Preparation of inorganic composites of chitosan/magnesium oxide nanoparticle for antibacterial activity

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Abstract

Natural polymer based nanocomposites have received attention in research due their biocompatible nature and their versatile application in medical field. In the current investigation, we report the synthesize of chitosan /magnesium oxide nanoparticle (CS/MgO) composite material. The prepared sample was characterized by analytical methods such as FTIR, XRD and HRSEM.The antibacterial activity of the CS/MgO composite was evaluated. FT-IR spectral results showed the CS/MgO composite has distinctive functional groups. HR-SEM images revealed that the formation of well dispersed MgO nanoparticles in CS and it is observed that the MgO nanoparticles are embedded between the functional moieties present in the chitosan which shows the good interaction between chitosan and MgO nanoparticles. The antibacterial activity of the composite was evaluated against *Staphylococcus aureus* and *Escherichia coli.* It is observed that the CS/MgO composite material showed comparatively higher inhibition against *E. coli* than *S.aureus.*

Keywords: Chitosan, magnesium oxide nanoparticles; spectral studies; antibacterial activity.

Introduction

In medical field, a novel biocomposite material is being developed to meet the demands of materials for regeneration and tissue engineering. Among these, polymer composites are made up of metal oxide nanoparticle and polymers materials exhibit distinctive structural and biological features comparable and used to hybrid biological systems in development of bones. Polymer nanocomposites are widely used in biomedical coatings which are designed using metal oxide nanoparticle components with their polymeric mate[1].

Chitosan (CS) is a naturally biopolymer and it possesses a potential to be utilised to generate films, fibres, beads, and dust is obtained by deacetylation of chitin found in crab shells [2,3]. Due to its antimicrobial qualities and biocompatibility, chitosan has gained prominence for use in biomedical applications, wound dressings, lipid-sequestering agents, hydrogels, blood anticoagulants, blood separation membranes, contact lenses, controlled drug release, and food packaging components [4-8]. Despite the presence of hydrogen interactions between amino and hydroxyl groups in the CS, certain challenges are encountered.Having limited strength, being unable to dissolve in common solvents, and lacking stability in the body are some of the drawbacks[9-12].

Polymeric materials are employed in biomedical application are often synthetic polymers such as poly(lactide-co-glycolic) acid, polydopamine, polylactic acid, poly(-caprolactone), polyurethane, and synthetic resin such as collagen, chitosan (CS), and cellulose[13-18].Metal oxide nanoparticleis used in the preparation of polymeric composites are carbon, ceramics, metal, and metal oxides.[19-24]. However, coatings comprising only polymers continually have significant downsides. For example, polymers are frequently adaptable but need mechanical strength and chemical stability. The other limited factors of the biomedical applications of polymers are usually not homogeneous, molecular weight distribution and functionalizable in nature[25,26]. Because has poor adhesion to surfaces, do not effectively create a protective layer, have a tendency to aggregate, may harm cells and they are not suitable for use in medical coatings[27].

In the present investigation, we discuss the fabrication of a composite film that includes CS and MgO nanoparticle as well as the assessment of its antibacterial effects. The film composite material was prepared by solvent casting method and characterized by Fourier

transform infrared spectroscopy, X-ray diffraction and High resolution scanning electron microscopy. Also, antimicrobial activity of the prepared composite material was evaluated.

Experimental

Materials

Magnesium sulphate heptahydrate ($MgSO_4$. $7H_2O$; 99%), sodium hydroxide, and acetic acid chemicals were provided by Sigma-Aldrich. Chitosan was purchased from Merck. De-ionized water that has undergone two distillations was used for the experiment. Each and every reagent used in the experiment was of analytical quality.

Preparation of magnesium oxide nano particles

The magnesium oxide nanoparticles were synthesized as the stabilizer by green synthesis approach as well as solution-combustion method[28]. The starting materials used for the synthesis were magnesium sulphate heptahydrate ($MgSO_4$. $7H_2O$) and acetic acid. In a MgO-NPs synthesis, 1:1 molar ratio of magnesium sulphate and acetic acid was dissolved in distilled water along with stirring for few minutes and 20 mL of *Plectranthus ambonicus (Karpuravalli)* extract and 5 mL of water were taken in a titration flask and the solution was heated up to 80 °C for efficient extraction. The reaction mixture was then allowed to cool down to 30 °C and 0.1 M solution of $MgSO_4$. $7H_2O$ was dropwise added into the reaction assembly. The reaction was allowed to proceed for 12 hrs. The acquired greenish white precipitates were filtered, washed and dried in an oven for 2 h to get the MgO-NPs. The prepared NPs were stored in an airtight sample bottle.

Extraction of chitosan from (*Penaeus monodon* shell)

The used raw black tiger shrimp shell (*P. monodon* shell) for the extraction of chitosan as reported in our previous paper[29].

Preparation of polymer nanocomposites

Synthesis of polymer nanocomposites chitosan - MgO nanoparticle was prepared by solution mixing method as follows: 0.6 g of chitosan was dissolved in 20 mL of CH_3COOH and 0.2 g of MgO nanoparticle was dispersed separately using magnetic stirrer. After an hour of dispersion, the MgO nanoparticle was added to chitosan solution. The stirring was continued for 24 h in order to get well dispersed chitosan - MgO nanocomposites. The obtained product

was poured into clean Petri dish and the nanocomposite was kept in an oven at 60°C to remove moisture and excess solvent for two days. The prepared chitosan - MgO nanocomposites was used for characterisation studies.

Preparation of antibacterial activity assay (Agar disc diffusion method)

For the preparation of inoculums, stock cultures were maintained at 4 ^{o}C on nutrient agar slant. A test tube was filled with nutrient broth. To this broth a loop full of culture was added and the contents of the test tube were incubated for about 24 h at 37 ^{o}C. Agar diffusion method was chosen to perform this assay. In the present study, Muller Hinton Agar (MHA) medium was taken. To a petri plate Muller Hinton Agar (MHA) medium was drizzled and allowed to get solidified. The inoculums were placed with the bacterial suspension on solid plates with sterile swab moistened. 20 µl of chitosan - MgO nanocomposite (Concentration: 1000 µg, 750 µg and 500 µg) was placed in the disk. The assay experiment had been incubated for 24 h at 37 ^{o}C.

Results and discussion

FT-IR spectroscopy of chitosan - MgO composite

The FTIR spectra of chitosan - MgO composite were recorded in the wave number range of between 4000 and 500 cm^{-1} (Fig. 1). In the MgO NPs, the broad peak (–OH) hydroxyl stretching vibration is observed at 3291 cm^{-1}, which corresponds to the absorbed water molecules[30]. The metal-oxygen peaks found in the wave number range of between 500 and 800 cm^{-1}. It confirms the stretching vibrations between magnesium and oxygen (Mg-O) [31, 32]. Mg-O stretching bands are observed at 682 and 598 cm^{-1}. The chitosan main broad -OH and -NH peaks with hydrogen bond at 3309 $cm^{-1}$and 1640 cm^{-1}. It revealing the amide group (C-O stretching along with the N-H deformation mode).The peak at 1375 cm^{-1} is due to the COO- group of carboxylic acid salt, 1047 cm^{-1} is attributed to the C-O-C bonds in glucose circle[33]. However, with the CS-MgO NPs, hydrogen bond is observed at 3318 cm^{-1} (-OH and -NH), 2934 cm^{-1} (C-H asymmetric), 2856 cm^{-1} (C-H symmetric stretching), 1591 cm^{-1} (N-H deformation groups), 1408 cm^{-1} (amine (C-N) axial deformation) and 1378 cm^{-1} (-COO carboxylic group)[34] and 667 and 548cm^{-1} (Mg-O stretching bands), respectively. From the results, it was decided that chitosan can form strong and high intermolecular hydrogen bond with MgO.

XRD analysis

The XRD design of CS/MgO is portrayed in Fig 3. A wide peak at 2θ = 19.92° within the XRD design of the compound. The polymer CS indicates amorphous in nature. The CS/MgO composite film exhibits an XRD pattern with a highest point occurs around 2θ = 20°, and this is caused by the amorphous CS. The XRD pattern of the CS/MgO film shows three peaks at angles 39. 97°, 58.91°and 62.15° in addition to the broad peak at 2θ = 20°. The arrangement of magnesium oxide particles is a cube-like structure. The planes of the oxide have a matching with the numbers (JCPDS No. 4-829) in the corresponding to (111), (110), and (220) planes. Pure MgO powder's XRD pattern shows crystalline peaks with high intensity 2θ at 37.72°, 42.76°, 58.81°and 62.08°. The MgO peaks migrated towards higher 2θ value of XRD pattern of the CS/MgO film exhibited distinct variations from that of pure MgO powder and the peak at 42.76° was not visible. Furthermore, compared to pure MgO,the CS/MgO film intensity of the MgO crests essentially diminished (Fig 3). According to the chitosan/MgO composite was successfully made by dispersing MgO nanoparticles into the CS matrix.

HR-SEM analysis

Using HR-SEM, surface morphology was determined of the chitosan - MgO compositefilm is shown in Fig. 4. The chitosan - MgO composite film can be seen in different magnifications through the images are presented. The CS/MgO film appeared to be uneven and wrinkled when observed at a low magnification. There were a multitude of small openings and slit shaped holes scattered across it. Fig.3a. MgO nanoparticles were described as needle-like platelets with an average diameter and thickness of 75 nm and 27 nm in Fig.3b. The HR-SEM image at a greater magnification of 50 k is remarkable for revealing the margins of multiple MgO nanoplates which proved that the MgO nanoplates were implanted within the CS framework.

Antibacterial activity assay

The antibacterial activity of chitosan - MgO nanocomposite was analysed against two microorganisms which are gram-negative bacteria (*E. Coli*) and one gram-positive bacteria (*S. aureus*) (Table 1). The antimicrobial activity was then determined by calculating the

inhibition zone diameter (Fig. 5). The observation indicates that CS/MgO composite have *E. coli* shows high inhibition zone than *S. aureus*[35].

Conclusion

In this investigation, we report the synthesis of a safe and highly biocompatible polymeric nanocomposite containing MgO nanoparticles embedded ina chitosan biopolymer. The composite film of CS/MgOvarious analytical techniques like Fourier transform infrared spectroscopy, X-ray diffractometry and Field-emission scanning electron microscopy were employed to examine the composite.The antibacterial performance of CS/MgO composite was screened against *E.Coli* and *S.aureus.* CS/MgO composite showed good antibacterial activity. The antibacterial activity of the composite was comparatively higher against *E.Coli* than *S.aureus.* Thus, the CS/MgO composite can serve as a good biomaterial for bone tissue engineering and clinical applications.

Figures

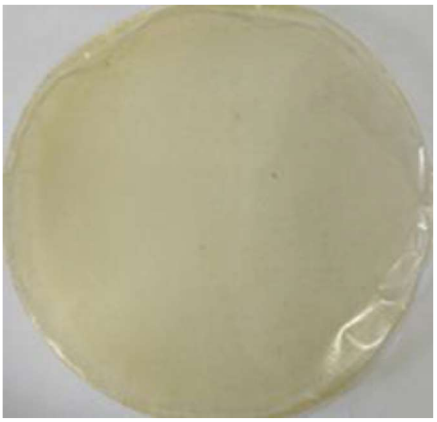


Fig.1 Photograph of CS/MgO composite film

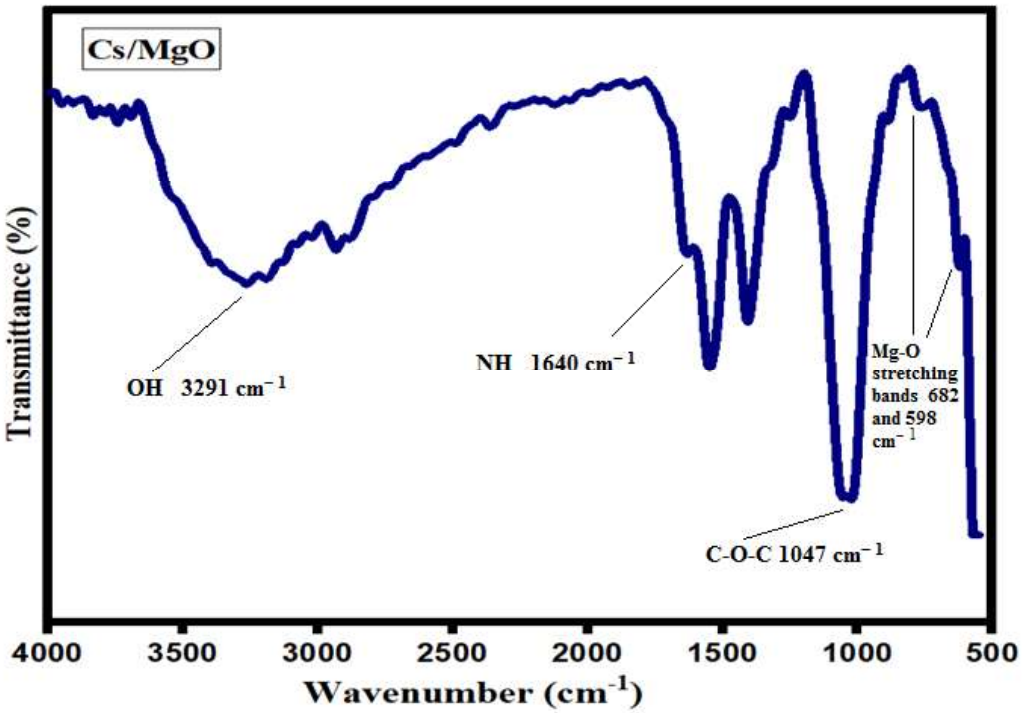


Fig. 2 FTIR spectrum of chitosan – MgO composite

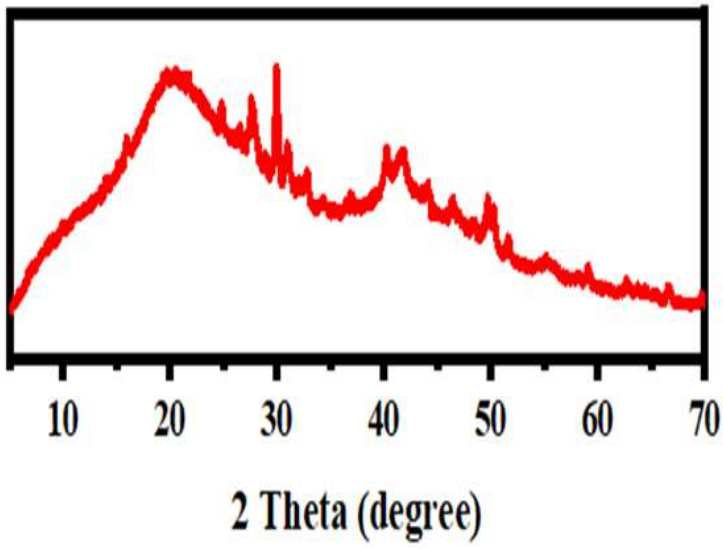
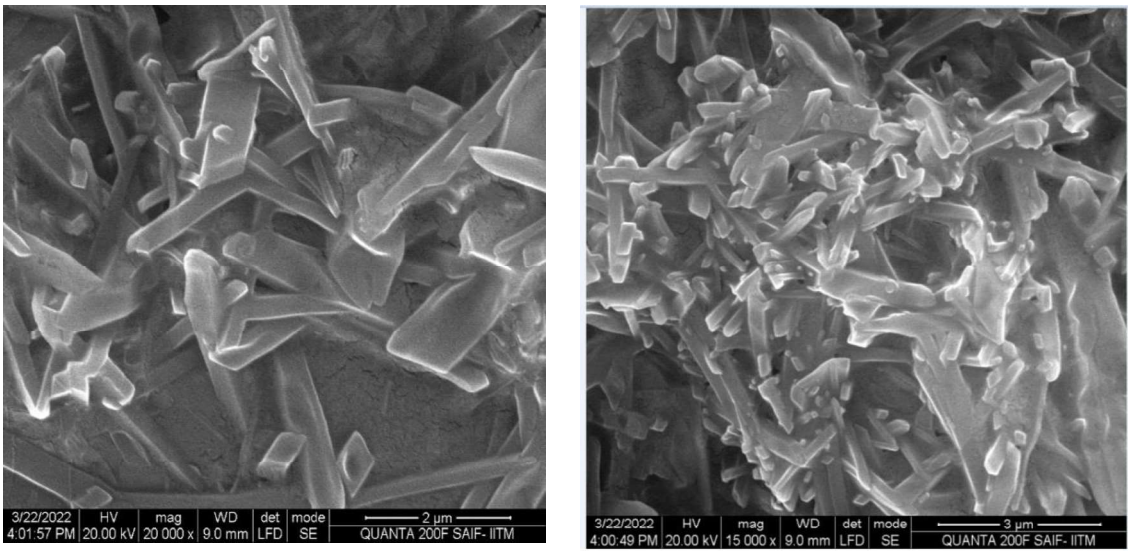
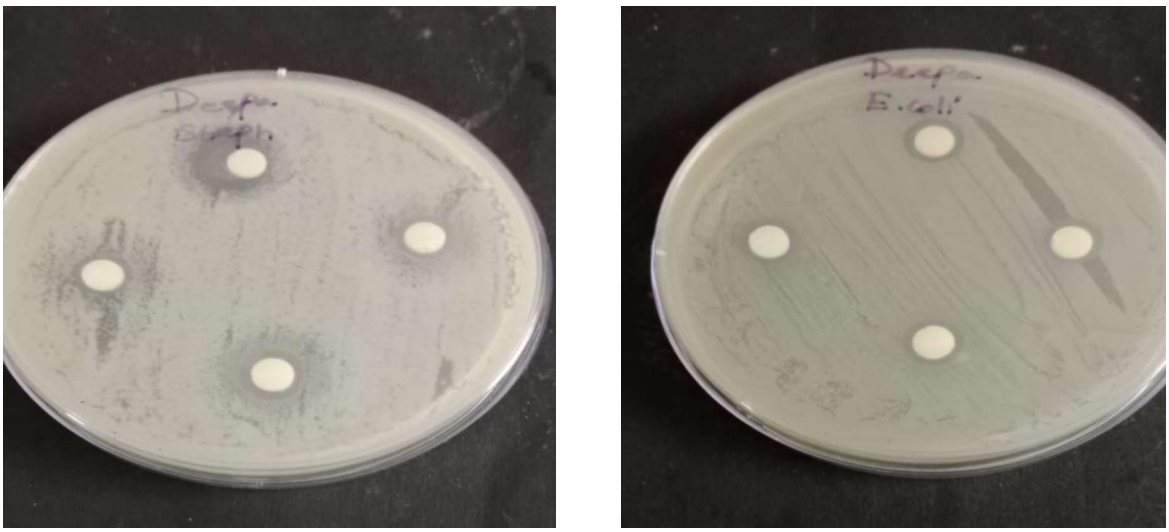


Fig. 3 XRD analysis of chitosan – MgOcomposite



(a) **(b)**

Fig. 4 (a) and (b) HR-SEM images of chitosan – MgO composite at 2µm and 3 µm resolution respectively



S. aureus ***E. Coli***

Fig.5.Antibacterial efficacy of CS/MGO composite

Table

Table: 1 Antibacterial activity of CS/MgO composite - zone of inhibition (mm)

Organisms	Zone of Inhibition (mm)			
	Sample (µg/ml)			Standard
	1000	750	500	Anti Anti (20µl/disc)
Staphylococcus aureus	10	10	9	14
Escherichia coli	10	10	10	10

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