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Evaluation of Antibacterial Activity, Biodegradability and Mechanical Properties of Chitosan Blended ZnO Biofilm for Food Packaging

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ABSTRACT

Chitosan and chitosan blended ZnO composite biofilms were developed by wet chemical method and the antimicrobial resistance of both the products have been studied. Chitosan powder, zinc acetate and sodium hydroxide with agar-agar (binding agent) were taken as starting materials for the above synthesis. Study on the surface functional groups has been carried out by Fourier Transformed Infrared (FTIR) Spectroscopy. The crystal structure along with phase characteristics and morphology has been investigated by X-ray diffraction (XRD). The mechanical and biodegradability measurements were performed to evaluate the strength of the prepared films. Antibacterial potency of the composite film has been studied towards both *Gram-negative* and *Gram-positive* bacteria such as *Escherichia coli* and *Staphylococcus aureus*. XRD measurement showed that the synthesized Chitosan/ZnO composite displays hexagonal wurtzite class of crystalline phase structure with size 62 nm. SEM study revealed that the homogenous and polymeric structure of the synthesized film. The bio-composite demonstrated improved and superior defensive properties to bacterial growth with higher zone of inhibition in comparison to pure chitosan case. It has also a greater tensile strength enhancing its application for antimicrobial packaging material.

Keywords: Chitosan, Biofilm, Antibacterial activity, Biodegradability, Mechanical property.

INTRODUCTION

Bionanocomposites (BNC) have displayed wide range of applications in the field of agriculture, drug delivery, food packaging and sensors[1-5]. These BNCs are the class of advance materials emerging out of the hybridization of biopolymers and inorganic nanoparticles. These materials show good biodegradability, biocompatibility, mechanical strength, and thermal stability[6]. Packaging industries extensively use these BNCs to avoid microbial contamination of food materials to maintain a good quality in terms of strength, better health and hygiene standard of customers[7]. But, the use of synthetic polymers in these composites as base material and their disposal have aroused a

crucial environmental concern and triggered the use for Generally Recognized As Safe (GRAS) material of polymer matrix for this purpose[8]. The introduction of bio-safe polymer packaging material has significantly increased the shelf life of products. In this regard, chitosan (CS) has been emerged as a suitable alternative biopolymer manifesting amazing properties due to its wide availability, biocompatibility, and biodegradability with excellent films producing ability[9,10]. It is a unique biopolymer having novel structural and physicochemical attributes with tremendous applicability in cosmetics, biomedicines, biosensors, food packaging, tissue engineering, bone and cartilage regeneration, wound healing and drug delivery[6,11,12]. Its cationic nature helps it to attach with negatively charged surfaces like cell membrane of bacteria[13]. But, there exists some limitation of its use due to some of its adverse features like high water vapor permeability and incompetent mechanical property[14]. So, fruitful exploitation of this gifted polymer is highly essential to maximize its role in industrial utilization. This is feasible because chitosan carries some chemically active functional groups like $^{-}NH_2$ and –OH which can assist in forming hybrid composites with other functional biomaterials[15]. Metals and metal oxide nanoparticles show excellent antimicrobial and nutritional properties which can be blended with the above biopolymers to form BNCs[16-17].

ZnO is regarded as a decent antibacterial agent with high inhibitory effect towards microorganisms[18]. It shows novel structural, optical and electrical properties due to some of its typical characteristics such as wide band gap, high excitonic binding energy. It is nontoxic, consumed as a nutrient and declared as GRAS compound by food and drug administration[19]. ZnO blended with CS will be having dominated effect in killing bacteria with an improved mechanical and barrier properties which is possible due to the reaction of hydroxyl and amino functional groups with chelating Zn^{2+} ions[20, 21]. CS-ZnO nanocomposites will be effective with diverse applicability in the areas of UV protection, dye degradation, biosensor, food packaging and drug releasing carriers[22-27]. To the best of our knowledge, a very few reports are available on the composite system explaining its role in the above fields. The present study is devoted to synthesize natural polymer based biofilms of Chitosan blended with ZnO and investigate their capability in defending the growth of pathogens like *Gram-negative* (*E. coli*) and *Gram-positive* (*S. aureus*) bacteria for effective use of these biofilms towards food packaging. The improved mechanical and biodegradable properties of the composite have also been studied. Utilization of renewable resources in a low cost blending procedure to prepare biofilms is also one of the main highlights of this work.

EXPERIMENTAL

Materials

The chemical reagents used for the experiment were zinc acetate, acetic acid, and sodium hydroxide along with nutrient agar which were purchased from M/s Sigma Aldrich and taken without further purification. Shrimp cells were collected from the local market near Chilika Lake, Odisha, India. Two bacterial strains *Escherichia coli* and *Staphylococcus aureus* were collected from Department of Pharmacy, Utkal University, Bhubaneswar, Odisha, India. Deionized water was used for preparing solution.

Preparation of chitosan powder

The collected shrimp shells were washed and dried in sunlight for two days and crushed into fine powder. Dried powder of shrimp shells were placed in plastic bottles and stored at ambient temperature. Chitosan(CS) powder was prepared by Brine process consisting of demineralization of shrimp shells, chitin processing (deproteinization) and chitosan processing (Deacetylation)[28].

Preparation of biofilms

Firstly, 1 g of chitosan flakes was dissolved in 50 ml of 2% (v/v) aqueous acetic acid using ultrasonication for 2 hours. Then, 0.4 g of agar powder was dissolved in 12 mL of warm water and slowly added to the above chitosan solution. 1 g of zinc acetate dehydrate was added to the above reaction mixture and subjected to sonication for 2 h to allow the dispersion of zinc acetate powder forming a viscous solution. The solution was then poured to plastic petri-dish without formation of air bubble for the preparation of film. The prepared film was kept for 24 h and dried in sunlight for 4 hours. The obtained films were kept inside a desiccator for future analysis. By following the above process, a pure chitosan film was also prepared without adding zinc acetate. The pure chitosan film was named as

C-1 and the chitosan/ZnO as C-2. The prepared biofilm at different stages of fabrication has been illustrated in Figure 1.

Fig.1. (a) Raw shrimp, (b) Demineralization, (c) Deproteination, (d) Deacytalytion (e) Chitosan Film (f) Chitosan: ZnO biofilm

Characterization of films

X-ray diffraction analysis of chitosan (C1) and chitosan blended ZnO composite film (C2) were carried out and the diffraction peaks were recorded in 20–80° of 2θ. The X-ray diffractometer (Model: Perkin Elmer) was equipped with Cu Ka source radiation (λ = 0.15406 nm). FTIR spectra of C1 and C2 samples were obtained by deploying a spectrometer (Perkin Elmer, Spectrum GX) in transmittance mode to analyze the existence of different functional groups. Surface morphology of samples was studied by SEM (Model: Hitachi SU-6600). Mechanical properties like tensile strength and elongation at break of films were evaluated by Universal Testing Machine (Model-3345, TFUC).

Antibacterial study

For these measurements, two types of pathogens such as; *Gram-negative* bacteria (*Escherichia coli*) and *Gram-positive* bacteria (*Staphylococcus aureus*) were selected and their defense mechanism against the prepared biofilms was investigated. For culture of these bacteria, a nutrient agar medium was established and agar well diffusion study method was adopted to study the activity of the pathogens[29]. The minimum inhibitory concentration (MIC) for both types of biofilms was determined by Broth dilution method[30]. In this study, four test tubes were taken with 10 mL of media and a loop full of culture was fed to all the test tubes. The test tube without bacterial suspension is considered as control. All the test tubes were kept for overnight incubation at 35°C temperature. The absorbance value was recorded for the MIC determination.

RESULT AND DISCUSSION

FTIR spectra of pure chitosan and chitosan blended ZnO (C1 and C2) composite biofilms are shown in Fig. 2. The spectra were recorded in 4000 – 400 cm^{-1} range in transmittance mode. It has been observed that two bands in the 3000–3500 cm^{-1} are observed for the sample C1 which have been ascribed to the OH and NH groups[6]. The same have been observed in the case for C2 but with different intensities. The bands at 2895 cm^{-1} and 2760 cm^{-1} present in the spectra of both C1 and C2 corresponds to the symmetric and asymmetric stretching vibrations of $-CH_2$ groups[6]. The band around 1632 cm^{-1} has been assigned to C=O stretching modes and the peak corresponding to 1585 cm^{-1} belongs to N-H bending modes. The sharp peak around 1370 cm^{-1} present is C1 indicates the existence of acetamide groups. This peak is absent in C2 which indicates that the chitosan sample is not fully deacetylated. The assimilation of ZnO nano fillers into the polymer matrix has modified the IR spectra. The same can also be understood from the changing width of the bands corresponding to $-NH_2$ and -OH groups in the composite sample as compared to pure chitosan. This can also be described by the decrease of hydrogen bonds between $-NH_2$ and -OH groups with the integration of ZnO particles into the polymer matrix. The skeletal stretch of C–O in polysaccharides is present in the region (1000 – 1090 cm^{-1}) in the FTIR patterns of both C1 and C2[31]. The sharp band at 480 cm^{-1} is ascribed to Zn-O-Zn bonds present in C2 which confirms the formation of ZnO in the composite.

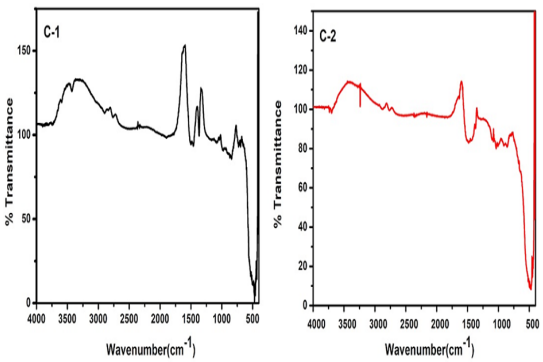

Fig. 2. FT-IR spectra of pure Chitosan (C1) and Chitosan blended ZnO (C2) composite film

The XRD patterns of pure chitosan (C1) and chitosan ZnO (C2) composites were recorded and have been illustrated in Fig. 3. The XRD pattern of C1 shows two major peaks around 11° and 21° which belong to chitosan. The structure of chitosan is basically crystalline polysaccharide in nature which is due to its regular chain[31]. The peak originated at a 2θ value of 11° is the signature of the water molecules into bounded to the crystal lattice[32]. The second peak at 2θ = 21° which is observed as broad peak and highest intensity is the characteristic peak of chitosan chains. This chitosan chain is aligned through hydrogen bonds which are strong intermolecular and intramolecular in nature[33]. On the other side, the diffraction pattern of C2 shows the presence of several peaks at positions such as; 32.14, 34.12, 36.99, 46.350 of 2θ which are related to the crystallographic planes (100), (002), (101) and (102) respectively of wurtzite ZnO as per the data base (JCPDS No. 36-1451)[34,35]. The average particle size of ZnO crystallites in the composite was calculated using Debye-Scherrer equation and found to be 62 nm. The characteristic peaks of chitosan are also observed in the composite but with reduced intensity. So, incorporation of ZnO nano-fillers may have extended the spacing between the chitosan chains by the interacting with the amino groups present in chitosan[6]. Introduction of ZnO nanoparticles has improved the semi-crystallinity of chitosan. The position and existence of diffraction peaks of both chitosan and ZnO in the composite highlight the dispersion of crystalline ZnO nanoparticles on the chitosan matrix.

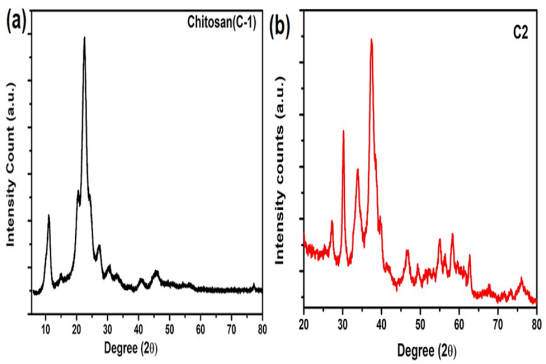

Fig. 3. XRD image of Chitosan (C1) and chitosan: ZnO (C2) biofilm

The surface morphology of films (C2) was evaluated using scanning electron microscopy. In the SEM photographs, presence of nano ZnO particles on the polymer surface was appeared as homogeneous with no interface layer as shown in Fig. 4. A high resolution image shows the ZnO nanoparticles in the form of nanosheets which are showing flowerlike structure. These nanoparticles were strongly adhered to the chitosan matrix and modified physical and chemical features of chitosan.

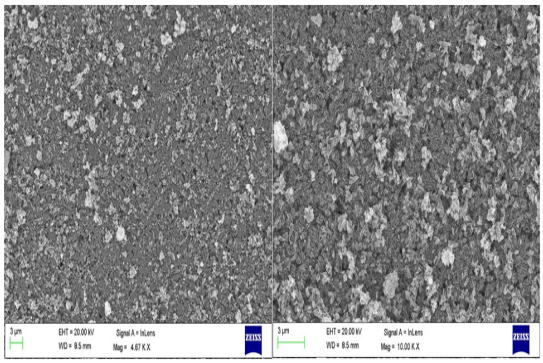

Fig. 4. FESEM images of C2 biofilm

The mechanical properties of the bionanocomposite film were analyzed to study its durability for useful packaging application. Mechanical properties such as, tensile strength and elongation at break have been calculated (Table 1). These properties are very important characteristics for packaging material. These features determine and specify the stretchability prior to breakage and film strength[36,37]. It has been observed that these properties significantly enhanced for the bionanocomposites as compared to pure chitosan sample. The values of tensile strength for C2 are found to be double as compared to C1 indicating the increased strength of the composite film. When ZnO nanoparticles are incorporated into the chitosan structure, an intermolecular cross link is formed between the polymer chains which increase the strength of the materials. The value of elongation at break (%) for C1 is found to be higher than C2 which indicates the formation of good blended film.

Table 1: Mechanical properties of films

Sample	Sample Tensile strength (MPa)	Elongation at break (%)
Chitosan Film (C1)	11.44±0.62a	25.42±0.95a
Chitosan :ZnO Film (C2)	26.1± 0.96b	22.62±0.577b

The biodegradable (BOD) properties of the composite biopolymer films were analyzed in aerobic liquid medium. This was followed by measuring the

BOD_5 by microorganisms present in 5 g of compost which was prepared according to the ISO standard 14851[38]. The BOD_5 values were studied after seven days of incubation. A multiplication and growth of aerobic bacteria present in the compost are observed on the prepared biofilm. It shows good BOD properties of the composite film which occurred due to the ability of microorganisms to assimilate on chitosan as an only carbon source. The effective degradation property of the biofilm with time has been depicted in Figure 5.

Fig. 5. Biodegradability of prepared bio film after 7 days in compost soil

The antibacterial assay of pure chitosan along with chitosan/ZnO composite films was tested against *Gram-negative* bacteria *E. coli* and *Gram-positive* bacteria *S. aureus* by agar well diffusion method at different concentration. The diameter of the inhibition zones around each well is measured in mm and given in Table 2. The results indicates that the degree of zone of inhibition is more against gram negative bacterial strains as compared to the gram positive bacteria for both the films (C1 & C2). But, the value of zone of inhibition is high in case of C2 film in comparison to C1 film. This may be due to the effect of nano sized ZnO fillers present in the composite film C2. ZnO nanoparticles having larger surface area can tightly bind to the surface of the bacterial cells to disrupt the membrane. Minimum inhibitory concentration (MIC) of the C1 and C2 films was determined by using broth dilution method. MIC was determined at different concentrations like 2 mg/mL, 4mg/mL, 6 mg/mL, 8 mg/mL of broth solution and control against *Gram-positive* and *Gram-negative* organisms. The microorganisms such as; *E. coli* and *S.aureus* demonstrated MIC at 6 mg/mL and 4 mg/mL for C1 and C2 films respectively. The results show a decrease in MIC value of 2 mg/mL for C2 in comparison to C1 between concentrations ranging from 2 mg/mL to 8 mg/mL. The details of the parameter obtained from the study have been given in Table 2. It shows that the ZnO nanoparticle induced bionanocomposite film is more effective in inhibiting and growth of pathogens as compared to pure chitosan film. So, these films will be more effective in application for food packaging.

Table 2: Zone of inhibition and Minimum inhibitory concentration of prepared film

Sample Name	*Escherichia coli* (zone of inhibition mm)				*Staphylococcus aureus*(zone of inhibition mm)				MIC
Different conc. of broth Soln	10 mg/mlL,	20 mg/mL,	30 mg/mL,	40 mg/mL	10 mg/mL,	20 mg/mL	30 mg/mL	40 mg/mL	mg/mL
C1	7 ± 0.25	15 ± 0.25	20 ± 0.25	28±0.25	5 ± 0.15	9±0.15	13 ± 0.15	17 ± 0.15	6
C2	9 ± 0.25	19 ± 0.25	25 ± 0.25	31±0.25	6 ± 0.25	12 ± 0.25	18 ± 0.25	21 ± 0.25	4

CONCLUSION

In the present study, chitosan and chitosan-ZnO composite biofilm were prepared by blending the chitosan powder with zinc acetate which has been found as an effective method for preparing bionanocomposites. Various properties of the composite film along with pure chitosan have been studied to understand the structural, mechanical and antibacterial properties. FTIR spectra demonstrate the formation of the composite film with the presence functional groups characteristic to both the samples. X-ray diffraction results represent the semicrystalline nature of chitosan upgraded to good crystallinity in composite with the presence of hexagonal shaped ZnO nanoparticles with size 62 nm. Introduction of ZnO nanofillers has extended the chain spacing in chitosan forming the composite structure. The mechanical and biodegradability measurement are performed to know the strength of the films and their effective biodegradation. The chitosan blended ZnO nanocomposite film shows better tensile strength and higher biodegradability in comparison to pure chitosan. Antibacterial activity of both the prepared films are studied and concluded that the biocomposite film is most effective to *Gram-negative E. coli* bacteria in comparison to *S. aureus*. The composite film also shows higher zone

of inhibition value in proportionate to pure chitosan film. Above properties of the bionanocomposite will be accountable for diverse applications in the field of agriculture and food packaging.

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Competing Interest

The authors do not have any conflict of interests with the contents in the paper.

REFERENCES

1. Hamidi, M.; Azadi, A.; Rafiei, P. *Adv. Drug Deliv. Rev.*, **2008**, *60*, 1638–1649.
2. Rhim, J.W.; Park, H.M.; Ha, C.S. *Prog. Polym. Sci.*, **2013**, *38*, 1629–1652.
3. Bortolin, A.; Aouada, F.A.; Mattoso, L.H.C. *J. Agr. Food Chem.*, **2013**, *61*, 7431–7439.
4. Nogi, M.; Yano, H. *Advanced Materials.*, **2008**, *20*, 849-1852.
5. Fernandes, E.M.; Pires, R.A.; Mano, J.F. *Prog. Polym. Sci.*, **2013**, *38*, 1415–1441.
6. Toiserkani, H. *Composite Interfaces.*, **2016**, *23*(3), 175–189.
7. Dashipour, A.; Razavilar, V.; Hosseini, H.; Shojaee-Aliabadi, S.; German, J.B.; Ghanati, K.; Khakpour, M.; Khaksar, R. *Food science and Technolo.*, **2015**, *72*, 606–613.
8. Gutierrez, L.; Sanchez, C.; Batlle, R.; Nerin, C. *Trends Food Sci. Technol.*, **2009**, *20*, 92–99.
9. Bourtoom, T.; Chinnan, M.S. *Food Sci. Technol.*, **2008**, *41*, 1633–1641.
10. Noshirvania, N.; Ghanbarzadeha, B.; Mokarrama, R.R.; Hashemib, M.; Comac, V. *International Journal of Biological Macromolecules.*, **2017**, *99*, 530–538.
11. Bowman, K ; Leong, K.W. *Int. J. Nanomed.*, **2006**, *1*, 117–128.
12. Liu, M.; Wu, C.; Jiao, Y. *J. Mater. Chem.* B., **2003**, *1*, 2078–2089.
13. Dhillon, G. S.; Kaur , S.; Brar , S. K.; *Int. Nano Lett.*, **2014**, *4*, 107-109.
14. Mujeeb Rahman, P.; Abdul Mujeeb, V.M.; Muraleedharan, K.; Thomas, S. K. *Arabian Journal of Chemistry.*, **2018**, *11*, 120–127.
15. Dutta, P.K.; Tripathi, S.; Mehrotra, G.K.; Dutta, *J. Food Chem.*, **2009**, *114*, 1173–1182.
16. Ghanbarzadeh, B.; Oleyaei, S.A.; Almasi, H. Crit. *Rev. Food Sci. Nutr.*, **2015**, *55*, 1699–1723.
17. Pantani, R.; Gorrasi,G.; Vigliotta, G.; Murariu,M.; Dubois, P. *Eur. Polym. J.*, **2013**, *49*, 3471–3482.
18. Sirelkhatim, A.; Mahmud, S.; Seeni, A.; Kaus, N.H.M.; Ann, L. C.; Khadijah, S.; Bakhori, M.; Hasan, H.; Mohamad, D. *Nano-Micro Lett.*, **2015**, *7*(3), 219–242.
19. Shahmohammadi Jebel, F.; Almasi, H. *Carbohydr. Polym.*, **2016**, *149*, 8–19.
20. Youssef, A.M.; El-Sayed, H.; El-Sayed, S.M.; Salama, H.H.; Dufresne, A. *Carbohydr. Polym.* **2016**, *151*(20), 9–19.
21. Bajpai, S.K.; Chand, N.; Chaurasia, V. *J. Appl. Polym. Sci.*, **2009**, *115*(2), 674–683.
22. Zhao, M.; Huang, J.; Zhou, Y. *Biosens. Bioelectron.*, **2013**, *43*, 226–230.
23. Youssef, A.M.; Abou-Yousef, H .; El-Sayed, S.M.; *Int. J. Biol. Macromol.*, **2015**, *76*, 25–32.
24. Wang, Y.; Zhang, Q.; Zhang, C.L. *Food Chem.*, **2012**, *132*, 419–427.
25. Haldorai, Y.; Shim, J. *J. Compos. Interfaces.*, **2013**, *20*, 365–377.
26. El Shafei A.; Abou-Okeil A. *Carbohydr. Polym.*, **2011**, *83*, 920–925.
27. Yuan Q.; Hein , S.; Misra, R.D.K. *Acta Biomater.*, **2010**, *6*, 2732–2739.
28. Alca Ahing, F.; Wid, N. *Transactions on Science and Technology.*, **2016**, *3*, 227–237.
29. Geoprincy, G.; Nagendhra G. N.; Renganathan S. *Int. J. Pharm. Pharm. Sci.*, **2012**, *4*, 544-548.
30. Umamaheswara Rao V.; Nagababu P. *Int. J. Rec. Sci. Res.*, **2015**, *6*, 2783-2789.
31. Leceta, I.; Guerrero, P.; Ibarburu, I. *J. Food Eng.*, **2003**, *116*, 889–899.
32. Ogawa, K.; Yui, T.; Miya, M. *Biosci. Biotechnol. Biochem.*, **1992**, *56*, 858–862.
33. Yamaguchi, I.; Tokuchi, K.; Fukuzaki, H.; *Biomed, J. Mater. Res. Exp.*, **2001**, *55*, 20–27.
34. Sahu, D; Panda, N.R; Acharya, B. S. *Mater. Res. Express.*, **2017**, *4*, 114001.
35. Dash, Debasrita Dash; Panda, N. R; Sahu, Dojalisa, *Appl. Surf. Sci.*, **2019**, *494*, 666-674.
36. Krochta, J.M.; Johnston, C.D.M. *Food Technology.*, **1997**, *51*, 61-74.
37. Pranoto, Y.; Salokhe, V.M.; Rakshit, S.K. *Food Research International.*, **2005**, *38*, 267-272
38. I.T. 61/SC 5/WG 22, ISO 14851:1999, Multiple. Distributed through American National Standards Institute (ANSI)., **2007**.