Antioxidant and antimicrobial studies of nanochitosan, carboxymethyl cellulose and graphene oxide binary and ternary composites

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Abstract

*The present study aimed to prepare binary and ternary composites of nanochitosan (NCS), carboxymethyl cellulose (CMC) and graphene oxide (GO) for the biological and biomedical application. The prepared NCS/CMC, CMC/GO and NCS/CMC/GO composites were characterized using FTIR, XRD and SEM studies. The results revealed the suitability of the material for biological application with increased amorphous nature for cell adhesion. Antimicrobial studies were carried out against selected bacterial and fungal species. DPPH scavenging activity was done for the prepared biocomposites. NCS/CMC/GO ternary composite have the highest action against *Bacillus* sp (21 mm) followed by *Klebsilla* sp. (19 mm) and *Proteus* sp. (16 mm). For selected fungal species of *Candida*, *Rhizopus* and *Aspergillus niger* the ternary composites shows 28 mm, 26 mm and 24 mm inhibitory zone. The overall results exhibit the potential antioxidant and antimicrobial activity of the prepared composites.*

Keywords: NCS/CMC/GO ternary composite, antibacterial studies, antifungal studies, antioxidant studies.

Introduction

Nanotechnology is a relatively new and rapidly developing technology that is gaining traction in a variety of fields as industry, agriculture, business, medicine and public health [1]. It will integrate the nanoscale structures into large components and systems by keeping their benefits [2,3]. A variety of nanomaterials have been produced and investigated for primary food packaging nowadays. Its major function is to improve the material's mechanical, barrier, antibacterial, and thermal properties [4]. Biopolymers in this regard acts as good antimicrobial agents. Biopolymers due to its low toxicity, biodegradability, and stability it can be used in a variety of pharmacological and medicinal applications.

Especially, chitosan biopolymer due to its OH and NH functionality it possess the properties including biodegradability, non-toxicity, high elasticity, good adhesion and sorption [5, 6]. These properties made chitosan (CS) as a promising material for antimicrobial applications [7, 8]. Thus, in the present work chitosan biopolymer was converted into its nanoform via ionic gelation method and mixed with carboxymethyl cellulose and graphene oxide to improve its biological properties.

Carboxymethyl cellulose (CMC) is an anionic polymer that is linear, long-chained, and water soluble [9]. Due to its high transparency, good film-forming property, large mechanical

strength, non-toxicity, and biodegradability, (CMC) is widely used as a packaging material (for films and coatings), medicine, flocculating agent, chelating agent, emulsifier, thickening agent, water-retaining agent, and sizing agent [10]. In this context, graphene oxide (GO) is one of the most appealing nanofillers in polymer nanocomposite technology because it improves and enhances the mechanical, thermal, and electrical properties of many nanocomposites, which could lead to novel solutions for a variety of applications [11].

GO has antibacterial properties, which has sparked interest in developing GO-based antimicrobial surfaces [12, 13]. The protection of hazardous biocides from being released was a key component in the design of these antimicrobial GO surfaces. It can induce bacterial cells to adhere together, causing cell integrity to be compromised and loss of cell viability, limiting bacterial growth on the surface [14 – 16]. As a result, these materials have significant applications in biomedicine, marine transportation, membrane-based water treatment, and heat exchangers [17, 18].

Hence, in the present work, the polymer composites of Nanochitosan (NCS), Carboxymethyl cellulose (CMC), and Graphene oxide (GO) in the form of binary and ternary composites were prepared by solution casting method. The prepared composites were subjected to FTIR, XRD and SEM studies and evaluated its antioxidant and antimicrobial properties.

Material and Methods

Chemicals and reagents

Chitosan (deacetylation 92% and MW 120,000) was obtained from India Sea Foods, Cochin, India, while AR grade tripolyphosphate and acetic acid were obtained from Finar chemicals, Ahmadabad, and Thomas Bakers chemicals Pvt. Ltd., Mumbai, respectively, and used without further purification. Carboxymethyl cellulose (CMC) was purchased from Molychem, Mumbai. Graphite fine power extremely pure was procured from Sisco Research Laboratories, Pvt. Ltd., Chennai.

Preparation of Nanochitosan

The nanochitosan was made using the sol-gel process, which involved dissolving 1 g of chitosan in 100 mL of 2% acetic acid. TPP solution (0.8 g in 107 ml DD water) was progressively added to this, stirring constantly. Nanochitosan was obtained as a suspension. The supernatant was discarded, and the nanochitosan was air dried for usage and analysis (19).

Preparation of Graphene oxide (GO)

The Hummers method was used to functionalize the graphite (20). 50 mL of cold H_2SO_4 (cooled under water bath till it reaches ice cold temperature of 0°C) was used to dissolve 1 g of graphite and 0.5 g of NaNO_3 . For 30 minutes, the mixture is stirred continuously. About 4g of KMnO_4 was gently added to the aforesaid mixture under steady stirring for further oxidation. The temperature was kept below 20°C for the KMnO_4 addition. By placing the beaker in an ice bath for about 1 hour, the temperature of the reaction mixture was decreased to ice cold. The addition of KMnO_4 was done slowly and carefully with control. The ice bath was then removed, and the reaction was allowed to continue by swirling the solution for 30 minutes at 35°C with magnetic stirring.

50 mL DD water was progressively added to this combination. The reaction was then continued at 98°C for 20 minutes, followed by the addition of 200 mL of DD water and 5 mL of

H₂O₂ (30%) to remove any remaining KMnO₄ and MnO₂. For the final purification of graphite oxide, the resulting mixture was filtered and washed with DD water and a 5 percent HCl solution (50mL). The graphene oxide (GO) powder was obtained after filtration and drying under vacuum at 70°C.

Preparation of Binary ternary composites

1g Under magnetic stirring, 1g of carboxymethyl cellulose dissolved in water and 1g of nanochitosan dispersed in water were mixed for about 30 minutes. The 1:1 CMC/NCS binary compound was poured into petridish and allowed to air dry. The CMC/GO binary composite was prepared using the same approach.

Preparation of Ternary composites

1 g of nanochitosan was combined with 1 g of CMC dissolved in water to make the NCS/CMC/GO ternary composite. This was followed by the addition of 0.5g of graphene oxide. The solution was well agitated for 30 minutes with moderate agitation before being placed into a petridish and allowed to air dry.

Characterization

Fourier Thermo NicoletAVATAR 330 spectrophotometer was used to perform FTIR spectrum studies of the produced blends in the 4000–400cm⁻¹ wave length region, utilising the KBr pellet method. X-ray diffraction (XRD) patterns were investigated utilising a Ni-filtered Cu K X-ray radiation source and an X-ray powder diffractometer (XRD –SHIMADZU XD – D1). At a scanning rate of 50min⁻¹, the relative intensities were obtained in the range of 5° – 90°. The ratio of crystalline area to total area of the peaks can be used to calculate the percentage of crystallinity (Xc). The composite was sliced into various sizes and wiped with a thin gold – palladium layer by a sputter coater unit (VG – microtech, UCK field, UK) for examination, and the cross section topography was examined with a Cambridge stereoscan 440 scanning electron microscope TESCAN VEGA3 SBH.

Antimicrobial activity binary and ternary composites

The antibacterial activity of NCS/CMC and CMC/GO binary composites, as well as NCS/CMC/GO (1:1:0.5) ternary composites, was investigated using the diffusion method on Muller Hinton Agar (MHA) medium with three bacterial strains: *Bacillus sp.*, *Klebsilla sp.*, and *Proteus sp.* gram positive and gram negative bacteria. *Bacillus sp.*, *Klebsilla sp.*, and *Proteus sp.* were inoculated on Muller Hinton Agar (MHA) medium and spread uniformly in Petri plate using sterile spreader. After the MHA medium had solidified, a small amount of the binary composites were placed on different cultured agar plates, which were then incubated on individual racks for 24 hours at 37 °C. The diameter of the zone of inhibition grown around the sample against the test microorganism was measured using the rules to assess the antibacterial activities of the prepared sample. Similarly the antifungal activity was done using the pathogenic fungi such as *Candidasp.*, *Rhizopus sp.*, and *Aspergillus Niger sp* on agar plates the samples.

Antioxidant Activity of binary and ternary composites

The DPPH scavenging experiment was used to test the composite's antioxidant properties. In antioxidant activity research, DPPH radical scavenging activity is a common test. The obtained composite's radical scavenging ability was tested by mixing (20–200 g/mL) with 4

mL DPPH (0.004 % in methanol) and incubating at room temperature for 30 minutes at the absorbance 517nm. When the colour changes, the absorbance drops as the DPPH is scavenged by the sample by hydrogen atom donation to create a stable DPPH complex. As a control, a DPPH solution with no sample is used.

The percentage of inhibition (I %) of free radical production from DPPH was calculated by using the following equation

$$\% \text{ of Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c – Absorbance of the control and A_s – Absorbance of the sample solution.

Results and Discussions

FTIR studies

Hydrogen bonding and miscibility of the composite can be analyzed using the FTIR spectroscopy technique, which determines the functional group present in a molecule as well as the chemical and structural bond information [21]. The FTIR spectrum of the NCS/CMC binary composite, CMC/GO binary composite, and NCS/CMC/GO ternary composite is shown in Figures 1–3. The existence of OH stretching and NH stretching vibrations in the H-bond formation is shown by a broad band at 3444.84 cm^{-1} , 3427.51 cm^{-1} , and 3439 cm^{-1} in the binary and ternary composites. Table 1 shows the numerous bands of polymer composites that reflect the respective functional group.

Figure 1: FTIR spectrum of NCS/CMC binary composite

Figure 2: FTIR spectrum of CMC/GO binary composite

Figure 3: FTIR spectrum NCS /CMC/GO ternary composite

Table 1: FTIR details of NCS, CMC and GO composites

Wavenumber (cm ⁻¹)			Functional Group
NCS/CMC binary composite	CMC/GO binary composite	NCS/CMC/GO ternary composite	
3444	3427	3439	OH Stretching and NH stretching vibration
2860	2922	2920	Asymmetric CH Stretching
2366	2362	2357,1859	NH ₃ ⁺ Stretching
1633	1633	1631	C=O Stretching (Amide Group)
1411	1417	1408	CH bending
1325	1319	1332	C-H and OH bending
1080.14	1112.93	1101	C-N and P=O stretching
889.18	894.97	896	C-O-Clinkage

The bands found in the binary and ternary composites at 2366 cm⁻¹, 2362 cm⁻¹, and 2920 cm⁻¹ confirm the existence of protonated NH₃⁺ groups, which would have played a role in the production of nanochitosan via electrostatic contact. In the binary and ternary composites, carbonyl stretching (amide I) is found at 1633.71 cm⁻¹, 1633.71 cm⁻¹, and 1631cm⁻¹, respectively. The FTIR spectra of NCS/CMC, CMC/GO, and NCS/CMC/GO composites shows a matching band shift and intensity shift, indicating that the composites were formed through strong interactions between the polymers.

XRD studies

Figure 4: XRD pattern of NCS/CMC binary composite

Figure 5: XRD pattern of CMC/GO binary composite

Figure 6: XRD spectrum NCS/CMC/GO ternary composite

The XRD pattern for the binary and ternary composites of NCS, CMC, and GO is shown in Figures 4–6. The amorphous nature of the produced composites is confirmed by the XRD pattern's broad feature. The binary composites NCS/CMC and CMC/GO have two short broad peaks in their XRD patterns $2\theta=28^\circ$ and 30° and $2\theta=19^\circ$ and 22° respectively, while the ternary composite displayed broad peaks $2\theta=25^\circ$ and 27° , indicating the amorphous nature of the composite. The XRD pattern of nanochitosan composites shows a single well-defined peak and a shoulder peak with a shift in peak position, indicating modification of polymers to form binary and ternary composite [22].

SEM

SEM is widely used to investigate both the surface morphology of materials and the biological response to them. Surface properties have a huge impact on whether a material device succeeds or fail, implying the necessity and need for accurate characterization of the material surface [23]. Figure 7a depicts the SEM morphology of the NCS/CMC binary composite, which is very favourable for since the surface area has been substantially enhanced while a very stable composite has been formed. The porous aspect of the binary composite is owing to the inclusion of GO, which increases the stiff structure of the material, as shown in Figure 7b. Figure 7c shows a SEM picture of the NCS/CMC/GO composite, which reveals a rough surface and an open pore structure with distinct needle walls and a high degree of pore interconnectivity in the created ternary composite material.

Figure 7: SEM images of (a) NCS/CMC; (b) CMC/GO and (c) NCS/CMC/GO composites

Antimicrobial study

Antibacterial activity

Table 2: Antibacterial Activity of binary composites and ternary composite

Organism (Bacteria)	Diameter of zone of Inhibition (cm)			
	Control Ciprofloxacin	NCS/CMC	CMC/GO	NCS/CMC/GO (1:1:0.5)
<i>Bacillus species</i>	22	19	20	21
<i>Klebsilla species</i>	17	18	19	20
<i>Proteus species</i>	15	17	16	18

Figure 8: Antibacterial activity of binary composites and ternary composites

The antibacterial activity of the prepared NCS/CMC and CMC/GO binary composite and NCS/CMC/GO ternary composites was tested using agar disk diffusion method and the results are shown in Figure 8 and Table 2. Ciprofloxacin was used as control. The zone of inhibition values revealed that the prepared NCS/CMC, CMC/GO and NCS/CMC/GO composites could inhibit the growth of both gram-negative and gram-positive bacteria species. These results revealed the existence of strong electrostatic interaction between the prepared biocomposite and the bacterial cell wall. Due to this interaction between nanochitosan composite and lipopolysaccharides (gram -ve) and teichoic acids (gram +ve) there will be the blockage of intra/extracellular exchanges and disruption in bacterial cell wall resulting in the leakage of cytoplasmic content. In comparison between selected bacterial strains, NCS/CMC/GO had the highest action against *Bacillus sp* (21 mm) followed by *Klebsilla sp.* (19 mm) and *Proteus sp.* (16 mm). It's possible that the discrepancy is due to their differing cell walls [24].

Antifungal Activity

NCS/CMC, CMC/GO and NCS/CMC/GO biocomposites was investigated against *Rhizopus sp.*, *Aspergillus niger*, and *Candida sp.* The measured zone of inhibition was given in the table 3 and figure 9.

Table 3: Antifungal Activity of binary composites and ternary composite

Organism (Fungi)	Diameter of zone of Inhibition (cm)			
	Control Amphotericin	CMC/NCS	CMC/GO	NCS/CMC/GO (1:1:0.5)
<i>Candida</i>	24	19	20	28
<i>Rhizopus</i>	23	18	19	26
<i>Aspergillus niger</i>	22	17	18	24

Figure 9: Antifungal Activity of binary composites and ternary composite

Higher inhibitory zone was recorded against *Aspergillus niger*, *candida sp.* and *Rhizopus sp.* NCS/CMC/GO biocomposite when compared with standard control Amphotericin. This superior antifungal activity was due to the change in physiological pH in the microbial cell and the formation of water proof layer. Hence, the prevention of transportation of food materials for the survival of microbial cells has taken place.

Antioxidant activity of Binary and ternary composites

The antioxidant activity of prepared binary composites and ternary composite was studied using the DPPH radical scavenging method. The DPPH radical shows strong absorption with NCS/CMC, CMC/GO and NCS/CMC/GO biocomposites at 517 nm using spectrophotocalorimeter for the existence of odd electrons. While DPPH free radical scavenging activity is used to assess substances for their ability to behave as free radical scavengers. The antioxidant activity of polysaccharides has been connected to the kind of monosaccharide, uronic acid content, sulfation degree, glycosidic linkage, and molecular weight [25].

The presence of OH and NH functional groups in NCS, CMC and GO exhibits radical scavenging activity and can protect a variety of biomolecular target molecules from oxidation [26]. The increase in antioxidant of ternary composite than binary was mainly depends on the

hydrogen donation capability of polymer composites. As shows in figure binary composites and ternary composite of exhibits good antioxidant activity

Figure 10: Antioxidant activity of prepared binary and ternary composite

Conclusion

Nanochitosan/Carboxymethyl cellulose, Carboxymethyl cellulose/Graphene oxide, and Nanochitosan/Carboxymethyl cellulose/Graphene oxide composites were effectively generated and characterised utilising FTIR, XRD, and SEM analyses in the current work. The physical and chemical interaction between the polymer composites taken is shown by the FTIR spectrum. XRD studies reveal a decrease in crystalline nature and an increase in amorphous character in ternary composites, which can improve mechanical strength over binary composites. SEM image indicate the composites was rough surface better than ternary composite very stable composite. The results of antimicrobial studies showed the potentate antimicrobial effect of binary composites NCS/CMC, CMC/GO and ternary composite NCS/CMC/GO against selected bacterial and fungal species. Thus, suggesting that it can be used for future application food packing material and wound healing applications.

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