Synthesis and Characterization Studies of Mesoporous Chitosan Coated Fe_3O_4 Nanoparticles with Folic Acid

Agnes J

St Xavier's College <https://orcid.org/0000-0002-2488-6884>

Ajith P

St Xavier's College

Sappani Muthu M

St Xavier's College

Premanand D (✉ dpremanand@yahoo.co.in)

St Xavier's College

Research Article

Keywords: Magnetic nanoparticles, Chitosan, Folic Acid, Iron Oxide (Fe_3O_4)

Posted Date: January 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1262551/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at International Journal of Engineering and Advanced Technology on April 30th, 2022. See the published version at <https://doi.org/10.35940/ijeat.C3367.0411422>.

Abstract

In this present work, Fe_3O_4 nanoparticles were obtained by a one-part Co-precipitation method. Secondly, a simple solvothermal method was employed to synthesis Chitosan (CS) coated Fe_3O_4 nanoparticles. Chitosan was used as a surface modification agent. The surface of Chitosan-coated Fe_3O_4 nanoparticles was conjugated with Folic Acid (FA). Various properties of the resultant products were performed by characterization studies. The structure and surface morphology of as-prepared nanoparticles were characterized by X-ray diffraction (XRD) and Scanning electron microscopy (SEM). The physical parameter such as strain and the crystallite size was evaluated for all the reflection peaks of the samples by using Williamson–Hall (W-H plot) method. Determination of the chemical component was marked by Fourier transform infrared spectroscopy (FTIR) and Energy-dispersive X-ray spectroscopy (EDAX) analyses. UV- Vis experiment was carried out to record optical absorbance and bandgap energy of the nanoparticles was determined by Tauc's plot. Thermogravimetric analysis (TGA) was conducted to recognize the thermal stability of the magnetic nanoparticles and endothermic peaks were observed from the first derivative of the TGA curve (DTG curve).

1. Introduction

The prime multidisciplinary branch of modern technology is Nanotechnology. In this current situation, nanotechnology is probably the most emerging area of research. Nano-based materials prepared by this excellent technology hold supreme potential. Nanomaterials mean a normal, manufactured material that is typically defined as any material with a containment range from 1 to 100 nm. To battle bulk materials, nanomaterials are commonly synthesized for their fascinating properties such as optical, mechanical, and magnetic properties. Magnetic Iron oxide nanoparticles are synthesized by various methods such as the Co-precipitation method [1–2], wet chemical reduction technique, and solvothermal method. But mostly Solvothermal method is utilized for producing magnetic nanoparticles because of its simple approach [3]. Magnetic nanoparticles [MNPs] are needed in a wide range of applications. Magnetic nanoparticles are less stable and appear to cause agglomeration due to their small particle size and large surface-to-volume ratio. Magnetic iron oxide (Fe_3O_4) with face-centered cubic forming oxygen has a cubic reverse spinal structure [4–5]. Due to the multifunctional properties such as small scale, superparamagnetism, and low toxicity, magnetic iron oxide (Fe_3O_4) nanoparticles have a broad variety of practical applications including physics, medicine, and biology [6]. The magnetic iron oxide nanoparticles showed that the synthesized NPs have a spherical shape and the magnetic ferrofluid contains single-domain magnetic particles and performs superparamagnetic behavior. By decreasing the temperature, the magnetic softness of ferrofluid decreases while its anisotropy increases [7]. Chitosan is a polymeric substance that is used for nanoparticles synthesis. It has outstanding special properties, such as biocompatibility, non-toxicity, biodegradability, much adhesively, and the ability to increase the transport of drugs by paracellular cells [8]. Many modifiers, such as poly (acrylic acid) PAA, polyethyleneimine (PEI), polyethylene glycol (PEG), and other biocompatible polymers, have been applied to the reaction mixtures to monitor the size of Fe_3O_4 nanoparticles and increase colloidal stability and biocompatibility. Chitosan

has crystalline nature and depending upon its physical state, exhibits polymorphism [9]. Due to its important biological and chemical properties, chitosan has been intensively studied as a base material for magnetic carriers compared to the types of polymers described above. It is desirable for the chitosan coating to change the surface properties of the core structure, either to enhance the contact pattern with the surrounding structure [10]. Behdokht Bahrami, Mousa Mohammadnia Afrouzi et al. have made an investigation for realizing the efficiency of various folate–conjugated nanoparticles in cancer therapy. From several findings, there was evidence that folate–conjugation increases the stability of NPs and it is useful for cancer imaging, detecting cancer cells, and for targeted drug delivery systems [11]. FA ligands grafted $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ mesoporous spheres are ferromagnetic and targeted to organs by an external magnetic field. The obtained results of this research confirm that the synthesized nanoparticles possess a high potential for targeted anticancer drug delivery [12]. A wide range of surveys has been done based on folate decorated chitosan, which has given a new perception in order to acknowledge the enhanced potential of these nanoparticles. This survey reveals that both cancer cells and cancer stem cells are killed by the folate chitosan NPs [13]. The surface-modified Fe_3O_4 magnetic nanoparticles with Folic acid enrich their capability of taking the anticancer drug to the targeted delivery system. Characterization studies reveal that these NPs exhibit high loading affinity for an anticancer drug [14]. The review has been carried out on Folic acid-functionalized NPs gives a clear idea about the development of active cancer diagnosis and therapy. Polymer-based NPs such as Biopolymers Chitosan, PLGA [poly (lactide-co-glycolide)], PLA (polylactic acid), Dextran, Hyaluronic acid, Chemopolymers NPs (Polyamidoamine, Polyethyleneimine), Lipid-based NPs (Bilayer lipid Nps, Monolayer lipid NPs), and Metal and- Nonmetal – based NPs made a great impact on Targeted drug delivery system. Surface-modified NPs by various anti-cancer drugs improve the specific drug delivery into cancer cells [15]. Various studies have been reported, drug loading Iron oxide NPs send to the affected area by an external magnetic field. Conjugation of Fe_3O_4 NPs with different modifiers has great potential in anticancer treatment [16– 17].

This present work was done based on detecting the changes on the surface of MNPs (Fe_3O_4) using modifiers Chitosan and Folic Acid. In this context Co-Precipitation and Solvothermal method was implemented for synthesizing Fe_3O_4 and composite MNPs. Accordingly, Surface modification of prepared MNPs was demonstrated by characterization studies XRD, FTIR, SEM, EDAX, UV-Vis, and TGA techniques.

2. Experimental

To accomplish new innovative work two phenomenons are essential 1. Materials, 2. Methods. Materials are the basic platform to launch a new adventure in the current situation. Methods are chosen should be efficacious and generate great interest in everyone involved.

2.1 Materials

Ferric Chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Ferrous Chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) were purchased from Spectrum Reagents and Chemicals Pvt. Ltd. Edayar, Cochin. Chitosan ($\text{C}_6\text{H}_{11}\text{NO}_4$)_n,

polyvinylpyrrolidone (C_6H_9NO)_n, and N-hydroxysuccinimide (NHS) [$(CH_2CO)_2NOH$] were collected from Himedia. Folic acid ($C_{19}H_{19}N_7O_6$) and N-ethyl-N-(3-dimethylamino propyl) Carbodimide (EDC) [$C_8H_{17}N_3$] were obtained from Loba Chemie Pvt. Ltd. Ammonium hydroxide, Ethylene glycol ($C_2H_6O_2$), Diluted Acetic Acid ($C_2H_4O_2$), Sodium Acetate (CH_3COONa). Dimethyl Sulphoxide (DMSO) (C_2H_6OS), Ethanol (C_2H_5OH) were of analytical grade, purchased from United Scientific Centre, and utilized without further purification.

2.2 Methods

The synthesizing process contains the following three steps. The first step was carried out by the Co-precipitation method and the other two steps were done by the Facile Solvothermal method [18].

2.2.1 Synthesis of pure Fe_3O_4 nanoparticles

The magnetite Fe_3O_4 nanoparticles were prepared by the Co-precipitation method [19–20]. The mixture of 12.0g of $FeCl_3 \cdot 6H_2O$ and 4.9g of $FeCl_2 \cdot 4H_2O$ were dissolved in 100 mL of deionized water and kept in a vigorous stirrer at 40° C constantly for 3 hours. Black precipitates were formed by adding 25 mL of ammonium hydroxide into the solution swiftly. This reaction mixture was stirred vigorously at 90 °C for 3 h. Final products were collected from the solution by filtration. The further purification process was carried out with deionized water. Ultimately slurry of obtained nanoparticles was dried in a microwave oven at 70 °C for 24 h.

2.2.2 Synthesis of CS-coated Fe_3O_4 NPs

The reaction mixture was prepared by dissolving 1.50 g of $FeCl_3 \cdot 6H_2O$, 0.75 g of Chitosan, 1.0 g of Polyvinylpyrrolidone, 3.6 g of Sodium Acetate (NaOAc) in 70mL of ethylene glycol, and stirred vigorously until the transparent solution is obtained. This mixture is kept under heating treatment in Autoclave at 300 °C for 6 h. Filtration was carried out for the obtained colloidal suspension and washed with 0.5% diluted acetic acid and deionized water several times. The obtained CS-Coated Fe_3O_4 NPs were calcinated at 60° C in an oven and collected with a magnet for further process.

2.2.3 Surface modification of CS-Coated Fe_3O_4 NPs with Folic Acid

The prepared CS-Coated Fe_3O_4 NPs were conjugated with Folic Acid as part of NHS and EDC activation. Generally, 0.1 g of FA, 0.10 g of NHS, and 0.158 g of EDC mixed with a suspension of 100 mL DMSO that contains 0.2 g of CS-coated Fe_3O_4 NPs. This admixture is kept under ultrasonication for 30 minutes and stirred vigorously. The resultant dark precipitates were collected by filtration and purged thrice with deionized water and ethanol. Finally dried at 80 °C for 16 h and collected to be characterized.

3. Instrumentations

Crystalline structure, and phases of prepared pure Fe₃O₄, CS-coated Fe₃O₄, and FA-CS-coated-Fe₃O₄ nanoparticles were examined employing X-ray diffraction (XRD) with a Philips PW-1800 diffractometer with CuK α radiation ($\lambda = 1.5406 \text{ \AA}$). From the obtained XRD pattern the crystallite size of the nanoparticles was calculated by using the Scherrer equation. The chemical bonds of the nanoparticles were recorded by Fourier Transform Infrared Spectroscopy (FTIR), Perkin Elmer Spectrum GX. The surface morphology of the produced nanoparticles was recognized by Scanning electron microscopy (SEM), JEOL-S-3400 N II equipped with EDAX was utilized to carry out elemental studies. UV-Vis spectra were obtained for the nanoparticles using Ultra Violet- Visible Spectrometer (Shimadzu UV-1800). Thermal behavior was characterized by using a thermoanalyser (STA-1500).

4. Results And Discussion

4.1 Structural analysis

The crystal structure, crystalline phases, and crystallite size of synthesized pure Fe₃O₄ and composite nanoparticles such as CS coated Fe₃O₄ and FA coated CS-Fe₃O₄ were identified by using an X-ray diffractometer. Fig.1. presents XRD pattern of prepared magnetic nanoparticles 1(a) Fe₃O₄ NPs, 1 (b) CS-coated Fe₃O₄ NPs, 1 (c) FA-CS-coated Fe₃O₄ NPs. The obtained characteristic peaks of NPs at $2\theta = 33.24^\circ, 35.71^\circ, 43.53^\circ, 57.57^\circ$ and 62.49° correspond to Bragg reflection planes (220), (311), (400), (333), and (440). The obtained diffraction peaks are indexed as an inverse spinel structure of Fe₃O₄ NPs having lattice parameter $a = 8.39 \text{ \AA}$ and results are in good agreement with the standard data (JCPDS: 19-0629) [21]. Manifestation of other peaks at different 2θ values is the evidence for the conjugation of chitosan and folic acid. The Williamson-Hall method was carried out to determine the crystallite size and the strain-induced in powders [22]. The instrument broadening (β) corrected for each diffraction peak was estimated by the relation

$$\beta_D = [(\beta^2)_{\text{measured}} - (\beta^2)_{\text{instrumental}}]^{1/2} \quad (1)$$

On the other hand, the crystallite size of the nanoparticles was calculated by Debye- Scherrer equation as

$$D = K\lambda / \beta_D \cos\theta \quad (2)$$

Where D is particle size (nm), K is the grain shape factor (0.9), λ is the wavelength ($\text{\AA}$), θ is the Bragg angle, and β_D is the peak width at half-maximum intensity.

The strain induced in powder associated to the crystal imperfections was determined by,

$$\epsilon = \beta_s / 4 \tan\theta \quad (3)$$

$$\beta_s = 4\epsilon \tan\theta \quad (4)$$

The above equation (4) indicates the strain-induced line broadening.

$$B_{hkl} = \beta_s + \beta_D \quad (5)$$

$$B_{hkl} = (4\epsilon \tan\theta) + (K\lambda/D \cos\theta) \quad (6)$$

By rearranging Eq. (6)

$$B_{hkl} \cos\theta = (K\lambda/D \cos\theta) + 4\epsilon \sin\theta \quad (7)$$

The above Eq.(7) is known as the Williamson-Hall equation. Therefore, this equation represents the UDM (Uniform Deformation Model), where the strain was assumed to be uniform in all crystallographic directions. The plot is drawn with $4 \sin\theta$ along the x-axis and $B_{hkl} \cos\theta$ along the Y-axis. The crystalline size was calculated from the Y-intercept and the strain (ϵ) from the slope of the linear fit to the data. The UDM analysis results for the nanoparticles are shown in Figure 2 (a-c). The crystallite size of the nanoparticles was calculated as 10.35 nm (a. Pure Fe_3O_4), 14.87 nm (b. CS-coated Fe_3O_4), and 15.77 nm (c. FA-CS-coated Fe_3O_4) respectively. Table 1. represents the Crystallite size, microstrain, and intercept value of pure Fe_3O_4 NPs, CS-coated Fe_3O_4 NPs, and FA-CS-coated Fe_3O_4 NPs using the W-H method.

4. 2 FTIR analysis

Conjugation of Chitosan and Folic acid with Fe_3O_4 NPs is very important for biotechnological purposes. Functional groups of prepared MNPs are analyzed by FTIR spectrometer. Fig. 3 depicts the FTIR spectra which confirm the chemical composition of naked Fe_3O_4 NPs and the surface-modified Fe_3O_4 NPs. In Fig. 3(a) the obtained strong band at 558 cm^{-1} attributed to the Fe-O group [23]. Fig. 3(b) presents broadband at 3414 cm^{-1} assigned to the -OH group and the peaks manifested at 2922 cm^{-1} and 2868 cm^{-1} corresponds to the C-H group. The weak band around 1064 cm^{-1} is the stretching vibrations of the C-O bond in the spectrum of the composite NPs [24]. Fig. 3(c) confirms the conjugation of FA with CS-coated Fe_3O_4 NPs by the characteristic bands around 817 cm^{-1} , 1270 cm^{-1} , and the spectrum at 1604 cm^{-1} assigned to the aromatic ring of FA [25]. The new strong band at 1626 cm^{-1} is the perfect indication for the unification of Chitosan and Folic acid with Fe_3O_4 MNPs [26] and a weak C=C stretching band was found at 1421 cm^{-1} [27].

4.3 Morphological analysis

Morphology and elements presented in the synthesis products of pure Fe_3O_4 , CS-coated Fe_3O_4 , and FA-CS-coated Fe_3O_4 nanoparticles were depicted in Fig. 4(a-c) & 5(a-c) respectively. According to SEM images, there is no agglomeration for pure Fe_3O_4 nanoparticles [Fig. 4(a)] and it presents the spunch like structure. From Fig. 4(b) & 4(c) it was identified that CS-coated Fe_3O_4 NPs and FA-CS-coated Fe_3O_4 NPs possess agglomerated spherical structures.

Elements presented in the nanoparticles were evaluated by Energy dispersive X-ray analysis (EDAX). From the EDAX spectra of prepared NPs, it is crystal clear that the peaks are around 0.8, 4.5, 6.3, and 7 keV are

assigned to the binding energy of Fe [28]. There is a peak approximately at 0.7 keV, corresponding to O. All the three EDAX spectrums of synthesized NPs not only exhibit Fe and O, however Mn, but Cl and Cs also manifested in less mass percentage.

4.4 Optical Study

The absorbance of chemical substances was determined by utilizing the quantitative technique UV/ Vis Spectroscopy. Fig. (6) represents the absorption spectra (fig. 6a-c) and Tauc's plot (fig. 6 e-f) of MNPs. This experiment was conducted between the wavelength 100 - 800 nm. The resultant cut of wavelength for all the three samples such as Pure Fe₃O₄ (6a), CS-coated Fe₃O₄ (6b), and FA-CS-coated Fe₃O₄ (6c) NPs were obtained around 200 nm. These samples reveal sharp absorption edges. Tauc's plot is a significant tool to determine the optical band gap energy, which is drawn by using Tauc's relation:

$$(\alpha h\nu)^n = A(h\nu - E_g)$$

where α is the absorption coefficient, A is a constant, ν is the photon frequency, h is Plank's constant, and E_g is the bandgap energy. The absorption coefficient (α) was calculated for each value based on the absorption data [29]. The exponent n's value is determined by the type of transition like $n = 1/2$ for indirect bandgap and allowed transition; $n=2$ for direct transition; and $n=3/2$ for directly forbidden transition. A plot was drawn between $(\alpha h\nu)^{1/2}$ along Y-axis and $h\nu$ along X-axis to obtain the E_g value of nanoparticles. The determined bandgap energy of the MNPs by using the Tauc's plot is shown in Fig. 6 (e-f) and the calculated values are 4.7 eV, 4.3 eV, and 3.4 eV respectively. From these resultants, it is known that the E_g value of CS-Fe₃O₄ and FA-CS-Fe₃O₄ nanoparticles have been reduced than that of pure Fe₃O₄ nanoparticles. This is due to the change in the particle size concerning the surface modification with chitosan and folic acid [30].

4.5 Thermal study

Thermogravimetric analysis was performed to identify the change in mass of materials as a function of temperature. The prepared samples were subjected to up to 900 °C in an air atmosphere for estimating the chemical composition of prepared magnetic nanoparticles. TGA curve and 1st derivative TG (DTG) curve of pure Fe₃O₄ and CS-coated Fe₃O₄, FA-CS-coated Fe₃O₄ nanoparticles were shown in Fig. 7(A) & 7(B). Multistage decomposition was observed from the TGA analysis. From the TGA curve, it is found that the decomposition of the compound begins at 46° C approximately. The first derivative curve was plotted to detect the distinct degradation event where it begins and ends. From the observation, it is clear that there is no degradation at any point of temperature for pure Fe₃O₄ NPs [curve an in Fig. 7(A) & 7(B)] [31]. From the attained thermogram, it is obvious that there are several endothermic peaks are exhibited at different temperatures [Fig. 7(B)]. The endothermic peaks for the samples CS-coated Fe₃O₄, FA-CS-coated Fe₃O₄ nanoparticles around 100 °C are due to the removal of water absorbed by the nanoparticles with a minimum weight loss [curve b & c in Fig. 7(A) & 7(B)] [32]. On the other hand, the broad endothermic peaks between 200-500 °C and the strong & broad endothermic peaks at 690 °C and

750 °C are the indication of decomposition of the physisorption and the chemisorption of the surface modification agent such as Chitosan and Folic acid [curve b & c in Fig. 7(A) & 7(B)] [33]. Significantly, the inflection point was observed at 690 °C which describes the greatest rate of change on the weight loss. [curve b in Fig. 7(B)]. Above 780 °C rapid decomposition takes place. From these resultants of the TG curve, it was analyzed that Fe_3O_4 MNPs are coated with chitosan and folic acid at an appropriate level.

Conclusion

In this present work, the Co-precipitation method was executed to produce magnetic Iron Oxide nanoparticles (Fe_3O_4). The solvothermal method was utilized to fabricate CS-coated Fe_3O_4 and FA-CS-coated Fe_3O_4 nanoparticles. From the observation of XRD pattern crystallite size, strain (W-H Plot method), and the structure of Fe_3O_4 NPs as found to be an inverse spinel structure. Surface morphology of pure Fe_3O_4 NPs and CS-coated Fe_3O_4 NPs, FA-CS-coated Fe_3O_4 NPs were recorded by SEM analysis. The resultant FTIR spectrum gave the assurance of an overlay of chitosan and folic acid on the surface of magnetic nanoparticles (Fe_3O_4). The appropriate elements of prepared MNPs are identified through the EDAX spectrum. The cut of wavelength was recorded around 200 nm and bandgap energy found to be 4.7 eV, 4.3 eV, and 3.4 eV using Tauc's relation. Thermal stability through distinct mass loss (TGA) and inflection point (DTG) was estimated for the nanoparticles. Significantly, the findings of this research would be of great assistance to promote these surface-modified MNPs for various applications.

Declarations

Author's contributions

Agnes. J performed the experiments and wrote the manuscript. Ajith. P and Sappani Muthu. M contributed to preparing the samples. Prem Anand. D reviewed the manuscript and corrected the language. All authors read and approved the final manuscript.

Author's details

1. Agnes. J

Department of physics,

St. Xavier's College (Autonomous), Palayamkottai,

India, Tamilnadu.

Mail.Id: cicagnes0@gmail.com

2. Ajith. P

Department of physics,

St. Xavier's College (Autonomous), Palayamkottai,
India, Tamilnadu.

Mail.Id: mosesajith96@gmail.com

3. Sappani Muthu. M

Department of physics,

St. Xavier's College (Autonomous), Palayamkottai,

India, Tamilnadu.

Mail.Id: ts.tamil7594@gmail.com

4. Dr. Prem Anand. D,

Assistant professor,

Department of physics, St. Xavier's College (Autonomous), Palayamkottai,

India, Tamilnadu.

Mail.Id: drpremanand@yahoo.co.in

Conflict of interest

There is no conflict of interest.

References

1. Wang B, Zhang PP, Williams GR, Branford-White C, Quan J, Nie HL, Li MZ (2013) A Simple Route to Form Magnetic Chitosan Nanoparticles from Coaxial-Electrospun Composite Nanofibers. *J Mater Sci* 48(11):3991–3998. doi: 10.1007/s10853-013-7208-x
2. Andhariya N, Chudasama B, Mehta RV, Upadhyay RV (2011) Biodegradable Thermoresponsive Polymeric Magnetic Nanoparticles: A New Drug Delivery Platform for Doxorubicin. *J Nanopart Res* 13(4):1677–1688. doi: 10.1007/s11051-010-9921-6
3. Pal D (2020) *Synthesis of Metal Oxide Nanoparticles-A General Overview*. Vol. 59
4. Hui C, Shen C, Yang T, Bao L, Tian J, Ding H, Li C, Gao HJ (2008) Large-Scale Fe₃O₄ Nanoparticles Soluble in Water Synthesized by a Facile Method. *J Phys Chem C* 112(30):11336–11339. doi: 10.1021/jp801632p
5. Prabhu YT, Venkateswara Rao K, Siva Kumari B, Kumar VSeshaS, Pavani T (2015) Synthesis of Fe₃O₄ Nanoparticles and Its Antibacterial Application. *International Nano Letters* 5(2):85–92. doi:

6. Khayat Sarkar Z, Khayat Sarkar F (2011) *Synthesis and Magnetic Properties Investigations of Fe₃O₄ Nanoparticles* *. Vol. 7
7. Amirabadizadeh A (2017) Amir Zelati, and Zahra Lotfollahi. "Studying the Temperature Effect on the Magnetic Behavior of Fe₃O₄ Water Based Ferrofluid." Pp. 468–72 in *Key Engineering Materials*. Vol. 744 744 KEM. Trans Tech Publications Ltd
8. Ji J, Wu D, Liu L, Chen J, Xu Y (2012) Preparation, Characterization, and in Vitro Release of Folic Acid-Conjugated Chitosan Nanoparticles Loaded with Methotrexate for Targeted Delivery. *Polym Bull* 68(6):1707–1720. doi: 10.1007/s00289-011-0674-x
9. Anon n.d. "European Journal of Interdisciplinary Studies Pharmaceutical Uses of Chitosan in the Medical Field.”
10. Unsoy G, Yalcin S, Khodadust R, Mutlu P, Gunduz U (2012) *IN SITU SYNTHESIS and CHARACTERIZATION OF CHITOSAN COATED IRON OXIDE NANOPARTICLES and LOADING OF DOXORUBICIN*
11. Bahrami B, Mohammadnia-Afrouzi M, Bakhshaei P, Yazdani Y, Ghalamfarsa G, Yousefi M, Sadreddini S Farhad Jadidi-Niaragh, and Mohammad Hojjat-Farsangi. 2015. "Folate-Conjugated Nanoparticles as a Potent Therapeutic Approach in Targeted Cancer Therapy." *Tumor Biology* 36(8):5727–42
12. Zhu Y, Ying Fang, and Stefan Kaskel (2010) Folate-Conjugated Fe₃O₄@SiO₂ Hollow Mesoporous Spheres for Targeted Anticancer Drug Delivery. *J Phys Chem C* 114(39):16382–16388. doi: 10.1021/jp106685q
13. John A, Aruna SK, Jaganathan M, Ayyar NP, Krishnasamy R, Rajasekar (2017) and Eko Supriyanto. *Folic Acid Decorated Chitosan Nanoparticles and Its Derivatives for the Delivery of Drugs and Genes to Cancer Cells*. Vol. 113
14. Rana S, Shetake NG, Barick KC, Pandey BN, Salunke HG, Hassan PA (2016) Folic Acid Conjugated Fe₃O₄ Magnetic Nanoparticles for Targeted Delivery of Doxorubicin. *Dalton Trans* 45(43):17401–17408. doi: 10.1039/c6dt03323g
15. Narmani A, Rezvani M, Farhood B, Darkhor P, Mohammadnejad J, Amini B, Refahi S, and Nouraddin Abdi Goushbolagh (2019) Folic Acid Functionalized Nanoparticles as Pharmaceutical Carriers in Drug Delivery Systems. *Drug Dev Res* 80(4):404–424. doi: 10.1002/ddr.21545
16. Wu H, Liu G, Zhang S, Shi J, Zhang L, Chen Yu, Chen F, and Hangrong Chen (2011) Biocompatibility, MR Imaging and Targeted Drug Delivery of a Rattle-Type Magnetic Mesoporous Silica Nanosphere System Conjugated with PEG and Cancer-Cell-Specific Ligands. *J Mater Chem* 21(9):3037–3045. doi: 10.1039/c0jm02863k
17. Kumar CSSR, and Faruq Mohammad (2011) Magnetic Nanomaterials for Hyperthermia-Based Therapy and Controlled Drug Delivery. *Adv Drug Deliv Rev* 63(9):789–808
18. Shen M, Wu C, Lin C, Fan G, Jin Y, Zhang Z, Li C, and Wenping Jia (2014) Facile Solvothermal Synthesis of Mesoporous Chitosan-Coated Fe₃O₄ Nanoparticles and Its Further Modification with

- Folic Acid for Improving Targeted Drug Delivery. *NANO* 9(7). doi: 10.1142/S1793292014500817
19. Samanta B, Fischer HYanNO, Shi J, Jerry DJ, and Vincent M. Rotello (2008) Protein-Passivated Fe_3O_4 Nanoparticles: Low Toxicity and Rapid Heating for Thermal Therapy. *J Mater Chem* 18(11):1204–1208. doi: 10.1039/b718745a
 20. Yusoff AHM, Salimi MN (2017) and Mohd Faizal Jamlos. “Synthesis and Characterization of Biocompatible Fe_3O_4 Nanoparticles at Different PH.” in *AIP Conference Proceedings*. Vol. 1835. American Institute of Physics Inc
 21. Chaki SH, Tasmira J, Malek MD, Chaudhary JP, Tailor, Deshpande MP (2015) Magnetite Fe_3O_4 Nanoparticles Synthesis by Wet Chemical Reduction and Their Characterization. *Adv Nat Sci NanoSci NanoTechnol* 6(3). doi: 10.1088/2043-6262/6/3/035009
 22. Ruíz-Baltazar Álvaro de Jesús, Simón Yobanny Reyes-López, María de Lourdes Mondragón-Sánchez, Anel Ivonne Robles-Cortés, and Ramiro Pérez. 2019. “Eco-Friendly Synthesis of Fe_3O_4 Nanoparticles: Evaluation of Their Catalytic Activity in Methylene Blue Degradation by Kinetic Adsorption Models.” *Results in Physics* 12:989–95. doi: 10.1016/j.rinp.2018.12.037
 23. Karimzadeh I, Aghazadeh M, Doroudi T, Ganjali MR, and Peir Hossein Kolivand (2018) Electrochemical Preparation and Characterization of Chitosan-Coated Superparamagnetic Iron Oxide (Fe_3O_4) Nanoparticles. *Mater Res Innovations* 22(6):352–360. doi: 10.1080/14328917.2017.1323991
 24. Arum Y, Oh YO, Kang HW, Ahn SH, and Junghwan Oh (2015) Chitosan-Coated Fe_3O_4 Magnetic Nanoparticles as Carrier of Cisplatin for Drug Delivery. *Fisheries and Aquatic Sciences* 18(1):89–98. doi: 10.5657/FAS.2015.0089
 25. Qu D, Lin H, Zhang N, Xue J, Zhang C (2013) In Vitro Evaluation on Novel Modified Chitosan for Targeted Antitumor Drug Delivery. *Carbohydr Polym* 92(1):545–554. doi: 10.1016/j.carbpol.2012.08.112
 26. Chen Q, Wang X, Chen F, Zhang Q, Dong B, Yang H, Zhu Y (2011) Functionalization of Upconverted Luminescent $\text{NaYF}_4:\text{Yb}/\text{Er}$ Nanocrystals by Folic Acid-Chitosan Conjugates for Targeted Lung Cancer Cell Imaging. *J Mater Chem* 21(21):7661–7667. doi: 10.1039/c0jm04468g
 27. Saravanakumar K, Chelliah R, MubarakAli D, Oh DH, Kathiresan K, and Myeong Hyeon Wang (2019) Unveiling the Potentials of Biocompatible Silver Nanoparticles on Human Lung Carcinoma A549 Cells and *Helicobacter Pylori*. *Sci Rep* 9(1). doi: 10.1038/s41598-019-42112-1
 28. Wulandari IO, Vita T, Mardila DJ, Djok H, Santjojo (2018) and Akhmad Sabarudin. “Preparation and Characterization of Chitosan-Coated Fe_3O_4 Nanoparticles Using Ex-Situ Co-Precipitation Method and Tripolyphosphate/Sulphate as Dual Crosslinkers.” in *IOP Conference Series: Materials Science and Engineering*. Vol. 299. Institute of Physics Publishing
 29. Eid MM, El-Hallouty SM, El-Manawaty M, Abdelzaher FH (2019) “Physicochemical Characterization and Biocompatibility of Spion@plasmonic @chitosan Core-Shell Nanocomposite Biosynthesized from Fungus Species.” *Journal of Nanomaterials* 2019. doi: 10.1155/2019/4024958

30. Sivakumar D, Rafi M, Balaraman Sathyaseelan M, Nazeer P (2017) and Ayisha Begam. *Synthesis and Characterization of Superparamagnetic Iron Oxide Nanoparticles (SPIONs) Stabilized by Glucose, Fructose and Sucrose*. Vol. 8
31. Tenório-Neto E, Taveira T, Jamshaid M, Eissa MH, Kunita N, Zine Géraldine Agusti, Hatem Fessi, Abdelhamid Errachid El-Salhi, and Abdelhamid Elaissari. 2015. "TGA and Magnetization Measurements for Determination of Composition and Polymer Conversion of Magnetic Hybrid Particles." *Polymers for Advanced Technologies* 26(10):1199–1208
32. Lesiak, Beata N, Rangam P, Jiricek I, Gordeev J, Tóth L, Kövér, Mohai M (2019) "Surface Study of Fe_3O_4 Nanoparticles Functionalized With Biocompatible Adsorbed Molecules." 7. 10.3389/fchem.2019.00642
33. Rehana D, Haleel AK, Aziz Kalilur Rahiman (2015) Hydroxy, Carboxylic and Amino Acid Functionalized Superparamagnetic Iron Oxide Nanoparticles: Synthesis, Characterization and in Vitro Anti-Cancer Studies. *J Chem Sci* 127(7):1155–1166. doi: 10.1007/s12039-015-0876-0

Tables

Table 1 is available in the Supplementary Files section.

Figures

Figure 1

XRD pattern of synthesized NPs (a) pure Fe_3O_4 NPs, (b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs.

Fig. 2. UDM plot for synthesized nanoparticles a) Pure Fe_3O_4 NPs, b) CS-coated Fe_3O_4 NPs and c) FA-CS-coated Fe_3O_4 NPs.

Figure 2

See image above for figure legend

Figure 3. Fourier transform infrared spectroscopy (FTIR) spectra of (a) pure Fe_3O_4 NPs, (b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs.

Figure 3

See image above for figure legend

Fig. 4 SEM images of Synthesized NPs. a) Pure Fe_3O_4 NPs, b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs.

Figure 4

See image above for figure legend

Fig. 5. EDAX spectrum of synthesized NPs. a) Pure Fe₃O₄ NPs, b) CS-coated Fe₃O₄ NPs, c) FA-CS-coated Fe₃O₄ NPs.

Figure 5

See image above for figure legend

Fig. 6. UV/Vis spectra of synthesized NPs. (a) Pure Fe_3O_4 NPs, (b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs and Tauc's plot for (d) Pure Fe_3O_4 NPs, (e) CS-coated Fe_3O_4 NPs, (f) FA-CS-coated Fe_3O_4 NPs.

Figure 6

See image above for figure legend

Fig. 7 (A)TGA spectra of synthesized NPs, (a) Pure Fe_3O_4 NPs, (b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs and **Fig. 7(B)** 1st derivative curve of TGA, a) Pure Fe_3O_4 NPs, (b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs.

Figure 7

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.jpg](#)