



Synthesis and Characterization of Chitosan encapsulated Iron oxide Nanoparticles and their Antimicrobial activity study

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Abstract

The chitosan-encapsulated Iron oxide nanoparticles were prepared by co-precipitation method. The formation of iron oxide nanoparticles (FeO NPs) and CS coated iron oxide nanocomposite (CS- FeO) were preliminarily confirmed by color change. The characterization of synthesized nanoparticles was performed by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The observed bands at 500-800 cm⁻¹ in the FTIR spectrum indicated the presence of metal-oxygen (FeO) bond whereas band at 1646 cm⁻¹ indicated the presence of amino groups (-NH₂) which confirms the CS in the prepared CS-FeO nanoparticles. SEM results demonstrated a spherical morphology. The antibacterial activity was assessed by zone of inhibition method against Staphylococcus aureus MTCC 87 and Escherichia coli 443 invitro.

Keywords: Chitosan, Iron oxide nanoparticle and antibacterial activity

Introduction

Chitosan is an interesting polymer that has been used extensively in the medical field it is partially or fully deacetylated chitin. Chitosan is a fully biodegradable and biocompatible natural polymer. It is used as a novel nasal delivery system for vaccines and also proved to prevent infection in wounds. Pharmacology is the study of therapeutic value and or potential toxicity of chemical agents on biological system. Over the past few decades nanoparticles are widely employed in biomedical /pharmaceutical field for applications such as contrast agent's tumor targeting /therapy drug etc (1). Biodegradable nanoparticles as effective drug delivery carriers have attracted the scientific community due to

their therapeutic benefits without any side effects (2). Iron oxides are chemical compound of iron and oxygen. Biomedical applications are cellular therapy, tissue repair, drug delivery, magnetic response, imaging hypothermia and magneto-fiction (3). Research area applications are biomedicine (4) catalyst (5) sensor (6) etc. In magnetic resonance imaging to provide enhanced contrast at very low concentrations in the nanomolar range for studying tumors.

II Materials and Methods

Preparation of chitosan

Crustacean waste crab shells were collected from seashore. The shells were scraped free to lose tissue washed, dried ground pass through a 0.3-0.5mm sieve and subjected to demineralization, deproteinization deacetylation and finally the chitosan powder and was obtained. The chitosan was stored at room temperature for further studies.

Biosynthesis of iron oxide nanoparticles

Iron oxide ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was purchased from Sigma-Aldrich chemicals. In typical synthesis of iron nanoparticles 0.1 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.50 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were taken and mixed with 20ml of Millipore water (double distilled). The mixture was stirred in a magnetic stirrer for 10 minutes. Ammonia 7% solution was prepared and added the mixture until PH 11 was reached. This mixture kept for drying in oven at 110° . The final black precipitate was collected and washed several time with water.

Synthesis of chitosan coated iron oxide nanoparticles

The synthesized iron oxide nanoparticles were dried and made into a powder. 200 mg chitosan 85% deacetylated was made into a gel using 2-5 ml of formaldehyde. The iron oxide powders 200mg which is in the form of gel and makes to mix under magnetic stirrer for 1 hour. Finally after obtaining a homogenous mixture the chitosan coated iron oxide particles were filtered and dried for further analysis.

Zone of inhibition Testing

Antimicrobial activity of the samples was determined by well diffusion method. Two pathogenic bacterial strains namely Escherichia coli and Staphylococcus aureus were examined. The test bacterial strains were inoculated into nutrient broth and incubated at 37°C for 24 hours. After the incubation clear zone was observed. Inhibition of the bacterial growth was measured in mm.

Characterization of synthesized nanoparticles

XRD Studies

The phase identification and crystalline structure of the nanoparticles was characterized by X-ray diffraction. The X-ray diffraction pattern obtained for iron nanoparticles synthesized using chitosan showed that there exist strong diffraction peaks with 2θ values of 29.69° , 35.76° , and 45.73° corresponding to the crystal plane of (111), (110), (250) (fig1)

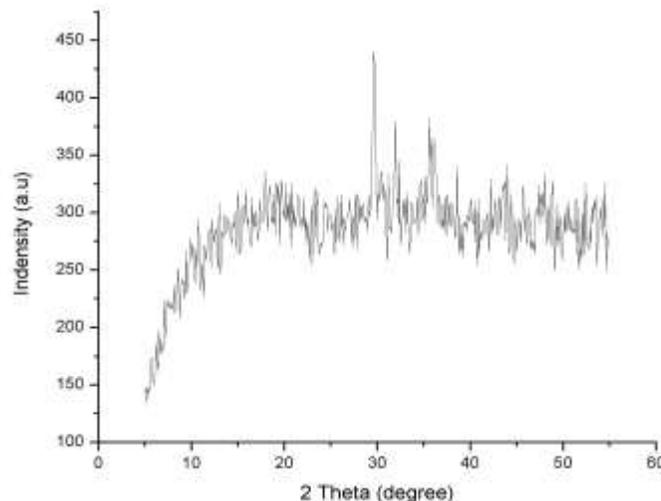


Figure 1: X- Ray Diffractogram of iron nanoparticles synthesized from Chitosan

Size determination from XRD

Using Debye-Scherrer formula the crystalline size for the nanoparticles was calculated.

$$D = K \lambda / \beta \cos \theta$$

Where D is the average particle size in nm, λ is the wave length of X-ray (0.15406nm), β is the full width at half maximum of the diffraction peak, K, is the Scherrer constant with the value of 0.9 to 1 and θ is the Bragg angle.

Scanning electron microscopy (SEM) micrograph of the chitosan nanoparticles

Scanning electron microscope is one of the powerful tools to identify the shape of nanoparticles. SEM image showed provided the morphology of the nanoparticles (figure 3). The scanning electrons microscopic image showed the high density of iron oxide nanoparticles synthesized by chitosan. The iron nanoparticles were relatively spherical and also individual nanoparticle was aggregated shows large nanoparticles. This aggregation

took place due to the presence of cell component and the surface of the nanoparticles act as the capping agent.

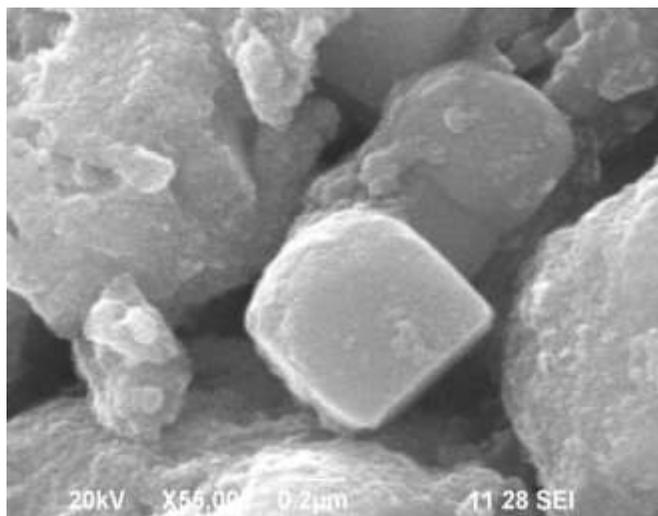


Figure: 2 SEM image of iron nanoparticle synthesized from chitosan

Energy dispersive X-ray Spectroscopy

To confirm the presence of the main elements in the synthesized materials an elemental composition analysis was done by energy dispersive X-Ray spectroscopy (EDX). Using this technique the elemental composition of the materials was analyzed with high resolution. EDX analysis data confirms the main components of materials. Chitosan powders have the weight percentage of iron as 37.75% (Figure 3)

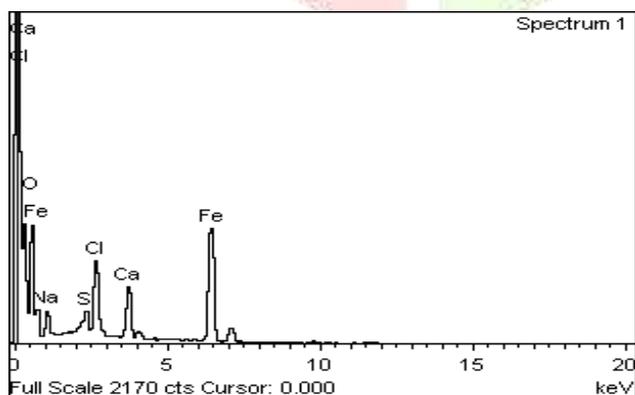


Fig 3: Energy Dispersive X-rays spectrograph of iron NPS from chitosan

FTIR Spectra Analysis

The FTIR spectrum of Fe NPs indicated that the NPS manifested absorption peaks at about 3132, 1631, 1402,

1118, 873, 615 cm^{-1} . The peaks near 3132 corresponded to the stretch vibrations of CH groups. The band at 1631 cm^{-1} corresponded to amide I due to carbonyl stretch in proteins. The peak at 1118 cm^{-1} corresponded to c-o-c ethers. The peak at 873 cm^{-1} belonged to the C-BR stretch of alkyl halides. The result clearly identified the involvement of primary amino groups in the interaction with metal surface and the amino groups were acted as capping sites for the FE NPs stabilization.

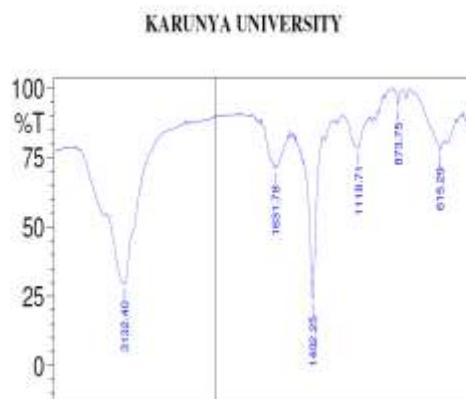


Fig-4: FT-IR spectrum of iron nanoparticles from chitosan

Antibacterial activity

From this study, the chitosan act as an antibacterial agent against Escherichia coli and Staphylococcus aureus. The result indicated that the iron nanoparticles synthesized from chitosan showed effective antibacterial activity in both Gram negative and Gram positive bacteria. When the concentration of standard Genmycin at 80mcg zone of inhibition for both S.aureus and E.coli were 25mm. similarly Getamysin at 400mcg zone of inhibition for S.auteus and E.coli were same of 12mm whereas when gentamycin at 800 mcg zone of inhibition for S.aureus is 14 mm and for E.coli 13. This shows that the zone of inhibition for S.Aureus is higher than the E.coli.



Fig-5: Antimicrobial activity of Iron oxide nanoparticles from chitosan

As per previous paper using Dioscorea tuber extract have the same zone of inhibition for nickel nanoparticles in *S.aureus* is 14mm and *E.coli* 13mm(7). Both are having same antibacterial activity.

Conclusion

In conclusion, the chitosan FeO nanoparticle was synthesized by co-precipitation method. Chitosan coated magnetic iron oxide nanoparticles were synthesized and characterized using XRD, FTIR, SEM and antimicrobial activity. Elemental composition analysis was done by energy dispersive X-ray spectroscopy. Antimicrobial activity of synthesized iron nanoparticles was studied against Gram positive *S.aureus* and Gram negative bacteria *E.coli* and their activity was measured. Zone of inhibition found for iron nanoparticle Gram positive bacteria is higher than Gram negative bacteria.

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