ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Structural and Antibacterial Properties of Silver Substituted Cobalt Ferrite Nanoparticles.

Sheena Xavier[1], Harry Cleetus[2], Nimila PJ[2], Smitha Thankachan[1], Rintu Mary Sebastian[1] and Mohammed EM[1]*.

[1]Research Department of Physics, Maharaja's College, Ernakulam, Kerala 682011, India.
[2]Research Department of Zoology, St. Albert's College, Ernakulam, Kerala 682018, India.

ABSTRACT

In the present study, silver substituted cobalt ferrite nanoparticles ($Co_{1-x}Ag_xFe_2O_4$, x=0.0, 0.025, 0.05, 0.075, 0.1) have been synthesized by sol-gel technique. The X-ray diffraction analysis shows the formation of the spinel structure in all the samples. The crystallite size of the samples was calculated from the prominent diffraction peak using Scherrer formula and it was found to be in the 14 to 20 nm range. The lattice parameter increases initially for x=0.025 and decrease thereafter. The antibacterial activity of the nanocomposite materials was investigated against selected Gram negative and Gram positive bacterial strains. An enhancement in the activity is observed with the addition of silver into cobalt ferrite nanoparticles. These materials with improved structural and antibacterial properties can find applications in therapeutics, textile industry and hygiene.

Keywords: Cobalt ferrite; silver doping; lattice parameter; crystallite size; antibacterial activity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Nanocrystalline ferrites have been the focus of intense research for more than a decade due to their unusual structural, magnetic and electrical properties. A resurgence of interest in magnetic nanoparticles is observed in recent years due to their promising applications in biomedicine. Ferrite nanoparticles offer potential applications in a variety of biomedical fields such as magnetically guided drug delivery, magnetic hyperthermia and magnetic resonance imaging [1-3]. These applications depend on the properties of ferrite which in turn are influenced by preparation conditions, size and shape of the nanoparticles. Therefore, based on the application an appropriate synthesis method has to be selected to achieve specific performance. Several physical and chemical methods are available for the synthesis of ferrite nanoparticles. The sol – gel technique allows good control over stoichiometry and produce nanoparticles with small size and narrow size distribution [4, 5]. Hence this method is selected for the synthesis of cobalt ferrite nanoparticles. Cobalt ferrite is an important magnetic material and is characterized by its high coercivity, moderate saturation magnetization and very high magneto-crystalline anisotropy. These properties along with their physical and chemical stability make them suitable for several technological applications [6, 7].

The application of magnetic nanoparticles as antimicrobial agents is gaining importance due to the fact that they can be easily manipulated by an external magnetic field. The iron oxide nanoparticles have been synthesized and tested for various applications in medicine such as magnetic hyperthermia, targeted drug delivery and bactericides [8-10]. Among the different ferrites, cobalt ferrite has special magnetic and physical properties which lead to its wide applications in medicine. The biomedical and clinical applications of silver nanoparticles are well established in the literature [11-13]. The addition of silver to cobalt ferrite will provide a new composite material with good magnetic behaviour and enhanced antimicrobial activity. Okasha et al. [14] studied the effect of silver substitution in magnesium ferrite and observed an enhancement in its thermal and electrical conductivity. Sun et al. [15] have shown that the biological activity of Fe_3O_4 nanoparticle was improved by coating a thin film of silver onto it and the magnetic properties were helpful in the recovery of the material from the site of action. The antibacterial activity of $Ag@CoFe_2O_4$ nanocomposite was examined by Kooti et al. [16] and they compared these results with that of silver and some antibiotics. Yamamoto et al. [17] enumerated the influence of particle size on the antibacterial activity of ZnO nanopowders and showed that the activity increased with decreasing particle size.

The evaluation of magnetic nanoparticles for targeted drug delivery, imaging and sensing applications are widely studied. However, the use of cobalt ferrite nanoparticles as antimicrobials against pathogenic and drug resistant microbes are not well investigated. Therefore, in the present study, attempts have been carried out to synthesize silver doped cobalt ferrite nanoparticles, hoping to achieve improvement in the antibacterial activity of cobalt ferrite. The $Co_{1-x}Ag_xFe_2O_4$ samples have been prepared by varying the doping concentration of silver from 0 to 10%. The structural characterization confirms the formation of spinel structure in all the samples. The biocidal activity was tested on Gram negative (*Escherichia coli, Pseudomonas aeruginosa and Serratia marcescens*) and Gram positive (*Staphylococcus aureus*) bacterial strains. The present investigation deals with the study of structural and antibacterial properties of silver substituted cobalt ferrite nanoparticles synthesized by sol-gel technique.

MATERIALS AND METHODS

Synthesis

Nanoparticles of silver substituted cobalt ferrite ($Co_{1-x}Ag_xFe_2O_4$, x=0.0, 0.025, 0.05, 0.075, 0.1) were synthesized by a facile and rapid sol-gel technique. Stoichiometric ratio of cobalt nitrate, silver nitrate and ferric nitrate (AR grade MERCK) were dissolved in minimum amount of ethylene glycol using a magnetic stirrer. The solution was heated at 333K until a wet gel of the metal nitrate was obtained. Further heating of the gel at 473K resulted in the self ignition and a highly voluminous and fluffy product is obtained. This powder was ground well using an agate mortar. The as synthesized powders were labelled as CA0, CA1, CA2, CA3 and CA4 based on the amount of silver in it.

The silver substituted cobalt ferrite samples were characterized by using X-ray powder diffractometer (XRD, Bruker AXS D8 Advance) using Cu-K_α radiation (λ = 1.5406 Å) at 40 kV and 35 mA. The optical density

(OD) measurements were made using a UV-visible spectrophotometer (VIS DOUBLE BEAM SPECTRO 1203, SYSTRONICS). A digital colony counter was used to determine the viable count of the bacterial strains.

Antibacterial study

This work explored the antimicrobial properties of as synthesized silver doped cobalt ferrite nanoparticles against microbes using solid agar plates and liquid broth medium. Various microorganisms *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Serratia marcescens (S. marcescens))* and *Staphylococcus aureus (S. aureus)* were used to evaluate the antimicrobial effect of the nanoparticles. The minimum inhibitory concentration of the cobalt ferrite nanoparticles was estimated by agar diffusion method and a test concentration of 1mg/ml was selected for the study. The aqueous suspension of nanoparticles was introduced into sterilized nutrient broth tubes and freshly grown bacterial cells were inoculated into the medium. These cultures were placed in a shaker at 200 rpm and then incubated at 310 K for 24 hours. A control (nutrient broth and culture only) and a blank (nutrient broth and nanoparticle only) were also prepared. The optical density (OD) measurements were made using a UV-visible spectrophotometer at 600 nm and the background was eliminated by taking blank readings. In order to determine the number of viable bacteria, the diluted overnight cultures were introduced into sterile petriplates containing the nutrient medium. These plates were placed in a shaker and then incubated at 310 K for overnight. The number of colony forming units (CFU) was counted using a digital colony counter. The survival percentage of the organisms was calculated using the following formula [3],

$$Survival\,(\%) = \frac{No.\,of\ CFU\ in\ the\ medium\ treated\ with\ nanoparticle}{No.\,of\ CFU\ in\ the\ control} \times 100 \qquad (1)$$

The qualitative assessment of the antibacterial effect was done using agar diffusion test. The sterilized nutrient medium was poured into agar plates and allowed to dry. Test cultures were inoculated over the dried surface of nutrient agar plate. For this the surface of the agar plate was seeded with the bacterial strain by streaking using L rod. This process was repeated three times, and then the plate was rotated to ensure an even distribution of inoculums. The appropriate number of wells was bored into the plates using sterile cork borer of 6mm in diameter. Cobalt ferrite nanoparticles with varying silver content were added to these wells and incubated for 24 hours. The contact biocidal property can be determined by measuring the diameter of the zone of inhibition (ZOI) around the well. The diameters of the ZOI for all the nanoparticles against the selected microbes were determined in the similar manner.

All the experiments were repeated three times and average values are presented to ensure the reliability of the results.

RESULTS AND DISCUSSION

Structural properties

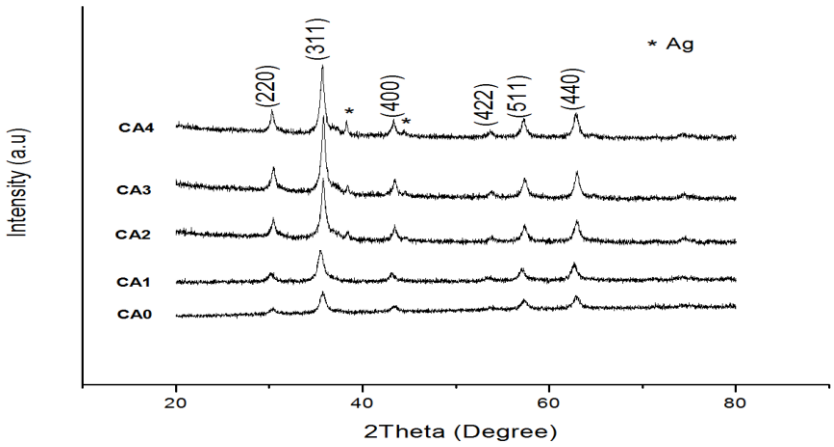

Figure 1: XRD pattern of $Co_{1-x}Ag_xFe_2O_4$

The XRD patterns of $Co_{1-x}Ag_xFe_2O_4$ nanoparticles are depicted in Fig. 1. The results obtained from XRD data agrees well with the standard values of cobalt ferrite (JCPDS PDF Card No.22-1086). The diffraction peaks corresponding to (220), (311), (400), (422), (511) and (440) reflection planes show that all the samples have attained cubic spinel structure. But, from x=0.05 onwards additional peaks are emerged which indicates the presence of metallic silver (JCPDS PDF Card No.04 - 0783).

The lattice parameter *a* for all the samples has been calculated using the following equation [18]

$$a = d_{hkl}\sqrt{h^2 + k^2 + l^2}, \quad (2)$$

where d_{hkl} is the interplanar spacing for the prominent peak indexed with (311). The lattice parameter of all the samples is given in Table 1. Though coarse grained cobalt ferrite exhibits inverse spinel structure, in the nano-regime it is reported that they adopt partially inverse structure. Therefore, both the divalent (Co) and trivalent (Fe) ions occupy the tetrahedral and octahedral sites of the spinel lattice. The lattice parameter of silver substituted cobalt ferrite is observed to increase initially and then it decrease for higher silver concentrations. An increase in lattice parameter with increase in silver content is expected because of the large ionic radius of Ag^{2+} (0.108 nm) compared to that of Co^{2+} (0.072 nm) ion. The unit cell of the spinel cobalt ferrite expands to accommodate larger silver ions so that lattice parameter increases. A possible explanation for the decrease in the lattice parameter for x > 0.025 can be the compression of the spinel lattice by the secondary phase formed on the grain boundaries. Similar observation was reported in the case of gadolinium doped lithium nickel ferrites [19]. Due to large ionic radius, silver ions cannot diffuse in the spinel lattice and hence they form secondary phases on the grain boundaries. For smaller concentration of silver such as x=0.025, it can be presumed that the cobalt ions in the octahedral sites are replaced by silver ions. However, when the concentration increases, the aggregation of silver occurs on the grain boundary and further expansion of the spinel lattice is hindered. Beyond x=0.025, most of the silver remains as metallic silver and this is evident in the XRD pattern. The study on silver doped $MgFe_2O_4$ nanoparticles showed the formation of metallic silver phase and suggested that iron ions change their valence from Fe^{3+} to Fe^{2+} to achieve charge neutralization [14]. Further investigations are needed to confirm the factual distribution of cations in silver doped cobalt ferrite samples. The crystallite size *D* of the samples has been estimated from the broadening of XRD peaks using the Scherrer equation [20].

$$D = \frac{0.9\lambda}{\beta \cos\theta}, \quad (3)$$

where λ is the X-ray wave length, β is the full width at half maximum (FWHM) and θ is the Bragg angle. It can be seen from Table 1 that the crystallite size of the samples increases with silver ion concentration. The X-ray (actual) density of the un-doped sample is in agreement with that reported for bulk cobalt ferrite.

Antibacterial Properties of $Co_{1-x}Ag_xFe_2O_4$ nanoparticles

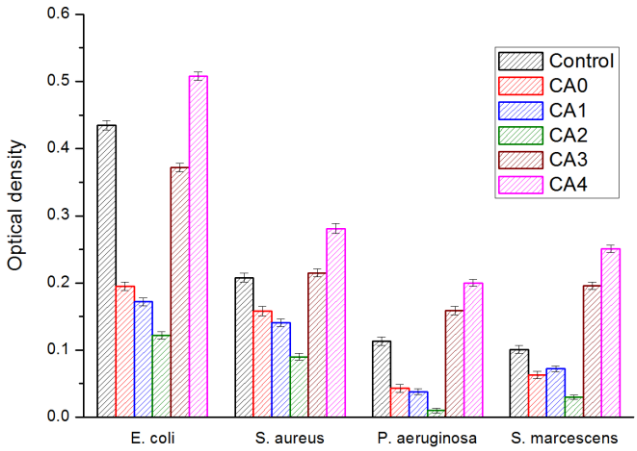

Figure 2: Optical density measurements of $Co_{1-x}Ag_xFe_2O_4$ nanoparticles

ISSN: 0975-8585

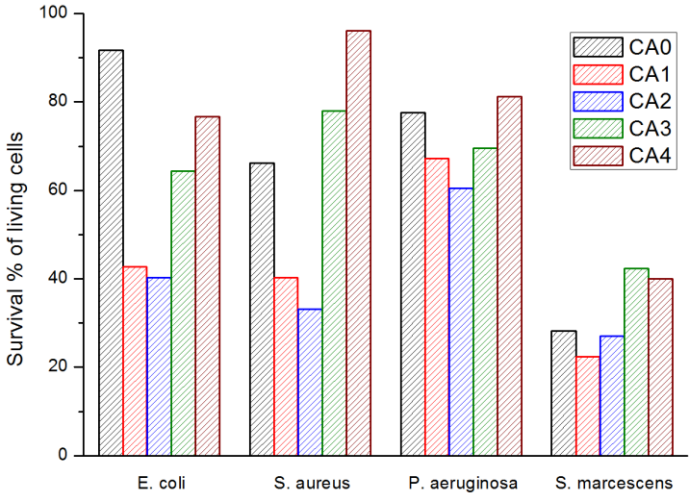

Figure 3: Survival % of living organisms

In this work, we have analyzed the antimicrobial efficacy of silver substituted cobalt ferrite nanoparticles against various microbes using liquid broth and plate based growth studies. The minimum inhibitory concentration in the present study was observed to be 1mg/ml. The optical density and viable count measurements of the bacterial strains in the culture media were examined as a function of silver content and these results are depicted in Fig.2 and Fig.3 respectively. From the OD measurements, it was observed that the antibacterial activity improved with increase in silver content up to a silver concentration of x=0.05.

In this work, we have analyzed the antimicrobial efficacy of silver substituted cobalt ferrite nanoparticles against various microbes using liquid broth and plate based growth studies. The minimum inhibitory concentration in the present study was observed to be 1mg/ml. The optical density and viable count measurements of the bacterial strains in the culture media were examined as a function of silver content and these results are depicted in Fig.2 and Fig.3 respectively. From the OD measurements, it was observed that the antibacterial activity improved with increase in silver content up to a silver concentration of x=0.05.

The viable cell measurements of the various organisms demonstrated that the number of recovered bacteria was reasonably less than that observed in the control. However, when the silver content exceeds beyond x=0.05, a stimulatory effect in the bacterial growth was observed in both the studies. Several authors reported that the nanoparticles exhibit particle size dependant antibacterial behaviour [21, 22]. The small size allows the nanoparticles to cross the cell wall of the bacteria disrupting the cell membrane and leading to cell death. Also the degree of dispersion of nanoparticles in water plays an important role in the antibacterial mechanism and it increases with decrease in particle size. Therefore, the improved antibacterial properties of the samples with smaller silver content can be attributed to the large surface to volume ratio which provides them better contact with the bacterial cell.

The antimicrobial activity of the silver doped cobalt ferrite nanoparticles was qualitatively measured by performing agar diffusion test against all microorganisms. The diameters of the ZOI are determined and these are tabulated in Table 1. The absence of growth around the nanoparticles is an indirect measure of the ability of the material to inhibit the growth. The ZOI produced by the nanoparticles against the Gram negative microbe *E. coli* and the Gram positive bacteria *S. aureus* are shown in Fig. 4 and Fig. 5 respectively. The results obtained from this study revealed that the samples efficiently inhibit the growth of the microbes. It can be seen that the contact biocidal property of the pure cobalt ferrite sample is insignificant compared to that of silver doped samples. The efficacy of the nanoparticles increased with silver content. The biocidal activity exhibited by the samples depends on the particle size and the silver content. It is well known that in the nano regime, the bactericidal activity exhibited by the smaller particles is higher than bigger particles. The activity shown by CA2 is higher than other samples and this may be due to the better contact of the nanoparticles with the bacterial cell membrane.

ISSN: 0975-8585

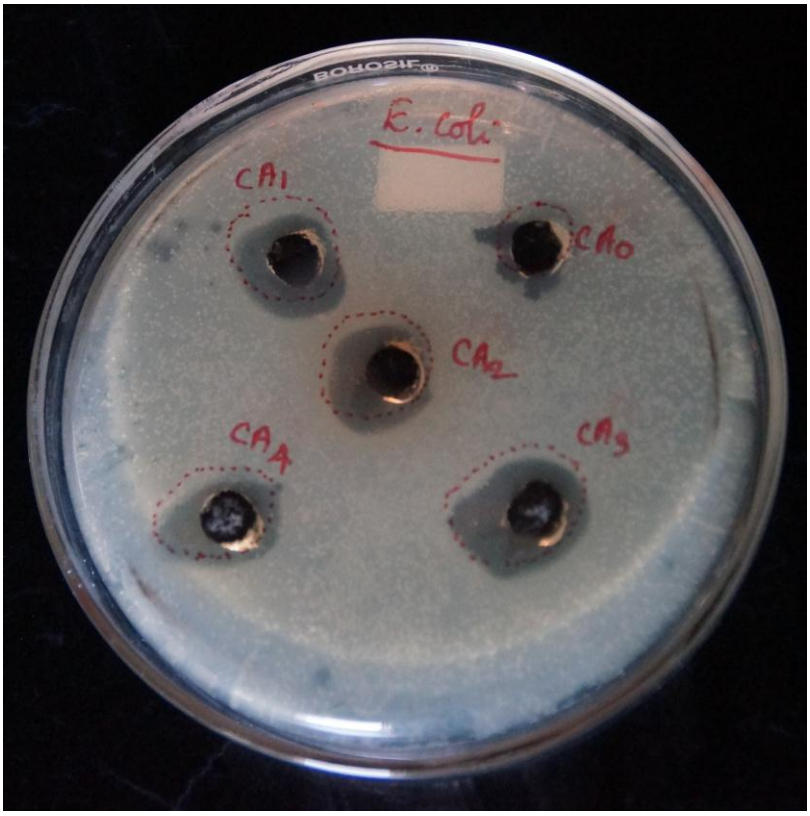

Figure 4: Agar diffusion assay for E. coli

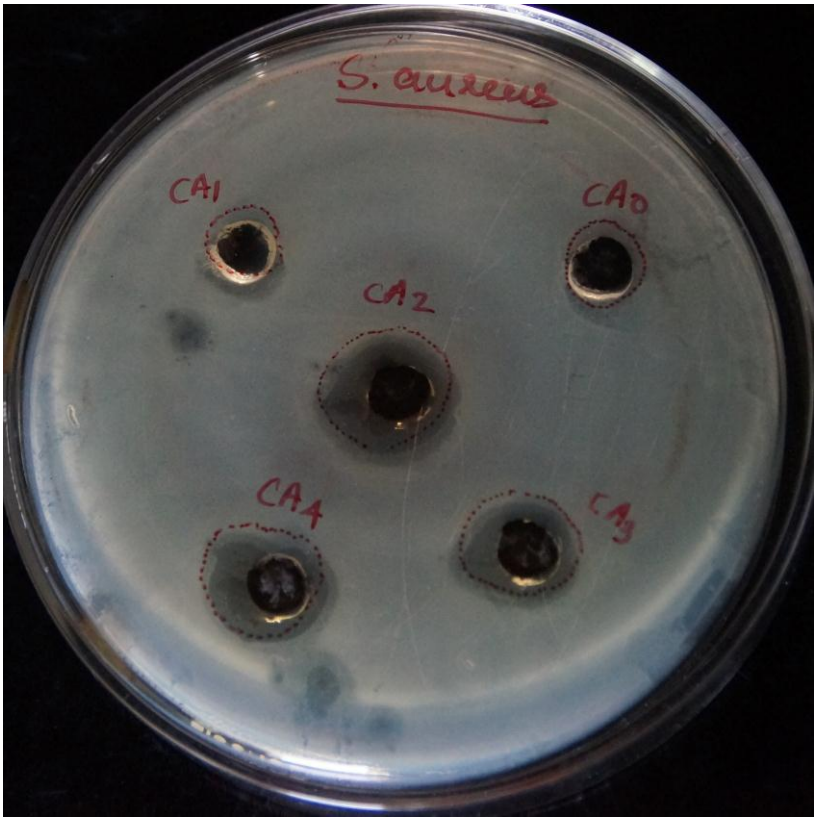

Figure 5: Agar diffusion assay for S. aureus

Table 1: Effect of silver substitution on the lattice parameter, crystallite size density and Zone of Inhibition (ZOI) of Co_{1-x} $Ag_xFe_2O_4$ system

Sample	Lattice parameter Å	Crystallite size (nm)	X- ray density (gcm^{-3})	ZOI E.coli (mm)	ZOI S.aureus (mm)
CA0	8.325	14.1	5.402	-	-
CA1	8.379	14.6	5.298	10	10
CA2	8.314	18.0	5.423	15	12
CA3	8.310	18.8	5.432	8	8
CA4	8.297	20.3	5.457	12	12

Several mechanisms have been suggested for the antibacterial action of nanopowders. However, they remain speculative and the actual inhibitory mechanism of the nanoparticles may require further studies [23]. Generally, the good antibacterial properties exhibited by the nanoparticles could be due to the large surface area to volume ratio which results in better contact with bacterial cell. It has been suggested that nanopowders bind to the membranes of microorganisms which can prolong the lag phase of the growth cycle and increase the generation time of the organisms. Consequently the organism takes more time to complete cell division [24]. Gao et al. [11] suggested that silver nanoparticles attach to the bacterial cell membrane and release silver ions which change the permeability of the cell membrane causing the death of the bacterial cell. Other mechanism contributing to the occurrence of antibacterial activity of nanoparticles is the release of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and super-oxide (O^{2-}) generated from the surface of nanoparticles [25, 26]. The penetration rate of an active oxide through the bacteria cell wall may play a part in the killing rate of nanopowders against bacteria. Hydrogen peroxide generated from the surface of ceramic nanopowders can easily penetrate the cell wall of bacteria and cause cell destruction. Therefore, we may speculate that the small size of the nanoparticles which results in the large release of active oxygen species may be playing a crucial role in the bacterial growth inhibiting character of the silver doped cobalt ferrite nanoparticles.

Thus the above studies demonstrate that the CA series nanoparticles have significant antibacterial effect on a wide range of microorganisms. However, cytotoxicity studies have to be conducted on this nanocomposite material before considering it for practical applications. Thus the silver substituted cobalt ferrite nanoparticles with good growth inhibitory capacity against microbes can be considered for possible applications as antimicrobials in the field of medicine.

CONCLUSIONS

Silver substituted cobalt ferrite nanoparticles, $Co_{1-x}Ag_xFe_2O_4$ have been successfully synthesized by sol-gel technique. The XRD analysis reveals that the prepared samples exhibit a spinel structure with sizes varying from 14 to 20 nm. The lattice parameter and crystallite size increase with silver content and this can be attributed to the large radius of the silver ions. The decrease observed in the lattice parameter for higher silver content may be due to the compression of the spinel lattice by the metallic silver phase formed on the grain boundaries.The antibacterial efficacy was tested against Gram negative and Gram positive bacterial strains and the results show an enhancement in the activity with the addition of silver into cobalt ferrite. However for higher concentrations of silver ion, a decline in the antibacterial behaviour is observed. The improvement in the biocidal activity is attributed to the increase in the surface to volume ratio of the nanoparticles which enhances the contact area with the microbes. Thus the silver substituted cobalt ferrite nanoparticles with good structural and antibacterial properties can offer great promises in biomedical and pharmaceutical applications.

ACKNOWLEDGEMENTS

SX acknowledges the University Grants Commission of India for providing teacher fellowship. EMM thanks DST and UGC for the financial support. Authors thank SAIF, CUSAT, Kochi for providing facilities for XRD measurements.

REFERENCES

[1] Goldman A. Modern Ferrite Technology, Van Nostrand, New York 1990.
[2] Amiri S, Shokrollahi H. Mater Sci Engg C 2013; 33: 1 – 8.
[3] Sanpo N, Berndt CC, Cuie W, James W. Acta Biomater 2013; 9: 5830 – 5837.
[4] Binu PJ, Smitha T, Sheena X, Mohammed EM. J Alloys Compd 2012; 541: 29 – 35.
[5] Smitha T, Binu PJ, Sheena X, Mohammed EM. J Magn Magn Mater 2013; 348: 140 – 145.
[6] Gul IH, Maqsood A. J Alloys Compd 2008; 465: 227 – 231.
[7] Zi Z, Sun Y, Zhu X Song W. J Magn Magn Mater 2009; 321:1251 – 1255.
[8] Chudasama B, Vala AK, Andhariya N, Upadhyay RV, Mehta RV. Nano Res 2009; 2: 956 – 965.
[9] Arokiyaraj S, Saravanan M, Udaya Prakash NK, M. Valan AM, Vijayakumar B, Vincent S. Mater Res Bull 2013; 48: 3323 – 3327.
[10] Liu CH., Zhou ZD, Yu X, Lv BO, Mao JF, Xiao D. Inorg Mater 2008; 44: 291 – 295.
[11] Gao M, Sun L, Wang Z, Zhao Y. Mater Sci Engg C 2013; 33: 397 – 404.
[12] Sondi I, Sondi BS. J Coll Inter Sci 2014; 275: 177 – 182.
[13] Das R, Gang S, Nath SS. J Biomater Nanobiotechnol 2011; 2: (472 – 475.
[14] Okasha N. J Mater Sci 2008; 43: 4192 – 4197.
[15] Sun S, Zeng H, Robinson DB, Raoux S, Rice PM, Wang SX. J Am Ceram Soc 2004; 126: 273 – 279.
[16] Kooti M, Saiahi S, Motamedi H. J Magn Magn Mater 2013; 333: 138 – 143.
[17] Yamamoto O. Int J Inorg Mater 2001; 3: 643 – 646..
[18] Cullity BD, Elements of X-Ray Diffraction, Addison – Wesley, California, 1978.
[19] Jing J, Liangchao L, Feng X. J Rare Earth. 2007; 25: 79 – 83.
[20] Binu PJ, Smitha T, Sheena X, Mohammed EM Phys. Scr. 2010; 84: 045702.
[21] Raghupathi KR, Koodali RT, Manna AC. Langmuir 2011; 27: 4020 – 4028.
[22] Sharma N, Kumar J, Thakur S, Sharma S, Shrivastava V. Drug Invention Today 2013; 5: 50 – 54.
[23] Moritz M, Moritz MG. Chem Eng J 2013; 228: 596 – 613.
[24] Yamamoto O, Sawai EJ. B Chem Soc Jpn 2001; 74 (9): 1761 – 1765.
[25] Hashim M, Alimuddin, Shirsath SE, Meena SS, Kotnala RK, Ameena P, Aashis SR, Shalendra K, Pramod B, Ravi K. J Magn Magn Mater 2013; 341: 148 – 157.
[26] Sawai J. J Microbiol Meth 2003; 54(2): 177 – 182.