ANTIFUNGAL ACTIVITY OF SILVER SUBSTITUTED COPPER FERRITE NANOPAWDER SYNTHESIZED BY SOL-GEL METHOD

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ABSTRACT

Silver doped $CuFe_2O_4$ nanoparticles prepared by sol gel process based on the citric acid at room temperature. The structure, microstructure of the Ag doped $CuFe_2O_4$ nanoparticles was studied under the effect of calcinations temperature. The prepared nanoparticles were characterized by X-ray diffraction (XRD), Fourier transform infrared spectra (FTIR) and transmission electron microscope (TEM) studies were carried out to investigate the formation of crystalline nanosized Ag doped $CuFe_2O_4$ spinel. The XRD result shows the crystalline inverse spinel structure with particle size 15.23 nm. The sharp peaks showed all-crystalline nature of single phase ferrite. The antimicrobial activity of the Ag doped $CuFe_2O_4$ nanoparticles tested against three fungus. The results show that Ag doped $CuFe_2O_4$ nanoparticles exhibit a marked degree of activity against fungus. It is clear from the tables that $CuFe_2O_4$ NPs silver doped with 1 wt%, 2 wt% and 3 wt% exhibit higher antifungal activities as compared to pure $CuFe_2O_4$. Furthermore, the antifungal result shows better inhibition for doped samples than pure samples. Ag doped Copper ferrite has high antimicrobial activity and it can also use for medicinal purpose.

Keywords: *Nanoparticles, Spinal ferrites, XRD, FTIR, Fungal Pathogens.*

Introduction:

Nanosized ferrite materials have attracted great attention in recent years due to their unique physical and chemical properties which differ significantly from their conventional counterparts [1]. Recent studies have demonstrated antimicrobial activity of various nanoparticles, including silver [2], Copper [3], titanium oxide and zinc oxide [4]. Metals and their compounds have been used as therapeutic agents from historical times until the present day. The material science research is focused on the invention of new materials with the enhanced properties. Transition metals of copper, zinc, chromium and nickel have shown very good antimicrobial properties [5]. Some researchers suggested that substitution of spinel with metals can help in enhancing the biomedical properties of the ferrite nanoparticles [6]. Magnetic nanoparticles are one of the most promising materials since they possess exceptional antibacterial properties because of their large surface area to volume ratio, which is of enthusiasm to researchers due to the developing microbial resistance against antibiotics, and the improvement of resistant strains [7 - 9]. Hence, interesting results were obtained by several methods for synthesizing nanosized magnetic spinel ferrite nanoparticles and to find its influence of dopant in magnetic and antibacterial properties, such as solid-state reaction [10], co-precipitation [11], hydrothermal [12], ceramic process [13] and sol-gel methods [14]. Among these methods, we have chosen sol-gel method, due to the fact that, with this method, significantly large amount of products can be produced within a short time. The sol–gel technique is a low temperature process which the formation of a three dimensional inorganic network [15]. As pointed out above, very few works have been found in literature on the Silver doped copper ferrite system and its antibacterial activity. Herein, we report the influence of doping on both Structural and antibacterial properties of copper-zinc ferrite nanoparticles prepared by sol-gel method.

Materials and Methods

Material

Copper nitrate tetra hydrate, Iron nitrate non hydrate and citric acid and (Ag $(NO_3)_2.4H_2O$) was obtained of analytical grade. All experiment was done by using ethyl alcohol. Undoped $CuFe_2O_4$ and Ag doped $CuFe_2O_4$ were synthesized by sol-gel method.

Synthesis of $CuFe_2O_4$ nanoparticle

Silver doped $CuFe_2O_4$ nanoparticles were synthesis by sol-gel method. All chemicals add in beaker and continuous stirring on magnetic stirrer at 80^0C for 2 hrs then form gel and heat at 131^0C in pressure bomb for 12 hrs and calcinite at temperature 350^0C.

Assay To Evaluate Antifungal Activity

Fungal Culture

Test Organism: *Aspergillus, Aspergillus flavus*

Medium Potato dextrose broth was used as a medium for Disc diffusion assay.

Preparation of Fungal Suspension

With the help of sterile wire loop, the test was inoculated into a test tube containing Potato dextrose broth. The concentration of the inoculum was adjusted to 0.5 McFarland's standards which is equivalent to 10^8 CFU/ml. This was used in assay.

Procedure

Disc diffusion assay antifungal activities of the synthesised NPs were evaluated by the standard disc diffusion method described by Bauer et al. [17] and modified according to clinical and laboratory standards institute guidelines.

1. As per the composition, 250 ml of Potato dextrose agar was prepared using sterile distilled water and it was sterilized at 121^0C at 15 lb pressure for 15 min in an autoclave.
2. The medium was cooled at room temperature and poured in sterile petri plates and were allowed to solidify.
3. Fungal culture inoculum adjusted at 0.5 McFarland's standards was swabbed over the medium using sterile cotton swab.
4. Two sterile disc were placed on each petrify plate with the help of sterile forceps and two antibiotic disc, one as positive control and other for combination. The 20 µl of sample, sample control (methanol) were poured on each sterile disc as well as on

one of the antibiotic disc (Amphotericin-B) and incubated at 27^0C in incubator for 48 hrs. This experiment carried out in triplicate set for avoiding any contamination.

5. Zone of inhibition were observed and measured with the ruler scale.

RESULT AND DISCUSSION

X-ray Diffraction Study (XRD)

Figure 1 shows the XRD patterns of copper ferrite samples prepared with various Ag substitutions (1wt %, 2 wt % and 3 wt %), annealed at 350ºC for 3 hrs. The size distribution of nano Ag doped $CuFe_2O_4$ is presented in figure 1. The diffraction peaks agree with the international standard diffraction data card JCPDS number 36-1451and provide a clear evidence of Ag doped $CuFe_2O_4$.The resulting size distribution was a good match for TEM and the XRD results. From fig.1. Ag doped $CuFe_2O_4$ nanoparticles have an average particle size of about 21nm. All peaks were consistent with the peaks of standard Ag doped $CuFe_2O_4$ with high crystalline. The diffraction planes are identified as the (111), (220), (311), (400), (422), (511), (440) with bragg angle 18.40^0, 30.00^0, 38.26^0, 44.09^0, 49.59^0, 58.22^0, 62.22^0 respectively which confirmed the formation of single phase cubic spinel structures. No impurities peaks like were observed, confirming the purity of the samples [18]. In fig. 1 (a, b, and c) showed slightly differences in their peaks, due to the various percentage of silver doping. As increases the doping percentage at the same temperature it affects on the crystalline nature of the sample. The average size of the nanoparticles can be estimated using the Debye–Scherrer equation [19].

$$D = \frac{0.89\lambda}{\beta\cos\Theta}$$

It is near about 21 nm. No peaks from any other phases of pure and Ag doped $CuFe_2O_4$ were observed. From fig. 1 it is clear that the positions of the reflection peaks for as dopen powders are almost identical to the corresponding peaks for the calcined material. This implies that the basic structure of the nano particles is essentially the same as that of the bulk material.

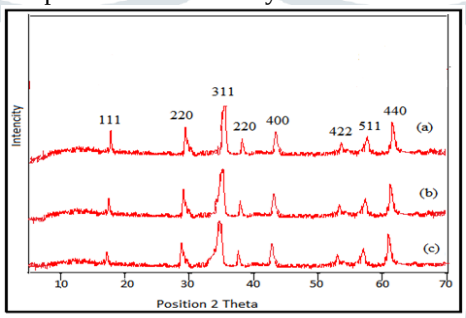

Figure 1 X-ray diffraction patterns for (a) 1 wt %, (b)2 wt% and (c)3 wt% Ag doped $CuFe_2O_4$ NPs calcinied at 350^0C.

Fourier Transform Spectroscopy (FTIR) Characterization:

The FTIR spectroscopy is used to identify their functional groups present in the ferrite composition. Fig. 2 shows the FTIR spectra in the range of 4000-400 cm^{-1} for the Ag doped $CuFe_2O_4$ samples sintered at 300^0C. The absorption band around ~3550 cm^{-1} indicates the presence of O-H group. The IR spectra show the two strong absorption bands in the range of 400 - 600 cm^{-1} typical to spinel structure characteristics, confirms that the samples prepared are spinel in structure. Normally, the higher frequency band is observed in the range of 600-500 cm^{-1}. And lower frequency band is observed in the range of 500-400 cm^{-1}. These two bands are common feature for all ferrites [20]. In the present study the absorption bands 600cm^{-1} and 400cm^{-1} for all the compositions. This reveals the formation of single-phase spinel structure having two sub-lattices tetrahedral (A) site and octahedral site [21]. In figure 2 the spectra exhibit two absorption bands at 543 and 443 cm^{-1}. These spectra represent characteristic features of ferrospinels of tetrahedral and octahedral M-O stretching frequency. The characteristic band at 1373 cm^{-1} is ascribed to the symmetric vibration of NO_3- group. The absorption peaks corresponding to 997 and 710 cm^{-1} are related to the presence of Fe ions in ferrites. The band at 3842.20 cm^{-1} shows O-H stretching and 2972.94 cm^{-1}.

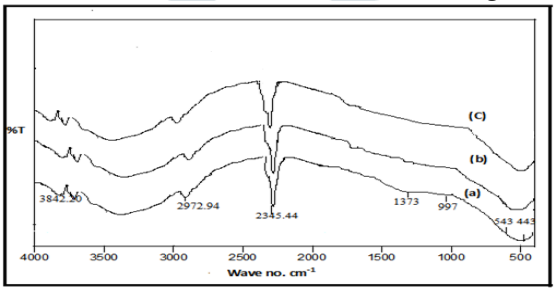

Figure 2 FTIR Spectra for (a) 1 wt% (b)2 wt% and (c) 3 wt% Ag doped $CuFe_2O_4$ NPs calcinate at 350ºC.

Transmission Electron Microscopy (TEM) Characterization:

The detailed morphology and crystalline structure of the undoped $CuFe_2O_4$ and Ag doped $CuFe_2O_4$ calcined at 350^0C for 3 hrs were further investigated by TEM, and the TEM bright- field images with corresponding selected area electron diffraction. Transmission electron microscopy images of the undoped $CuFe_2O_4$ and Ag doped $CuFe_2O_4$ samples are depicted in figure- 3 (a, b, c and d) respectively. From the TEM images, the pure and Ag -doped $CuFe_2O_4$ nanoparticles are nearly in equal size of 21 nm. The evaluated particles size from the XRD pattern, which were in good agreement with the TEM results. In figure the corresponding selected area electron diffraction (SAED) pattern indicates the crystalline and preferential orientation of the Ag doped $CuFe_2O_4$ samples pattern without any additional diffraction spots of Ag and Cu clusters, and which is in good agreement with the Quartzite structure of the XRD results and the standard data card JCPDS 36-1451.

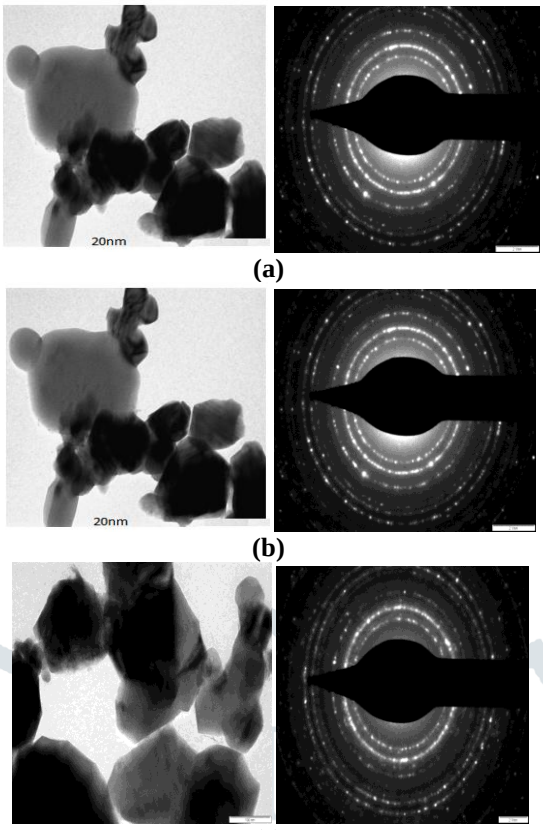

Figure 3 TEM images with corresponding SAED patterns of the (a) 1 wt % (b) 2 wt % and (c) 3 wt %Ag doped $CuFe_2O_4$ NPs calcinate at 350^{0}C.

ANTIFUNGAL ACTIVITY

Antifungal activity of Ag doped (1 wt%, 2 wt% and 3wt%) $CuFe_2O_4$ NPs at 350^{0}C calcination temperature by using Disc diffusion method

Disc diffusion assay Antifungal activities of the synthesized NPs were evaluated by the standard disc diffusion method and modified according to clinical and laboratory standards institute guidelines. The antimicrobial activities of pure and doped $CuFe_2O_4$ NPs are carried out against three fungal pathogens i.e. *Aspergillus flaves* and *Aspergillus niger*. The zone of inhibition is given in tables 1 and figure 4 (a,b,c) and fig.5 (a,b,c). It is clear from the tables that $CuFe_2O_4$ NPs silver doped with 1 wt%, 2 wt% and 3 wt% exhibit higher antifungal activities as compared to pure $CuFe_2O_4$. The experiment results indicate that doping in the nanomaterials plays a significant role in the antimicrobial activity. Thus, in this report, silver doped $CuFe_2O_4$ NPs have shown the best antifungal behavior compared to $CuFe_2O_4$ NPs. Our results are well supported by the earlier studies reported that transition metal enhances the antifungal activity. The obtained results indicated that active oxygen species generated from transition metal oxides of pure and doped ferrites have more potential to penetrate the cell wall and decrease the cell wall division. Furthermore, the antifungal result shows better inhibition for doped samples than pure samples. The results reported here are better than the previous reports [22]. With an increase in concentration of doping antimicrobial activity increased. Our data are in accordance with the previous studies, dealing with the antimicrobial effects of NPs [23]. If the concentration of doped metals in nano- $CuFe_2O_4$ increases in culture medium, interaction between oxygen and dehydrogenise increases too which enhances antimicrobial activity [24]

Aspergillus Niger

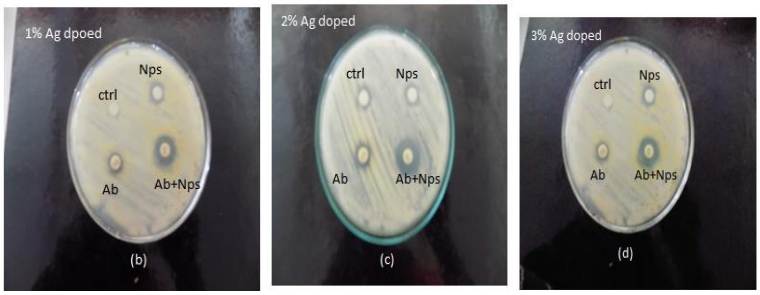

Figure 4 Zone of inhibition of the antifungal activity of (a) 1 wt%, (b) 2 wt% and (c) 3 wt% doped Ag in $CuFe_2O_4$, against A. Niger.

Aspergillus Flaves

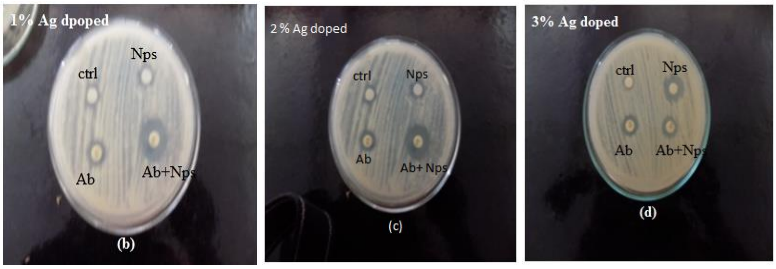

Figure 5 Zone of inhibition of the antifungal activity of (a) 1 wt% doped Ag, (b) 2 wt% and (c) 3 wt% doped Ag in $CuFe_2O_4$, against A. Flaves.

Fungal pathogenes	1wt% Ag doped	2 wt% Ag doped	3 wt% Ag doped
A. flavus	17mm	19mm	21mm
A. niger	15mm	18mm	20mm
Control	8mm	6mm	8mm

Table 1 Zone of inhibition of the antifungal activity of 1 wt%, 2 wt% and 3 wt% Ag doped $CuFe_2O_4$ against fungal pathogens.

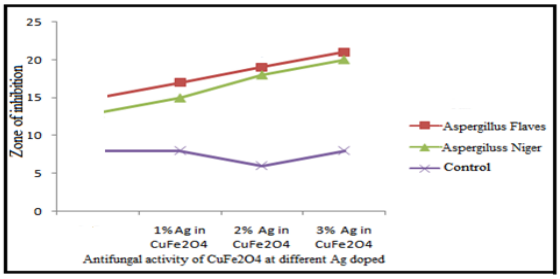

Figure 6 Antifungal activities of 1 wt%, 2 wt% and 3 wt% Ag doped $CuFe_2O_4$ against fungal pathogens.

Fungal pathogens	1 wt% Ag doped	2 wt% Ag doped	3 wt% Ag doped
A.flavus			
Antibiotic	22mm	23mm	24mm
Antibiotic + Nps	23mm	24mm	25mm
A. niger			
Antibiotic	20mm	22mm	23mm
Antibiotic + Nps	21 mm	23 mm	25mm

Table 2 Zone of inhibition of the antifungal activity of 1 wt%, 2 wt% and 3 wt% Ag doped $CuFe_2O_4$ with Antibiotic.

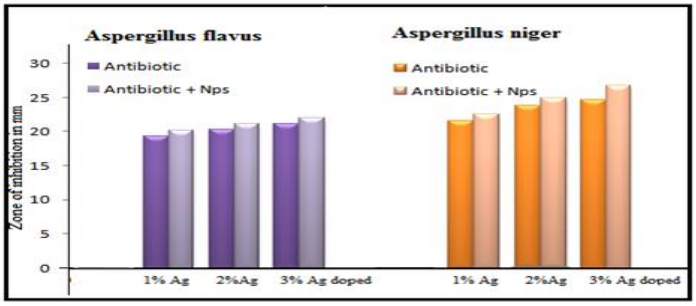

Figure 7 Antifungal activity of antibiotic and antibiotic + Nps against Aspergillus flavus and Aspergillus niger.

CONCLUSION

Influence of 1wt%, 2 wt% and 3 wt% Ag doped copper ferrite synthesized by sol-gel method. The XRD confirmed that all the peaks belong to the single-phase spinel cubic structure with no impurities of secondary phases of $CuFe_2O_4$. From FTIR vibrations shows octahedral and tetrahedral sites in the spinel structure, respectively. TEM images show most of the nano particles to be spherical in shape and agglomerated. The particle sized is 21nm and it calculated from XRD and cinfirmed by TEM. Further, this tested against different fungal pathogens and observed that due to doping of silver it shows excellent inhibition zone at 3 wt% Ag doped $CuFe_2O_4$ NPs.

ACKNOWLEDGEMENT

The authors are also indebted to Principal, Dr. V. G. Thakare, Shri Shivaji Science College Amravati, India for his kind cooperation during this research work. Authors gratefully acknowledge Institute of pharmaceutical Education and Research, Borgaon, Dist. Wardha for the support to carry out the antibacterial activity.

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