

Synthesis, Characterization and applications of Gentamicin functionalized Fe₃O₄ nano hybrid

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ABSTRACT

Recently the applications of functionalized magnetic nano particles are increased in a linear fashion. This is due to the ease of synthesis and controlled growth of magnetic nano particles. In the present investigation gentamicin drug functionalized Fe₃O₄ nanoparticles were synthesized by co-precipitation method. Thus obtained nano hybrid was characterized by Fourier transform infrared (FTIR) spectroscopy, UV-visible spectroscopy, X-ray diffractogram (XRD), Field emission scanning electron microscopy (FE-SEM), and Vibrating sample magnetometer (VSM) like methods. The above synthesized nano hybrid is sensitive to magnetic force and gravitational force. This principle is used here to increase the settling rate of starch under three different pHs namely acid (pH-3.5), alkaline (pH-12.5) and neutral (pH-7.1). It was found that while increasing the percentage loading of nano hybrid the sediment height of starch is smoothly increased. The VSM value of starch is smoothly increased. The VSM value of the starch/Fe₃O₄-gentamicin nano composites were reduced due to the encapsulation effect. Thus obtained nano composites were characterized by FTIR, DSC, TGA and SEM. The settling velocity of starch under three different pHs were calculated and critically compared.

Keywords- Starch, Functionalization, Gentamicin, Sedimentation, nano hybrid.

I. INTRODUCTION

Extensive research works have been done in the synthesis, characterization and application of magnetic nanoparticles. This is because of their ease of preparation, size control and their wide applications in various science and engineering fields. For example mera et al., [1] synthesized Fe₃O₄ without surfactants at room temperature. Sun and co-workers [2] synthesized the bio-compatible Fe₃O₄ nanoparticle by co-precipitation method. Fe₃O₄ thin film for super capacitor application by hydro thermal method was reported by chen and research team[3]. Template free mono dispersed magnetic nanoparticles were reported in the literature [4]. Spinel

type Fe₃O₄ was prepared by cabanas et al., [5]. Hollow magnetite microsphere were prepared and used as drug carriers [6]. Magnetic nano particle are used in pharmaceutical science as drug delivery and gene therapy material [7]. Even then, its applications are restricted to some extent due to the absence of functional groups on its surface. Hence, the main objective of the present investigation is synthesis of -OH and -NH₂ functionalized Fe₃O₄. In 2005 He et al., synthesized amino and carboxyl functionalized Fe₃O₄ [8]. PLA decorated Fe₃O₄ nanocrystals were synthesized in various methodologies were reviewed [9]. Dendrimer functionalized Fe₃O₄ for specific targeting and imaging of cancer cell was reported by wang et al., [10]. PEG and PVP coated Fe₃O₄ was synthesized and their cytotoxicity was studied [11]. Thiol functionalized magnetite/graphene oxide nano hybrid for Hg²⁺ removal was prepared [12]. Salunkhe and co-workers [13] reported about the PVA functionalized cobalt Fe₃O₄ nanoparticle for bio-medical applications. Acrylic acid functionalized Fe₃O₄ nano particles was prepared by 1,3-dipolar cycloaddition reaction [14]. Oleic acid stabilized Fe₃O₄ nanoparticle was synthesized [15]. By through literature survey we could not find any report based on the drug functionalized particularly gentamicin functionalized Fe₃O₄ nanoparticle.

Gentamicin is an aminoglycoside, heat stable, bactericidal antibiotic with hydroxyl, amino functional groups linked through other linkages [16]. Gentamicin is very much useful in the bio-medical field particularly in orthopedic surgery [17]. High antimicrobial activity with less side effect and with more functionality urged us to do the present investigation. i.e. surface functionalization of Fe₃O₄ by gentamicin.

The above literature survey indicates that generally the functionalized Fe₃O₄ nanoparticle were used in the biological field as a bio-imaging agent. Application of functionalized magnetic particle in the purification and isolation of starch is not reported. The novelty of present investigation is the gentamicin functionalized Fe₃O₄ was used for the settling of starch in an aqueous medium under three different pH's.

Starch is an essential food material for human beings with high carbohydrate content. During the isolation and purification process around 5% of starch materials becomes waste. This is due to the slow settling of starch under different pH condition. Generally the starch is settled under the influence of gravitational force. In 1999, Closs et al., [18] studied about the phase separation and rheology of starch in the presence of gallytomannan pectinase enzyme was used for the industrial extraction of starch from cassava in Ghana [19]. Phosphorylation of potato starch was made in order to increase the percentage yield of starch [20]. Other authors also explained about the isolation and purification of starch [21-25]. The increase in settling velocity of starch by the gravitational force and magnetic force is not so far reported by other research team. We took this job as a challenge and successfully did the same.

II. EXPERIMENTAL

1. Materials

The raw materials required for the preparation of Fe₃O₄ and its hybrid are as follows: ferrous sulphate (FeSO₄), ferric chloride (FeCl₃) and sodium hydroxide (NaOH) were purchased from CDH, India. The double distilled (DD) water was used for the solution preparation work. The ferrite nanoparticle was functionalized in the presence of Gentamicin (Gentamicin, Ranbaxy, India). The sedimentation velocity of Starch (Himedia, India) was studied under three different pHs like 2.5, 7.1 and 12.0.

2. Synthesis of Fe₃O₄ functionalized Gentamicin nano hybrid

5 g ferrous sulphate and 10 g ferric chloride in 100 mL water were mixed thoroughly; 2 g gentamicin in 25 mL water was added slowly. The slow addition was continued for 30 min at 45°C under nitrogen atmosphere under mild stirring condition; 5 g NaOH in 25 mL water was added to the content slowly. The appearance of dark black color confirmed the formation of gentamicin-functionalized ferrite. The stirring was continued for another 1 h. Then the beaker was kept on a magnetic bar to isolate the gentamicin-functionalized ferrite and to purify the nanohybrid material. The purification process was continued three times with 50 mL fresh water. After the purification process the contents were filtered and dried at 110°C for 8 h. The dried sample was weighed and stored in a zipper lock cover.

3. Synthesis of Fe₃O₄–Gentamicin/starch nanocomposite

Then 2 g potato starch was dissolved in 100 mL water. The above-synthesized gentamicin-decorated ferrite hybrid was added slowly under vigorous stirring condition. The gentamicin-functionalized ferrite can be loaded at different percentage weights. After the reaction, the contents were evaporated to dryness. The final product, starch/Fe₃O₄–gentamicin systems were dried, weighed and stored in a zipper lock cover.

III. CHARACTERIZATION

Characterization Fourier transform infrared (FTIR) spectrum was taken by using Shimadzu 8400 S, Japan model instrument from 4000 to 400 cm⁻¹ by KBr pelletization method. Photoluminescence spectrum was measured with the help of PL, Jasco Model FP-6000, Japan, instrument from 300 to 700 nm. DSC and TGA were measured by using Universal V4.4A TA Instruments (simultaneous DSC and TGA analyzer) under nitrogen atmosphere at the heating rate of 10 K/min from room temperature to 373 K. X-ray diffraction (XRD, XS08, BRUKER, USA) was recorded with an advanced instrument and scanning from the 2θ value of 2–60° at a scanning rate of 2°/min.

IV. RESULT AND DISCUSSION

1. Characterization of Gentamicin/Fe₃O₄ nano hybrid

1.1. FTIR study

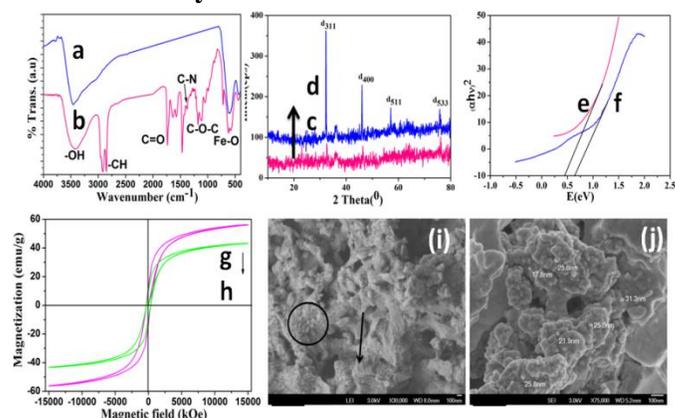


Fig. 1: FTIR spectrum of (a) ferrite, (b) ferrite–gentamicin hybrid; XRD of (c) ferrite, (d) ferrite–gentamicin hybrid; Band gap energy of (e) ferrite, (f) ferrite–gentamicin hybrid; VSM of (g) ferrite, (h) ferrite–gentamicin hybrid; SEM image of (i) ferrite, (j) ferrite–gentamicin hybrid

The aim of the present investigation is functionalization of Fe₃O₄ by a drug called gentamicin. Gentamicin contains hydroxyl, ether, primary amine and secondary

amine like functional groups. The amino group of gentamicin interacts with the Fe_3O_4 surface. Fig. 1a indicates the FTIR spectrum of Fe_3O_4 . A sharp peak at 604 cm^{-1} confirmed the M-O stretching. A broad peak around 3463 cm^{-1} confirmed the OH stretching. This is due to the intercalated water molecules. After the surface functionalization this spectrum shows some new peaks. A doublet peak around 624 cm^{-1} declared the presence of M-O stretching. The important point noted here is the M-O stretching was splitted into a doublet peak. This authenticated the presence of Fe^{2+} and Fe^{3+} can interact with the amino group of gentamicin and hence the M-O stretching was splitted into a doublet in the Fe_3O_4 . A twin peaks at 1110 and 1180 cm^{-1} explains the both linear and cyclic ether linkage. The C-N stretching of gentamicin is appeared at 1378 cm^{-1} . The CH bending vibration can be seen at 1460 cm^{-1} . The C-O stretching from the moisture is appeared at 1725 cm^{-1} . The CH symmetric and antisymmetric stretchings were appeared at 2866 and 2917 cm^{-1} respectively. The OH stretching is appeared at 3423 cm^{-1} as a broad peak (Fig. 1b). Hence the appearance of new peaks confirmed the surface functionalization of Fe_3O_4 by the gentamicin.

1.2. XRD study

Fig. 1c indicates the XRD pure Fe_3O_4 . The XRD shows the peak corresponding to d_{311} , d_{400} and d_{533} crystal planes. This confirmed the poly crystalline nature of pristine Fe_3O_4 . Fig. 1d exhibits the XRD of Fe_3O_4 after the surface functionalization with gentamicin. Here also one can see the above said peaks. Even then the crystallinity of the various crystal planes were improved. This is purely due to the surface functionalization reaction. The further increasing crystallinity of Fe_3O_4 can be confirmed by noting a new peak at 56.7°C corresponding to the d_{511} crystal plane. In comparison after the surface functionalization reaction the crystallinity of the Fe_3O_4 was increased due to the controlled crystal growth and self assembly nature.

1.3. Energy band gap study

The band gap plot of Fe_3O_4 before and after surface functionalization is given in Fig. 1e and f respectively. The band gap energy values were determined as 0.436 and 0.63 eV respectively. For the pristine Fe_3O_4 and functionalized Fe_3O_4 the band gap energy study indicated that the Fe_3O_4 has the lower band gap energy.

1.4. VSM study

Fig. 1g and h confirmed the VSM loop of Fe_3O_4 before and after functionalization reaction with gentamicin. The

VSM value of pure pristine was determined as 54.8 emu/g whereas after the surface functionalization reaction the VSM value was declared to 42.0 emu/g . The decrease in magnetization value can be explained on the basis of surface coating reaction.

1.5. FE-SEM analysis

The FE-SEM image of Fe_3O_4 before and after functionalization is given in Fig. 1i and j respectively. Before surface functionalization the Fe_3O_4 shows different size and shapes. The size of the spheres were calculated as less than 100 nm . But after the surface functionalization reaction one can see the spherical nanoparticles only with the size ranging from $15\text{--}35\text{ nm}$. This showed that after the surface functionalization reaction both the size and shape were altered. In the presence of gentamicin the particle size of Fe_3O_4 was found to be reduced.

2. CHARACTERIZATION OF STARCH/ Fe_3O_4 GENTAMYCIN NANOCOMPOSITES

2.1. FTIR study

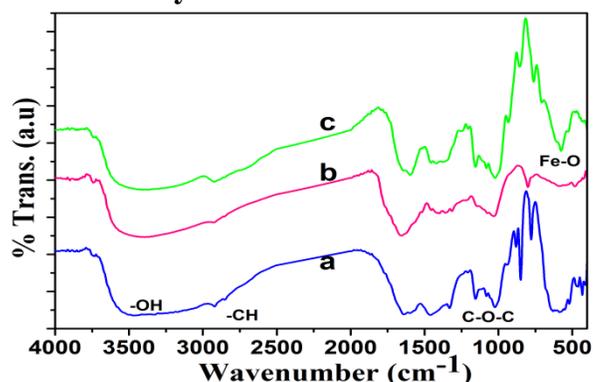


Fig. 2: FTIR spectrum of starch/ferrite-gentamicin nano composite under (a) acidic, (b) alkaline, (c) neutral

FTIR study starch/ Fe_3O_4 -gentamicin nano composites under acidic (Fig. 2a), alkaline (Fig. 2b) and neutral (Fig. 2c) pH were shown in Fig. 2. As usual the M-O stretching is appeared around 600 cm^{-1} as a broad peak. The CH out of plane bending can be seen at 774 cm^{-1} . The C-O-C pyranose linkage of starch is appeared at 1018 cm^{-1} . A broad peak at 1460 cm^{-1} is due to the CH bending vibration. The OH bending vibration of starch is appeared at 1643 cm^{-1} . The CH symmetric and antisymmetric stretchings of starch is appeared at 2859 and 2913 cm^{-1} respectively. A broad peak at 3457 cm^{-1} is ascribed to the OH stretching of starch. Fig 2b exhibits the FTIR spectrum of starch/ Fe_3O_4 nano composite synthesized under alkaline condition. Here also the above said peaks

are appeared. A broad peak at 1656 cm^{-1} is due to the $\text{O}^- \text{Na}^+$ like structure of starch. Under alkaline pH the OH group of starch was converted into $\text{O}^- \text{Na}^+$ like ionic structure. The M-O stretching around 584 cm^{-1} was broadened. This confirmed the highly nucleated structure of Fe_3O_4 .

The FTIR spectrum of starch/ Fe_3O_4 nano composite synthesized under alkaline pH is given in Fig. 2c. Here the FTIR spectrum is entirely different from that of starch composite prepared under acidic and alkaline pH. The shape of the M-O stretching (577 cm^{-1}) was sharper. The OH bending vibration is appeared as a doublet peak. This is due to the OH bending vibration of adsorbed water molecules and OH group of starch molecules. This indicated that under neutral pH more number of water molecules were adsorbed by the starch through the hydrogen bonding.

2.2. DSC study

The phase transition in the starch/ Fe_3O_4 nano composite prepared under three different pH shown in Fig. 3.

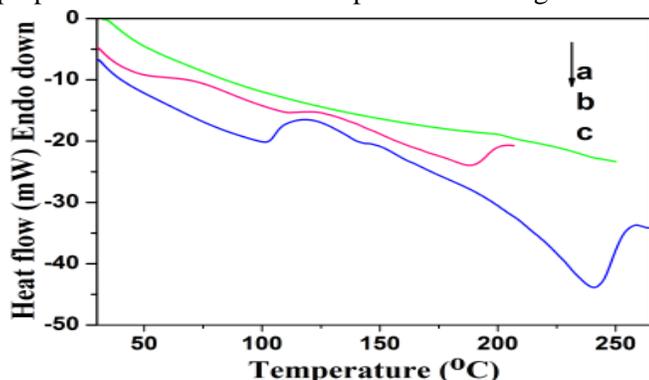


Fig.3: DSC of starch/ferrite-gentamycin nano composite under (a) acidic, (b) alkaline, (c) neutral
 Fig. 3a indicates the nano composite under neutral pH. The thermogram doesn't show any endo or exothermic transition up to 250°C . Fig. 3b exhibits the DSC of nano composite prepared under acidic condition. The first transition around 46.8°C is corresponding to the removal of physisorbed water molecules. The second transition at 111.2°C is ascribed to the complete removal of moisture, physisorbed and chemisorbed water molecules. The transition at 188.3°C is due to the degradation of starch backbone.
 Fig. 3c indicates the DSC thermogram of starch nano composite synthesized under alkaline pH. Two endothermic transitions were observed. The first transition at 100.8°C due to the removal of moisture, physisorbed and chemisorbed water molecules. The second transition at 241°C is associated with the degradation of starch backbone. In comparison starch

nano composite prepared under neutral pH exhibited the highest thermal stability as starch nano composite prepared under acidic pH exhibited the lowest thermal stability due to the degradation of starch backbone. Under acidic pH the ether linkage between two glucose structures was hydrolyzed and has decrease of molecular weight. This leads to the lower degradation of temperature of starch.

2.3. TGA study

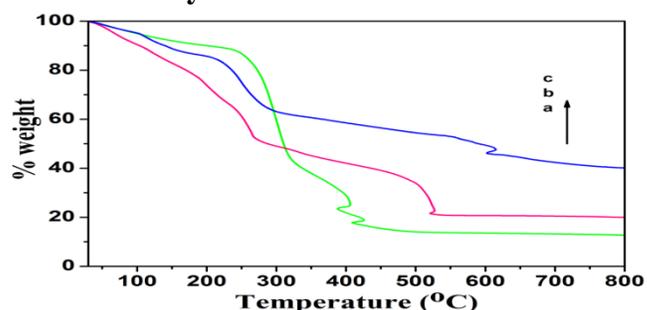


Fig. 4. TGA of starch/ferrite-gentamycin nano composite under (a) acidic, (b) alkaline, (c) neutral

The TGA thermogram of starch/ Fe_3O_4 nanocomposite is shown in Fig. 4. Fig. 4a indicates the TGA thermogram of starch nano composite under neutral pH. The thermogram exhibits a three step degradation process. The first minor weight loss up to 220°C is due to the weight loss corresponding to moisture, physisorbed water molecules. The second major weight loss around 290°C is due the breaking of ether linkage between the glucose units. As a result there will be a sudden decrease in molecular weight. The third weight loss step around 400°C is due to the degradation of glucose units. Above 750°C the system shows 12.7% weight remained. This is due the added Fe_3O_4 and carbanaceous matter. Fig 4b indicates the TGA thermogram of starch nano composite prepared under acidic condition with two step degradation process. The first minor weight loss up to 400°C is due to the weight loss corresponding to the removal of moisture, physisorbed and chemisorbed water molecules and also with the weight loss due to the breaking of ether linkage between the two glucose units. The second weight loss step is associated with the degradation of glucose units. Above 750°C the system exhibits 20% weight residue. Fig. 4c represents the TGA thermogram of starch nano composite prepared under alkaline pH. Again the system shows three steps degradation process the first minor weight loss up to 100°C is due to the removal of moisture and physisorbed water molecules. The second minor weight loss up to 200°C is due to the removal of chemisorbed water molecules. The third major weight

loss around 270⁰ C is due to the degradation of starch backbone. Above 750⁰C the system exhibits 40.4% weight residue remained. This is due to the ionic structure of starch i.e., the formation of O⁻Na⁺ like ionic structure. Moreover under alkaline pH the Fe₃O₄ nanoparticles were nucleated and become more crystalline nanoparticles as a result exhibited the highest thermal stability. In overall comparison the acid mediated nano composite exhibited lowest thermal stability where as the alkaline mediated nano composite exhibited the highest residue remained above 750⁰ C.

2.4. Settling velocity

Fig. 5 indicates the plot of time against the sediment height.

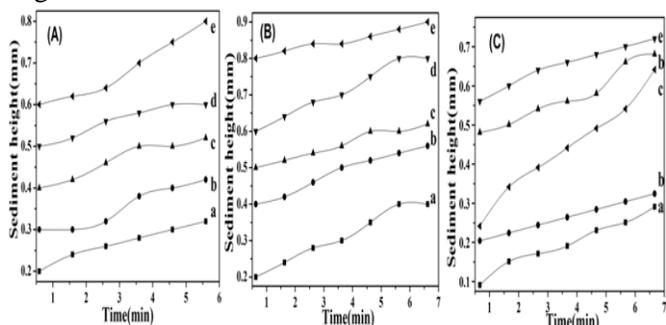


Fig.5: Settling behavior of starch/ferrite-gentamycin nano composite under (a) acidic, (b) alkaline, (c) neutral

Fig. 5A is ascribed to the starch/ Fe₃O₄ synthesized under acidic pH at different percentage weight loading of nano hybrid (Fig. 5A, plots a-e). While increasing the percentage weight loading of nano hybrid the sediment height was increased with the time. Under acidic pH the settling velocity was calculated as 0.37×10^{-6} m/s. Fig. 5B indicates the plot of sediment height against time under alkaline pH. Here also one can see the above said trend while increasing the percentage weight loading of nano hybrid (Fig.5B, plots a-e). The settling velocity was calculated as 0.911×10^{-6} m/s. Fig. 5C confirms the plot of time against settling height starch/ Fe₃O₄ nano composite synthesized under neutral pH. While increasing percentage weight loading of nano hybrid the settling height was also increased (Fig. 5C, plots a-e). The settling velocity was calculated as 0.216×10^{-6} m/s. In overall comparison the alkaline system exhibited the highest velocity due to the bigger size of starch molecules.

VI. CONCLUSION

From the above results and discussion part the important points are summarized here as conclusion. The appearance of C-N stretching in the FTIR spectrum

confirmed the surface functionalization of starch by gentamicin. The increasing crystallinity after the surface functionalization was confirmed by a noting a peak corresponding d₅₁₁ crystal plane. The band gap energy value was found to be increased after the surface functionalization reaction to 0.643ev. After the surface functionalization reaction both the size and shape of the Fe₃O₄ nano particle were changed with the decrease in VSM value. The starch nano composite prepared under acidic condition exhibited lowest thermal stability. The alkaline system exhibited the highest settling velocity due to the bigger size of starch. The present investigation is very much useful for the separation and purification process of starch.

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