Synthesis and Characterization of Biocompatible $Zn_{0.4}Co_{0.6}Fe_2O_4$ Nanoparticles

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Abstract **Magnetic nanoparticles have found many applications including biomedicine. However, factors such as cost-effectiveness, ease of synthesis and biocompatibility of the synthesized nanoparticles still need to be improved for the practical application. In the present study, we have synthesized $Zn_{0.4}Co_{0.6}Fe_2O_4$ superparamagnetic nanoparticles with a simple hydrothermal method and characterized using XRD, FTIR, FESEM, EDS, and VSM. Further, evaluation of the cytotoxicity against Astrocytes cell lines has been carried out using MTT Assay.**

Keywords- Superparamagnetic Nanoparticles, Hydrothermal Synthesis, Cytotoxicity

I. INTRODUCTION

Superparamagnetic nanoparticles (SPMNPs) are an important class of materials owing to their diverse applicability. The superparamagnetic property makes these nanoparticles (NPs) controllable inside the body by the application of an external magnetic field, which brings a paradigm shift to their application. SPMNPs have found application in photocatalysis [1], photodegradation of dyes [2], electrochemical energy storage [3], remote control of single cell functions [4], hyperthermia [5], MRI imaging [6], anticancer drug delivery [7], antibacterial activity [8] and radiotherapy [9].

Presently, researchers are working on improving the SPMNPs stability, heating efficiency and biocompatibility to take them into preclinical and clinical studies. Therefore, many studies are being performed on the effects of size, chemical composition, applied magnetic field and frequency, synthesis method, capping agents and doping to improve these properties [10]. Out of these, a big challenge is to obtain SPMNPs with high heating efficiency. Specific loss power (SLP) is the measure of heating efficiency and the conventional SPMNPs have very low SLP value depending on the SPMNPs size, chemical composition and applied magnetic field and frequency. Furthermore, the doping of some elements with different percentage has shown to improve the heating efficiency of the SPMNPs to a considerable extent. Amongst them, $Zn_{0.4}Co_{0.6}Fe_2O_4$ SPMNPs have shown a very high SLP value [5].

There are many techniques available to synthesize $Zn_{0.4}Co_{0.6}Fe_2O_4$ SPMNPs. However, the hydrothermal synthesis technique offers better size control and allows large-scale production of NPs, which makes them cost-effective [11]. Therefore, in the present study, we report a synthesis of $Zn_{0.4}Co_{0.6}Fe_2O_4$ SPMNPs by a simple hydrothermal method and evaluation of their biocompatibility in Astrocytes cell lines (mouse neuron cells) by MTT assay.

II. MATERIALS AND METHODS

A. *Synthesis of $Zn_{0.4}Co_{0.6}Fe_2O_4$ SPMNPs*

To synthesize $Zn_{0.4}Co_{0.6}Fe_2O_4$ NPs, the $Zn(NO_3)_2{\bullet}6H_2O$, $Fe(NO_3)_3{\bullet}9H_2O$, $Co(NO_3)_2{\bullet}6H_2O$ and NaOH of analytical grade were used. 0.133 M of $Fe(NO_3)_3{\bullet}9H_2O$, 0.026 M of $Zn(NO_3)_2{\bullet}6H_2O$, and 0.04 M of $Co(NO_3)_2{\bullet}6H_2O$ were dissolved in 50 ml double distilled water (ddw) and stirred for 15 minutes. Then, 1.04 M of NaOH dissolved in 10 ml ddw was added drop wise to the above mixture and the solution was stirred for 30 more minutes. After that, the mixture was transferred to Teflon autoclave (100 ml) and heated in a hot air oven at 160°C for 12 hours. The obtained NPs were washed three times in ddw to remove ionic impurity and two times in ethanol to remove organic impurity. Furthermore, the nanoparticles were dried in hot air oven at 70 °C for 6 hours. Later, in order to remove the defects in the crystals and to increase the crystallinity, the NPs were calcinated in a furnace at 450 °C for 10 hours.

B. *Characterization*

The structure and crystallite size of the synthesized SPMNPs were studied by X-ray powder diffraction with Rigaku Miniflex 600 X-Ray Diffractometer, with CuKα radiation (λ= 1.54060 A°, 35 kV, 10 mA). Fourier transform infrared spectroscopy (FTIR) data were recorded in the wavenumber range 4000 – 500 cm^{-1} using Shimadzu FTIR spectrometer. Field emission scanning electron microscopy (FE-SEM) and Energy dispersive spectroscopy (EDS) study was performed using Carl Zeiss Sigma FE-SEM attached with EDS. Magnetic characterization was performed by Quantum design vibrating sample magnetometer (VSM).

C. *MTT Assay*

For toxicity evaluation, MTT assay [12] was performed for the NPs concentration range of 0-200 µg/ml. MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide is a water-soluble salt of tetrazolium. 10,000 cells were added to each well of 96 well plate. NPs treatment was given for 24 hours. The final MTT concentration was 5 mg/ml. Cells were incubated for 4 hours. After that, the medium was removed and 100 µl of DMSO was added, and

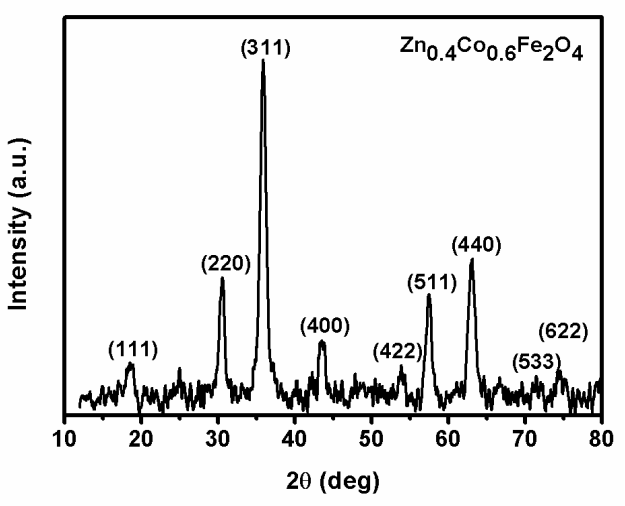

Fig. 1 The X-Ray diffraction pattern of $Zn_{0.4}Co_{0.6}Fe_2O_4$ NPs

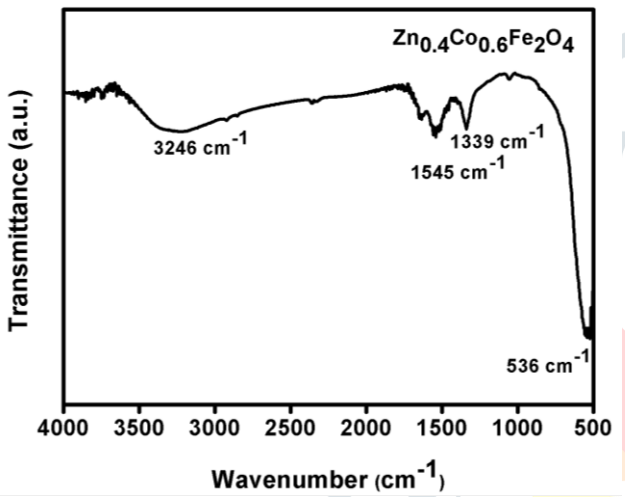

Fig. 2 The FTIR spectrum of $Zn_{0.4}Co_{0.6}Fe_2O_4$ NPs

the plate was incubated for 20 minutes until the formazan was dissolved completely. Optical density was measured by a microplate reader at a wavelength of 590 nm. The cell viability was determined as the ratio of the optical density (OD) of the sample to the OD of the control solution and expressed as a percentage.

III. RESULTS AND DISCUSSION

The x-ray diffraction pattern of SPMNPs is shown in Fig. 1. The formation of a homogeneous single-phase cubic spinel structure was confirmed from the obtained XRD pattern. The Fig.1 shows diffraction peaks corresponding to (111), (220), (311), (400), (422), (511), (440), (533), and (622) reflection planes. The average crystallite size of the sample was estimated by the Scherrer equation

$$D = \frac{0.9\lambda}{\beta\cos\theta}$$

where D is the crystallite size, λ the wavelength of the radiation, θ is the Bragg diffraction angle and β is the full-width at half maximum (FWHM) of the diffraction peaks in radians. The calculated average crystallite sizes of the samples were 8.3 nm.

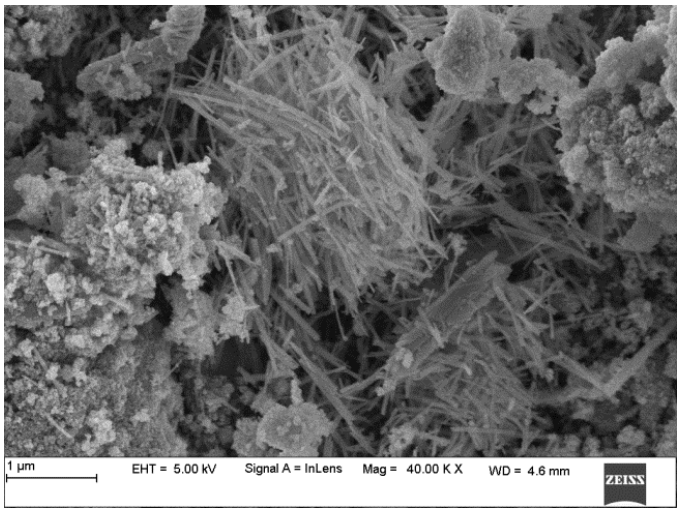

Fig. 3 The FE-SEM image of $Zn_{0.4}Co_{0.6}Fe_2O_4$ NPs

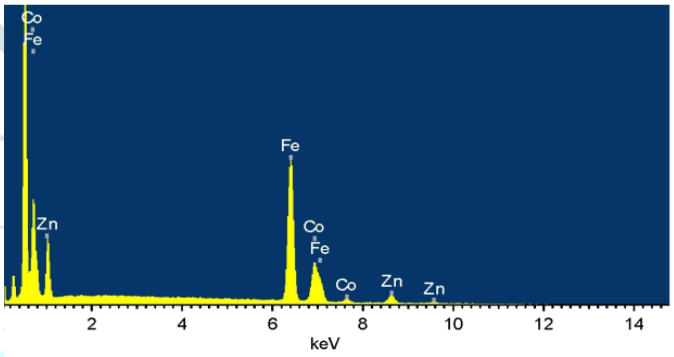

Fig. 4 The EDS spectra of $Zn_{0.4}Co_{0.6}Fe_2O_4$ NPs

FTIR analysis was done in order to study the nature of different bonds in the NPs and the FTIR spectrum is shown in fig. 2. The peak at 536 cm^{-1} confirms the metal-oxygen bond. The peak at 3246 cm^{-1}, 1545 cm^{-1} and 1339 cm^{-1} correspond to the O-H stretching, N-O asymmetric and N-O symmetric stretching on the surface of the NPs respectively. The symmetric and asymmetric N-O stretching could be due to the impurities present in the sample.

The FE-SEM image of the zinc doped cobalt ferrite NPs obtained from FE-SEM is shown in Fig. 3. The image shows the formation of a mixture of rod- and sphere-shaped nanomaterials. Slight agglomeration of the nanoparticles can also be seen due to permanent magnetic moment. Zn substitution leads to higher magnetic moment causing clustering.

The elemental composition of $Zn_{0.4}Co_{0.6}Fe_2O_4$ was obtained from EDS spectrum which is shown in Fig. 4. The analysis confirms that the observed elemental composition is the same as the calculated elemental composition in the NPs.

Fig. 5 shows the magnetic property of the $Zn_{0.4}Co_{0.6}Fe_2O_4$ nanoparticles studied using VSM technique at room temperature with a maximum magnetic field of 4kOe. From the figure, it can be seen that the synthesized nanoparticles are superparamagnetic and exhibit a good saturation magnetization (Ms) of 68 emu/g with no remanence and coercivity.

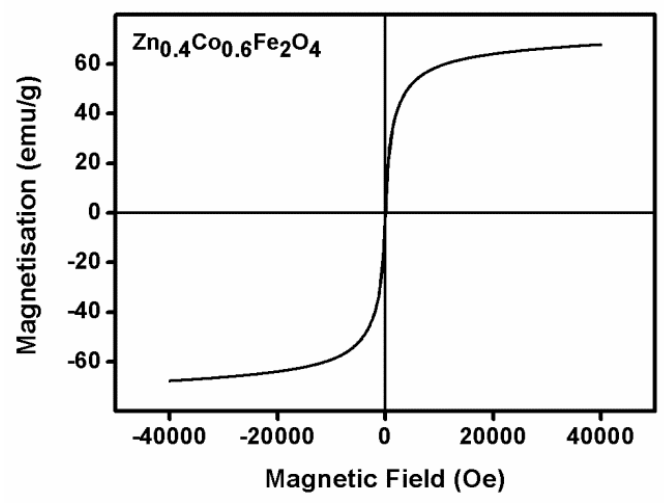

Fig. 5 The VSM data of $Zn_{0.4}Co_{0.6}Fe_2O_4$ NPs

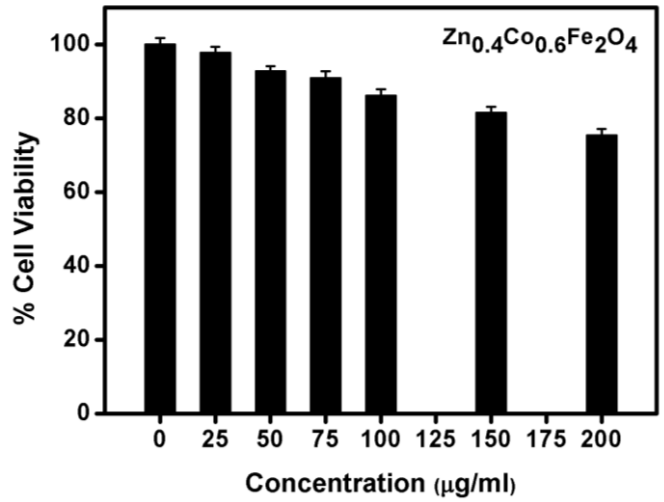

Fig. 6 Cell viability study of $Zn_{0.4}Co_{0.6}Fe_2O_4$ NPs on astrocytes.

The MTT assay results presented in the Fig. 6 show that the studied NPs are non-toxic to the Astrocytes cell lines (Normal cell lines) up to the concentration of 150 µg/ml and NPs become slightly toxic as the % cell viability drops below 80%. This implies that the NPs are biocompatible and can be used for the anticancer studies.

IV. CONCLUSIONS

In the present study, we prepared the $Zn_{0.4}Co_{0.6}Fe_2O_4$ SPMNPs using a simple hydrothermal method. The prepared nanoparticles were characterized using XRD, FTIR, FE-SEM, EDS, and VSM. These studies showed the formation of a mixture of rod and sphere-shaped nanoparticles with good crystallinity and magnetization value of 68 emu/g. The synthesis technique reported in this paper offers better size control, low cost of preparation and large-scale production of nanoparticles. The MTT study performed to evaluate the cytotoxicity showed that the prepared SPMNPs are nontoxic and hence can be used in cancer therapy applications.

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