pH 7, 8, 9 by co-precipitation method and its subsequent characterization by using X-ray diffraction (XRD). The synthesized cobalt ferrites were evaluated for their antibacterial and antifungal activity by agar-well diffusion method. The cobalt ferrite of pH 7 exhibits antibacterial effect with MIC-25 $\mu\text{g}\cdot\text{ml}^{-1}$ against gram positive *S. aureus* bacterial strain compared to standard ciprofloxacin drug (MIC-6.25 $\mu\text{g}\cdot\text{ml}^{-1}$). The cobalt ferrite of pH 8 exhibits antifungal effect with MIC-25 $\mu\text{g}\cdot\text{ml}^{-1}$ against *Candida albicans* and *Rhizopus oryzae* fungal strain compared to standard fluconazole drug (MIC-12.5 $\mu\text{g}\cdot\text{ml}^{-1}$).

Keywords: Cobalt ferrite, X-ray diffraction, agar-well diffusion, antibacterial and antifungal.

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Introduction

Magnetic nanoparticles have been broadly studied and exploited for various basic and advanced technological applications, such as catalysts, electronic and magneto-optic devices, sensors and data storage materials.^{1,2} Spinel ferrites $M(\text{Fe}_2\text{O}_4)$, where M represent metal (Co, Zn, Ni, Mn, etc).³ Cobalt ferrites are very efficient magnetic materials owing to their high coercivity, mechanical hardness, moderate saturation magnetization (80emu/g) and high chemical stability, it is regarded as a potential candidate in the field of recording media.^{4,5} In the spinel structure of Cobalt ferrites, Co^{2+} ions occupy octahedral B-sites while Fe^{2+} ions are evenly allotted among tetrahedral A-sites and octahedral B-sites.^{6,7} Cobalt ferrite is a rigid ferrimagnetic material since it has high squareness ratio, high saturation magnetization and greater magneto-crystalline anisotropy with a positive anisotropy constant.⁸

Recent advances in nanotechnology, chiefly the development of nanoparticles for drug delivery, have produced noteworthy impact in healthcare and medicine.^{9,10} Nanomaterials are used for the development of diagnostic tools like bio-imaging, bio-sensing, and many others.¹¹ In general nano range particles exhibit a higher surface area-to volume ratio. The specific surface area is significant for catalytic activity such as antimicrobial activity of nanoparticles.^{12,13} With increase in the specific surface area of nanoparticles, their biological efficiency can also increase on the account of a rise in surface energy.

The problems of multidrug-resistant (MDR) microorganisms have arrived at an alarming level in many countries around the world. The variety of clinically significant species of bacteria has developed resistance to a number of anti-microbial agents and this is becoming increasingly important

global problem.¹⁴ Due to the alkalinity and active oxygen species of CoO, they provide strong antibacterial activity.¹⁵ The antimicrobial nanoparticle formulations are increasingly investigated and many are under various stages of pre-clinical and clinical tests.^{16,17} *Candida albicans* a dimorphic fungi cause 90 % candidacy in mammals and humans.^{18,19} It is also causing nasocomial infection and approximate vaginal infection.²⁰ *Escherichia coli* is a major organism of water borne infection and nosocomial with higher mortality and morbidity rates is the other organism used in this study.²¹ Over view of literature survey, the importance of nanoparticle cobalt ferrites in the biological systems may result in antimicrobial activity.

In this present work, we report the synthesis of nano cobalt ferrite with pH 7, 8, 9 by environmental friendly, low cost co-precipitation method, the structural properties of cobalt ferrite (CoFe_2O_4) by XRD and studied the influence of pH 7, pH 8 and pH 9 on *in vitro* antibacterial and antifungal activities.

Experimental

Synthesis of cobalt ferrite

The nano cobalt ferrite (CoFe_2O_4) is synthesized by environmental friendly and low cost chemical co-precipitation method by choosing the analytical grade (molychem) $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and FeCl_3 . These precursors are weighed and mixed in desired stoichiometric ratio in distilled water. The reactants are stirred for nearly one hour to get clean solution. In order to maintain pH of 7, 8 and 9 drop wise ammonia solution is added and again stirred for one hour. The reddish solution so obtained contains precipitate which is filtered to get the cobalt ferrite using AR grade filter paper. The paste from precipitate is dried at room temperature and annealed around 800°C in muffle furnace for 5 hr which will yields the nano cobalt ferrite.^{24,25} The

process of synthesis of nano cobalt ferrite by co-precipitation method is schematically as shown in Figure 1.



The X-ray diffraction patterns of the samples have been obtained on Bruker AXS D8 Advance Diffractometer (XRD) with Cu-K α radiation, wavelength, $\lambda = 1.5406 \text{ \AA}$.

Results and discussion

XRD

The sample structure is interpreted using the XRD analysis. The XRD image of cobalt ferrite (CoFe_2O_4) at pH = 7, 8 and 9 is presented in Figure 1. The lattice parameter of series is calculated using equation (2) and tabulated in Table 1 - 4.

$$d = \frac{a}{(h^2 + k^2 + l^2)^{1/2}} \quad (2)$$

where d – interplanar spacing, a – lattice parameter and $(h \ k \ l)$ – Miller indices. Average crystalline size can be calculated by Debye-Scherrer's formula.²²

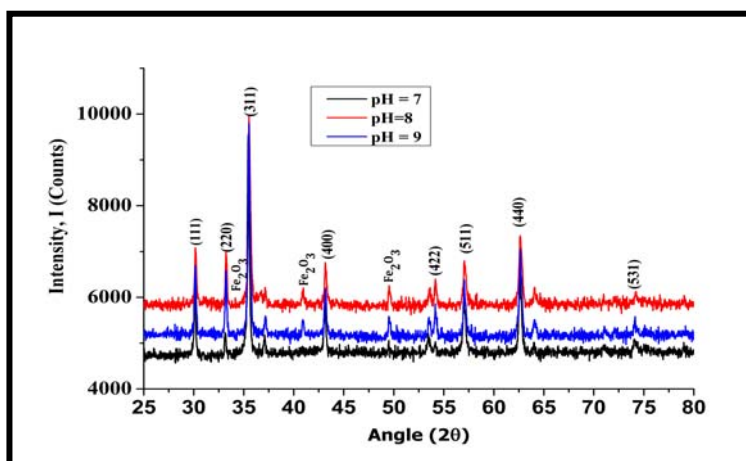


Figure 1. XRD comparative intensity plot of cobalt ferrite (CoFe_2O_4).

$$D = \frac{K \cdot \lambda}{\beta \cdot \cos \theta} \quad (3)$$

where, D- crystalline size, K- crystalline shape factor which is taken to be 0.9 in this case considering the particles to be spherical in common, λ - wavelength of X-ray used (1.5406 Å), β_D –pure diffraction broadening.

Table 1. Calculated values of Lattice parameter (a) and crystallite size (D) for CoFe_2O_4 samples (pH = 7).

Angle 2 θ	30.052	35.328	43.034	53.379	57.052	62.534
Lattice parameter (a) (Å)	8.4089	8.4242	8.4080	8.4066	8.3866	8.4004
Crystallite size (D) (Å)	442	359	348	266	280	272

Table 2. Calculated values of Lattice parameter (a) and crystallite size (D) for CoFe_2O_4 samples (pH = 8).

Angle 2 θ	30.207	35.483	43.19	54.103	57.052	62.69
Lattice parameter (a) (Å)	8.3637	8.3846	8.3728	8.2993	8.3824	8.3781
Crystallite size (D) (Å)	413	326	351	307	275	233

Table 3. Calculated values of Lattice parameter (a) and crystallite size (D) for CoFe_2O_4 samples (pH = 9).

Angle 2 θ	30.052	35.483	43.034	54.103	57.052	62.69
Lattice parameter (a) (Å)	8.4056	8.3846	8.4025	8.2993	8.3824	8.3781
Crystallite size (D) (Å)	426	319	337	435	309	279

Table 4. Calculated average values of Lattice parameter (a) and crystallite size (D) for CoFe_2O_4 samples (pH = 7, 8, 9).

pH	7	8	9
Lattice parameter (a) (Å)	8.4057	8.3561	8.3754
Crystallite size (D) (Å)	328	318	351

From X-ray figure we can observe a maximum (311 peak) at 2θ , 35.328 degrees for pH 7, 35.483 degrees for pH 8 and 35.483 degrees for pH 9 that can be attributed to spinel structure. The Miller indices (111), (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), (440) and (531) confirms the cubic phase²². The peaks in the XRD graphs at 33.3° , 40.9° and 49.5° corresponds to Hematite $\alpha\text{-Fe}_2\text{O}_3$ with JCPDS number 33-0664($\alpha\text{-Fe}_2\text{O}_3$)²³. The Crystallite size (D) of synthesized cobalt ferrites is lowest for pH 8 with 318 Å and highest for pH 9 with 351.1 Å. The lattice parameter values are highest for cobalt ferrites with pH 7 when compared with pH 8 and pH 9. The X-ray diffraction confirms the structure of cobalt ferrites.

Pharmacological screening

In vitro antibacterial screening

The nano cobalt ferrites were screened for *in vitro* antibacterial activity by agar-well diffusion method²⁴ against two Gram-positive (*Bacillus subtilis* (ATCC no. 23857) and *Staphylococcus aureus* (ATCC-29213)) and two Gram-negative (*Escherichia coli* (ATCC-25922) and *P. aeruginosa* (ATCC No. 25619)) bacterial strains. The minimum inhibitory concentration (MIC) of the synthesized compounds (1-3) and ciprofloxacin was compared, it revealed that few of the synthesized compounds exhibited good antibacterial activity against Gram-positive *S. aureus* bacterial strain. Screening results are summarized in Table 5. The best antibacterial effect was observed for cobalt ferrites of pH 7 and pH 8 with MIC $25 \mu\text{g}\cdot\text{ml}^{-1}$ each against Gram-positive *S. aureus* bacterial strain. While cobalt ferrites of pH 9 exhibited MIC $25 \mu\text{g}\cdot\text{ml}^{-1}$ against Gram-negative *P. aeruginosa* bacterial strain. The results are also represented in bar diagram of Figure 2.

Table 5. *In-vitro* antibacterial screening for compounds.

CoFe ₂ O ₄ pH	Micro-organisms used for Antibacterial Activity (MIC µg/mL)			
	Gram positive		Gram negative	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
7	25	25	50	100
8	100	25	100	50
9	50	50	100	25
Ciprofloxacin ^a	6.25	6.25	3.125	6.25

^a Ciprofloxacin was used as a positive control against bacteria species.

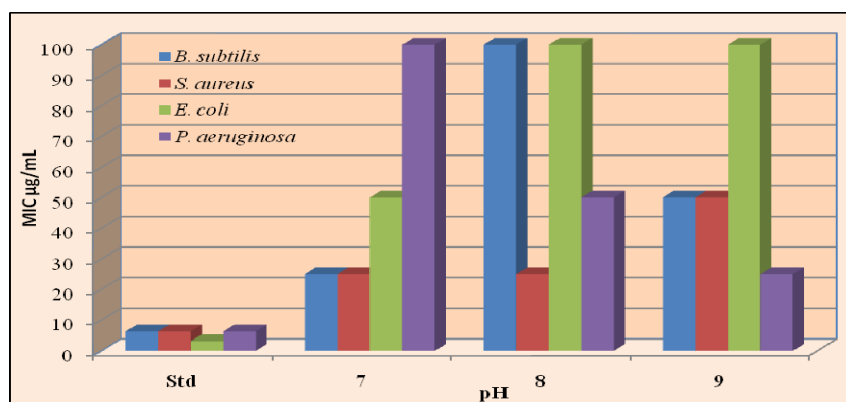


Figure 2. Graphical representation of Minimum inhibitory concentrations (MIC) (µg/mL) of all cobalt ferrites against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *P. aeruginosa*.

***In vitro* antifungal screening**

The antifungal activity was evaluated against fungal strain such as *Aspergillus fumigatus*, *Candida albicans*, *Penicillium chrysogenum* and *Rhizopus oryzae*. Fluconazole was used as a standard for the comparison of antifungal activity and Minimum inhibitory concentration (MIC) values were determined. The antifungal screening data revealed that, the synthesized compounds exhibited good antifungal activity against

Penicillium chrysogenum fungal strain. Screening results are summarized in Table 6. The best antifungal effect was observed for cobalt ferrites of pH 8 with MIC $25 \mu\text{g}\cdot\text{ml}^{-1}$ each against *Candida albicans* and *Rhizopus oryzae* fungal strain. The results are also represented in bar diagram of Figure 3. Finally, these compounds could be further optimized for future development of more potent and selective antimicrobial agents.

Table 6. *In-vitro* antifungal screening for compounds (1-3).

CoFe ₂ O ₄ pH	Micro-organisms used for Antifungal Activity (MIC $\mu\text{g}/\text{mL}$)*			
	<i>Aspergillus fumigatus</i>	<i>Candida albicans</i>	<i>Penicillium chrysogenum</i>	<i>Rhizopus oryzae</i>
7	50	100	25	100
8	100	25	50	25
9	100	50	25	50
Fluconazole ^a	25	25	12.5	12.5

^a Fluconazole was used as a positive control against fungal species.

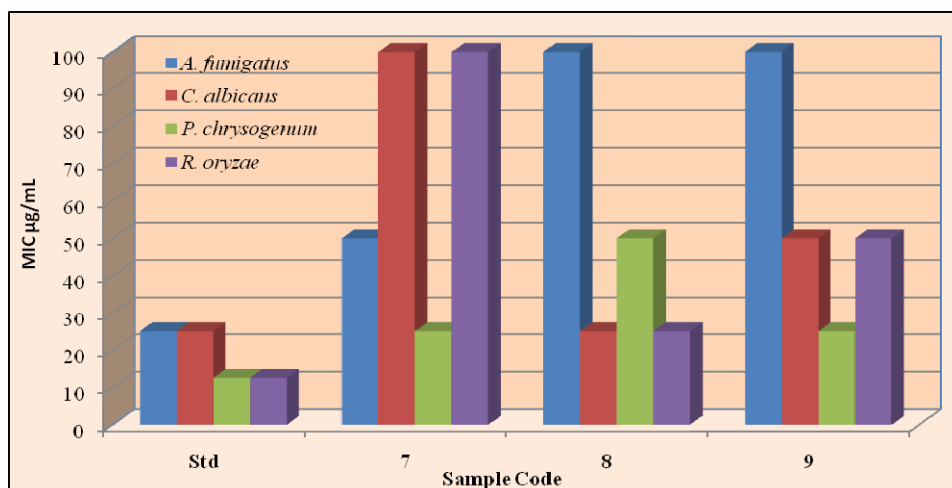


Figure 3. Graphical representation of Minimum inhibitory concentrations (MIC) ($\mu\text{g}/\text{mL}$) of all cobalt ferrites against *Aspergillus fumigatus*, *Candida albicans*, *Penicillium chrysogenum* and *Rhizopus oryzae*.

*Biological assay methods**Antibacterial activity*

The nano cobalt ferrites pH 7, 8 and 9 were screened for *in vitro* antibacterial activity by agar-well diffusion method²⁴ against two Gram-positive (*Bacillus subtilis* (ATCC no. 23857) and *Staphylococcus aureus* (ATCC-12598)) and two Gram-negative (*Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC No. 25619)) bacterial strains. In this experiment, antibiotic ciprofloxacin was used as reference standard to compare antibacterial activities. The synthesized compounds were dissolved in dimethyl sulfoxide (the stock solution 1 mg·ml⁻¹). Furthermore, the dilutions were prepared at the required quantities of 100, 50 and 25 µg·ml⁻¹ concentrations. To ensure that the solvent had no effect on bacterial growth, control test was also performed containing disc loaded with only DMSO at the same dilution used in our experiment. Test compound solutions prepared in DMSO were serially diluted and loaded (10 µl) to sterile filter paper discs (6mm diameter), which finally contained (25, 50 and 100 µg·ml⁻¹) of the compound per disc, respectively. Impregnated discs were then dried for 1h and placed on inoculated plates. The seeded plates were incubated at 37 °C for 16 h. The radii of inhibition zones (in mm) were measured and the percentage inhibition of test compounds was related to the standard drug whose zone of inhibition was taken as 100%. The results of minimum inhibitory concentrations (MICs) of the synthesized compounds against bacterial species are determined.

Anti-fungal activity

The nano cobalt ferrites pH 7, 8 and 9 were screened for *in-vitro* anti-fungal activity by agar-well diffusion method²⁴⁻²⁷ against fungal strain such as *Aspergillus fumigatus*, *Candida albicans*, *Penicillium chrysogenum*

and *Rhizopus oryzae*. In this experiment, Fluconazole was used as a standard for the comparison of antifungal activity. Minimum inhibitory concentration (MIC) values were determined using standard disc diffusion method. The tests were carried out at a concentration of $100 \mu\text{g}\cdot\text{mL}^{-1}$, $50 \mu\text{g}\cdot\text{mL}^{-1}$, $25 \mu\text{g}\cdot\text{mL}^{-1}$ and $12.5 \mu\text{g}\cdot\text{mL}^{-1}$. After 48 hrs of incubation at 37°C , the zone of inhibition was measured in millimeters. The percentage inhibition of test compounds was related to the standard whose zone of inhibition was taken as 100%.

Conclusion

In summary, we have synthesized nano cobalt ferrites with pH 7, 8 and 9 by co-precipitation method. The X-ray diffraction confirms the structure of cobalt ferrites. *In vitro* antibacterial activity assays revealed cobalt ferrites of pH 7 (MIC $25 \mu\text{g}\cdot\text{mL}^{-1}$) with highest antibacterial activity and this series of compounds also possesses selective antibacterial inhibitory activity toward Gram-positive bacteria *S. aureus* over Gram-negative bacteria. The antifungal activity showed cobalt ferrites of pH 8 (MIC $25 \mu\text{g}\cdot\text{mL}^{-1}$) with good antifungal activity.

Acknowledgments

The authors thank B.V.B. College of Engineering and Technology, Hubli, University Sophisticated Instrumentation Centre (USIC), Karnatak University Dharwad and Indian Institute of Science (IISc), Bangalore, India for providing spectral analysis.

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