Research Article

Evaluation of the Zinc Ferrite Nano particles for Bio-applications

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Received: 29-05-2017; **Revised:** 18-07-2017; **Accepted:** 22-08-2017.

ABSTRACT

Zinc ferrites are successfully nano particles were prepared by the hydrothermal method. In this method PEG used as a capping agent. The structure and morphology of ensuring product was characterized by powder X-ray Diffraction, Fourier transform infrared and UV-visible spectroscopy. The XRD result confirms the formation of spinel cubic structure with Fd3M space group matches with JCPDS No.82-1042. The average particle size calculated by Debye Scherer formula is 3.54 nm and the X-ray density of the powder is 1.6466 g/cc3. In this study, quenching study, anti-bacterial and *in vitro* anticancer properties of zinc ferrite nano particles were evaluated. Antibacterial activity of zinc ferrite was studied against *E.coli* pathogenic bacteria and *In vitro* anticancer activity was screened by MTT assay against MCF cell line. The molecular interaction study had been performed using Bovine Serum Albumin. The result shows that the zinc ferrite nano particles have been used for the bio-applications like antimicrobial activity, anticancer properties, and bio molecule interaction. It reveals that the zinc ferrite nano particles have biological applications.

Keywords: Antibacterial, Anticancer, Zinc ferrite, Quenching, Biological applications.

INTRODUCTION

The most promising feature of nanotechnology is the magnetic nano particles (MNPs) of different compositions [1]. They offer remarkable applications in the fundamental and technological field such as biomedical, bio processing and catalysts among many others [2-5]. Zinc belongs to a class of microelements that is considered to play an important role in many vital biochemical reactions and physiological processes: growth and development of the cells, stimulation of the gene transcription and cell proliferation, slowing down the oxidation processes, optimization of the human immune system [6-8]. Therefore, to get more information about zinc ferrite nano particles ($ZnO{\cdot}Fe_2O_3$) and to improve their applications or develop new ones, careful studies related to their functionality, particle sizes and also their antimicrobial behavior are essential. Among the spinel ferrite compounds Zinc Ferrite ($ZnFe_2O_4$) exhibits super paramagnetic behavior and it has potential application in many fields, such as photocatalysis, magnetic resonance imaging (MRI), Li-ion batteries and gas sensors. Various synthesis methods are proposed to prepare $ZnFe_2O_4$ nano particles such as co-precipitation, combustion, thermal decomposition, solvothermal, hydrothermal, ball milling, and ceramic route techniques. Among the synthesis methods the hydrothermal method has been widely used, because of its simplicity, low cost, nontoxic route and yields crystalline nano materials in a short time. In this work super paramagnetic $ZnFe_2O_4$ nano particles were synthesized by hydrothermal method using the capping agents [9-17].

In this work, zinc-doped magnetite nano particles are synthesized through by hydrothermal method using PEG as capping agent. To characterize structure and morphology of nano particles by Powder X-ray Diffraction (P-XRD), Fourier Transform Infrared (FT-IR) and UV-visible spectroscopy. To evaluate the properties of synthesized nano particles by quenching study, anti-bacterial and *in vitro* anticancer activity. This method may be the most promising one because of its simplicity and productivity. It is widely used for biomedical applications because of the ease of implementation and the need for less hazardous materials and procedures.

MATERIALS AND METHODS

Synthesis and characterization

Zinc ferrite ($ZnFe_2O_4$) nano particles was synthesized and characterized with applications. $ZnFe_2O_4$ nano particles were prepared by hydrothermal method using PEG ass capping agents. 4.87 g of ferric nitrate ($Fe(NO_3)_3$), 3.75 g of zinc nitrate ($Zn(NO_3)_2$) and 3.28 g of sodium hydroxide (NaOH) were mixed together and dissolved in 40 ml of distilled water. Then 2 ml of PEG was added drop wise in the solution under constant stirring at room temperature and the pH level was maintained at 11. The mixture was continuously stirred for half an hour and transferred to 50 ml of Teflon lined autoclave. The autoclave was sealed and maintained at 165°C for 16 hours in the furnace and then allowed to reach the room temperature. Finally the brown precipitated solution was washed several times with distilled water and absolute ethanol. Then the brown precipitates were collected and dried at 60°C for 6 hours in hot air oven and characterized by Powder X-ray Diffraction (P-XRD), Fourier Transform Infrared (FTIR) ,

UV-visible spectroscopy, SEM and Quantitative Analysis by Energy Dispersive X-ray Spectrometry (EDAX).

***In vitro* anticancer activity**

In vitro studies of anti-cancer activity against MCF cell line (Breast cancer cell line) MCF (Michigan Cancer Foundation) procured from NCCS Pune was maintained at 10% heat inactivated FBS (Foetal Bovine Serum) in carbon dioxide incubator. The cells may be in confluent stage. So the cells were trypsinised using 0.025% trypsin (cell culture grade HIMEDIA) upon reaching confluences. Following which, the cells were sub-cultured on to micro culture plates and used for further studies. Anticancer effect of zinc ferrite was determined on MCF cell lines. A standard concentration of 500 µg/ml was added and incubated. The Anti-proliferative effect was determined by standard MTT assay. The cell culture suspension was washed with 1 X PBS (Phosphate Buffered Saline) and then added with 200 µl MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5-diphyhyl tetrazolium Bromide] solution to the culture flask (MTT 5 mg/volume dissolved in PBS). It is then filtered through a 0.2 µm filter before use. It is then incubated at 37°C for 3 hours, removed all MTT solution, washed with 1 X PBS and added with 300 µl DMSO to each culture flask and incubated at room temperature for 30 minutes until all cells get lysed and homogenous color was obtained. The solution was then transferred to centrifuge tube and centrifuged at top speed for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD was measured at 540 nm using DMSO blank.

Quenching study (bio molecule interaction study)

The bio molecule interaction was studied using bovine serum albumin with $ZnFe_2O_4$ nano particles. The solution was allowed to interact with different size and concentration of $ZnFe_2O_4$ nano particles and the interaction was studied by absorption and fluorescence studies. Here 2 µM BSA was prepared by using phosphate buffer saline (pH-7.4) and mixed with zinc ferrite nano particles for their absorption studies. Also 0.02 µM BSA was prepared and mixed with different concentration of zinc ferrite nano particles for fluorescent studies. 20µM solution of BSA was prepared and the pH was found to be 7.2. Previously the same experiment was tried with BSA of 0.2µM, 0.02µM, and 2µM and 20µM concentration. 2µM and 20µM concentration was considered to be suitable for interaction studies. Before carrying out the experiment, the nano particle concentration was matched with the concentration of 20µM BSA by matching the optical density of absorption spectra by UV-Vis spectroscopy.

Antibacterial activity

The antimicrobial activity was studied against *Escherichia coli* by well diffusion method on Muller-Hinton agar. In this experiment we used three types of discs: (1) paper disc (10 mm) saturated with 10 µL of $ZnFe_2O_4$ and nitro (a commercial antibiotic); (2) blank discs with distilled water. *E. coli* strains were suspended in saline solution and plated in the culture medium, and then the discs were placed. The plates were incubated at 35 °C for 24 hours, after this time the inhibition zones were measured[18].

RESULTS AND DISCUSSION

Structural characterization

The absorption spectrum of $ZnFe_2O_4$ nano particles in UV-light region was studied. A ZnFe2O4 nano particle exhibits the characteristic absorption peak at 315 nm (Fig.1). FT-IR spectrum of $ZnFe_2O_4$ nano particles (Fig.2) showed significant absorption peaks at 541 cm^{-1}. The absorption band at 541 cm-1 represents Fe–O stretching vibrations characteristic peak of the spinal structure of zinc ferrite [19]. The FT-IR band at 1385cm-1 is due to stretching vibration of C-H bond [20]. The peak at 3445 corresponds to the vibration of O-H and the light band at 1646 could be attributed to the adsorbed water or humidity [21]. The crystallographic structure of the synthesized $ZnFe_2O_4$nano particles was identified by powder X-Ray Diffraction (XRD) measurement. The powder XRD patterns of $ZnFe_2O_4$ presented in Fig. 3. The XRD peaks of the prepared samples were indexed by comparing with the JCPDS card No: 82-1042which confirms the formation of $ZnFe_2O_4$ XRD pattern shows that synthesized nano particles exhibit mixed phase of ZnO and $ZnFe_2O_4$ where the $ZnFe_2O_4$ belongs to face centered regular spinel cubic structure. The XRD peak indexed at 2θ=37.19° and at 2θ= 35.14° in corresponds to (3 1 1) plane of $ZnFe_2O_4$ (Fig.4). The diffraction peak of ZnO was indexed by comparing with the JCPDS card no: 82-1042. The predominant (1 0 1) XRD peak at 2θ=36.3° corresponds to the ZnO belonging to the primitive hexagonal structure. In the figure (220),(311),(101),(400),(511),(440)represents the zinc ferrite other peaks represents the ZnO. The volume of the prepared powder is 617.66 the density of the prepared powder was found to be 1.6466 g/cc3. The value of crystallite size calculated for the zinc ferrite nano particle was 3.54 nm.

The FE-SEM images exhibit agglomerated bunch of square shaped nano particles. The small size of nano particles leads to high agglomeration because of its high surface energy. The FE-SEM image shows that the agglomeration of $ZnFe_2O_4$ nano particles because of the effect of capping agent on modifying crystal sizes. It is difficult to determine the exact size of the particle using FE-SEM because most of the particles are agglomerated (Fig. 5). The EDAX spectrum confirms the presence of Zn, Fe and O in the synthesized nano particles with atomic percentage of 26.85, 46.76 and 26.39 respectively (Fig.6).

Anticancer activity

The anticancer activity shows that the amount of $ZnFe_2O_4$ nano particle along with camptothecin increases the cancer cell death increases. The addition of 50 µg/ml of $ZnFe_2O_4$ nano particle with camptothecin expressed cell viability of 95.2381%; 150 µg/ml with 80.95238 % cell

viability ; 250 µg/ml with 57.30428 % cell viability; 350µg/ml with 27.5222 % cell viability and 450µg/ml with 14.60856 % cell viability(Fig.7). This shows as the concentration of $ZnFe_2O_4$ nano particle increases, the breast cancer cell death increases (Fig 8).

Bio molecule interaction of $ZnFe_2O_4$ nano particle

The quenching assay shows the UV- Vis absorption spectra of $ZnFe_2O_4$ nano particles with BSA (concentration of 2µM). It is observed that the spectral signature for protein shows increase in absorption intensity and no shift in the wavelength. This enhancement was due to absorption of BSA which is present partly on the surface of $ZnFe_2O_4$ nano particles .The change in the intensity of the absorption peak at 280nm explicitly shows the formation of surface complex. However, there is a considerable variation in the peak absorption position of $ZnFe_2O_4$ nano particles with respect to particle size (Fig. 9).

Antibacterial activity

Antimicrobial activity of zinc-ferrite nano particles was comparatively equal with the commercial antibiotic nitro. The zone of inhibition of zinc ferrite nano particles and nitro discs against *E.coli* was found to be 200 mm and 300 mm (Fig. 10). The water control disc expressed absence of inhibition. From the above results it is confirmed that the zinc ferrite prepared by hydrothermal method shows good antibacterial activity.

CONCLUSION

Zinc ferrite nano particles were successfully prepared by hydrothermal method using PEG as capping agent. Bio-molecular interaction study has been done using UV- Vis Spectroscopy with Bovine Serum Albumin, in which the material showed good interaction with the bio molecule. The XRD confirms the crystal structure and phase purity of the sample. The SEM of $ZnFe_2O_4$nanoparticles shows the spherical agglomerated particles. T*In vitro* cytotoxic and antibacterial activity showed the application of Zinc ferrite nano particle in inflammation and cancer therapy.

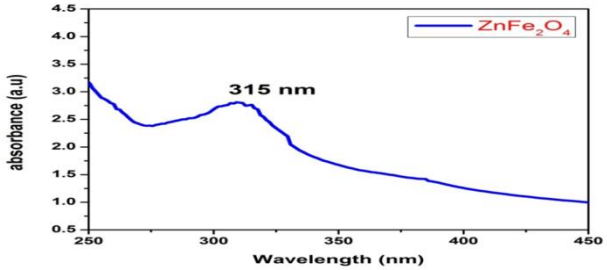

Figure 1: UV-Visible spectrum - absorption peak of $ZnFe_2O_4$ at ~315 nm

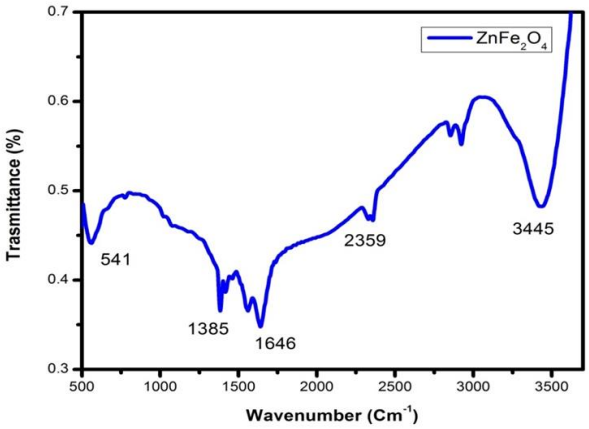

Figure 2: FTIR spectrum- 541cm^{-1}represents Fe–O stretching vibrations characteristic peak of the spinal structure of zinc ferrite

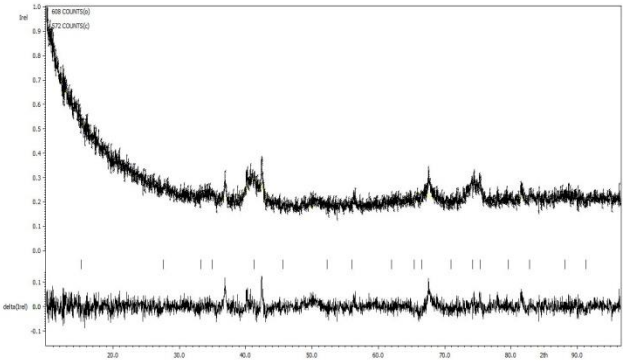

Figure 3: XRD pattern - diffraction peak of ZnO was indexed by comparing with the JCPDS card no: 82-1042.

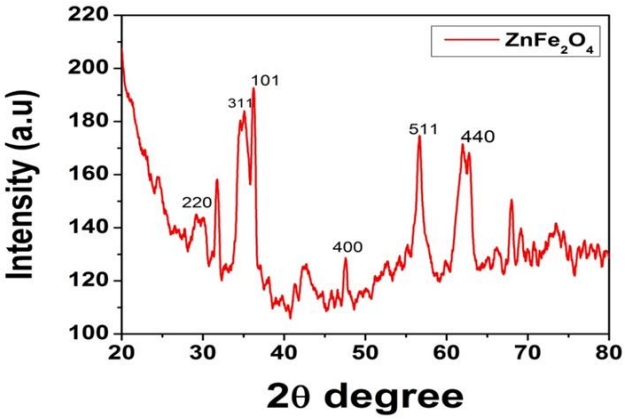

Figure 4: XRD pattern of Zinc ferrite nano particles - XRD peak indexed at 2θ=37.19° and at 2θ= 35.14° in corresponds to (311) plane of Zinc ferrite. (220),(311),(101),(400),(511),(440) represents the zinc ferrite other peaks represents the ZnO.

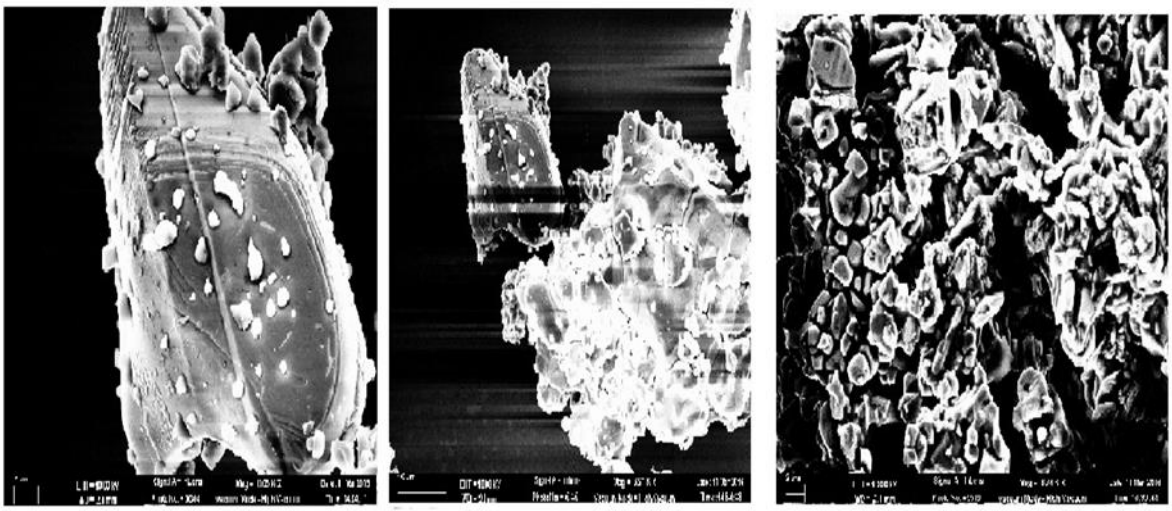

Figure 5: FE-SEM images - zinc ferrite nano particle

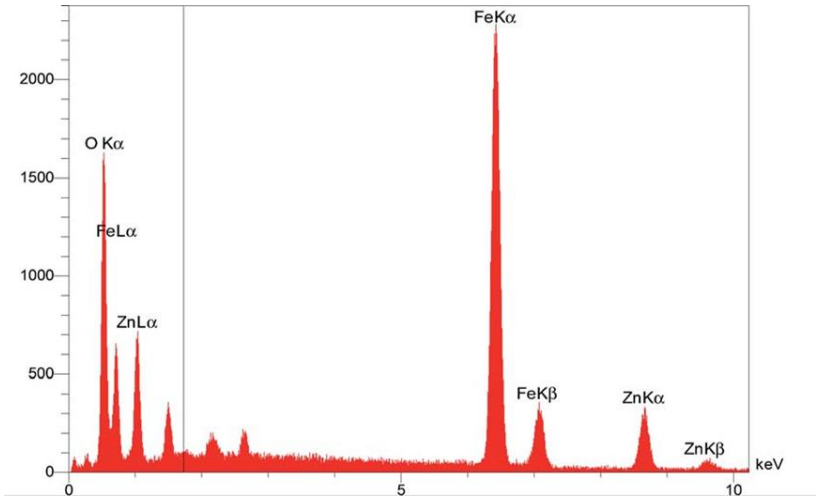

Figure 6: EDAX Measurement of zinc ferrite nano particle -elemental composition -Zn (26.85%), Fe (46.76%), O (26.39%)

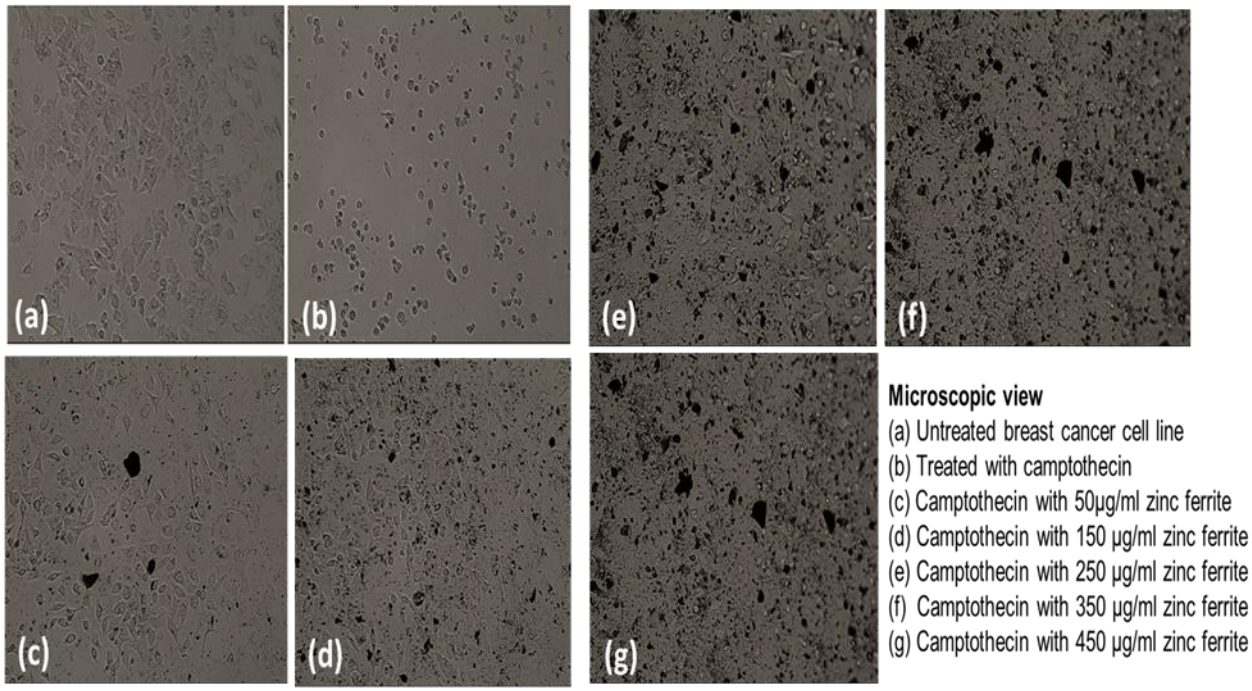

Figure 7: Microscopic view of untreated and treated cell line

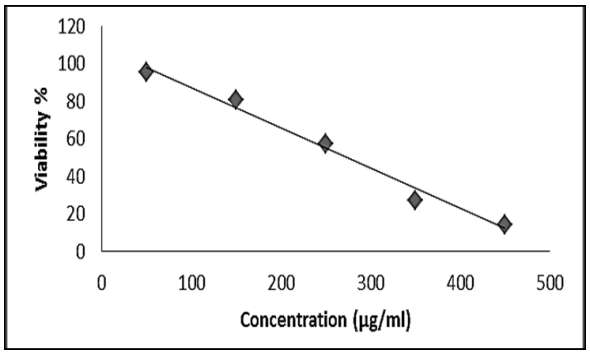

Figure 8: Cell viability percentage of MCF-7 vs zinc ferrite

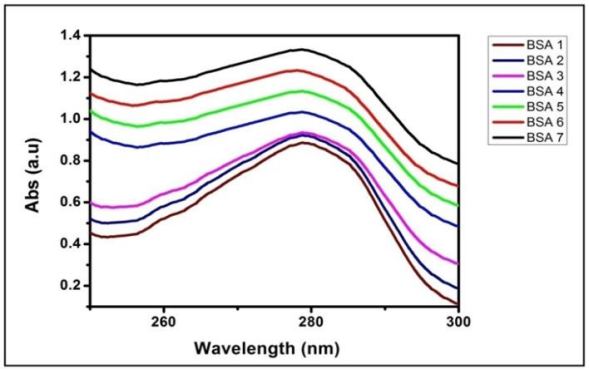

Figure 9: UV-Visible spectra- quenching study

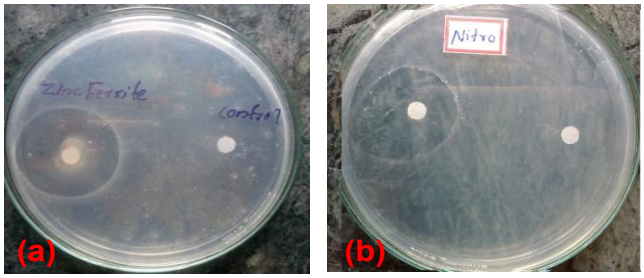

Figure 10: Antibacterial activity of zinc-ferrite nano particle and nitro –*E.coli* (a) Zone of inhibition by zinc-ferrite nano particle - 120 mm (b) Zone of inhibition by commercial antibiotic agent (Nitro) – 150mm

REFERENCES

1. Mudshinge SR, Deore AB, Patil S, Bhalgat CM. Nano particles: emerging carriers for drug delivery. Saudi Pharm J 19, 2011, 129-41.
2. Krishnan K. Biomedical nano magnetics: a spin through possibilities in imaging, diagnostics, and therapy. IEEE Trans Magn 46, 2010, 2523-58.
3. Tartaj P, Morales M, Veintemillas-Verdaguer S, Gonz´alez T, Serna C. The preparation of magnetic nanoparticles for applications in biomedicine. J Phys D: ApplPhys 36, 2003, 182-97.
4. Faraji M, Yamini Y, Rezaee M. Magnetic nanoparticles: synthesis, stabilization, functionalization, characterization, and applications. J Iran ChemSoc 7, 2010, 1-37.
5. Indira TK, Lakshmi MK. Magnetic nanoparticles–a review. Int J Pharm SciNanotechnol 3, 2010, 1035-42.
6. Chu A, Foster M, Hancock D, Bell-Anderson K, Petocz P, Samman S. TNF-a gene expression is increased following zinc supplementation in type 2 diabetes mellitus. Genes Nutr 10, 2015, 1-10.
7. Miceli MV, Tatejr DJ, Alcock NW, Newsome DA. Zinc deficiency and oxidative stressin the retina of pigmented rats. Invest Ophthalmol Visual Sci 40, 1999, 1238-44.
8. Harréus U, Baumeister P, Zieger S, Matthias C. The influence of high doses of vitamin C and zinc on oxidative DNA damage. Anticancer Res 25, 2005, 3197–202.
9. Song Sun, Xiaoyan Yang, Yi Zhang, Fan Zhang, Jianjun Ding, Jun Bao , Chen Gao, Enhanced photocatalytic activity of sponge-like ZnFe2O4 synthesized by solution combustion method, Progress in Natural Science: Materials International, 2012, 22, 639–643.
10. Wan J, Jiang X, Li H, Chen K, Facile Synthesis of Zinc Ferrite nano particles as non-lanthanide T1 MRI contrast agents, J. Mater. Chem. 22, 2012, 13500-13505.
11. Lin Lian, LinruiHou, Lu Zhou, Lishi Wangb and Changzhou Yuan, Rapid low-temperature synthesis of mesoporous nanophase ZnFe2O4 with enhanced lithium storage properties for Li-ion batteries, RSC Adv., 2014, 4, 49212–49218.
12. Shurong Wang, XuelingGao, Jiedi Yang, Zhenyu Zhu, Hongxin Zhang, and Yanshuang Wang, Synthesis and gas sensor application of ZnFe2O4 – ZnO composite hollow microspheres, RSC Advances, 2014, 1-10.
13. Kalpanadevi Kalimuthu, Sinduja C. Rangasamy, Manimekalai Rakkiyasamy, Characterization of ZnFe2O4 nanoparticles obtained by the Thermal Decomposition of ZnFe2(cin)3(N2H4)3, Acta Chim. Slov. 60, 2013, 896–900.
14. 8. Blanco-Gutierrez V, Torralvo MJ, Saez-Puche R, Bonville P, Magnetic properties of solvothermally synthesized ZnFe2O4 nanoparticles, Journal of physics, 200, 2010, 1742-6596.
15. 9. RameshbabuR,Ramesh R, Kanagesan S, Karthigeyan A, Ponnusamy S, Synthesis and study of structural, morphological and magnetic properties of ZnFe2O4 nanoparticles, J.supercond Nov Magn, 27, 2014, 1499-1502.
16. 10. Mozaffari M, Masoudi H, Zinc Ferrite nanoparticles: New preparation method and magnetic properties, J Supercond Nov Magn, 27, 2014, 2563–2567.
17. 11. Mohammad Niyaifar, Effect of preparation on structure and magnetic properties of ZnFe2O4, Journal of Magnetics, 19(2), 2014, 101-105.
18. Mallmann EJ, Cunha FA, Castro BN, Maciel AM, Menezes EA, Fechine PB. Antifungal activity of silver nanoparticles obtained by green synthesis. Rev Inst Med Trop Sao Paulo. 57, 2015, 165–167.
19. Rao, G.S., C.N.R. Rao, J.R. Ferraro, 1970. Infrared and Electronic Spectra of Rare Earth Perovskites: Ortho-Chromites, -Manganites and -Ferrites. Appl. Spectrosc, 24, 436.
20. Ibrahim, M., M. Alaam, H. El-Haes, A.F. Jalbout, 2006. A. de Leon, Analysis of the structure and vibrational spectra of glucose and fructose. Ecletica Quimica, 31(3).
21. Jalaly, M., M.H. Enayati, F. Karimzadeh, and P. Kameli, 2009. "Mechanosynthesis of nanostructured magnetic Ni-Zn ferrite," Powder Technology, 193, 150-153.

Source of Support: Nil, **Conflict of Interest:** None.