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

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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 synthesize 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 the 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).***

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

I. 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 [1].

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Magnetic Iron oxide nanoparticles are synthesized by various methods such as the Co-precipitation method [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 [4]. Magnetic iron oxide (Fe_3O_4) with face-centered cubic forming oxygen has a cubic reverse spinal structure [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]. 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 [7]. FA ligands grafted Fe_3O_4@ 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 [8]. A wide range of surveys has been done based on folate decorated chitosan, which has given a new perception 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 [9]. 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 a high loading affinity for an anticancer drug [10]. The review has been carried out on Folic acid-functionalized NPs giving a clear idea about the development of active cancer diagnosis and therapy [11]. 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 [12]. Surface-modified NPs by various anti-cancer drugs improve the specific drug delivery into cancer cells. Various studies have reported, that 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 [13].

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.

II. EXPERIMENTAL

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

A. Materials

Ferric Chloride hexahydrate ($FeCl_3.6H_2O$), Ferrous Chloride tetrahydrate ($FeCl_2.4H_2O$) were purchased from Spectrum Reagents and Chemicals Pvt. Ltd. Edayar, Cochin. Chitosan $(C_6H_{11}NO_4)n$, 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.

B. 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 [14].

C. Synthesis of pure Fe_3O_4 nanoparticles

The magnetite Fe_3O_4 nanoparticles were prepared by the Co-precipitation method [15]. The mixture of 12.0g of $FeCl_3.6H_2O$ and 4.9g of $FeCl_2.4H_2O$ 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 [16].

D. Synthesis of CS-coated Fe_3O_4 NPs

The reaction mixture was prepared by dissolving 1.50 g of $FeCl_3.6H_2O$, 0.75 g of Chitosan, 1.0 g of Polyvinylpyrrolidone, and 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.

E. 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 are 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.

III. INSTRUMENTATIONS

Crystalline structure, and phases of prepared pure Fe_3O_4, CS-coated Fe_3O_4, and FA-CS-coated-Fe_3O_4 nanoparticles were examined employing X-ray diffraction (XRD) with a Philips PW-1800 diffractometer with CuKα radiation (λ =1.5406 Å). 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).

IV. RESULTS AND DISCUSSION

A. Structural analysis

The crystal structure, crystalline phases, and crystallite size of synthesized pure Fe_3O_4 and composite nanoparticles such as CS-coated Fe_3O_4 and FA coated CS-Fe_3O_4 were identified by using an X-ray diffractometer. Fig.1. presents XRD pattern of prepared magnetic nanoparticles 1(a) Fe_3O_4 NPs, 1 (b) CS- coated Fe_3O_4 NPs, 1 (c) FA-CS-coated Fe_3O_4 NPs. The obtained characteristic peaks of NPs at 2θ = 33.24°, 35.71°, 43.53°, 57.57°, and 62.49° correspond to Bragg reflection planes (220),

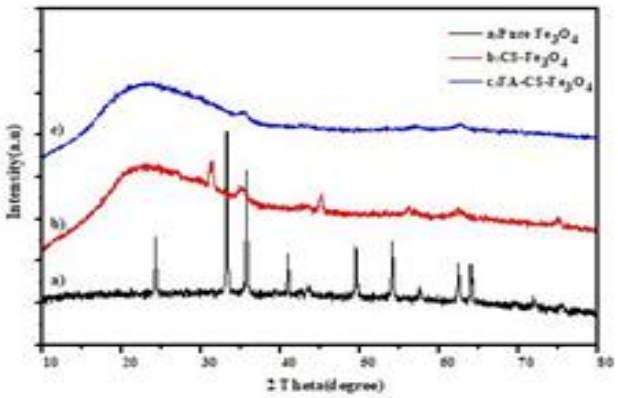

Figure 1. XRD pattern of synthesized NPs (a) pure Fe_3O_4 NPs , (b) CS- coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs.

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(311), (400), (333), and (440). The obtained diffraction peaks are indexed as an inverse spinel structure of Fe_3O_4 NPs having lattice parameter a = 8.39 Å and the results are in good agreement with the standard data (JCPDS: 19-0629) [17]. Manifestation of other peaks at different 2θ values is evidence of 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 [18]. 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 (Å), θ is the Brag 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,

$$\varepsilon = \beta s/4 \tan\theta \quad (3)$$

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

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

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

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

By rearranging Eq. (6)

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

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 sinθ along the x-axis and B_{hkl} cosθ 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.

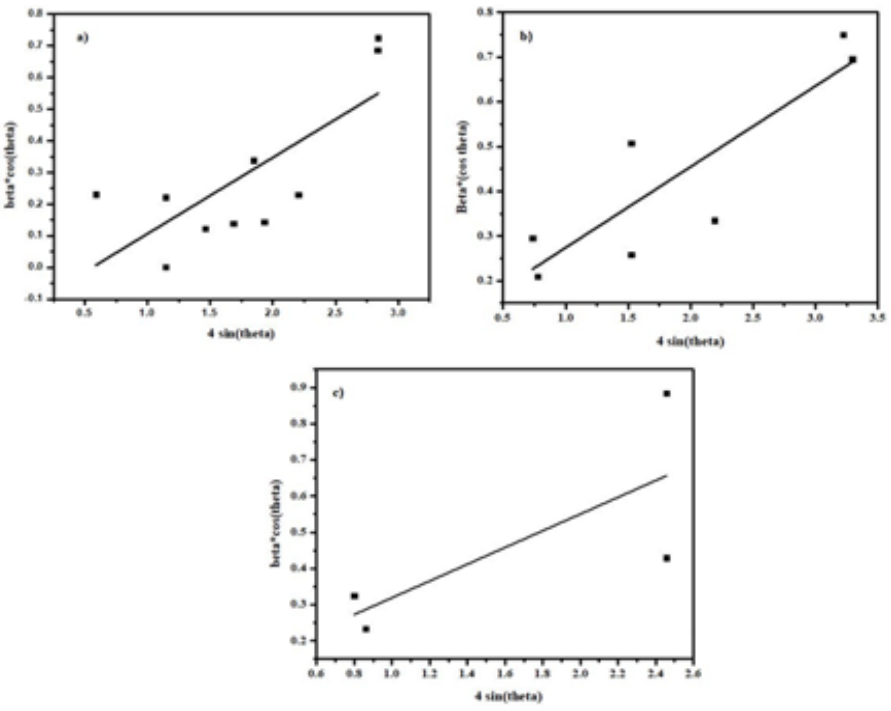

Fig. 2. UDM plot for synthesized nanoparticles a) Pure Fe_3O_4 NPs, b) CS-coated Fe_3O_4 NPs and c) FA-CS-coated Fe_3O_4 NPs.

B. 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} is attributed to the Fe-O group [19]. 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 [20]. Fig. 3(c) confirms the conjugation of FA with CS-coated Fe_3O_4 NPs by the characteristic bands around 817cm^{-1}, 1270 $cm^{-1,}$ and the spectrum at 1604 cm^{-1} assigned to the aromatic ring of FA [21]. 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 [22] and a weak C=C stretching band was found at 1421 cm^{-1} [23].

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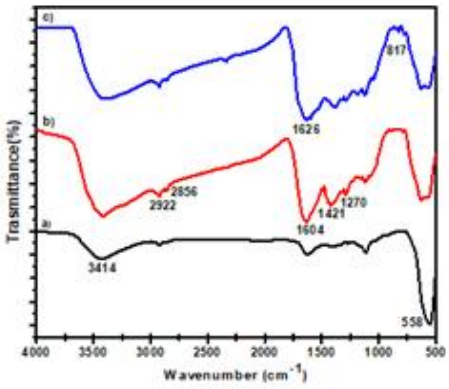

Figure 3. Fourier transform infrared spectroscopy (FTIR) spectra of (a) pure Fe_3O_4 NPs, (b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs.

C. 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.

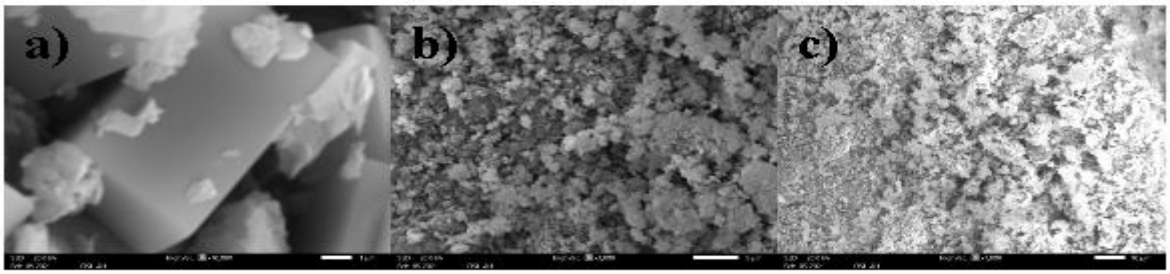

Fig. 4. SEM images of synthesizd NPs (a) Pure Fe_3O_4, b) CS-coated Fe_3O_4 NPs, c) FA-CS-coated Fe_3O_4 NPs.

D. 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 Fe_3O_4 (6a), CS-coated Fe_3O_4 (6b), and FA-CS-coated Fe_3O_4 (6c) NPs was obtained at 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:

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

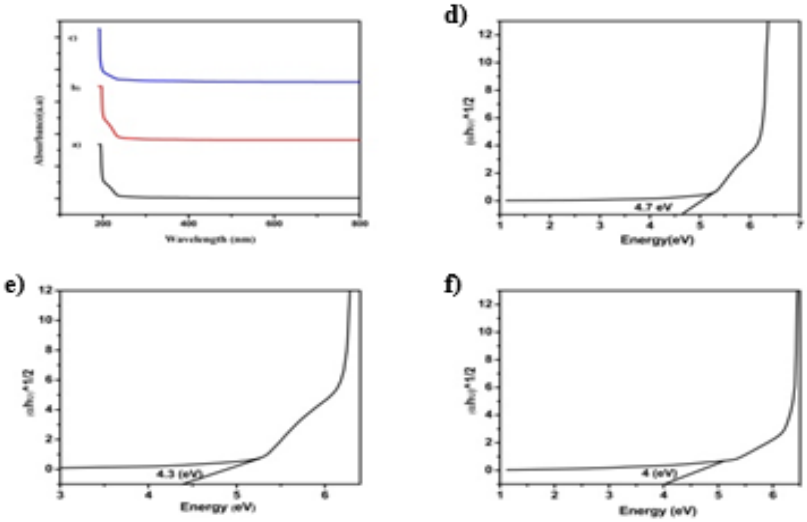

Fig. 5. UV-Vis Spectra and Tauc's plot of synthesized NPs (a,d) Pure Fe_3O_4 NPs, (b,e) CS-coated Fe_3O_4 NPs, (e,f) FA-CS-coated Fe_3O_4 NPs.

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 [24]. 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 hν 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 4 eV respectively. From these resultants, it is known that the E_g value of CS-Fe_3O_4 and FA-CS-Fe_3O_4 nanoparticles have been reduced than that of pure Fe_3O_4 nanoparticles. This is due to the change in the particle size concerning the surface modification with chitosan and folic acid [25].

E. 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 900ºC in an air atmosphere for estimating the chemical composition of prepared magnetic nanoparticles. TGA curve and 1st derivative TG (DTG) curve of pure Fe_3O_4 and CS-coated Fe_3O_4, FA-CS-coated Fe_3O_4 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 46ºC 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 Fe_3O_4 NPs [curve an in Fig. 6(A) & 6(B)] [26]. From the attained thermogram, it is obvious that there are several endothermic peaks are exhibited at different temperatures [Fig. 6(B)]. The endothermic peaks for the samples CS-coated Fe_3O_4, FA-CS-coated Fe_3O_4 nanoparticles around 100ºC are due to the removal of water absorbed by the nanoparticles with a minimum weight loss [curve b & c in Fig. 6(A) & 6(B)] [27].

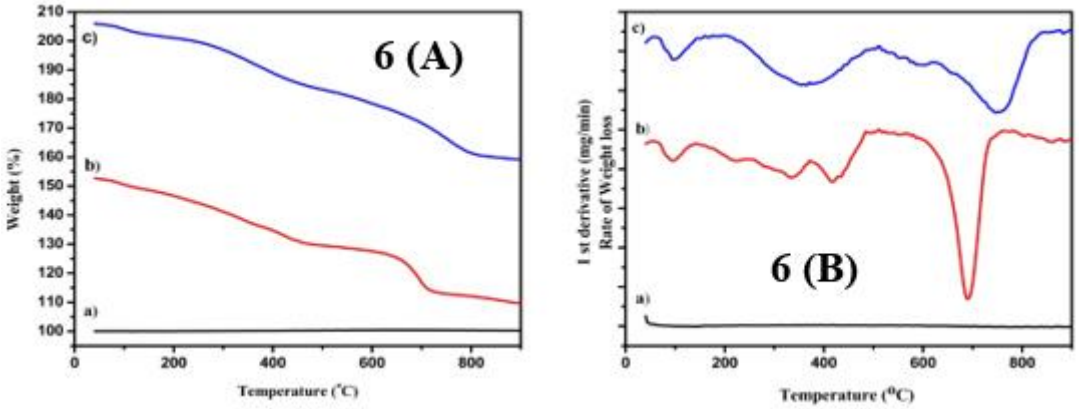

Fig. 6 (A) TGA and DTA Fig. 6 (B) spectra of synthesized NPs (a) Pure Fe_3O_4 NPs, (b) CS-coated Fe_3O_4 NPs, (c) FA-CS-coated Fe_3O_4 NPs.

On the other hand, the broad endothermic peaks between 200-500ºC and the strong & broad endothermic peaks at 690ºC and 750ºC are the indication of the decomposition of the physisorption and the chemisorption of the surface modification agent such as Chitosan and Folic acid [curve b & c in Fig. 6(A) & 6(B)] [28]. Significantly, the inflection point was observed at 690ºC which describes the greatest rate of change in the weight loss. [curve b in Fig. 6(B)]. Above 780ºC 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.

V. 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 the 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 was found to be 4.7 eV, 4.3 eV, and 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.

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