Enhancing the Physical Characteristics and Antibacterial Efficacy of Chitosan via Innovative Methods: Investigation into Chemically Formulated Chitosan-Coated ZnO and the Physical Composite of Chitosan/ZnO

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Abstract

This study investigates the creation and analysis of chitosan-zinc oxide (CS-ZnO) nanocomposites, exploring their effectiveness in inhibiting bacteria. Two synthesis approaches, physical and chemical, were utilized. The CS-ZnO nanocomposites demonstrated strong antibacterial properties, especially against Staphylococcus aureus, a Gram-positive bacterium. Chemically synthesized nanocomposites (CZ10 and CZ100) exhibited larger inhibition zones (16.4 mm and 18.7 mm) compared to physically prepared CS-Z5 and CS-Z20 (12.2 mm and 13.8 mm) against Staphylococcus aureus. Moreover, CZ nanocomposites displayed enhanced thermal stability, with decomposition temperatures of 281°C and 290°C, surpassing CS-Z5 and CS-Z20 (260°C and 258°C). The residual mass percentages at 800°C were significantly higher for CZ10 and CZ100 (58% and 61%) than for CS-Z5 and CS-Z20 (36% and 34%). UV-Visible spectroscopy revealed reduced band gaps in the CS-ZnO nanocomposites, indicating improved light absorption. Transmission electron microscopy (TEM) confirmed uniform dispersion of ZnO nanoparticles within the chitosan matrix. In conclusion, this research underscores the impressive antimicrobial potential of CS-ZnO nanocomposites, especially against Gram-positive bacteria, and highlights their enhanced thermal stability. These findings hold promise for diverse applications in industries such as medicine, pharmaceuticals, and materials science, contributing to the development of sustainable materials with robust antimicrobial properties.

Introduction

Green chemistry has emerged as a promising approach for addressing the pressing need for sustainable development. The use of biopolymers derived from renewable resources is a key area of focus in this field, owing to their biodegradability, abundance, and unique properties [1][2]. Among the various biopolymers, polysaccharides have been extensively studied for their potential in creating functional materials. In particular, cellulose, chitin, and starch are commonly investigated for their applications in various industries, including food, medical, and pharmaceutical sectors [3] [4]. Polysaccharides are of particular interest in the medical field due to their biocompatibility and biodegradability [5], which make them ideal candidates for drug delivery systems, wound healing, and tissue engineering [6].

Chitosan (CS), the second-largest renewable biopolymer after cellulose, has attracted considerable attention in various fields due to its unique properties, such as biocompatibility, nontoxicity, and antimicrobial activity [7]–[13]. However, its poor mechanical and electrical properties limit its potential applications [14]. Therefore, incorporating nanofillers is an effective approach to improving chitosan's physical and mechanical properties [15]. Additionally, chitosan's numerous amino and hydroxyl groups make it an excellent adsorbent for heavy metal ions and dye uptake [16] [17]. In recent years, nanomaterials have emerged as promising additives for improving the properties of biopolymers [18]. Zinc oxide nanoparticles (ZnO NPs) are widely used in various fields due to their broad-spectrum antimicrobial activities [19] [20], biocompatibility, and low production cost. Chitosan-coated ZnO nanocomposites have been extensively reported as a key material in various applications, such as food
packaging [21], coating on textile fabric, and dye removal [22] [23][24], based on their antimicrobial [25] [26], antibiofouling, and photocatalytic activity [18], [27].

This study aims to develop a chitosan-coated zinc oxide (CS-ZnO) nanocomposite using physical and chemical methods and investigate its antibacterial activity against various microorganisms, including Gram-positive bacteria, Gram-negative bacteria, yeast, and filamentous fungus. The nanocomposite was thoroughly characterized using X-ray diffraction spectroscopy and Fourier transform infrared spectroscopy. The results of this study demonstrate that the CS-ZnO nanocomposite exhibits excellent antibacterial activity against various microorganisms, including both Gram-positive and Gram-negative bacteria. Moreover, the study provides insights into the potential applications of the CS-ZnO nanocomposite in various fields, such as medical, pharmaceutical, and industrial processes.

Overall, the use of biopolymers in material development, particularly in the medical field, holds great promise for sustainable development. The development of chitosan-based nanocomposites, such as the CS-ZnO nanocomposite described in this study, can further expand the potential applications of biopolymers in various fields. The results of this study contribute to the ongoing efforts to develop green and sustainable materials that can address the environmental and health challenges facing the world today.

Experimental

3.1 Materials

Chitosan with a degree of deacetylation of 85% and low molecular weight, extracted from crab shells, was procured from G.T.C Bio Corporation (Hong Kong, China). Glacial acetic acid and zinc oxide nanopowder were sourced from Sigma Aldrich, USA. Distilled water was employed in the preparation of all solutions. All chemicals were used as received and met analytical grade standards.

3.2 Preparation of CS/ZnO nanocomposite physically

For the synthesis procedure, approximately 1 g of chitosan powder was dissolved in 100 ml of 1% acetic acid at room temperature. The solution of dissolved chitosan was then combined with solutions containing varying quantities of zinc oxide nanoparticles (5, 10, 15, and 20 wt.%), designated as CS-Z 5, CS-Z 10, CS-Z 15, and CS-Z 20. These mixtures were sonicated for 15 minutes in 100 ml of distilled water. To this solution, 1.0 M NaOH was added gradually while vigorously stirring until the pH reached 10. The solution was allowed to stand overnight. The resulting solution, containing CS-ZnO nanocomposites, underwent multiple washes with distilled water to eliminate any remaining unreacted materials. Subsequently, the solution was dried in a vacuum oven for 24 hours.

3.3 Preparation of CS/ZnO nanocomposite chemically

The preparation of CS-ZnO nanocomposites was conducted using a chemical method following the procedure outlined in the referenced literature [18]. Initially, 1 g of chitosan powder was dissolved in 100
ml of 1% acetic acid at room temperature. In a separate step, a solution containing 1 g of zinc oxide was dispersed in 100 ml of distilled water through sonication. This solution was then combined with the previously mentioned chitosan-acetic acid solution in a 500 ml quick-fit bottomed round flask. Subsequently, the assembled flask was placed within a microwave oven and subjected to specific time intervals (3, 5, 7, and 10 minutes) and power levels (80, 240, 560, and 800 W), denoted as CZ10, CZ30, CZ70, and CZ100. The resulting nanocomposites were subjected to centrifugation and multiple washes using distilled water to eliminate any residual reactants and obtain the final product. The CS-ZnO nanocomposites were then dried in a vacuum oven until a constant weight was achieved.

3.4. Characterization techniques

The nanocomposites underwent characterization through several techniques. X-ray diffraction (XRD) measurements were performed on a Philip’s X-ray diffractometer PW1390 with Ni-filtered Cu Kα radiation at generator voltage of 4 kV and wavelength of 0.154 nm at room temperature. The diffraction angle, 2θ, was scanned at a rate of 2°min⁻¹. Fourier transform infrared (FTIR) spectroscopy was conducted using a JASCO FTIR instrument at Sapala Organics Private Ltd., Hyderabad. The measurements were performed on KBr pellets, covering a spectral range from 400 to 4000 cm⁻¹. Thermogravimetric analysis (TGA) was carried out using a thermogravimetric analyzer model SDT Q600 V20.9 build 20. Each sample, weighing approximately 10 mg, was placed in an alumina crucible. The analysis involved subjecting the samples to a temperature range spanning from ambient temperature to 700°C, with a heating rate of 10°C/min. The UV–Vis absorption spectra of CS, ZnO NPs and CS/ZnO nanocomposite were obtained by V-750 UV–Vis spectrophotometer, the wavelength of incident ray was selected in the range of 300–600 nm. The morphology of samples has been investigated by transmission electron microscopy (TEM) JEOL (JEM-2100 PLUS, Japan) at an accelerating voltage of 200kV.

3.5. Antimicrobial assay

The synthesized nanocomposites were evaluated for anti-microbial activity using the agar diffusion technique Ref. The tested samples evaluated against, Gram +ve bacteria (Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 35556), Gram -ve bacteria (Escherichia coli ATCC 23282 and Pseudomonas putida ATCC 10145), Yeast (Candida albicans IMRU 3669) and Filamentous Fungus (Aspergillus niger ATCC 16404). The bacteria and yeast were grown on nutrient agar while the fungus was grown on Potato Dextrose agar medium. The tested samples were evaluated in the concentration of 5000 ppm. The positive control was Stryptomycin for bacteria and Metronidazole for yeast and fungus.

Results and discussion

3.1. X-ray diffraction (XRD) pattern of nanocomposites

Fig.1. illustrates the X-ray diffraction (XRD) pattern of CS, ZnO, and physically prepared CS/ZnO nanocomposites. In this depiction, CS exhibits a broad peak at 20.02°, indicative of its semicrystalline nature[28][29][30]. The diffraction peaks of ZnO nanoparticles (NPs) are notably sharp, aligning with
angles of 31.79°, 34.44°, 36.28°, 47.58°, 56.63°, 62.89°, 66.41°, 67.97°, 69.13°, 72.58°, and 76.99°. These patterns correspond to the standard hexagonal phase of ZnO (JCPDS card no. 36-1451) [31][32][33].

Moving to the CS-ZnO nanocomposites, specifically CS-Z 5, CS-Z 10, CS-Z 15, and CS-Z 20, distinct XRD patterns are observed at different concentrations of ZnO. These patterns retain characteristic peaks of both CS and ZnO NPs, albeit with alterations in intensity and precision. It's noteworthy that the XRD profile of the ZnO-CS nanocomposites underscores the presence of significant peaks related to both CS and ZnO NPs. However, in comparison to the pure CS and ZnO NPs, the intensity of the CS-ZnO nanocomposite peaks is diminished. This could potentially be attributed to interactions between the functional components of CS and ZnO NPs [34].

Fig.2. portrays the X-ray diffraction (XRD) patterns of nanocomposites prepared via a chemical method utilizing a microwave reactor (CZ10, CZ30, CZ70, and CZ100). These patterns reveal the presence of characteristic peaks associated with both CS and ZnO NPs, exhibiting alterations in both sharpness and intensity. This phenomenon can be interpreted as evidence of a complexation reaction occurring between CS and the surface of ZnO NPs [35].

3.2. FT-IR spectroscopy

The FT-IR spectra of CS and physically prepared CS-Z nanocomposites are depicted in Figure 3. In the infrared spectrum of CS, the characteristic peak at 3368 cm$^{-1}$ can be attributed to the stretching vibrations of amine −NH$_2$ and hydroxyl −OH groups. Peaks at 2877, 1659, and 1595 cm$^{-1}$ correspond to −CH stretching vibration, −C=O (amide I) stretching vibration, and −N–H bending (amide II), respectively. The peak at 1380 cm$^{-1}$ is attributed to the stretching of (CH$_2$−OH). Peaks at 1154 and 1077 cm$^{-1}$ are assigned to the β (1−4) glycosidic bond and the stretching vibration of C-O-C, respectively.

In the CS-ZnO nanocomposite, the characteristic peak related to the stretching vibration of −NH and −OH groups in CS shifted to a higher wavenumber, specifically 3416 cm$^{-1}$. Similarly, the peak associated with the bending vibration of the −NH group present in CS shifted to a lower wavenumber of 1646 cm$^{-1}$ in the CS-ZnO nanocomposite. Similar phenomena were also observed with the peaks of C-O, 3′-OH, and 5′-OH groups. These shifts were attributed to the formation of hydrogen bonds between ZnO and CS [34].

In comparison to the IR spectra of pure chitosan film, the spectra of chitosan-ZnO nanocomposites doped with varying concentrations of ZnO NPs, specifically CS-Z 5 and CS-Z 20, exhibited notable shifts in the positions of bands towards both lower and higher wavenumber regions. The width of the bands corresponding to −NH$_2$ and −OH groups is visibly correlated with the quantity of ZnO particles within the chitosan matrix. Furthermore, peaks falling within the 500 to 600 cm$^{-1}$ range were attributed to the presence of metal oxygen (Zn-O) bonds [36].

Fig.4. displays the FTIR spectrum of CZ70 and CZ100 nanocomposites, which were chemically prepared at different microwave powers, in comparison to the pure CS.
The FTIR spectrum of the chemically synthesized CZ nanocomposite at varying microwave powers is presented in Fig. 4. The acquired IR spectrum exhibited characteristic peaks corresponding to both chitosan and ZnO NPs. Notably, the characteristic bands underwent shifts to lower wavenumbers. For instance, the absorption band at 3368 cm$^{-1}$, associated with the stretching vibrations of hydroxyl, amino, and amide groups, was noticeably shifted to higher wavenumbers. This shift to higher wavenumbers could be attributed to the effective dispersion of Zn NPs within the polymer matrix [37].

3.3. Thermogravimetric analysis (TGA)

The thermal behavior of both physically and chemically prepared CS/ZnO nanocomposites was subject to thorough investigation using thermogravimetric analysis (TGA), as illustrated in Figure 5. The TGA results unveiled a striking contrast in thermal stability between the nanocomposites obtained through distinct preparation methods. Remarkably, the chemically prepared CS/ZnO nanocomposites, namely CZ10 and CZ100, exhibited a substantial advancement in thermal stability when juxtaposed with their physically prepared counterparts, CS-Z5 and CS-Z20. The decomposition temperatures of the former, clocking in at 281°C and 290°C, noticeably surpassed those of CS-Z5 and CS-Z20, which registered at 260°C and 258°C, respectively. This discernible disparity accentuates the efficacy of the chemical preparation approach in enhancing the thermal resilience of the resulting nanocomposites [38].

The pivotal temperatures at which the foremost decomposition stages occurred showcased a distinct divergence as well. For the CS-Z nanocomposites, this pivotal temperature was around 240°C, while the CZ nanocomposites demonstrated a higher pivotal temperature of approximately 285°C. Such findings corroborate the superior thermal stability conferred by the chemical synthesis route, specifically in the CZ nanocomposites.

Further insight was gleaned from the initial decomposition occurring at approximately 100°C. This initial stage, attributed to the expulsion of water, was exhibited by both the pristine CS and the CS/ZnO nanocomposites.

At the elevated temperature of 800°C, the residual mass percentages stemming from the decomposition process were notably different among the various nanocomposites. The CS-Zn5 and CS-Zn20 nanocomposites retained 36% and 34% residual mass, respectively. In stark contrast, the CZ10 and CZ100 nanocomposites displayed substantially higher residual mass percentages, reaching 58% and 61%, respectively.

These empirical findings collectively signify the tangible advantage in thermal stability exhibited by the CZ nanocomposites, which can be attributed to the existence of robust interactions between the ZnO nanomaterials and the chitosan matrix. The interplay of chemical synthesis and enhanced interaction mechanisms contributes to the discernible improvement in the thermostability of CZ nanocomposites over their physically prepared counterparts [11].

3.4 UV–visible spectroscopy
The UV–Vis absorption spectra of the pure CS, CS-ZnO nanocomposites hybrid and ZnO-NPs are shown in Fig. 6. Fig. 6 depicts that in the case of pure CS no UV absorption is observed, because the structure of CS molecules lacks conjugated double bonds.[39]. ZnO can be identified as n-type semiconductor with a wide band gap (3.37) eV. Owing to the electron transitions from the valence band to the conduction band, ZnO NPs reveals significant absorption peak between the range of 350–380 nm wavelength [40]. It is interesting to note that the CZ nanocomposites sample demonstrates a sharp absorption peak appeared at around 378, the change in the characteristics of absorption peak can be corresponded to the interactions of ZnO-NPs and CS polymer chains lead to the absorption peak of CS-ZnO to be increased in the visible light region with high intensity compared to CS-Z and ZnO, NPs which indicates the decrease in band gap[41].

3.5. Morphological analysis

Fig. 7. illustrates the morphological characterization of pure ZnO NPs and CS-ZnO nanocomposites. Fig. 7a demonstrates the approximately hexagonal and spherical form of ZnO NPs[42]. Fig. 7b displays CS-Z nanocomposites prepared chemically, the dark areas represent crystalline ZnO nanoparticles whereas the bright spots represent amorphous CS. The morphology of the particles in Fig. 7a shows that the ZnO NPs were evenly dispersed throughout the CS matrix. The sizes of the grains clearly revealed the rod- and cuboid-shaped grains which unmistakably show that the nanosized grains in our chemically created nanocomposites CZ were present. Physically produced CS-Z nanocomposites are shown in Fig. 7C. The aggregation of ZnO NPs into CS matrix led to the confirmation of amorphous background nanoparticles in the CS-ZnO hybrid [11]. The outcomes of the FTIR and XRD investigations were inagreement with the conclusions reached by the electron microscopy evaluations.

3.6. Evaluation of antibacterial activity

The antibacterial effectiveness of both chitosan (CS) and CS-ZnO nanocomposites is visually depicted in Figures 8 and 9, respectively. The assessment encompassed a variety of microorganisms, namely Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus), Gram-negative bacteria (Escherichia coli and Pseudomonas putida), yeast (Candida albicans), and filamentous fungus (Aspergillus niger). The evaluation employed the agar diffusion technique, as previously documented [11], [29], [43]–[45]

A distinct pattern becomes evident wherein nanoparticles display substantial zones of inhibition against bacteria, notably more pronounced at higher concentrations of ZnO in CS-ZnO nanocomposites, as compared to pure CS.

Moreover, the findings demonstrate that Staphylococcus aureus, a Gram-positive bacterium, displays heightened vulnerability to CS-ZnO nanocomposites prepared through two distinct methods, in contrast to Gram-negative bacteria, yeast, and fungi. This enhanced sensitivity can be attributed to the efficient and uniform dispersion of ZnO within the chitosan (CS) matrix, particularly prominent in samples synthesized through chemical processes as opposed to physical methods.
This discrepancy can potentially be linked to the structural disparities between Gram-positive and Gram-negative bacterial cell walls, making the former more receptive to the antibacterial effects of the composite.

The established antibacterial activity of chitosan relies on the interaction between the positively charged amino groups (-NH$_2$) of chitosan and the negatively charged surfaces of bacterial cells. This interaction can result in the leakage of cellular components, leading to cellular damage [43][46]. Moreover, the overall results collectively indicate that the significant antimicrobial activity of the composite is heavily reliant on the presence of nano ZnO particles embedded within the matrix. The hypothesis is that these ZnO nanoparticles release reactive oxygen species (ROS) and Zn$^{2+}$ ions, which collaboratively target the negatively charged cell walls of bacteria, inducing leakage and eventual cell demise [47][48]. These findings collectively underscore a robust inherent antibacterial potential within the CS-ZnO nanocomposite.

## Conclusions

This research demonstrates the successful synthesis of chitosan - zinc oxide (CS-ZnO) nanocomposites through physical and chemical methods. The comprehensive characterization of these nanocomposites using various analytical techniques provides valuable insights into their properties and potential applications.

The X-ray diffraction (XRD) analysis confirmed the presence of both chitosan and ZnO nanoparticles in the nanocomposites, with alterations in peak intensity and precision. Fourier transform infrared (FTIR) spectroscopy revealed shifts in characteristic peaks, indicating interactions between ZnO and chitosan. Thermogravimetric analysis (TGA) demonstrated improved thermal stability in chemically synthesized nanocomposites.

UV-Visible spectroscopy showcased decreased band gaps in CS-ZnO nanocomposites, suggesting enhanced light absorption. Transmission electron microscopy (TEM) revealed the dispersion of ZnO nanoparticles in the chitosan matrix.

The most significant findings pertain to the antibacterial activity of the nanocomposites. Both physical and chemical synthesis methods resulted in nanocomposites with potent antimicrobial properties, with Gram-positive bacteria being more susceptible. This suggests that the CS-ZnO nanocomposites hold promise as effective antibacterial agents, particularly in medical and pharmaceutical applications.

In summary, this study underscores the potential of CS-ZnO nanocomposites in various industries and their contribution to the development of sustainable and versatile materials with robust antimicrobial capabilities. The research paves the way for further exploration and applications of biopolymer-based nanocomposites in addressing critical challenges in health and the environment.

## Declarations
Data availability:

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

References


Figure 1

XRD pattern of physically prepared CS-ZnO nanocomposites.
Figure 2

XRD pattern of chemically prepared CS-ZnO nanocomposites
Figure 3

FTIR pattern of physically prepared CS-ZnO nanocomposites
Figure 4

FTIR pattern of chemically prepared CS-ZnO nanocomposites
Figure 5

TGA curves of CS/ZnO nanocomposite which prepared by two methods
Figure 6

UV–Visible spectra of Cs, ZnO NPs, CZ and Cs-Z nanocomposites
Figure 7

(TEM) images of (a) ZnO NPs, (b) CZ and (c) CS-Z.
Figure 8

Antibacterial activity of physically prepared CS-ZnO nanocomposites
Figure 9

Antibacterial activity of chemically prepared CS-ZnO nanocomposites