STRUCTURAL, MORPHOLOGICAL AND MAGNETIC PROPERTIES OF FUNCTIONALIZED MANGANESE IRON OXIDE NANOPARTICLES FOR BIOLOGICAL APPLICATIONS

Priti Ghutepatil[1,*] and Priya Oghe[2]

Received: March 21, 2024; Revised: May 13, 2024; Accepted: June 12, 2024

Abstract

The chitosan functionalized $MnFe_2O_4$ nanoparticles were synthesized using thermal decomposition method. The morphological, structural and magnetic properties of obtained chitosan functionalized magnetic nanoparticles were studied to assess the feasibility for biomedical applications. The crystallite size of nanoparticles observed using XRD was 15nm. Functional group attachment to surface of nanoparticles was analyzed using FTIR. Particle size of nanoparticles was studied by TEM analysis and it showed that particles were roughly spherical. Thermal stability and bonding strength of ligands to the surface of material was determined using TGA. The magnetization of chitosan functionalized nanoparticles observed by VSM is 56emu/g and VSM showed no coercivity and remanence existence, stipulating the super-paramagnetic behavior. The cell viability of chitosan functionalized nanoparticles was above 85% at concentration up to the 1.0mg mL^{-1} on mouse fibroblast cell line i.e. L929 cell line. These studies revealed that, $MnFe_2O_4$ nanoparticles are potential materials for biomedical applications.

Keywords: Magnetic nanoparticles; Chitosan; Super-paramagnetic; Biomedical application

Introduction

Magnetic nanoparticles (MNPs) have been recognized as promising material due to their outstanding properties (physical and chemical) in countless fields. It has been researched with interest due to its intrinsic characteristics in order to use it in medical applications such as magnetic resonance imaging (MRI), magnetic cell separation (MCS), contrast agent, drug delivery and magnetic hyperthermia (Zhao *et al*., 2006, Phadatare *et al*., 2013). Magnetic nanoparticles have controllable sizes in the range of few to tens of nanometers. Magnetic nanoparticles can be operated by applying external magnetic field. Manganese ferrite ($MnFe_2O_4$) is noteworthy ferrite constituent. It demonstrates outstanding characteristics such as high resistivity, high magnetization, and superparamagnetic nature. Due to these properties, manganese ferrite ($MnFe_2O_4$) becomes noteworthy material for biomedical applications (Sahoo *et al*., 2012). The suitable surface chemistry is an

[1] *Department of Applied Science, Pimpri Chinchwad College of Engineering & Research, Pune-412101 India. E-mail: pritighutepatil@gmail.com*
[2] *Baburaoji Gholap College, PWD Colony Rd, Defence Area, Old Sangvi, Pune, Pimpri-Chinchwad, Maharashtra 411027 India.*
[*] *Corresponding author*

DOI: https://doi.org/10.55766/sujst-2024-04-e03952

Suranaree J. Sci. Technol. 31(4):030210(1-6)

important factor considered for biomedical applications. The coating of magnetic particle is accomplished through chemical and physical interactions. The many studies have described or demonstrated that coatings of magnetic nanoparticles with polymers, such as pluronic(F127), carboxymethyl dextran, 21 dextran, polyvinyl alcohol, polyethylene glycol, fatty acids, chitosan, etc. (Khot *et al*., 2012). In past few years, the magnetic chitosan nanocomposites have attracted great interest because of it's potential in biomedical applications (Wu *et al*., 2009). The chitosan showed the chelating, unique polycationic and film-forming properties due to the existence of active amino and hydroxyl functional groups, which is favorable micromolecule to coat magnetic nanoparticles (Liu *et al*., 2009). The functionalization of MNPs with biopolymer enables banishment of drug molecules, enhances biocompatibility and offer provides that allows attachment of targeting moieties. Agnihotri etc al. studied the functionalized chitosan and demonstrated that it is promising biomaterial for biomedical applications (Agnihotri *et al*., 2004; Xiao *et al*., 2013). Many methods are developed for the synthesis of MNPs including co-precipitation, sol-gel, microwave, combustion and thermal decomposition method (Salunkhe *et al*., 2014). However, thermal decomposition method is impressive method as it gives smaller size, monodispersity and high magnetized nanoparticles. Biocompatibility of magnetic nanoparticles is significant factor to use them for biological applications. Nanoparticles can be functionalized with polymers to reduce toxicity, increase the compatibility and prevent particles from oxidation. Here, chitosan polymer is used to functionalize the magnetic nanoparticles as chitosan is non-toxic in nature, biocompatible, biodegradable and exhibits high affinity to the cell membrane.

The present work reports the structural and morphological study of chitosan functionalized MnFe2O4 MNPs synthesized by thermal decomposition method. The biocompatibility study of chitosan functionalized nanoparticles is also reported.

Materials and Methods

The chitosan functionalized $MnFe_2O_4$ magnetic nanoparticles were synthesized using thermal decomposition approach. Mixture of $MnCl_2$ (5mmol), $FeCl_3$ (10mmol), chitosan (1g), sodium acetate (1.3g) were incorporated into 30mL diethylene glycol to form transparent solution via stirring. Chemicals used without further purification and were of analytical grade. The whole reacting materials are stirred strenuously and refluxed for 180 minutes at 200°C. Blackish colored colloidal suspension of particles become visible in flask having round bottom, cooled at RT. The obtained particles were rinse multiple times with ethanol. After this, particles separation has been done magnetically and dried at room temperature (Shen *et al*., 2014).

Results and Discussion

The X-ray Diffraction - Philip-3710 technique with Cu-Kα radiation (λ = 1.5418 Å) in the 2θ range from 20° to 80° is intended for structural study and phase purity of chitosan functionalized magnetic nanoparticles (Figure 1). The diffraction peaks obtained with (hkl) values (220), (311), (400), (422) and (511) matches with the peaks of the diffraction pattern of the standard material (JCPDS 38-0430). The FWHM (Full width at half maxima) was determined by using Gaussian fit method and crystallite size (D) is calculated by using Scherer's formula;

$$D = (0.9\,\lambda) / \beta \cos\theta$$

where "D" denotes crystallite size, λ is wavelength of radiation, β is FWHM of intense peak, 0.9 is the Scherer constant, θ is diffraction angle. Crystallite size of chitosan functionalized magnetic nanoparticles was 15nm.

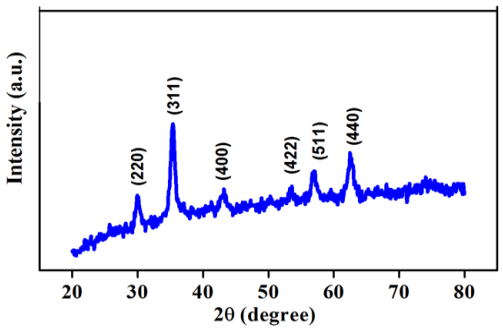


Figure 1. XRD pattern of chitosan functionalized $MnFe_2O_4$ nanoparticles

It is observed that coating of magnetic nanoparticles was affirmed by Fourier transform infrared (FTIR) and thermogravimetric analysis (TGA). FTIR spectra were recorded for MNPs with the spectrometer (Alpha ATR Bruker Eco Model) over the spectral range of 450 to 4,000cm^{-1}

(Figure 2). Functional group attachment to surface of core MNPs was analyzed by FTIR. The sample (P) shows a characteristic spectrum at around 538 and 665cm^{-1} attributed to the Fe-O structure and characteristic band at 1056cm^{-1} was assigned to C-O-C stretching vibration. The spectrum at 1,406cm^{-1} was assigned to -C-O stretching of primary alcoholic group in chitosan and the band around 1625 and 3,400cm^{-1} was designated to N-H bending vibration and O-H stretching respectively. This confirms the existence of chitosan molecules on the surface of magnetic nanoparticles.

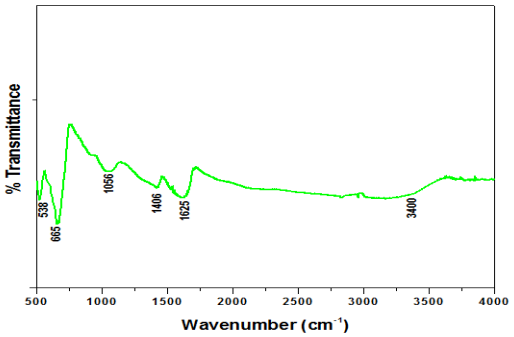


Figure 2. FTIR spectra of chitosan functionalized $MnFe_2O_4$ magnetic nanoparticles

Figure 3 shows the TGA analysis of coated magnetic nanoparticles carried out using trans-analytical instrument model (SDT 2960) to decide the adhesion of ligands to the magnetic nanoparticles surface and thermal stability. TGA curve for the chitosan functionalized MnFe2O4 magnetic nanoparticles were noted down in temperature ranging from 27 to 900℃ with a heating rate of 10℃ in nitrogen atmosphere. The observed weight loss is in 3 steps. Figure 3. demostrates the weight loss in 3 stages in temperature ranges of room temperature to 200℃, 200-400℃ and 600-800℃. The weight loss in beginning step having to the evaporation of adsorbed water and moisture, the weight loss in second step corrosponds the decomposition of diethylene glycol from the surface of sample and weight loss in third step from 600-800°. It is quite steady step due to the further decomposition of sample to form pure $MnFe_2O_4$. A drastic drop can be seen in the range 150-400℃ and it is contributed from the thermal decomposition of chitosan occured.The first weight loss was because of removal of moisture content. The second weight loss was observed because of decomposition of diethylene glycol and last loss in weight was observed due to the bonding of chitosan molecules to the surface of nanomaterials (Li *et al*., 2008; Guoa *et al*., 2012; Wang *et al*., 2013).

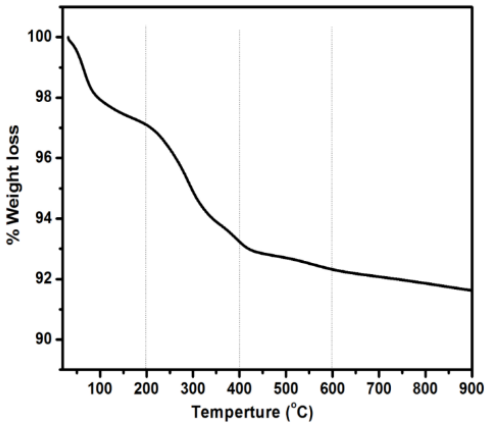


Figure 3. Thermogravimetry curve of chitosan functionalized $MnFe_2O_4$

The particle size and morphology of Magnetic nanoparticles were studied with the help of SEM (Scanning Election Microscope) TEM (Transmission Electron Microscope) which are shown in Figure 4 and Figure 5. Morphological analysis of synthesized nanoparticles is performed using FESEM (Scanning Election Microscope) and TEM (Transmission Electron Microscope). FE-SEM (Hitachi S-4800) were used to determine the morphology which shows distinct nanoclusters which are roughly spherical in shape. TEM (Philips CM 200 model) having operating voltage 20-205kV with resolution 2.4°A used to determine particle size.TEM images show roughly spherical nanoparticles having some irregularities, with slightly wide size distribution. Saturation magnetization and coercivity were measured by VSM (Vibrating sample magnetometer) at room temperature with M-H curve. Figure 5(a) depicts TEM image of chitosan functionalized $MnFe_2O_4$ MNPs and it shows the nearly spherical formed nanoparticles with few irregularities (Ebrahiminezhad *et al*., 2013). Average particle size is 17 nm with standard deviation 3.2. which is in conformity with diameters obtained from XRD data. Particle size meets with crystallite size measured from XRD. Figure 5(b) depicts selected area electron diffraction (SAED) patterns and it shows vivid ring patterns suggesting crystalline nature of MNPs as specified by XRD. The ring patterns correspond to (220), (311), (400), (511) and (440) planes, which can be clearly observed in XRD results.

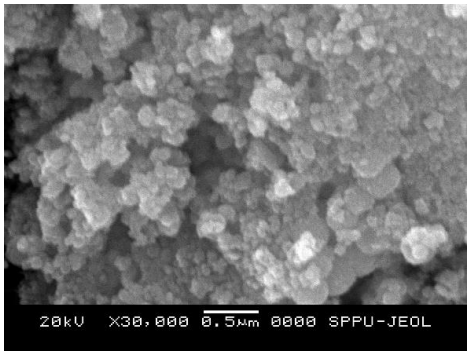
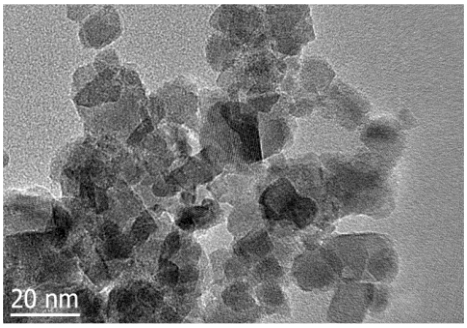
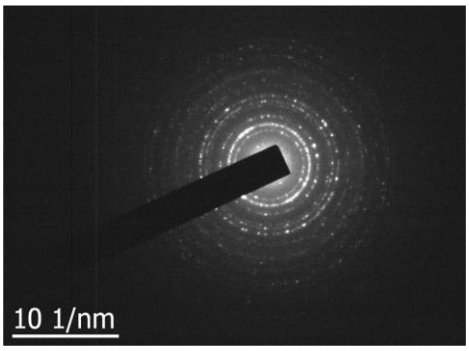


Figure 4. SEM image of chitosan functionalized $MnFe_2O_4$ nanoparticles



(a)



(b)

Figure 5. (a) SEM image of chitosan functionalized $MnFe_2O_4$ nanoparticles. (b) SAED Pattern of chitosan functionalized $MnFe_2O_4$ magnetic particles

Saturation magnetization and coercivity were measured by VSM (Vibrating sample magnetometer) at room temperature with M-H curve. The study of Magnetic properties of synthesized chitosan functionalized $MnFe_2O_4$ magnetic nanoparticles calculated by VSM. The M vs H measurements at 300K were carried out as shown in Figure 6. No coercivity and remanence existence observed from hysteresis curve of functionalized $MnFe_2O_4$ magnetic nanoparticles at 300 K indicating the superparamagnetic behavior. The saturation magnetization of chitosan functionalized $MnFe_2O_4$ is observed 56emu/g. (Taavoni-Gilan, 2018) synthesized chitosan coated $MnFe_2O_4$ naoparticles by co-precitation method in two steps which was showing saturation magnetization around 39.5emu g-1.

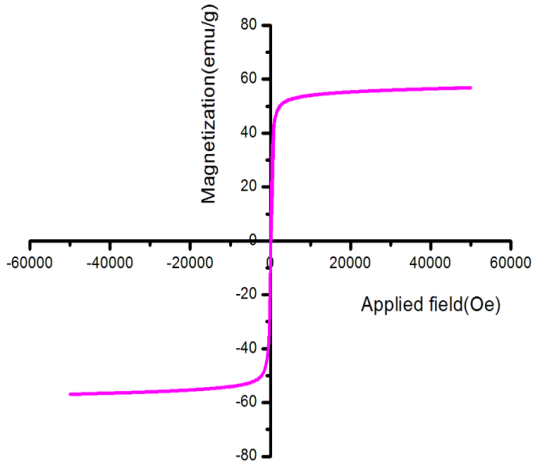
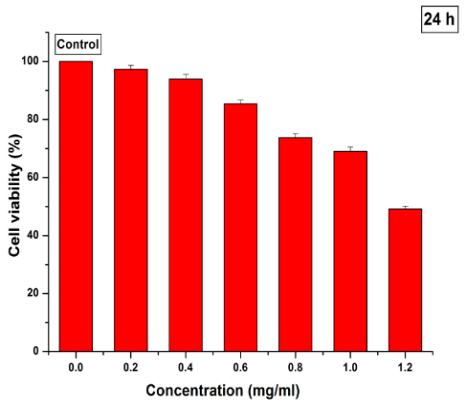
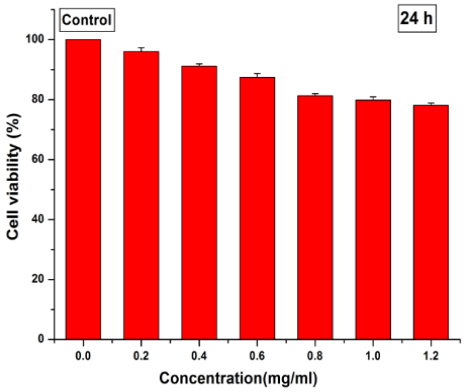


Figure 6. M-H curves of chitosan functionalized $MnFe_2O_4$ magnetic nanoparticles

Biocompatibility of magnetic nanoparticles is an important parameter regarding their use for biomedical applications. The MTT assay was accomplished to examine cytotoxic effect of magnetic nanoparticles. Cytotoxicity study of bare $MnFe_2O_4$ and chitosan functionalized $MnFe_2O_4$ was done on the L929 cell line and the procured data is shown in Figure 7(a) and Figure 7(b). The L929 cell line was incubated with both bare and functionalized magnetic nanoparticles for 24 h with the concentrations of 0.2, 0.4, 0.6, 0.8 and 1.2mg mL^{-1} at 37°C in a 5% CO_2 atmosphere. Results show that cell viability of bare $MnFe_2O_4$ is upto 50% but for chitosan functionalized $MnFe_2O_4$ it is showing above 80% up to 1.2mg mL^{-1} it means that functionalized $MnFe_2O_4$ nanoparticles are promising material for biomedical applications.



(a)



(b)

Figure 7. (a) Biocompatibility study of bare $MnFe_2O_4$ at 24 h on L929 cell line. (b) Biocompatibility study of chitosan functionalized $MnFe_2O_4$ at 24 h on L929 cell line

Conclusions

In summary, the chitosan functionalized $MnFe_2O_4$ nanoparticles were synthesized using thermal decomposition method. Average size of obtained nanoparticles was 17nm. The detailed study about structural properties, magnetic properties and morphological properties of $MnFe_2O_4$ nanoparticles have been analyzed in detail. Experimental result exhibited that magnetic nanoparticles exhibited superparamagnetic behavior and high saturation magnetization at room temperature. MTT assay proved that prepared magnetic nanoparticles are biocompatible up to the 1.2mg/mL concentration on L929 cell line. Thus, magnetic nanoparticles prepared by thermal decomposition method are smaller size, superparamagnetism, higher magnetization and low cytotoxicity which can be potential material for biomedical applications.

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