

Silica Coated Magnetic Nanoparticles for Biological Applications

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(Received: 05 November 2019, and Accepted: 16 April 2020)

Abstract

The research paper describes the synthesis, characterization of $Fe_3O_4@SiO_2$, $BiFeO_3@SiO_2$, $ZnFe_2O_4@SiO_2$, $BiFe_{0.9}Zn_{0.1}O_3@SiO_2$ and $BiFe_{0.75}Co_{0.25}O_3@SiO_2$ nanoparticles. The materials were synthesized by chemical co-precipitation technique and are characterized by X-ray diffraction, Transmission electron microscope with EDS and Vibrating sample magnetometer. Further, the biocompatibility studies were performed on THP-1 cells. The results indicated that the developed nanoparticles have considered being good biocompatible materials.

Keywords: Magnetic nanoparticles, Silica coating, Biocompatibility, Cytotoxicity.

1. INTRODUCTION

Magnetic based nanoparticles such as iron oxide nanoparticles and other ferrites are attractive materials for their vital applications in targeted drug delivery, magnetic fluids and water purification etc [1,2,3]. But the limiting factors for their usage in biomedical field are agglomeration, durability and toxic nature [4]. Therefore there is need of hour for preparing the non-toxic biocompatible magnetic nanoparticles. The preparation of non-magnetic & biocompatible coating over magnetic nanoparticles has been very interesting for addressing issues like aggregation, toxic nature of nanoparticle, surface functionalization, poor durability etc which control the magnetic nanoparticles for direct application in the biomedical field [5,6,7]. The coated magnetic nanoparticles are employed as catalysts in the biomedical field because of their sensitivity and easy separation to magnetic field. Among various surface modification approach such as polymers, organic monolayers etc, one of the best ways is to coat magnetic nanoparticles with nonmagnetic silica layer which is

significant in technology perspective. Silica coating has the advantages like its chemically inert, non-toxic, and avoidance of agglomeration, temperature resistant, highly biocompatible, resistant to decompose and have been widely used to improve the stabilization of nanoparticles in an alkali atmosphere [8,9].

Furthermore, the silica surfaces can be effortlessly functionalized with carboxyl, thiol and amine groups which can be modified with drugs and enzymes for biomedical applications [10,11]. At the same time, silica coated magnetic nanoparticles find potential applications in bioseparation [12], enzyme immobilization [13] and diagnostic analysis [14]. Several researchers found the biological applications of transition metal magnetic nanoparticles [15]. Whereas, silica covered magnetic nanoparticles are very active in biological and magnetic applications [16,17]. But there is still opportunity to address many issues as discussed earlier. Non-toxic nature is an issue to be addressed which has a variance in the literature reports. But, the progress in

magnetic nanoparticles cytotoxic assessment is inadequate. To address this silica coated on to the magnetic nanoparticles needs a sincere attention to identify the appropriate material with excellent cytotoxicity.

The present work focused on the preparation, magnetic study and biocompatibility of the $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{BiFeO}_3@\text{SiO}_2$ and $\text{ZnFe}_2\text{O}_4@\text{SiO}_2$ and $\text{BiFe}_{0.9}\text{Zn}_{0.1}\text{O}_3@\text{SiO}_2$ and $\text{BiFe}_{0.75}\text{Co}_{0.25}\text{O}_3@\text{SiO}_2$ nanoparticles. The materials are achieved by chemical double coprecipitation technique and are characterized by X-ray diffraction (XRD), Transmission Electron Microscope (TEM) and Vibrating Sample Magnetometer (VSM). The cell viability measurements are evaluated on THP-1 cells. The results of the nanoparticles indicate that the silica coated nanoparticles may find potential applications in biomedical field.

2. EXPERIMENTAL PROCEDURE

2.1. Materials and Methods

Ferric nitrate nonahydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, 99.99%), bismuth nitrate pentahydrate ($\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$, 99.99%), barium chloride hexahydrate ($\text{BaCl}_2 \cdot 6\text{H}_2\text{O}$), zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 98%), ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 98%), tetraethylorthosilicate (TEOS) are bought from Sigma Aldrich company, sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), ethanol, nitric acid, ethylene glycol and potassium hydroxide are acquired from Merck organization, Mumbai, India (Merck), are purchased and used as received. Penicillin–streptomycin and Roswell Park Memorial Institute medium (RPMI) 1640 were acquired from Thermo Fisher Scientific, USA. Fetal bovine serum is acquired from American Type Culture Collection (ATCC), USA. Cell Counting Kit- 8 (CCK-8) is acquired from ENZO life advancements, USA.

2.2.1. Synthesis of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ Nanoparticles (S-1)[18]

In a reaction vessel, the equal mole proportion volume of ethanol and tetra ethyl orthosilicate mixed for 5 minutes pursued by adding ammonium hydroxide. The blending proceeded for 15 minutes pursued by the addition of as prepared iron oxide nanoparticles. The reaction is further continued for 12 hours to finish the hydrolysis and condensation of tetra ethyl orthosilicate. The obtained composite is thoroughly cleaned with deionized water, ethanol and dried up at 60°C in hot air oven over the night.

2.2.2. Synthesis of Silica Coated Bismuth Ferrite ($\text{BiFeO}_3@\text{SiO}_2$) Nanoparticles (S-2)[19]

At first, calculated quantities of bismuth nitrate pentahydrate and iron nitrate nonahydrate are dissolved and stirred in 1N HNO_3 at 60°C for 2 hours to get a homogeneous mixture. To this, stabilizing agent (ethylene glycol) is added and potassium hydroxide is added as precipitating agent and the pH is accustomed to ~ 12 to finish the precipitation of BiFeO_3 nanoparticles and finally neutralized to pH ~ 7 . The obtained nanoparticles are dried up in hot air oven at 80°C for 8 hours. Then, the nanoparticles are calcinated at 500°C for 3 hours to get the clean crystalline BiFeO_3 nanoparticles. Further, the as prepared bismuth ferrite nanoparticles are dispersed in ethanol under sonication. To above blend 6 mL ammonium solution ($\text{NH}_3 - 25 \text{ wt } \%$) is added under stirring condition for 45 minutes. To above mixture tetra ethyl orthosilicate (TEOS) is added. The blend is additionally held under steady mixing form for 24 hours. The as prepared Silica covered bismuth ferrite nanoparticles are permitted to settle at the bottom are centrifuged and washed a few times with water lastly with ethanol. The washed nanoparticles are dried in vacuum to gather powder.

2.2.3. Synthesis of Silica Coated Zinc Ferrite ($\text{ZnFe}_2\text{O}_4@ \text{SiO}_2$) Nanoparticles (S-3)

The S3 sample is prepared by the chemical double coprecipitation procedure as reported in the literature [20]. The short methodology includes the equal mole proportions of zinc nitrate hexahydrate and iron nitrate nonahydrate are blended and proceed with the mixing for 1 hour at 60°C by adding ethylene glycol about 50 mL as a stabilizing agent. In this manner, the mixture is nucleated with potassium hydroxide solution, pH is changed in accordance with ~ 12 and the mixing is proceeded for 2 hours at 70°C . The product is thoroughly washed with deionized water, ethanol and separated. The acquired nanocomposites are dried up at 80°C for 10 hours and calcinated for 3 hours at 500°C . Further, as synthesized nanocomposites are dispersed in ethanol under sonication. To above blend 6 ml ammonia solution (NH_3 -25 wt %) is included under stirring condition. The blend is held under mixing conditions for 45 minutes. Then 3 mL tetraethyl orthosilicate (TEOS) is added drop wise to above blend. The blend is additionally held under consistent mixing form for 24 hours. The abundance SiO_2 is tapped and covered particles are further centrifuged and washed a few times with water lastly with ethanol to evacuate excess silica and other products. The washed item is dried in vacuum to gather powder.

2.2.4. Synthesis of Silica Coated Zinc Doped Bismuth Ferrite ($\text{BiFe}_{0.9}\text{Zn}_{0.1}\text{O}_3@ \text{SiO}_2$) Nanoparticles (S-4)

4.8 g of bismuth nitrate, 3.63 g of iron nitrate and 0.12 g of zinc nitrate are disintegrated in 150 mL deionized water under consistent mixing with help of stirrer at 60°C for 1 hour and ethylene glycol 50 mL is added as a stabilizing agent. Accordingly, the reaction blend is precipitated with aqueous potassium hydroxide, accordingly pH is changed to ~ 12 and the mixing is proceeded for

2 hours at 70°C . The resultant is thoroughly washed with deionized water, ethanol and separated to get $\text{BiFe}_{0.9}\text{Zn}_{0.1}\text{O}_3$ nanoparticles. The acquired nanoparticles are dried for 10 hours at 80°C and calcinated for 3 hours at 500°C . Further, the as synthesized nanoparticles (1g) are taken in flask having three necks and scattered in 500 mL of ethanol with the help of sonicator. To above blend 6 mL liquor ammonia (NH_3 -25 wt %) is added under mixing condition. The blend is held under mixing conditions for 45 minutes. To above mixture is added to 3 mL tetraethyl orthosilicate (TEOS) drop wise. The blend is additionally held under consistent mixing condition for 24 hours. The as prepared SiO_2 covered $\text{BiFe}_{0.9}\text{Zn}_{0.1}\text{O}_3$ nanoparticles are permitted to gravity sediment. The sediment is carefully cleaned with deionized water and ethanol to expel excess silica and other products. The washed item is dried in vacuum to gather powder.

2.2.5 Synthesis of Silica Coated Cobalt Doped Bismuth Ferrite ($\text{BiFe}_{0.75}\text{Co}_{0.25}\text{O}_3@ \text{SiO}_2$) Nanoparticles (S-5)

The planning of $\text{BiFe}_{0.75}\text{Co}_{0.25}\text{O}_3$ nanoparticles are accomplished by reported method [21]. 4.8 g of bismuth nitrate, 3.02 g of iron nitrate and 0.32 g of cobalt chloride are mixed in 1N nitric acid under consistent stirring for 1 hour at 60°C and stabilizing agent ethylene glycol 50 mL is added. Then the reaction blend is precipitated with potassium hydroxide solution, pH is changed in accordance with ~ 12 and the mixing is proceeded for 2 hours at 70°C . The precipitated nanoparticles are washed with deionized water, ethanol and separated. The acquired product is dried up at 80°C for 10 hours and calcinated at 500°C for 3 hours. Further, the readied nanoparticles 1 g is taken in round bottom flask and dispersed in 500 mL ethanol with the help of sonicator. To above blend 6 mL ammonia solution (NH_3 -25 wt %) is added. The blend is held under mixing conditions for

45 minutes. Then 3 mL tetraethyl orthosilicate (TEOS) is added drop wise to above blend. The blend is additionally held under steady mixing form for 24 hours. The as prepared silica covered $\text{BiFe}_{0.75}\text{Co}_{0.25}\text{O}_3$ nanoparticles are permitted to gravity sediment. The sediment is carefully cleaned with deionized water and finally with ethanol to expel excess silica and other products. The washed nanoparticles are dried in vacuum.

2.3. Characterization techniques

The structures of prepared nanocomposites are affirmed by X-ray diffraction, Transmission Electron Microscope with EDS, moreover, the Vibrating Sample Magnetometer is used to find magnetic properties of nanocomposites. X-Ray Diffraction measurements are completed utilizing a Bruker model ENDEAVOR D4 diffractometer with $\text{Cu-K}\alpha$ radiation and utilizing a scan rate of 0.02°S^{-1} . Transmission electron microscope results are taken on JEOL, JEM1200EX at 200 kv, the samples are dispersed in a 1:1 methanol and water arrangement and kept on a 3 mm copper grid and dried at ambient temperature remove the excess solution by using filter paper. The thermo scientific multiskan GO microplate spectrophotometer is utilized to measure the absorbance of culture plate.

2.4. Cytotoxicity Assessment

To culture the THP-1 cells, RPMI1640 medium having penicillin-streptomycin and 10% fetal bovine serum is used. The 100 mm cell culture dishes were utilized to this cell culture. The culture dishes are kept up in humidified incubator with 5% CO_2 and 95% air at 37°C for the development of cells. Before utilize the as prepared nanoparticle solutions for in vitro cytotoxicity tests, and are cleaned the sterilized nanoparticle solutions by UV light treatment for 2 hours. Then, the resultant nanoparticle solutions are

dispersed using sonication to forestall the total of particles.

The CCK-8 test is utilized to assess the suitability of cells (in vitro cytotoxicity test) for the treatment with the as prepared nanoparticles, such as, $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{BiFeO}_3@\text{SiO}_2$ and $\text{ZnFe}_2\text{O}_4@\text{SiO}_2$, $\text{BiFe}_{0.9}\text{Zn}_{0.1}\text{O}_3@\text{SiO}_2$ and $\text{BiFe}_{0.75}\text{Co}_{0.25}\text{O}_3@\text{SiO}_2$. The cells with the density of $\sim 3 \times 10^4$ cells for each well is put in 96-well tissue culture plates. These plates are kept with 5% CO_2 and air 95% at 37°C in an incubator and permitted to grow the cells in the wells for 8 hours. Subsequently, 10 μL of prepared nanoparticle solution are added to into the plates and incubated culture plates for testing times of 12 and 48 hours. The control is taken as cell suspension without the nanoparticles.

At the end of 12 and 48 hours, 10 μL of (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt) WST-8 solution in the CCK-8 kit is added to each well of the culture plate and incubated for 3 hours at 37°C according to the directions of manufacturers. The absorbance of culture plate is measured at 450 nm by micro plate spectrophotometer (Thermo Fisher). The cell viability of the nanoparticles for samples is articulated as a percentage of without nanoparticle samples, assumed to be 100 %.

3. RESULTS AND DISCUSSIONS

3.1. Structural Studies

The structural pattern of synthesized materials is achieved by XRD experiments. Figure 1 represents the XRD patterns of developed nanomaterials S1-S5 at room temperature. The representative peaks of S1-S5 samples are observed at 2θ 23.84, 30.09, 32.72, 42.02, 46.9, 51.89, 56.72; 30.30, 35.66, 43.28, 57.35, 62.71; 29.94, 35.08, 42.91, 56.46, 61.76; 23.84, 30.15, 32.72; 23.95, 26.00, 30.20, 32.88, 47.16, and 56.99; respectively. From the figure it is noticed that the particles have polycrystalline nature and the silica coating over the nanoparticles is clearly

observed. The silica coating over the magnetic nanoparticles attested the peak overlapping and reducing the intensity of the nanoparticles. These overlapping results the broader XRD peaks and thus reduces the crystalline sizes. The crystalline sizes of samples S1-S5 are 35.18, 41.12, 19.89, 27.56 and 45.93 nm respectively.

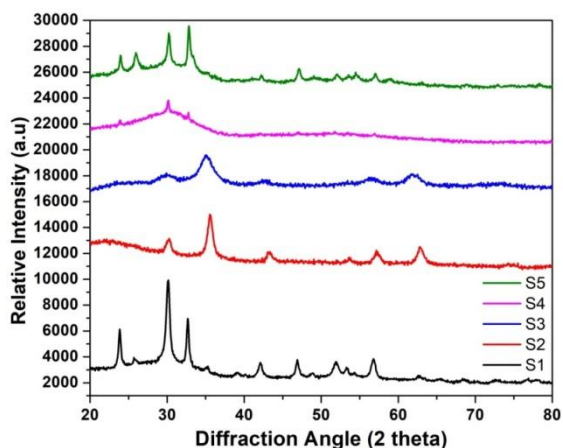


Figure 1. X-Ray Diffraction patterns of synthesized silica coated magnetic nanoparticles $Fe_3O_4@SiO_2$, $BiFeO_3@SiO_2$, $ZnFe_2O_4@SiO_2$, $BiFe_{0.9}Zn_{0.1}O_3@SiO_2$ and $BiFe_{0.75}Co_{0.25}O_3@SiO_2$.

3.2. Morphological Studies

The surface morphology and nature of the nanocomposites are determined by TEM studies. The results of TEM for the samples S1-S5 are presented in Figure 2 to 6. From the figures it is clearly noticed that the silica coating over the surface of nanoparticles and the compositions elemental analysis are conformed by EDS experiments done for synthesized nanocomposites revealed the absence of impurities. The data obtained in TEM studies is well matched with the XRD data. Also, it is observed that the particles are in poly crystalline nature. The Figure 7 represents the polycrystalline nature of the iron oxide nanoparticles and the planes are clearly observed.

3.3. Magnetic studies of composites

The room temperature magnetic properties of developed products are

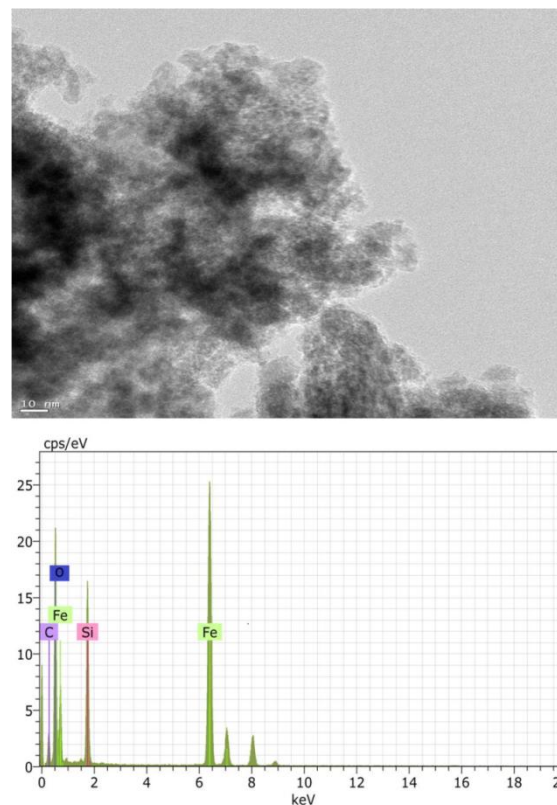


Figure 2. TEM and EDS images of $Fe_3O_4@SiO_2$.

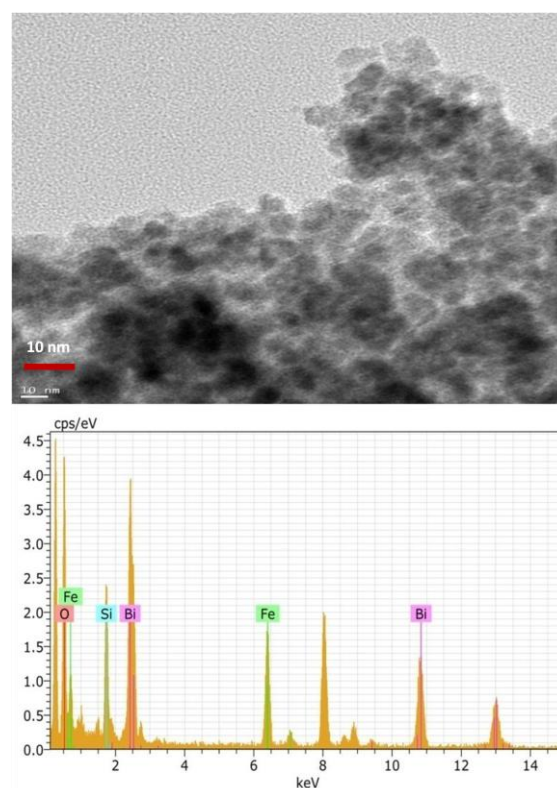


Figure 3. TEM and EDS images of $BiFeO_3@SiO_2$.

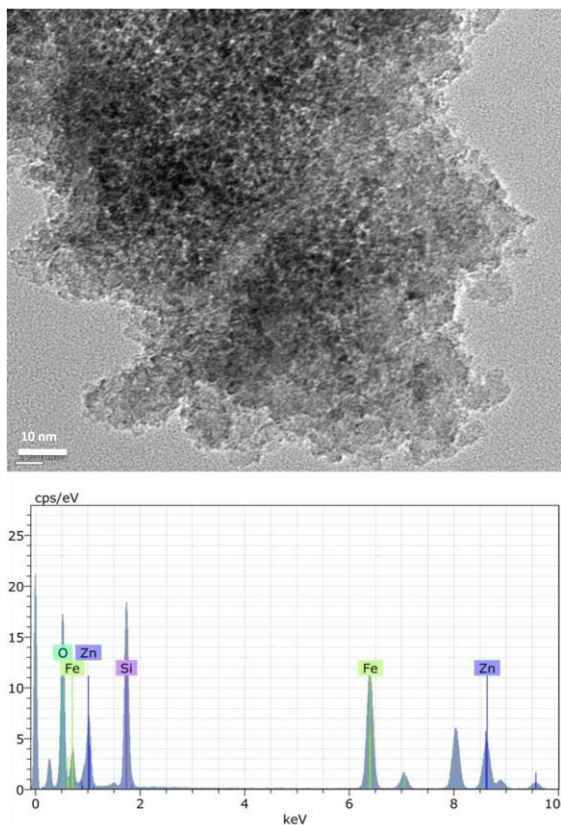


Figure 4. TEM and EDS images of $ZnFe_2O_4 @ SiO_2$.

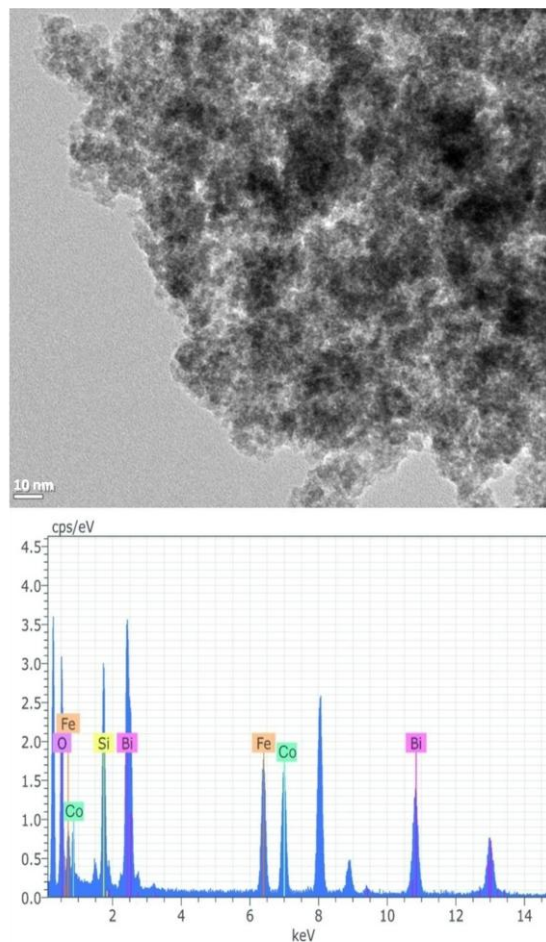


Figure 6. TEM and EDS images of $BiFe_{0.75}Co_{0.25}O_3 @ SiO_2$.

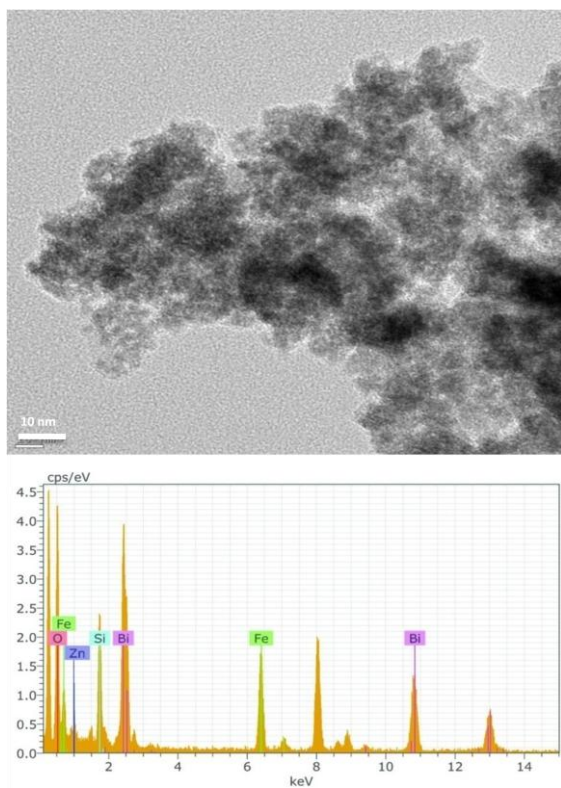


Figure 5. TEM and EDS images of $BiFe_{0.9}Zn_{0.1}O_3 @ SiO_2$.

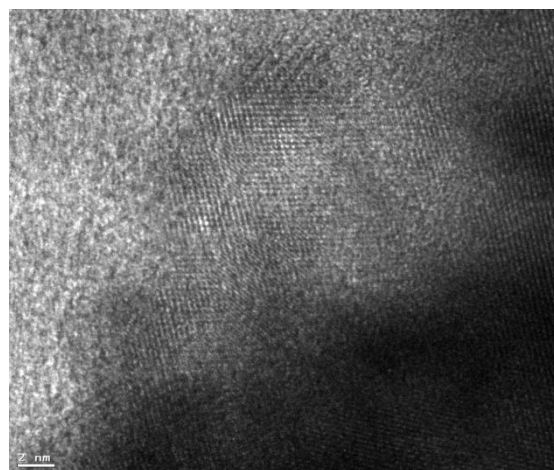


Figure 7. TEM image of polycrystalline nature of iron oxide.

measured in the range of -4T to 4T magnetic field and the graph was presented in Figure 8, from the figure it is noticed that the S1 sample shows superior super paramagnetic phenomenon of the iron

oxide nanoparticles even with silica coating. Also it is observed that the other particles magnetization is greatly reduced with silica coating compared with bare samples as reported in the literature [22]. From the figure it is noticed that the S3 and S4 samples showed very weak ferromagnetism representing the concentration of silica coating over the magnetic nanoparticles surface. The other samples S2 and S5 are almost showing diamagnetic nature.

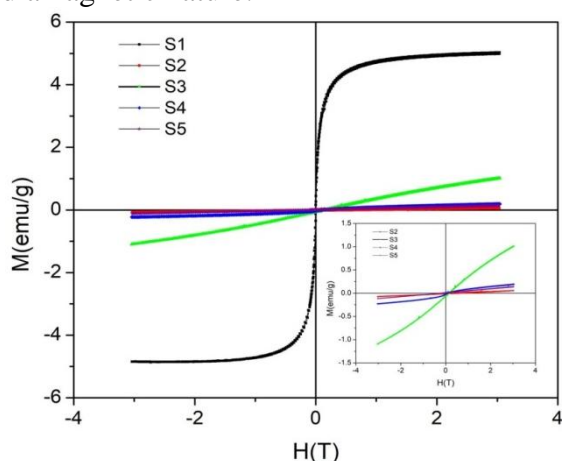


Figure 8. Room temperature M - H curves of nanocomposites.

3.4. *In vitro* cytotoxicity studies

The cytotoxic examination of developed nanoparticles is achieved by testing with THP-1 cells. The cells are incubated with two different concentrations (0.1 and 0.5 mg/mL) of the samples S1-S5 at time periods 12 and 24 hours. In CCK-8 assay, the dehydrogenase activities in cells are reduced the WST-8. Consequently, the WST-8 changes to formazan dye (yellow color) that soluble in culture media. In the each well of the culture plate the amount of dye is directly proportional to the number of living cells. In the culture plate the determination of amount of formazan dye can be achieved by measuring the absorbance spectrum at 450 nm with a micro plate spectrometer. The results of cytotoxic studies are depicted in Figure 9. The THP-1 cells treated with the two different concentrations (0.1 and 0.5 mg/mL) of prepared samples S1-S5 at time periods 12 hours and 24 hours Figure 9

(a&b). At two time points, the THP-1 cells exhibited an excellent viability ($\geq 100\%$) in all samples even in high concentration of 0.5 mg/mL of nanoparticle samples [23]. In view of the obtained results, the synthesized ferrite samples are considered to be good biocompatible materials.

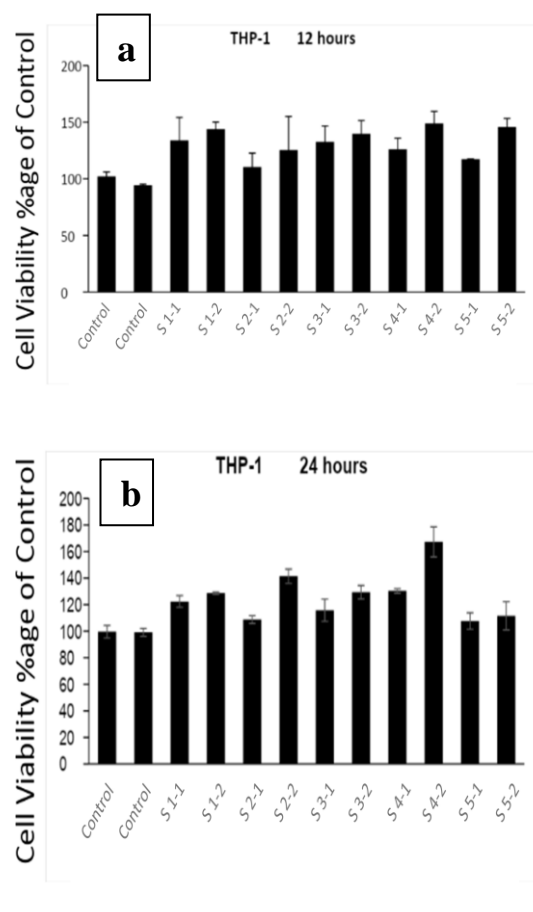


Figure 9. *In vitro* cytotoxicity tests of 0.1 mg/mL $Fe_3O_4@SiO_2$ (S1-1) and 0.5 mg/mL $Fe_3O_4@SiO_2$ (S1-2), 0.1 mg/mL $BiFeO_3@SiO_2$ (S2-1) and 0.5 mg/mL $BiFeO_3@SiO_2$ (S2-2), 0.1 mg/mL $ZnFe_2O_4@SiO_2$ (S3-1) and 0.5 mg/mL $ZnFe_2O_4@SiO_2$ (S3-2), 0.1 mg/mL $BiFe_{0.9}Zn_{0.1}O_3@SiO_2$ (S4-1) and 0.5 mg/mL $BiFe_{0.9}Zn_{0.1}O_3@SiO_2$ (S4-2) as well as 0.1 mg/mL $BiFe_{0.75}Co_{0.25}O_3@SiO_2$ (S5-1) 0.5 mg/mL $BiFe_{0.75}Co_{0.25}O_3@SiO_2$ (S5-2) samples with THP-1 cells at time periods of (a) 12 hours and (b) 24 hours. The culture of THP-1 cells without nanoparticles treatment considered as control (2 fold experiments).

4. CONCLUSION

In this chapter, silica coated iron oxide nanoparticles have been presented for biomedical applications. The nanomaterials are achieved by simple chemical double coprecipitation technique and are characterized by different techniques. The magnetic property attested for weak ferromagnetism compare to the silica coated iron oxide nanocomposites. The cytotoxicity data of nanocomposites on THP-1 cell line showed that all the nanoparticles are non toxic and out of all, the S4 sample

exhibited better biocompatible nature. This sample may be useful for biomedical applications.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal and Management SRKR Engineering College, Bhimavaram and the Principal, JKC College, Guntur for their encouragement and support. The authors are grateful to Dr. Venu Reddy for valuable discussions and providing necessary lab facilities.

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