Agriculture-derived Cellulose Nanocrystal Hybrid Composite of Chitosan and Polyethylene Glycol for Enhanced Antimicrobial Properties

Shivani Chaudhary (✉ schaudhary0006@gmail.com)
Dr. Bhim Rao Ambedkar University, Agra, India

Gautam Jaiswar
Dr. Bhim Rao Ambedkar University, Agra, India

Research Article

Keywords: Cellulose nanocrystals, chitosan, biocompatibility, antibacterial, nanocomposite

Posted Date: August 28th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3286965/v1

License: ☒ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

The biocompatible nanocomposite of cellulose nanocrystals / Chitosan / Polyethylene glycol was successfully prepared by the solution casting method. The prepared nanocomposite was characterized by UV-visible, Scanning Electron Microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), and X-Ray diffraction (XRD). UV-visible revealed the composite peaks formed by the absorbing wavelength. SEM images showed the uniformity of the composite formed. In XRD peaks obtained helps in comparative study of the pristine and composite of the material. The nanocomposite obtained had enhanced biocompatibility and antimicrobial activity. The variation study was also done with the help of various percentage composite prepared. The Cellulose nanocrystals/ Polyethylene glycol/chitosan (CPC1%) have different antimicrobial properties in comparison to CPC3% and CPC5% these composites have enhanced antimicrobial properties in comparison to CPC1%. These studies were done against gram-negative bacteria. Various percentages and different bacteria had different properties.

1. INTRODUCTION

The most abundant biomaterial of the earth is cellulose and it is found from last many decades on the earth (Yang et al. 2021). It is used as a fuel, in the textile, fibre, and weaving industry, clay bricks for construction in rural areas, pottery making, and in a wide range of fields. In modern times the industrial use of cellulose has been expanded to advanced chemicals, cellophane films, fibres used in dietary and for this reason, cellulose is a crucial abundant raw material among other organic materials present in the world (Lynd et al. 2005). Nanoscale materials are in discussion nowadays because of their tremendous properties like the formation of composite. One of these materials is cellulose nanocrystal (CNC) which has amazing properties like biodegradable, renewable, nontoxic as well as low-cost biomaterial (Farooq et al. 2020). CNC are highly crystalline, needle-shaped, or rod-like nanostructures and have one dimension less than 100 nm. The crystallinity provides marvellous properties to the CNC like low thermal expansion, gas impermeability, lightweight, sustainability, and surface which is adaptable to the chemistry reactions and easily forms composites (Emenike et al. 2023). The research of the CNC turns around cellulose nanofiber and cellulose nanocrystals (CNC).

Chitosan is a natural polymer obtained from shrimps or crabs in alkaline water by the deacetylation of chitin (poly N-deacetyl glucosamine) (Yampakdee et al. 2022). It is soluble in aqueous acids like acetic acid but insoluble in water because it does not form bonds with water molecules.

Polyethylene glycol (PEG) is a synthetic hydrophilic polymer and ethylene oxide is the monomer unit and its potency referred to the number of ethylene oxide units present in each different molecule (Bourke et al. 2003). It is a water-soluble macromolecule in comparison to chitosan which is insoluble and has applications in the field of pharmaceuticals, industrial, cosmetics, and biomedical. The main focus of this article is on the antibacterial activity of the polymer in the composite form (Abd El-Hack et al. 2020).
The PEG is a low molecular weight polymer and it has -OH group and an oxygen atom for donating so it forms a bond with water molecules beside this it can expand its cyclic structure and chain structure as well so that it can easily interact with the CNC structure network but chitosan has amine group (-NH₂), acetyl amine (-NHCOC₂H₃) and hydroxyl (-OH) functional groups in its structure for the bond formation in actual these functional groups acts as the binding group or as the active sites for upcoming groups for bond formation (Lutz et al. 2008).

The composite formed with these polymers acts as the potential antibacterial agent against various bacteria. Previous studies were done but not with this combination and not with this number of microbes (Jain et al. 2014). In this article, the study was expanded to gram-negative bacteria with t hybrid combination of these polymers. CNC has an additional benefit in that it has less cytotoxicity it can also be used as a wound dressing agent and inside the human body itself for removing bacteria and with a higher percentage of varying concentration as a disinfectant also (Park et al. 2014). So, from this research article study, it can be concluded that this composite emerges as a potential antibacterial agent.

Thus, in this research work the nanocomposite (CNC/Chitosan/Polyethylene glycol) was prepared by solution casting method. It was characterized by UV visible, SEM, FTIR, and XRD methods. The nanocomposite prepared in this research can be used as an antibacterial agent against bacteria. We studied the antimicrobial property of the prepared nanocomposite in various percentages.

2. EXPERIMENTAL SECTION

2.1. MATERIALS

The raw materials were taken starting with sodium chlorite (99% purity) from Sigma Aldrich, sodium sulphite (96%) from Merck, sodium hydroxide (98%) from CDH, sulphuric acid (99% purity) from Fisher, acetic acid (99% purity) from Thomas scientific, a dialyzing membrane purchased from Himedia of 60mm of capacity 1.99ml/cm, chitin already prepared and purified from shrimp shell, polyethylene glycol (99%) from Merck. Onion skin was chosen as the agricultural waste. It was used by washing under running water from tap and then from distilled water several times at last by grinding in a powder form.

2.2. SYNTHESIS OF CNC

Onion skin was used as agricultural waste. For removing dust from the onion skin, it was washed under running water after washed with distilled water and dried in an air oven at 105°C for 24 hours and grinded in powder form. It was bleached by using sodium chlorite of 0.7% w/v solution and the pH was maintained at 4 by using acetic acid. The mixture obtained was boiled for 2 hours with continuous stirring for removing the lignin part of the waste (Tillotson et al. 1997). The impurities were removed by filtering and washing the solution several times with distilled water. The residue obtained was boiled in 5% w/v sodium sulphite solution for about 5 hours. After boiling the holocellulose composed of hemicellulose and α-cellulose was obtained. This holocellulose was treated with 17.5% sodium hydroxide solution at 20°C for 45 minutes. This solution was filtered and treated with acetic acid to remove excess
sodium hydroxide solution and washed with distilled water as well. Cellulose microfibre (CMF) would be obtained after drying extracted cellulose at almost 105°C and 5 gm prepared CMF was taken and treated with sulphuric acid with continuous heat and stirring for 60°C for 3 hours. For cooling down the reaction temperature excess amount of distilled water was added to bring down the temperature of the reaction to room temperature. This obtained suspension was centrifuged at 4000 rpm for 20 min until the supernatant becomes cloudy at pH 5. The suspension would be sonicated and dialyzed against distilled water and deep freeze (-70°C) for obtaining CNCs.

2.3. PREPARATION OF CHITOSAN AND PEG SOLUTIONS

Using acetic acid as a medium the 1% solution of chitosan was prepared in it. For this process, 1% v/v acetic acid was prepared in distilled water and then 1 gm of chitosan was added to 0.25 gm of acetic acid solution with 2 hours of continuous stirring, and similarly, 1% w/v solution of PEG was prepared in deionized water with 1 hour of continues stirring. After preparation, both solutions were mixed with mechanical stirring by a glass rod and stirred for 4 hours for uniformity at room temperature.

2.4. DIFFERENT CONCENTRATIONS OF CNC SOLUTIONS AND THEIR NANOCOMPOSITES

The different concentration of CNC solutions was prepared with a variation of 1%, 3%, and 5% for the variation study of different properties related to it, and its composite was prepared with chitosan and PEG by casting solution method with distilled water at room temperature. The solution was stirred continuously for 8 hours for getting a homogenous and uniform solution. After completing the stirring of the solution, it was casted in a petri dish and dried in an oven at 37°C for 2 days. The obtained thin sheets were peeled off from the petri dish and the samples were named according to their concentrations like CPC 1% and CPC 3% shown in Table 1. Different concentrations of composite CNC would show different antimicrobial properties on the plate method.

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>CONCENTRATION /AMOUNT</th>
<th>CONCENTRATION /AMOUNT</th>
<th>CONCENTRATION /AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC</td>
<td>1% gm/vol</td>
<td>3% gm/vol</td>
<td>5% gm/vol</td>
</tr>
<tr>
<td>CHITOSAN</td>
<td>0.3 gm</td>
<td>0.3 gm</td>
<td>0.3 gm</td>
</tr>
<tr>
<td>PEG</td>
<td>0.3 gm</td>
<td>0.3 gm</td>
<td>0.3 gm</td>
</tr>
</tbody>
</table>

2.5. CHARACTERIZATION

2.5.1. SEM

SEM of the composite was performed for its images. It was done with a Zeiss device of magnification 550 to 100000 times by using different concentrations of composite (Prochon et al. 2021). The process
includes immersion of freeze-dried sample in liquid nitrogen for an average of 3 min before coating with gold. After this morphological study was done using these pictures by using a 10 kV secondary electron beam (Read et al. 1983).

### 2.5.2. FTIR

A Bruker of the Alpha II category with a range of 4000–400 cm\(^{-1}\) was used with a decreased total reflectance assembly. To determine the molecular functional groups of the prepared hydrogels (Das et al. 2021).

### 2.5.3. XRD

Bruker X-ray diffractometer was used to determine the XRD diffraction of the composite samples. The maximum output power of 3 KW or higher was obtained continuously (Zhao et al. 2006). The system should be fitted with a cooling system - external chiller/heat exchanger etc. for avoiding overheating and for smooth and stable running of the system.

### 3. RESULTS AND DISCUSSIONS

#### 3.1 UV-Spectroscopy

The UV spectrum of the nanocellulose composite shows a peak at 320 nm shown in Fig. 1. The nanocellulose begins to absorb strongly below about 200 nm, with indications of some absorption between 200 and 300 nm; and a weak absorption peak at 260 nm has been reported (Boopasiri et al. 2023). A weak absorption at about 300nm would expect from a synthesis of the individual spectra of the functional groups present in the molecule. The UV–vis results show that a strong absorption edge before 400 nm is significant and their absorption of ultraviolet light in the ultraviolet region shows a peak in that region (Abazari et al. 2014).

#### 3.2 FT-IR

The shifts in the peaks of CNC due to the incorporation of chitosan and PEG were to be estimated by the FT-IR spectra demonstrated in Fig. 2 (Pal et al. 2019). The spectra of three different concentrations were to be determined. The variation was in the concentration of CNC with chitosan and PEG fixed in concentration. The peaks at different wavelengths were obtained. The CNC/chitosan peaks were at 1161 cm\(^{-1}\) which can be ascribed to C-O an anti-symmetric stretching of bridge type. The stretching of PEG spectrum characteristics absorption bands of the -CH\(_2\) and -CH\(_3\) at 2883 cm\(^{-1}\) and -C-O-C- stretching at 1103 cm\(^{-1}\) and 960 cm\(^{-1}\) for both symmetrical and asymmetrical stretching (Gaina et al. 2016). Three different spectra were obtained for different concentrations of composite showed different potential antimicrobial activity.

#### 3.3 XRD
The crystalline structure of the nanocomposite can be studied by the X-ray diffraction method. The XRD pattern of the CNC composite had almost the same pattern and the results are shown in figure. The XRD results also revealed that the compound enzymatic hydrolysis treatment was such a mild process that the crystal structures of CNCs were not altered, and the strength of CNC would also be increased because the amorphous region of the composite decreased and crystalline region increased to a certain amount shown in Fig. 3 (Tong et al. 2020). The CPN 1%, CPN 3%, and CPN 5% exhibited their characteristic semicrystalline peaks around \(2\theta = 15, 16, \) and \(23^\circ\) respectively. Figure 3 presents the diffractograms for CNC/Chitosan/PEG composite films. The characteristic peak was obtained at about 18 and \(20^\circ\) which corresponds to 110 to 200 planes of crystallography and an additional peak was recorded at \(37^\circ\). There was an observed difference between the peaks pattern of CNC and CNC/chitosan/PEG composite which is significant in the sense of antibacterial activity, it showed that the composite had better properties for stopping the growth of bacteria in the medium. The pure chitosan had 20 and \(15^\circ\) peaks and the CNC/Chitosan/PEG composite had 23 and \(16^\circ\) variation. When the CNC fraction increased in the composite the peak shifts to a new position of \(23^\circ\) and it reveals that the composite is formed successfully in the matrix of chitosan and PEG. When the CNC composite was formed it increases the crystallinity of the composite and that of chitosan and PEG which also improves its quality as an antibacterial agent and the crystallinity index of 1%, 3%, and 5% may increase to 20, 21 and 36% with respect to the pristine form (Kargarzadeh et al. 2017). The results were in agreement that the composition had an effective increase in antimicrobial activity which can be observed from the studied results in the ahead section.

### 3.4 SEM OBSERVATIONS

The stability of the nanocomposite depends upon its aspect ratio, the ability of the solvent and groups present on surface to balance the attraction of hydrogen bonds of water and hydroxyl groups present in the composite, and the functionalization of the surface groups. This ability is present in CNC composite which is very important for its stability. Acid hydrolysis changed the crystalline micro fibrillated cellulose to nanocrystalline cellulose. Unmodified CNC films are brittle and can be easily fractured. So, the modification and functionalization of CNC is necessary for its commercial use. In the figure, it is seen that the surface of CNC gets harder and more stable with increasing concentration. In the composite of chitosan and CNC with PEG, it shows that the increased concentration of CNC in the casting solution leads to increased viscosity of the casting solution but this composite shows better results. SEM images showed a uniform distribution of the matrix of the composite in the images and a proper uniformity of the bonds in the composite shown in Fig. 4. This composite with these crucial properties has better antimicrobial properties (Vahedikia et al. 2019).

### 3.5 ANTIMICROBIAL PROPERTIES OF THE COMPOSITE

The antimicrobial property of the CNC-functionalized membrane was assessed by a plate counting method (Shin et al. 2022). A colony of different bacteria was grown in a Hilton Agar medium and was contacted with the membrane surface for 3 h at room temperature. Anti-microbial is important for
checking the activity of our prepared composite so that a new option may be given for the work. The bacteria suspension was spread on agar plates and incubated overnight at 37°C. Additionally, the cell morphology of bacteria following 3 h contact was observed under light. The CNC-PEG/Chitosan nanocomposite inhibits a variety of microorganisms, of which gram-negative bacteria (Escherichia coli) are more easily inhibited than other microorganisms, more prominently because the surface of the chitosan due to its zeta potential has a positive charge on its surface and is in contact with the negatively charged cell wall and these opposite charges neutralize each other and destroy the cell wall of bacteria because of this bacteria loss the viability and survival rate decrease. On the other hand, CNC surface has a negative charge on their surface so they can work against gram-positive bacteria. The antibacterial activity was determined by the ring formed around the discs in the medium present i.e., it shows the inhibition zone for the growth of bacteria and also depends on the type of bacteria used in the medium. Different bacteria have different activities in the medium shown in Fig. 5.

The bacterial cell wall is made up of lipopolysaccharide and this wall prevents the entry of any active component in the bacterial cell and prevents cell destruction. But these nanocomposites change the topography of the bacterial cell wall and develop an adhesion effect on their surface which might prove helpful in destructing the cell wall and membrane of the bacteria. Many parameters are the resulting factor for any kind of antibacterial performance of the prepared nanocomposite of CNC it includes the structure of the cell wall of bacteria, surface topography like bacteria is flagellated or non-flagellated and most important the release of antibacterial active compound from the wall of the composite which inhibits the growth of the bacteria. The bacteria used in this study i.e., E. coli has flagellated structure so it might be possible that the contact or adhesion of composite with cell wall was quite difficult but it may increase after a long-time exposure of both composite and bacteria with each other and this increases the inhibition chances of the bacterial cell by composite (Ahmad et al. 2021). For non-flagellated bacteria, it might be possible that it directly contacts the composite wall as a result the penetration of the component of the composite occurs directly which leads to the leakage of intracellular components and as a result death of bacteria.

4. CONCLUSION

A novel composite was prepared using natural and synthetic polymer in the form of bio nanocomposite film by using the solution casting method and it would be used as an antimicrobial agent. The structural characterizations showed that the chitosan and PEG are perfectly bio-compatible and miscible polymers because of hydrogen bond interactions between the carbonyl groups of PEG and amino and hydroxyl groups of chitosan, resulting in the formation of new biocompatible homogeneous mixture matrix for bio nanocomposite development with CNC.

When the CNC had been added to the chitosan/PEG blend, some additional hydrogen bonding can occur between the hydroxyl groups in nanocellulose and amino and hydroxyl groups of chitosan and between the carbonyl groups of PEG. Therefore, an interconnected structure is assumed to be formed in CS/PEG strengthened by nanocellulose. It was observed that the special two-dimensional morphology of the
nanocellulose, as well as its functionalized surface, provides well-homo dispersion, which leads to a high contact area in the CS/PEG matrix.

Bio nanocomposites of CPC1%, CPC3%, CPC5% exhibit superior antibacterial activity against gram-positive and gram-negative bacteria pathogens. Therefore, the prepared bio-nanocomposite membrane with features of good tensile, thermal property and have potential applications as an eventual antimicrobial material. The potential activity of the composite was shown by the inhibition zone seen in the petri dish coated with medium and the disc placed in them.

5. STATEMENT AND DECLARATIONS

5.1 Ethics approval and consent to participate

The authors declare that this submitted work is original and have not been published elsewhere in any form or language and all authors have consent to participate.

5.2 Consent for publication

All authors declare that they have consent for publication.

5.3 Availability of data and materials

No availability of data and materials.

5.4 Competing interests

“The authors have no relevant financial or non-financial interests to disclose and have no competing interest.”

5.5 Funding

“The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.”

5.6 Author Contributions for the publication.

“All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shivani Chaudhary. The first draft of the manuscript was written by Shivani Chaudhary and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

5.7 Acknowledgement

The authors wish to acknowledge The Head, Department of Chemistry, Dr. Bhimrao Ambedkar University, Agra, India to provide the necessary facilities, a healthy and clean environment for conducting the
research work.

REFERENCES


3459-3470.


Figures
Figure 1

UV spectrum of nanocellulose composites with polymers.
Figure 2

FTIR spectra of the composite of CNC with chitosan and PEG in three different concentrations 1%, 3%, and 5%.
Figure 3

XRD analysis of CNC/PEG/Chitosan

Figure 4
SEM images of CNC and chitosan solution with
(a) 1% CPC concentration composite SEM images
(b) 3% CPC concentration composite SEM images
(c) 5% CPC concentration composite SEM images

Figure 5

Antimicrobial activity on the petri dish plate with gram-negative bacteria.